

Salivary mental stress proteins

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Abstract

Of the major diagnostic specimen types, saliva is one of the most easily collected. Many studies have focused on the evaluation of salivary proteins secreted by healthy people and patients with various diseases during responses to acute mental stress. In particular, such studies have focused on cortisol, α -amylase, chromogranin A (CgA), and immunoglobulin A (IgA) as salivary stress markers. Each of these salivary stress markers has its own strengths and weaknesses as well as data gaps related to many factors including collection technique. In this review, we summarize the critical knowledge of the positive and negative attributes and data gaps pertaining to each salivary stress marker.

Keywords

salivary stress markers; mental stress; salivary cortisol; salivary α -amylase; salivary chromogranin A (CgA); salivary immunoglobulin A (IgA)

Abbreviations

CgA: chromogranin A, IgA: immunoglobulin A, ALS: amyotrophic lateral sclerosis

Introduction

Of the major diagnostic specimen types, saliva is perhaps the most easily collected [1, 2]. It is secreted from three major glands, namely, the parotid gland, the glandula submandibularis, and the glandula sublingualis [3], and the secretion process is regulated by the autonomic nervous system [4, 5]. The basis of the theory of saliva secretion is that the sympathetic and parasympathetic branches of the autonomic nervous system innervate the salivary glands. Sympathetic stimulation increases salivary protein secretion, whereas parasympathetic stimulation increases salivary flow rate [6]. As stress symptoms are induced by the sympathetic nervous system and the reactions of the hypothalamic-pituitary-adrenal axis as shown in Figure 1 [7, 8], saliva is considered to be a good material for evaluating stress conditions, especially in a depressive state, and the use of salivary biomarkers to evaluate stress in humans has received much attention in the last 30 years or so [9]. Some studies have focused on subfields such as the evaluation of salivary mental stress protein secretion during menstruation or pregnancy [10, 11]. Other studies have focused on the evaluation of these proteins during stress responses to acute mental stress in patients with various diseases, such as recurrent major depression, early life stress depression, and long-standing posttraumatic stress disorder [12].

In particular, such research has focused on cortisol [13-23], α -amylase [20, 22-33], chromogranin A (CgA) [9, 33], and immunoglobulin A (IgA) [18, 21, 23, 26-36] as salivary stress markers. These four proteins are very common not only in saliva but also in plasma, serum, and other fluids, but for each of them the largest variability in concentration due to acute mental stress occurs in saliva. It may be surprising, therefore, that it is actually very difficult to assess data on the concentrations of these proteins. Differences in subjects' sex, age, circadian rhythms, meals, drugs, autonomic function, salivary gland function, and salivary flow rate can affect the levels of salivary stress

proteins [37-43]. Moreover, sample collection methods may need to be carefully designed to minimize the impact of certain related factors on measurement validity for these salivary stress markers [44-47].

In this review, we summarize critical knowledge on the strengths and weaknesses of each salivary stress marker and the data gaps related to a variety of factors including collection technique.

1. Strengths and weaknesses of four salivary stress proteins

Table 1 shows a comparison of the four major salivary stress markers.

1) Salivary cortisol

Cortisol can be measured reliably and accurately in saliva. Correlations between salivary and plasma cortisol levels have been reported at about 0.70 in adults [48-50] and at about 0.67 in preterm infants [51]. Thus salivary cortisol could serve as a useful quantitative biochemical marker of affective states for patients of all ages [13-23].

Using salivary cortisol as a marker offers three major benefits. First, saliva flow rate has no impact on salivary cortisol levels. Naumova *et al.* reported that acute short-term mental stress did not influence salivary flow rate dynamics [52]. Second, physical stress has only a small impact on salivary cortisol levels. Toda *et al.* reported that there was no significant difference in cortisol levels measured before and after 40 min of walking [14]. Third, salivary cortisol has good shelf stability. Salivary samples are often required to be stored for long periods of time either because of the protocol of the project or because of a lack of funding for analysis. However, cortisol is less affected by long-term storage, even at room temperature, than are other potential markers. Centrifuged saliva samples for analysis of cortisol may be stored at 5 °C for up to three months or at -20 °C or -80 °C for at least one year [53].

Four major weaknesses of salivary cortisol as a marker must also be pointed out. The first is the effect of drugs and diseases [42, 54]. Treatments such as type 5 phosphodiesterase inhibitor administration amplify salivary cortisol responses in healthy humans [42]. A second weak point is the existence of a time lag between changes in plasma cortisol and associated changes in salivary cortisol. Although this lag is only one or two minutes long [55], salivary cortisol levels peak about 20 min after a mental stress event [18]. A third weak point is the fact that age and sex differences affect salivary cortisol. Age and sex interactions were observed for cortisol, with higher responses in older males [56]. Finally, a clear pattern related to circadian rhythm exists in human salivary cortisol levels [39, 57-60]. A straightforward comparison of salivary cortisol measurements taken at different times of day is unfortunately impossible.

