Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research

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Summary
Development of new biomarkers is a constantly evolving field of research endeavor in psychoneuroendocrinology. Salivary biomarkers have received special attention since they are readily accessible and easily obtained. Salivary alpha-amylase (sAA) has been proposed as a sensitive biomarker for stress-related changes in the body that reflect the activity of the sympathetic nervous system (SNS), and a growing body of research is accumulating to support the validity and reliability of this parameter. However, questions remain to be answered before sAA can be accepted as an index of SNS activity. This review describes sAA as an emerging biomarker for stress and provides an overview of the current literature on stress-related alterations in sAA. It critically discusses how sAA might reflect changes in the autonomic nervous system. Finally, current and future fields for the application of sAA measurement are outlined.

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1. Introduction

In recent years, salivary measures have become increasingly important in psychoneuroendocrinological research. In the early 1970s, Brown suggested that changes in saliva parameters could be regarded as an "index of specific states of psychopathology" (Brown, 1970, p. 66). While research is not yet quite at the point to use salivary measures in the way Brown suggested, the scientific understanding of various salivary parameters is progressing rapidly. However, apart from the analysis of hormones such as cortisol and DHEA (see, e.g. Vining and McGinley, 1987; see, e.g. Kirschbaum and Hellhammer, 1994), few other salivary components have been taken into consideration as meaningful physiological markers in psychoneuroendocrinological research. A specific focus of interest within this field of research is on stress. In stress research, subjects might be examined either in the field or in the laboratory. Whichever setting is chosen, valid and reliable measures of changes associated with stress must be applied. Moreover, the simple handling and easy sampling of a stress measure is of utmost importance. A wide array of possible parameters indicating stress-related changes has been proposed over the years. Some have disappeared into oblivion; others have endured for decades and are still being used. Since stress is a multi-faceted phenomenon, it requires a multidimensional measurement approach. As a consequence, research can gain from additions to the canon of psychobiological parameters. One parameter that has been suggested to reflect stress-related changes in the body is the salivary enzyme alpha-amylase (Chatterton et al., 1996; Nater, 2004; Rohleder et al., 2004; Granger et al., 2007). Salivary alpha-amylase (sAA) release is known to be elicited by activation of the autonomic nervous system (ANS) which controls the salivary glands.

The aim of this review is to assess whether sAA should be incorporated in the canon of psychoneuroendocrinological parameters measuring stress. The accompanying review by Rohleder and Nater (2009) provides a detailed account of methodological issues that arise when measuring sAA.

2. What is salivary alpha-amylase?

sAA (α-1,4-α-D-glucan 4-glucanohydrolase; EC 3.2.1.1) is one of the most important enzymes in saliva. The enzyme was first described in saliva by Leuchs in 1831 (Zakowski and Bruns, 1985). It consists of two families of isoenzymes, of which one set is glycosylated and the other contains no carbohydrate. The molecular weight of the glycosylated form is about 57,000; that of the non-glycosylated form is about 54,000. sAA accounts for 40% to 50% of the total salivary gland-produced protein, most of the enzyme being synthesized in the parotid gland (80% of the total) (Zakowski and Bruns, 1985; Makinen, 1989). It is a calcium-containing metalloenzyme that hydrolyzes the α-1,4 linkages of starch to glucose and maltose. It is known to be mainly involved in the initiation of the digestion of starch in the oral cavity. However, sAA has also been shown to have an important bacterial interactive function (Scannapieco et al., 1993).

2.1. Anatomy and physiology of the salivary glands

In this section, we review the salivary glands and their major product, saliva. A clear understanding of how and where saliva is produced and how this process is controlled is essential for gaining insight into the relationships between salivary parameters and stress.

There are three major salivary glands on each side of the face: the parotid, submandibular, and sublingual glands. In addition, numerous minor glands contribute to salivary outflow. The salivary glands are part of the digestive tract. Glands are comprised of different types of cells: acinar cells, various duct system cells, and myoepithelial cells (Humphrey and Williamson, 2001).

