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The shape of stress: the use of frequent sampling to measure temporal variation in S-IgA levels during acute stress

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Abstract

Previous studies have indicated that statistically significant increases in Secretory immunoglobulin A (S-IgA) can be achieved in as little as 5 min as a result of mental stress. However, the temporal resolution of these changes is low and therefore the rate and pattern of changes during the stress task and during subsequent recovery is unclear. A within-subjects design was used to examine levels of S-IgA before, during and after a short (8 min) mental stress task. S-IgA was measured from saliva samples obtained every 2 min during the entire 30-min session. Significant increases in S-IgA concentration were observed as early as the task instruction period, with additional increases during the stress task itself. The data also show a rapid recovery of S-IgA, with a return to baseline levels within 6 min. Results suggest that S-IgA changes can occur very rapidly and that the observed increases are short-lived. Copyright © 2007 John Wiley & Sons, Ltd.

Key Words

secretory IgA; baseline; acute stress; psychoneuroimmunology; recovery

Introduction

Secretory immunoglobulin A (S-IgA) is one of five classes of immunoglobulins found in serum and secretory fluids. S-IgA is the major class of immunoglobulins in mucosal secretions and is considered to be a major effector of host defense against micro-organisms that cause illnesses such as upper respiratory tract infections (Tomasi & Plaut, 1985). Recent research suggests that low basal S-IgA concentration may be a contributing factor to the development of upper respiratory tract infections during stressful periods (Volkmann & Weekes, 2006).

While chronic stressors, such as extended periods of academic examinations, have been shown to decrease S-IgA levels (Deinzer, Kleineidam, Stiller-Winkler, Idel, & Bachg, 2000; Jemmott & Magloire, 1988; Mouton, Fillion, Tawadros, & Tessier, 1989; Phillips et al., 2006), acute stress in both naturalistic and laboratory settings have been shown to *increase* S-IgA (e.g. Bosch, Brand, Ligtenberg, & Bermond, 1996; Bosch et al., 1998; Bristow, Hucklebridge, Clow, & Evans, 1997; Carroll et al., 1996; Huwe, Hennig, & Netter, 1998; Wetherell, Hyland, &

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Harris, 2004; Willemsen et al., 1998; Willemsen, Ring, McKeever, & Carroll, 2000; Zeier, Brauchli, & Joller-Jemelka, 1996). Researchers have suggested that these conflicting findings can be largely explained by examining the duration of the stressor (Bosch, de Geus, Ring, & Amerongen, 2004; Bosch, Ring, de Geus, Veerman, & Amerongen, 2002; Evans, Clow, & Hucklebridge, 1997). This general distinction is somewhat complicated by issues surrounding the nature (e.g. passive versus active stress, Bosch et al., 2001; Isowa, Ohira, & Murashima, 2004) and intensity (Wetherell & Sidgreaves, 2005) of the acute stressor, but serves as a useful primary system for classification of S-IgA studies.

There has been some variability in the way acute stress is characterized. Early studies demonstrated that changes were observable within 24–120 min (Carroll et al., 1996; Evans, Bristow, Hucklebridge, Clow, & Pang, 1994; Kugler, Reintjes, Tewes, & Schedlowski, 1996; Ohira, Watanabe, Kobayashi, & Kawai, 1999; Zeier et al., 1996), but researchers have more recently demonstrated increases in as little as 5–8 min (e.g. Ring et al., 1999; Ring et al., 2000; Wetherell et al., 2004; Wetherell & Sidgreaves, 2005; Willemsen et al., 1998; Willemsen et al., 2000; Winzer et al., 1999). However, the temporal resolution of these changes is low (generally pre- and post-task) and therefore the rate and pattern of changes during the stress task and during subsequent recovery is unclear.

The aim of the present study is to determine the pattern of changes in S-IgA during a commonly employed mental arithmetic acute stress task. Specifically, the aim is to demonstrate the rapidity of stress-induced increases in S-IgA and to examine the rate of S-IgA recovery post-stress. The rapidity of these changes has important methodological implications for studies employing S-IgA measures. Additionally, this is the first published study to apply a frequent sampling methodology to the analysis of stress-induced changes in S-IgA and thus the study examines the feasibility of such an approach for studies using salivary biomarkers.