2) Salivary α -amylase

Although a few studies have failed to observe changes in salivary α -amylase in response to stressful stimuli including noise, the heel prick test in neonates, or a strange situation paradigm [61-63], salivary α -amylase has been suggested as an index of autonomic activity because it is locally produced by salivary glands in the oral mucosa and because its levels are positively correlated with the acute sympathetic nervous system stress response in children and adults [20, 23-34, 64].

There are three major advantages to using salivary α -amylase as a biomarker. First, saliva flow rate has no impact on salivary α -amylase levels, as is the case with salivary cortisol. Rohleder *et al.* reported that the psychosocial stress-induced increase in salivary α -amylase is independent of saliva flow rate [65]. A second strong point is this protein's high sensitivity to stress. Changes in salivary α -amylase levels are more remarkable than those in salivary cortisol after the same mental stress event [66]. Finally, age has no impact on salivary α -amylase levels [67].

On the other hand, there are three major disadvantages to using salivary

α -amylase. The first is the low durability of its stress-induced increases. The time lag between the occurrence of a mental stimulation event and the moment of peak salivary α -amylase is only one to three minutes [68], but the recovery of normal salivary α -amylase levels after stress reduction is very rapid: the duration of α -amylase elevation is only about 10 min [67]. A second weak point is the existence of sex differences. Usually, men show higher salivary α -amylase levels than women do at baseline [67]. Finally, physical stress has an impact on salivary α -amylase levels. For example, Groza *et al.* reported that the physical stress associated with a surgical operation affected salivary α -amylase levels in children [68].

3) Salivary CgA

CgA is an acidic glycoprotein that is released along with catecholamine from the adrenal medulla and the sympathetic nerve endings [69, 70]. It has received attention as a novel stress marker in saliva not only in healthy subjects but also in patients with certain chronic diseases [13, 35, 71, 72]. Obayashi *et al.* reported a relationship between salivary CgA levels and affective state in amyotrophic lateral sclerosis (ALS) patients. As symptoms of disturbed autonomic function are rare in ALS, the salivary CgA level may be particularly useful as an index of affective state in these patients [72].

The major advantage of salivary CgA as a biomarker is its proper durability. The time lag between the occurrence of a mental stimulation event and the time of peak salivary CgA is small, on the scale of that of α -amylase. Moreover, salivary CgA remains elevated even in the recovery phase, up to 60 min after stimulation [73]. From a durability point of view, salivary CgA may be the most useful biochemical marker of chronic mental stress.

Yet three major disadvantages of salivary CgA must be pointed out. The first is the effect of oral diseases on salivary CgA levels. Shigeyama *et al.* reported that

significant associations between salivary CgA levels and symptoms of oral dryness and reduced salivary flow were observed [55]. Second, physical stress has an impact on salivary CgA levels as it does on salivary α -amylase levels [74]. Finally, human salivary CgA levels follow a clear circadian rhythm-based pattern throughout the day as do salivary cortisol levels [74].

4) Salivary IgA

Salivary IgA has also been reported as a potential stress marker in humans [15, 17, 20, 23, 33]. However, several studies have suggested a negative correlation between the level of salivary IgA and mental stress [75-77]. Salivary IgA is powerfully affected by oral contamination and saliva flow rate [78, 79]. In addition, its half-life is too long to assess psychological stress in real time [79]. Moreover, the stability of salivary IgA is lower than that of salivary cortisol. IgA is more easily affected by bacteria even under refrigeration. Centrifuged saliva samples for analysis of IgA may be stored at -30 °C for up to three months [80].

5) Combinations of multiple salivary mental stress proteins

Mental stress is a multi-dimensional construct. Associations may be masked given that different systems operate on different time courses. Thus multiple salivary markers should be assessed at the same time for a complete picture. Engert *et al.* reported that salivary α -amylase and cortisol stress responses are reliably associated at various time lags throughout a stressful situation [81]. The stress reactions at the beginning of a stressful event (primary protest) reflect the activation of the sympathetic nerve system and can be monitored by measuring α -amylase levels. Subsequent reactions (secondary protest) reflect the activation of the HPA and can be monitored by measuring salivary cortisol levels [81]. On the other hand, Gordis *et al.* reported that salivary alpha amylase and cortisol were asymmetrical in maltreated youth [82]. These

results suggest that a connection between the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system may exist and may underlie the association between salivary α -amylase and cortisol.

2. Proper collection methods for saliva

As specimen collection is both easy and non-invasive, saliva can be seen as a stress-free alternative to blood collection. One problem with saliva as a specimen is that the content of any collected sample is subject to variation. The analytes in saliva can vary greatly in composition and concentration.