2.1.1. Saliva

The components of saliva are produced primarily by acinar cells. Saliva is the main product of the salivary glands. It is a clear, slightly acidic mucouser exocrine fluid comprising a complex mixture of secretions from major and minor salivary glands and gingival crevicular fluid (Humphrey and Williamson, 2001; Kaufman and Lamster, 2002). This mixture of fluids derived from different glands is called "whole saliva", whereas the fluid secreted by single glands is called "duct saliva" (Edgar, 1992). The constant flow of saliva from the mouth into the gut has a protective function. This flushing effect transfers, for example, food debris and exogenous and possibly noxious agents into the gut (Tenovuo, 1998). Saliva is routinely categorized as resting (unstimulated) or stimulated. Under unstimulated conditions, 20% of saliva is derived from the parotid, 65% from the submandibular, and 7–8% from the sublingual glands. The minor glands contribute about 10% to whole saliva. Under stimulation, the contributions of each
gland change, with the parotid contributing more than 50% of total salivary secretions (Sreebny, 2000; Humphrey and Williamson, 2001).

2.1.2. Secretory pathways
For a detailed understanding of the secretory pathways of the salivary glands, a distinction must be drawn between neural and cellular mechanisms of secretion. This section provides a detailed account of these pathways.

Acinar cells are innervated by both the sympathetic and the parasympathetic branches of the ANS (Emmelin, 1987). Autonomic nerves are adjacent to both acinar and ductal cells. The afferent pathways for taste are via the facial and glossopharyngeal nerves to a solitary nucleus in the medulla. There is also input from higher centers in response to smell, sight, etc. The parasympathetic efferent pathways for the sublingual and submandibular glands are from the facial nerve via the submandibular ganglion; for the parotid gland they are from the glossopharyngeal nerve via the otic ganglion. The sympathetic postganglionic pathways are from the cervical ganglion of the sympathetic chain. Neurotransmitters are the first messengers in the communication pathway between nerves and secretion. Neurotransmitters exert their activity at the cell membrane; they communicate with intracellular second messengers that have direct control of secretory processes (Smith, 1996). Released in response to secretory stimuli, they bind to specific receptor proteins on the basolateral membrane, causing acute elevation of intracellular Ca. This results in large-scale fluid and electrolyte transport, and modest exocytosis of stored protein. Norepinephrine from sympathetic neurons binds to both alpha- and beta-adrenergic receptors on the acinar cell. Alpha-receptor activation is linked to elevation of intracellular Ca, while beta-receptor activation causes elevation of intracellular cyclic adenosine monophosphate (cAMP), which is linked to the secretion of salivary proteins that are stored in membrane-bound secretory granules (Baum, 1993; Castle and Castle, 1998).

The secretory process of salivary proteins may be divided into the three stages: (1) synthesis, (2) packaging and storage, and (3) release. Each of these stages is regulated by phosphorylation of target proteins brought about by a protein kinase such as cyclic AMP-dependent protein kinase (protein kinase A) (Smith, 1996).

2.2. Pathways of alpha-amylase secretion
In acinar cells, release of salivary components is under control of neuronal stimuli. Classic neurotransmitters and specific bioactive peptides serve as the main stimuli for sAA secretion. This section presents studies in rats and humans that used blocking or stimulating pharmacological agents or electrical stimuli to gain a better understanding of sAA secretion.

2.2.1. Rat studies
The findings of Batzri and co-workers are among the first to indicate the specific involvement of the ANS in the secretion process of sAA. In studies on slices of the parotid gland in rats, they found that beta-adrenergic receptors caused secretion of sAA (Batzri and Selinger, 1973; Batzri et al., 1973; Selinger et al., 1973). In a subsequent study, Anderson et al. (1984) examined differential contributions of the two branches of the ANS to sAA secretion. They found that sympathetic stimulation in unconscious rats led to the secretion of parotid saliva characterized by low salivary flow rate and high total protein and amylase concentrations. In contrast, parasympathetic stimulation induced a rich flow of saliva with low protein content, with mean concentrations of sAA approximately 1% of those measured in sympathetically stimulated saliva. After performing adrenalectomy, the authors observed significant reductions of sAA levels upon parasympathetic stimulation of the parotid gland, suggesting that circulating catecholamines (originating from the adrenals) might play a specific role in sAA secretion. Asking (1985) set out to compare sympathetic and parasympathetic stimulation of the parotid gland in the rat both separately and concomitantly. As in the Anderson et al. study, sympathetic stimulation caused a low flow of saliva containing amylase in very high concentration, whereas parasympathetic stimulation produced saliva with a low concentration of amylase. After combined sympathetic and parasympathetic stimulation, however, sAA secretion was much higher than the sum of the two separate stimulation concentrations. Using the beta-1-antagonist pafenolol, Asking (1985) showed that the higher sAA secretion due to sympathetic stimulation superimposed on parasympathetic background stimulation was elicited via beta-1-adrenoceptors. In a subsequent study (Asking and Gjorstrup, 1987), these findings were replicated in that sympathetic and parasympathetic stimulation led to equal sAA concentrations and the combination of the two drastically increased sAA concentrations. The authors found that sAA secreted on sympathetic activation consisted of pre-formed amylase, stored in granules, that was not replaced during secretory activity under these conditions. Ongoing sympathetic stimulation led to an attenuation of sAA concentration. Asking and Proctor (1989) studied the effects of prolonged parasympathetic nerve stimulation in rat parotid glands on alpha-amylase content in saliva and glands. The concentration and total output of sAA were measured throughout a stimulatory period of 120 min, as were glandular concentrations of sAA. The results suggest that amylase stores are much more rapidly replenished by synthesis during parasympathetic than during sympathetic activity, whereas sympathetic nerve excitation causes a pronounced loss of amylase-containing acinar granules. No such loss could be detected on parasympathetic stimulation, although the output of amylase was about the same as during sympathetic stimulation.