Methods

Participants

Participants were 25 female undergraduate students [20.2 (2.1) years] from the University of Texas-Pan American. All participants were instructed to refrain from exercising, smoking, eating or drinking anything but water for at least 1 h prior to their scheduled appointment. The study was approved by the university's Institutional Review Board and each student provided informed consent prior to participation.

Procedure

All participants attended an individual testing session scheduled during the mid-afternoon in a sound-attenuated, temperature controlled room. Participants were fitted with a skin-conductance sensor, a heart rate monitor and a respiration sensor (Procomp Infiniti, Thought Technology, Ltd., NY).[1] Participants were then given general information about the nature of the session, but were not given specific instructions regarding the stress task. All participants then sat quietly for 10 min (baseline), received 2 min of instruction about the stress task, were administered the 8-min stress task, and then sat quietly for another 10 min (recovery). During the 30-min session, all participants passively collected saliva in their mouths, which they spat into 15 consecutively numbered 50-mL vials at 2-min intervals. Thus, each participant provided five baseline samples, one task instruction sample, four stress task samples and five recovery samples.

Mental stress task

The mental arithmetic task was the paced auditory serial arithmetic task (PASAT, Gronwall, 1977; Willemsen et al., 1998), an 8-min task in which participants are required to add two sequentially presented single-digit numbers, from one to nine, while retaining the latter of the two numbers in memory for subsequent addition to the next number presented. Numbers are delivered via an audio tape player, and participants are instructed to write down their answers on a tabulated sheet of paper. The task consists of four 2-min series of 50, 60, 75 and 100 digits at presentation rates of 2.4, 2.0, 1.6 and 1.2 s, respectively. Instructions for the task were provided

[1] Physiological data are not reported in the current article, but will be analysed and published separately.

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immediately after the baseline period. Participants were told that at no point should they give up on the task; that should they lose track of the numbers being presented they should immediately pick up from the next number that they hear. In order to increase the social-evaluative elements of the task (Dickerson & Kemeny, 2004), the researcher stood over the shoulder of the participant as they wrote down their answers, ostensibly monitoring the participant's performance.

S-IgA analysis

Saliva samples were analysed by a research assistant who was blind to the purpose of the study. Saliva samples were centrifuged for 10 min and supernatants were aliquotted and stored in microcentrifuge tubes at –20°C. S-IgA assays were performed by placing 5 µL of saliva from participants into single wells on radial immunodiffusion plates containing a single antibody (Hycor Biomedical, Garden Grove, USA). Specificity of the assay was over 99 per cent and sensitivity was 20 µg/mL with an upper limit of 1130 µg/mL. All plates were incubated in a moist chamber for 24 h at room temperature (22°C) and then examined for immunoprecipitation rings. Immunoprecipitation rings were measured and S-IgA concentration was read from a standard curve.

Data reduction

Data from one participant was not included because six of her 15 S-IgA levels were over 2.5 standard deviations (SD) above the mean. Twelve of the 375 saliva samples (3.2 per cent) were too small in volume to be adequately analysed and were entered as missing values. Two samples from one participant (samples 12 and 13, taken during the recovery stage) were over 2.5 SD from the mean and were replaced with an S-IgA value equal to 2.5 SD above the mean. S-IgA concentrations (µg/mL) were normalized using natural logarithmic transformation prior to analysis.

Results

Means and SD for S-IgA at each of the sample collection periods are presented in Table I. A graphical representation of the data, based on natural log S-IgA concentration (µg/mL), is presented in Figure 1.

Table I. Means (standard deviations) for S-IgA concentration (µg/mL) for each of the 15 samples.

Condition	Baseline				TI		Stress			Recovery				
Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sig. Diff.					a	a, b	b, c	c		d	d				
Mean	203	214	205	196	199	221	243	271	296	293	271	248	215	215	197
(SD)	(106)	(122)	(108)	(106)	(102)	(107)	(118)	(141)	(156)	(162)	(172)	(179)	(136)	(125)	(115)

Note: Matching letters denote significant differences ($p < 0.05$) between sample means (based on planned comparisons). TI refers to the 2 min period of stress task instructions.