Figure 2 shows a summary of proper collection methods for saliva.

1) Before collection

Saliva should be collected after more than six hours of fasting. The consumption of dairy products has a particularly strong effect on cortisol data and so should be avoided. If IgA is to be assessed, alcohol should also be avoided for more than 24 hours prior to collection to avoid causing a change in saliva flow rate [83]. Caffeine can affect α -amylase. Smoking is also shown to affect salivary cortisol and α -amylase [84]. Toothbrushing should likewise be avoided for at least two hours before saliva collection in order to avoid contamination of the saliva with blood, but subjects should rinse their mouths 10 min before sampling.

2) Choice of collection devices

Previous studies on saliva collection have used a commercial device known as the Salivette (Sarstedt Inc., Newton, MA, USA) which permits the extraction of saliva into a dental cotton roll placed in the mouth [85-87]. But this method can yield a low collection rate, which is particularly problematic when IgA is to be measured [88, 89]. Cotton collection devices should be used only in situations where a small sample

collection volume is suitable, that is, only for cortisol and α -amylase measurement [90, 91]. Another saliva collection method described in the literature involves the suctioning of saliva using a small plastic feeding tube or a suction catheter attached to a syringe [92, 93]. This suction method is more invasive, however, and carries a risk of damage to the delicate mucous membranes of preterm infants, resulting in contamination of the sample with blood. Thus a new and more appropriate saliva collection system has been developed recently. Strazdins *et al.*, Harmon *et al.*, and de Weerth *et al.* reported the successful use of hydrocellulose microsponges (Sorbettes) for the collection of saliva [39, 94, 95]. Separately, Ng *et al.* invented a novel, safe, non-distressing and effective method of saliva collection using eye spears that measure 2 cm in length by 1 cm in width [96]. This technique had a success rate of 85% when implemented by trained staff even in premature infants, and no adverse events were recorded.

3) Storage of saliva

As bacteria are present in saliva, all samples should be refrigerated within 30 min and frozen within four hours to slow or stop bacterial growth. Although cortisol levels are not strongly affected by storage even at room temperature, IgA is more easily affected even under refrigeration, as described above.

4) Reducing viscosity

To prevent the effects of mucin, cell debris, food particles, and bacteria, all saliva samples should be frozen once before assay and vortexed after thawing.

Conflict of interest statement

No conflicts of interest to declare.