In a further examination of the adrenergic mechanisms involved in sAA secretion, Skov Olsen et al. (1988) found that stimulation of the beta-adrenergic receptors in rats increased the concentration of sAA by a factor of 30, while stimulation of the alpha-adrenergic receptors increased the concentration of sAA by a factor of 10. Experiments with several substances showed that sAA secretion was mainly contributed by beta-1-adrenergic mechanisms. Following up on these studies, Busch et al. set out to investigate the differences in release of sAA by the parotid and the submandibular gland in rats. They found that submandibular sAA did not respond with an increase to the administration of isoproterenol, a beta-adrenergic agonist, whereas parotid
sAA did. This effect was inhibited by the selective beta-1-antagonist atenolol, but not by the beta-2-antagonist butoxamine (Busch et al., 2002).

In sum, these findings demonstrate the involvement of the ANS, and the sympathetic nervous system in particular, in the release of sAA, with beta-adrenergic mechanisms as the main contributing factor in sAA secretion. However, findings from animal studies are not readily transferable to humans. Furthermore, stimulation of the sympathetic nerves in isolation, as used to elicit autonomic activation in the studies described above, cannot be considered a physiological reflection of secretory processes in vivo. In humans, this approach is not feasible. Use of pharmacological agents may be a more appropriate methodological approach for determining the role of the ANS in the secretion of sAA in humans, as the following studies show.

2.2.2. Human studies
Speirs et al. (1974) provoked a sympathetic response either by immersing subjects up to the waist in cold water (4–5 °C) or by administering isoprenaline and propranolol (both are beta-adrenergic blockers). Exposure to cold water and isoprenaline raised sAA concentrations in the parotid gland, whereas propranolol led to a reduction of sAA concentrations. These results offered first evidence for the sympathetic control of sAA secretion in humans (Speirs et al., 1974). As shown in a number of the animal studies described above, stimulation of beta-adrenergic receptors modulates the synthesis and the release of sAA. Laurikainen et al. (1988) studied the effects of timolol maleate, a widely used beta-blocking agent, on the quantity and quality of saliva secretion controlled by beta-adrenoceptors in healthy subjects. Alpha-amylase concentrations in parotid saliva decreased significantly after drug intake, thus corroborating similar findings from rat studies. Nederfors and coworkers investigated the effects of therapeutic doses of the selective beta-1-antagonist atenolol and the non-selective beta-antagonist propranolol on stimulated glandular saliva. Atenolol, but not propranolol, resulted in a decrease of parotid amylase in the morning, and in a decrease of submandibular/sublingual amylase both in the morning and at lunch time (Nederfors et al., 1994). The authors were able to replicate their findings in a well-controlled study on human hypertensive subjects with the selective beta-1-antagonist metoprolol (Nederfors and Dahlöf, 1996). Thus, these studies demonstrate the importance of beta-adrenergic mechanisms of sAA secretion in humans, as already shown in rats. Following up on these findings, van Stegeren et al. (2006) conducted a placebo-controlled double-blind study using propranolol in a stress–rest protocol. While the placebo group showed a substantial increase in sAA subsequent to the stress test, the sAA response in the propranolol group was attenuated. Finally, Ehler et al. (2006) proposed that sAA increases might reflect the interaction of stress-dependent sympathetic and parasympathetic stimulation via central nervous noradrenergic input. To examine this hypothesis, they assessed the indirect effect of yohimbine hydrochloride, an alpha-2-adrenergic receptor antagonist, on sAA release in a randomized placebo-controlled study. The results showed significant increases in sAA concentration in the yohimbine condition relative to the placebo condition.

2.2.3. Summary
The results of studies on animals and humans indicate that the ANS plays a powerful role in the secretion of sAA, with contributions of both the alpha-adrenergic and the beta-adrenergic mechanisms. These findings suggest that sAA might be regarded as an indirect indicator of autonomic activation.

3. Stress-induced salivary alpha-amylase secretion
As outlined in the previous sections of this review, the release of sAA is governed by activation of the ANS. Thus, an increase in sAA may be expected during psychological stress, when autonomic activation is high. The following section reviews findings on sAA responses due to psychological stress.

3.1. Early findings
The idea that sAA may serve as indicator of psychological stress emerged in the late 1970s. To our knowledge, the relationship between sAA and psychological stress was first examined in a study by Gilman et al. (1979a), in which subjects were exposed to eight days of hyperbaric pressure. Findings showed increased concentrations of sAA. The authors concluded that these increases were not only due to the hyperbaric exposure and its effects on the ANS, but also due to the psychological stress caused by the procedure itself. In the 1980s, Donald Morse and his group undertook a series of experiments with the goal of examining the impact of stress on various salivary parameters. Their findings proved quite counterintuitive for researchers interested in salivary alpha-amylase: Morse et al. found that relaxation, not stress, leads to increases in sAA (Morse et al., 1983a,b). However, these studies were methodologically flawed in that there was no control condition or random allocation to treatment groups. Furthermore, the “relaxation condition” always followed immediately after the “stress condition,” suggesting that the increases in sAA might be attributable to a stress response occurring after the stressor. Since Morse concluded that salivary parameters were valid and reliable indicators for psychological stress and relaxation, attempts were made to re-evaluate this statement by comparing the usefulness of salivary indicators with other known psychophysiological parameters (Borgeat et al., 1984). Borgeat et al. exposed subjects to relaxation (Jacobson’s progressive muscle relaxation) and to stress (written exercises, mental arithmetic task). In addition to salivary parameters such as sAA, they measured frontal muscle EMG, skin conductance, heart rate, and skin temperature. The interventions resulted in clear and distinct reaction patterns in the “classical” psychophysiological parameters, but no salivary changes were observed. Thus, initial findings on stress-related changes in sAA did not correspond with the physiological literature outlined above. However, further studies conducted under more rigorous conditions and employing scientifically advanced designs, e.g. including control groups etc., showed clear stress-related increases in sAA, as summarized in the next section.
3.2. Effects of psychological stress on salivary alpha-amylase

Whereas other stress-related factors in saliva, such as salivary cortisol (Kirschbaum and Hellhammer, 1989, 1994), received widespread scientific attention in the following decade, interest in sAA as a stress marker was not sparked again until Chatterton and colleagues published their findings of increases in sAA in a variety of stressful conditions (Chatterton et al., 1996). Beyond this seminal study, a number of studies applying psychological stress protocols have shown that sAA is highly sensitive to stress-related events. This section briefly reviews these studies.

In a study of subjects exposed to an academic examination, Bosch et al. measured several salivary parameters including sAA (Bosch et al., 1996). Whole unstimulated saliva was taken 30 min before the examination, 2 weeks later, and 6 weeks later. Results indicated an increase in concentration and output of sAA during the stress condition, while the salivary flow rate did not change. In a separate analysis, the authors found that the number and severity of critical life events was related to sAA activity, thus suggesting that everyday stress also contributes to stress-dependent changes in salivary parameters (Bosch et al., 1998). Chatterton et al. (1997) studied subjects preparing for skydiving and found increased sAA prior to the jump than in control subjects who did not jump. The highest levels were observed immediately after landing. Using a stressful video game to induce laboratory stress, Skosnik et al. found a significant increase in sAA after the 15-min stressor (Skosnik et al., 2000). In a further study employing a laboratory task to induce acute stress (Bosch et al., 2003), whole unstimulated saliva was measured before, during, and after an active memory task, passive viewing of a gruesome video, and a control condition. sAA output differed significantly between the three conditions, with highest levels being measured during the passive video task. A decrease in sAA output was found in the active memory task, suggesting that the sAA response depends on the nature of the stressor and the active or passive coping capabilities of the subjects. Another research group used a stressful and a relaxing video to induce stress and rest conditions and directly compared the effects of both on salivary cortisol and alpha-amylase responses in whole unstimulated saliva (Takai et al., 2004, 2007). The stressful video induced marked increases in both cortisol and sAA, whereas the relaxing video produced no changes in salivary cortisol, but significant decreases in sAA concentrations. Noto and colleagues examined sAA levels during a mental arithmetic task (Noto et al., 2005) and observed significant increases in sAA.