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Figure Legends

Figure 1

Mental stress response and salivary mental stress proteins

Figure 2

Summary of proper collection methods for saliva

Footnote

Table 1

Comparison of four major salivary stress markers

+: positive, -: negative

Figure 1

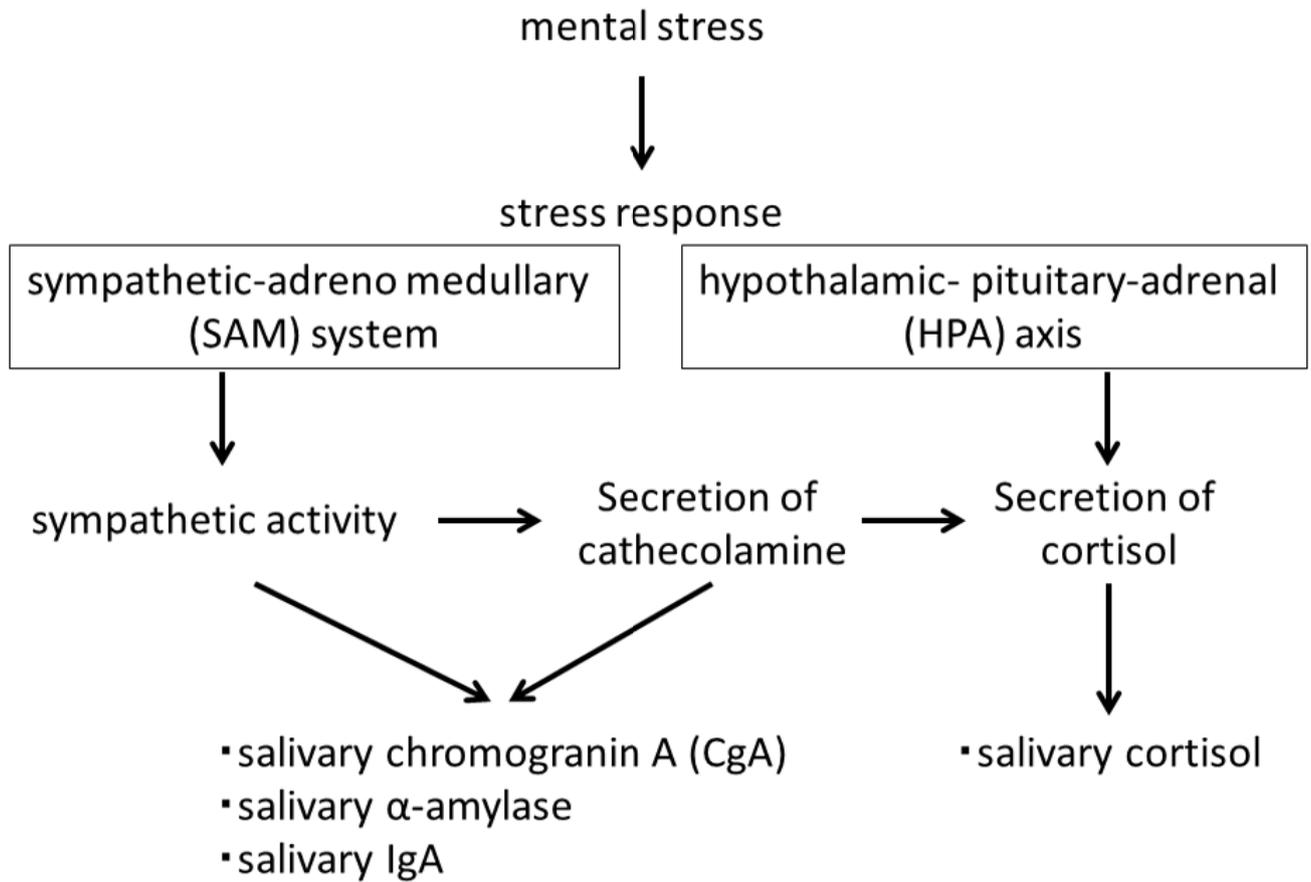


Figure 2

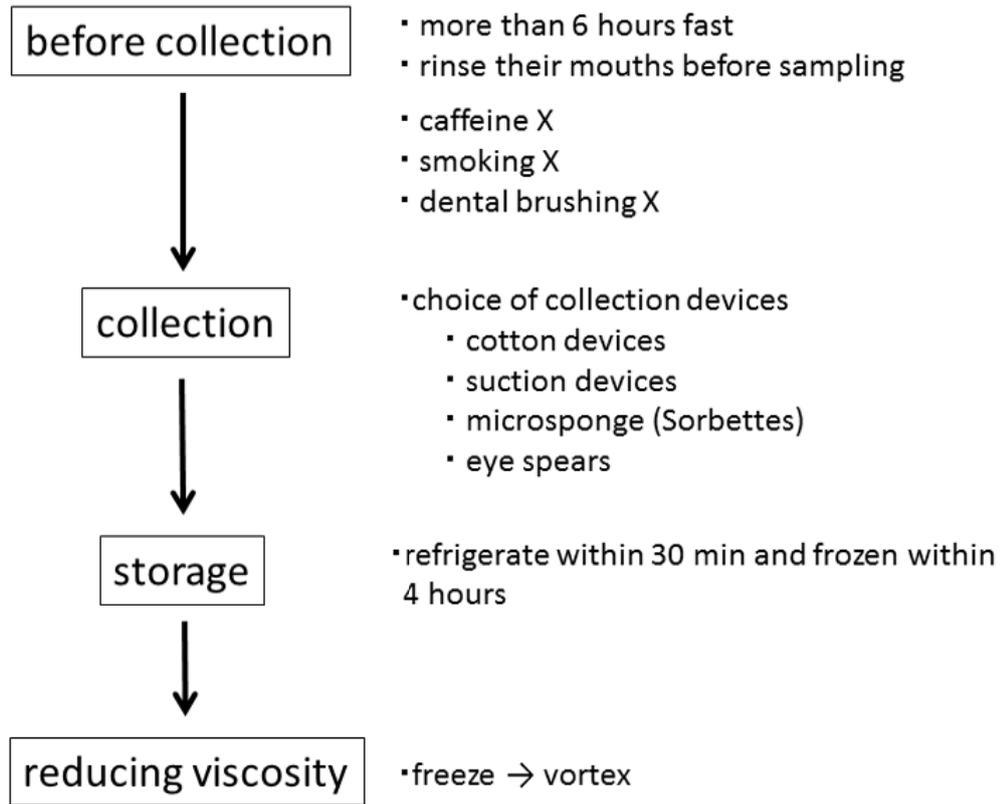


Table 1

	salivary flow rate	physical stress	sensitivity and stability	effect of drugs and diseases	time lag	age and sex interactions	circadian rhythm
Cortisol	no impact	small impact	less affected in temperature	big	big	+	+
α -amylase	no impact	big impact	high sensitivity, but low durability	small	big	-	-
Chromogranin A (CgA)	no impact	big impact	less affected in temperature	big	small	-	+
Immunoglobulin A (IgA)	big impact	small impact	affected in temperature	big	big	-	-