sAA increases have also been reported in response to other psychologically stressful conditions, such as experience of medical procedures (Yamaguchi et al., 2006a), adverse musical stimuli in men (Nater et al., 2006a), mothers watching their children being exposed to a stressful task (Granger et al., 2006), the cold pressor test (West et al., 2006; van Stegeren et al., 2008), achievement and interpersonal stress (Stroud et al., 2006), a driving simulation (Yamaguchi et al., 2006b), use of a noise burst and infant arm restraint in depressive mothers (Shea et al., 2006), execution of neck/face surgery by medical trainees (Yamakage et al., 2007), a mental arithmetic task (Goi et al., 2007), oral academic examination (Schoofs et al., 2007), a standardized test battery in toddlers (Fortunato et al., 2008), affective picture viewing (van Stegeren et al., 2008), and in a peer rejection paradigm in adolescents (Stroud et al., 2009). Interestingly, only a few studies have found no changes in sAA in response to stressful stimuli including noise (Morrison et al., 2003), the heel prick test in neonates (Schaffer et al., 2008), or a strange situation paradigm (Hill-Soderlund et al., 2008). The scarcity of non-significant changes suggests that sAA is indeed a highly sensitive parameter reflecting changes caused by psychological stressors.

A number of studies have used a standardized psychosocial stress test, i.e., the Trier Social Stress Test or TSST (Kirschbaum et al., 1993), to assess the usefulness of sAA as a biomarker for psychologically induced stress. The TSST comprises a mock job interview and a mental arithmetic task, both performed in front of an audience, leading to marked increases in both psychological and physiological stress indicators. In a pilot study, Rohleder et al. (2004) found marked increases in sAA due to the TSST. These findings were corroborated in a subsequent study employing the TSST and a rest condition (Nater et al., 2005) (see Fig. 1). The TSST led to significant increases in sAA, while the rest condition did not impact sAA levels.

A third study using the same study design again showed marked increases in sAA due to the TSST (Nater et al., 2006b). Other studies using the TSST and measuring sAA as a stress marker have reported sAA increases in response to the TSST in healthy children (Granger et al., 2006), healthy adults (Rohleder et al., 2006a,b; Grillon et al., 2007; Het and Wolf, 2007; Payne et al., 2007; Schoofs et al., 2008), postpartum mothers (Tu et al., 2006), and adolescents (Gordis et al., 2006, 2008). Nierop et al. (2006) found that pregnant women showed lower sAA responses than non-pregnant women when exposed to the TSST.

In conclusion, the discrepancies between earlier and more recent findings on the effects of stress on sAA may be attributed to the different stressors and stress paradigms used in the respective studies. Results have consistently shown changes in sAA in response to psychological stress.
Thus, sAA can be regarded as an excellent indicator of stress-related body changes. Whereas the studies outlined above measured changes in sAA in response to acute stress stimuli, only a handful of studies have reported associations of chronic stress and sAA. In one study, chronic stress was found to be associated with diurnal profiles of sAA (Nater et al., 2007). Subjects reporting relatively high chronic stress had higher momentary sAA activity than subjects reporting low chronic stress levels. Rohleder et al. recently showed that daily sAA secretion is associated with self-reported shame and depression in young women (Rohleder et al., 2008). Another study reported higher basal values of sAA activity in women exposed to Hurricane Katrina than in non-exposed controls (Vigil et al., in press). Finally, Wolf and colleagues found lower diurnal sAA in children with asthma than in healthy controls (Wolf et al., 2008). The latter two studies highlight the potential of measuring sAA as an index of chronic stress in selected high-risk populations.

3.3. Effects of exercise on salivary alpha-amylase

Another strong activator of the sympathetic nervous system is physical activity. A variety of studies have examined the effects of exercise on sAA. Gilman and co-workers observed a significantly higher concentration of sAA during exercise than in a control period. The authors discussed sAA as a valid measure of sympathetic activity via adrenergic receptors (Gilman et al., 1979b). Nexo and co-workers examined the impact of a 2-h long cross-country race, collecting stimulated whole saliva before and after the race. A marked increase in sAA was found after the race; the median level increased almost sevenfold compared to the beginning of the race (Nexo et al., 1988). In two studies reported by Chatterton et al. (1996), sAA was determined in subjects participating in a running and a bicycle exercise task. Both protocols resulted in increases of sAA. Another study investigated salivary components before, during and 1 h after a 2-h marathon. sAA activity increased significantly due to the strenuous activity, and levels remained high 1 h after the marathon (Ljungberg et al., 1997). In another study, sAA was used as an indicator for the anaerobic threshold in subjects performing a treadmill exercise test. Since the correlation between sAA and the anaerobic threshold was almost 1 \( r = 0.93 \), the authors concluded that sAA was a good and valid measure of the anaerobic threshold (Calvo et al., 1997). In a study of triathletes, several salivary parameters in unstimulated whole saliva were measured before and after a race consisting of swimming, cycling, and running. Mean sAA activity increased significantly during the triathlon (Steeneberg et al., 1997). Another study examined eight well-trained athletes during a high-performance 60-min cycle exercise task. Unstimulated whole saliva was collected before exercise, immediately after the task, and 1, 2.5, 5 and 24 h after exercise. A significant increase in sAA activity was found after exercise (Walsh et al., 1999). Similar results were obtained in a more recent study (Bishop et al., 2006) examining the influence of caffeine intake on sAA measures in an exercise paradigm. The authors found that caffeine intake resulted in an additional increase in sAA that was not observed in a placebo condition. A study examining the impact of exercise on a rowing ergometer on sAA levels showed increased levels after exercise (Kivlighan and Granger, 2006). Interestingly, sAA measures were positively associated with performance in this study. Finally, in a study of exercise-induced sexual arousal in women, exercise produced significant sAA increases relative to a non-exercise condition (Hamilton et al., 2008).

Taken together, a marked increase in sAA can be observed during exercise. Exercise is known to increase sympathetic activity, and the high protein level in saliva following exercise may be due to increased adrenergic activity in the salivary glands.

3.4. Is salivary alpha-amylase an indicator for the sympathetic nervous system?

The evidence summarized above supports the view that sAA may be a useful indicator for activity of the sympathetic nervous system. Some studies have tested this hypothesis by directly testing associations of sAA with various established measures of sympathetic activity.

A series of studies with this specific aim was undertaken by Chatterton and colleagues. In these studies, subjects were exposed to physical (running, exercise, exposure to heat and cold) and psychological (examination) stressors (Chatterton et al., 1996). Increases observed in both sAA and plasma catecholamines (norepinephrine and epinephrine) in response to physical and (in some cases) psychological stressors led Chatterton to conclude the two parameters might be measured as substitutes for each other. Indeed, the authors found significant correlations between sAA and both plasma norepinephrine and epinephrine \( (r = 0.64 \) and \( r = 0.49 \), respectively) in the exercise conditions of their study. The authors therefore suggested that sAA might serve as an indicator for plasma catecholamines (specifically, norepinephrine). A close inspection of this study, however, shows that only a small and non-significant correlation \( (r = 0.17 \) was observed in the psychological stress condition (examination). The results therefore indicate that similar mechanisms underlie increases in both catecholamines and sAA during physiological stress experience. A pilot study using a standardized stress protocol reported correlations between increases in sAA and plasma norepinephrine \( (r = 0.54) \) (Rohleder et al., 2004). In contrast, a study using the same stress protocol in a bigger sample found no associations when the plasma catecholamines norepinephrine and epinephrine were measured concomitantly with sAA levels (Nater et al., 2006b). Another study found increases in both plasma norepinephrine and sAA after carbon dioxide inhalation, but these two parameters did not correlate significantly (Wetherell et al., 2006). Thus, it seems doubtful that there is a 1:1 association between plasma catecholamines and sAA in the body’s response to stressful conditions.

Further attempts have been made to relate sAA to other indicators of sympathetic or parasympathetic activity. In a study of subjects exposed to a stressful video showing a surgical procedure or to a memory task, Bosch et al. (2003) measured both sAA and various cardiovascular parameters. The authors found negative correlations between left ventricular ejection time and sAA (in the video condition) and between the square root of the mean squared differences of normal-to-normal intervals (RMSSD, as an index of parasympathetic tone) and sAA during the memory task. These
findings suggest a predominant role of the sympathetic nervous system in the secretion process of sAA, together with vagal withdrawal, under psychological stress. They were complemented by a study using a standardized psychosocial stress protocol (Nater et al., 2006b), which reported a positive correlation ($r = 0.39$) between sAA and the low frequency/high frequency ratio thought to reflect sympathetic tone. In another study, a positive association between sAA and respiratory sinus arrhythmia (RSA) was observed ($r = 0.36$), indicating an association of increased sAA with vagal suppression (Granger et al., 2006). Finally, a recent study revealed a moderate positive association ($r = 0.26$) between sAA and basal skin conductance level (SCL), which is regarded as a sympathetic activity marker (El-Sheikh et al., 2008).

3.5. Summary

Taken together, it seems that sAA response patterns to both physical and psychological stressors correspond to the response patterns of the sympathetic nervous system. Whereas no correspondence of plasma norepinephrine and sAA could be established, associations between other markers of the ANS, such as cardiovascular parameters, have been found. It should be noted, however, that the correlations between sAA and sympathetic markers are relatively small. Based on the pharmacological and electrophysiological literature reviewed above, the pathways that lead to secretion of sAA are clearly sympathetic/parasympathetic in nature.

4. Applications of salivary alpha-amylase measurement

The findings on the association of sAA and the sympathetic nervous system summarized above indicate that sAA can function as a useful biomarker in acute and chronic stress studies. An overview of previous and potential applications in stress research is given below.

4.1. Salivary alpha-amylase as an indicator of autonomic dysregulation

Based on the knowledge that has been accumulated about the role of stress and its underlying physiological mechanisms in the secretion of sAA, it seems reasonable to conclude that sAA concentration can serve as an index for pathological dysregulation of the ANS in specific clinical and subclinical conditions. Anxiety-related conditions are accompanied by autonomic changes (Friedman et al., 1993; Shalev and Rogel-Fuchs, 1993; Southwick et al., 1993; Lang et al., 2000; Laederach-Hofmann et al., 2002; Coupland et al., 2003). sAA measurements might provide additional information on the autonomic changes occurring in anxiety patients. Some preliminary findings indicate that sAA measurement may be useful not only as an indicator of autonomic changes but also as a marker for anxiety reports. Takai et al. (2004) used a stressful video to induce stress and found sAA peak levels and state anxiety measures to correlate highly ($r = 0.535$). A similarly high correlation between state anxiety and sAA was found in another study, in which sAA levels were examined in healthy participants during a mental arithmetic task (Noto et al., 2005). The task induced significant increases in both state anxiety and sAA, with a correlation of $r = 0.589$ between the two variables. These examples demonstrate the potential of sAA measurement in a psychiatric context. To date, however, few studies with psychiatric patients have employed sAA as an indicator for autonomic changes. A notable exception is a recent study by Labudda et al. (2007), who measured sAA in pathological gamblers and found significant increases only in a subgroup of patients who showed less disadvantageous decision-making patterns.

However, sAA might be also measured in the context of somatic disorders in which autonomic dysregulation might be present. Exaggerated autonomic responses to different stimuli can be observed in hypertensive patients (Fredrickson et al., 1991; al’Absi and Wittmers, 2003) and patients with HIV (Cole et al., 2001). It might be of interest to measure sAA concentrations in these groups of patients. ANS dysregulation can also be found in atopic diseases. In a study of patients with dermatitis, Crespi and co-workers measured parotid secretory response to intra-oral citric acid. Protein concentration as well as sAA activity was significantly decreased in patients with atopic dermatitis. The authors interpreted these findings as a reflection of impaired beta-adrenergic mediated responses in atopic dermatitis (Crespi et al., 1982). In another study, Wolf et al. (2008) examined diurnal sAA in patients with asthma and in controls. The found lower daily sAA output in patients with asthma than in the control group.

4.2. Measurement of salivary alpha-amylase in treatment studies

Given these findings of increased or attenuated sAA in clinical populations, use of sAA to measure the effects of psychotherapy, e.g. stress management training (Gaab et al., 2003) seems warranted. A recent study has used sAA to assess stress levels in healthy women administered Relora (a medication including extracts of Magnolia officinalis and Phellodendron amurense) (Kalman et al., 2008). Another study defined sAA as the endpoint of a treatment study using reflexology in nursing home residents with dementia (Hodgson and Andersen, 2008). Finally, a third study found decreased sAA levels in patients undergoing surgery while exposed to natural sounds than in patients who were not exposed to any sounds (Arai et al., 2008). These examples show that sAA measurement is a promising approach for studies of treatment effects.

Taken together, sAA has already been used as a marker in a variety of conditions. More recent developments suggest that it may also be a useful marker in the context of pain (Shirasaki et al., 2007) or sleep (Seugnet et al., 2006) research. It may be particularly useful in ambulatory settings, such as field studies, where assessment of sAA through saliva as an indicator of autonomic functioning might present an easy, non-invasive, and efficient sampling method.

5. Conclusion

The aim of this review was to evaluate the role of sAA activity as a potential indicator for sympathetic activation in response to stress. Although first findings on the role of sAA in stress processes were published almost three decades
ago, no attempts were made to scientifically elucidate this relationship until the mid-1990s. Numerous studies have since shown that changes in sAA are indeed dependent on stressful stimuli, either physiological or psychological in nature. The biological meaning of this phenomenon remains to be clarified, however. In this context, it may be worthwhile reconsidering the main property of alpha-amylase, namely its digestive action. Alpha-amylase hydrolyzes starch to glucose and maltose, initiating the digestion of starch in the oral cavity; it has also been shown to have a bacterial interactive function (Scannapieco et al., 1993). More studies are needed to examine the cellular processes occurring after initiation of stress-related responses in the oral environment. It is difficult to deduce the function of short-term increases in alpha-amylase while the biological meaning of transient rises in the anti-bacterial action of the enzyme remains unclear. However, such short-term increases may be useful to the body in that energy is made available by increased digestive action in response to stress. Physiological stress reactions comprise orchestrated actions throughout the body, putting the organism in a state of overall preparedness to engage in fight or flight. Thus, increases in amylase activity may be one of many actions involved in activating the body’s resources to cope with stressful events or threats to homeostasis. However, this explanation applies only to reactions to short-term, acute stressors. Further studies are needed to examine long-term changes in sAA concentrations. If clinical or subclinical conditions associated with an increase of activity in the ANS result in chronically elevated concentrations of sAA, the sustained higher sAA activity and therefore digestive action may prove detrimental to oral health.

The studies summarized in this review clearly show that sAA is increased in states of stress, i.e. when autonomic activation is increased. We can therefore conclude that increases in sAA may reflect changes in the ANS. Further studies are needed to conclusively determine whether sAA can be included in the canon of biological stress markers. In particular, the mechanisms underlying elevations in sAA in response to stress warrant examination. Although a variety of studies have examined the physiological mechanisms of sAA production and secretion in animals, studies in humans are scarce. Use of pharmacological agents that inhibit or activate the ANS may prove particularly useful here, providing more detailed insights into the branches of the ANS responsible for increases in sAA. Clearly, more invasive techniques are available in animal studies. Experimental damaging of nerve fibers or direct stimulation of nerve cells is not feasible in a basic research context. It may be interesting, however, to use electrical stimulation techniques in awake or anaesthetized humans, e.g. in the clinical context of a hospital. Measurement of direct sympathetic nerve activity via microneurography is considered to be the most accurate technique for assessing sympathetic activation (Grassi and Esler, 1999). Beyond peripheral measurements, the relationship between central parameters and changes in sAA might prove very interesting. Since cerebrospinal fluid concentrations of norepinephrine reflect other mechanisms than do peripheral catecholamines, it would be worthwhile comparing sAA and central norepinephrine concentrations (Goldstein, 1998).

In summary, assessment of sAA as a non-invasive biomarker for the sympathetic nervous system offers a multitude of possibilities in different research areas. If further evaluated, sAA may well become an important parameter in stress research.

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Conflicts of interest

The authors have no conflicts of interest and declare no financial interests.

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