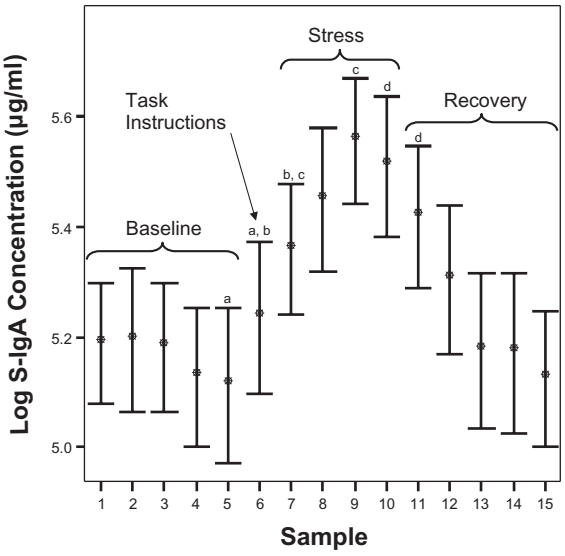

Figure 1. Error bars (±1 SE) of natural log S-IgA concentration (µg/mL) during baseline, stress and recovery. *Note:* Matching letters above error bars denote significant differences ($p < 0.05$) between sample means (based on planned comparisons).

Stability of baseline S-IgA measures

A repeated measures analysis of variance (ANOVA) comparing baseline S-IgA measures was not significant, suggesting that the first 10 min of the session showed a relatively stable level of S-IgA (see Figure 1). To examine this further, a paired *t*-test was conducted between the first and last baseline measures, which again revealed no significant difference ($t(22) = 1.08$, n.s.). Given this, the baseline measure immediately preceding the stress task instructions was used in all further analyses.

Increase in S-IgA during the stress task

A repeated measures ANOVA of task instruction and stress task S-IgA values revealed a significant effect for the stress task ($F(4, 76) = 8.21$, $p < 0.001$, $\eta^2 = 0.302$). To determine the point at which S-IgA levels increased above baseline, planned comparisons were performed beginning with the first measure. A paired *t*-test revealed that significant increases in S-IgA over baseline were obtained during the 2-min task instruction period ($t(22) = 3.67$, $p < 0.001$), and the increase remained significantly higher than baseline during the remainder of the stress task.

To examine the extent to which S-IgA continued to increase during the mental arithmetic task, paired *t*-tests were conducted for incremental pairs. The results indicated that 2 min into the stress task S-IgA had increased from task instruction levels ($t(20) = 3.57$, $p = 0.001$), did not increase significantly during the subsequent 2 min, but reached a significant increase above the first 2 min of the task by the 5- to 6-min period ($t(20) = 2.89$, $p = 0.006$). No increases were demonstrated during the last 2 min of the stress task.

S-IgA recovery following stress task

A repeated measures ANOVA of recovery S-IgA values revealed a significant effect ($F(4, 88) = 4.19$, $p = 0.004$, $\eta^2 = 0.160$]. Within 2 min of sitting quietly, S-IgA levels had dropped significantly below the final stress task levels ($t(23) = 2.21$, $p = 0.019$). To determine the point at which S-IgA levels had recovered to baseline, planned comparisons were performed beginning with the first recovery measure. Within 6 min of sitting quietly post-stress, S-IgA values did not differ significantly from baseline S-IgA levels ($t(23) = 0.75$, n.s.).

Discussion

The findings from this study indicate that levels of S-IgA are relatively stable when frequently measured during a 10-min resting baseline, but increase rapidly as a result of acute stress. S-IgA levels increased significantly from stable baseline levels within 2 min of a mental arithmetic task. Indeed, S-IgA was found to increase during the presentation of task instructions. The levels of S-IgA continued to increase during the stress task, until levelling off during the final 2 min of the 8-min task. These findings replicate and extend the work of previous researchers who have shown increases in S-IgA following an acute mental stress task (Bosch et al., 2001; Bristow et al., 1997; Carroll et al., 1996; Evans et al., 1994; Kimura, Isowa, Ohira, & Murashima, 2005; Ring et al., 2000; Wetherell et al., 2004), and also suggest that the effects are short-lived, with levels of S-IgA having returned to baseline levels within 6 min of task completion. Such rapid recovery of

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S-IgA is in keeping with prior research; for example, Bosch et al. (2001) showed recovery within 13 min and Wetherell et al. (2004) within 7 min.

Data from the present study suggest that inconsistencies in the timing of saliva sampling may increase the variability of data between different studies. Given the rapid response and recovery of S-IgA concentration during and following acute stress, respectively, timing differences could overestimate or underestimate the magnitude of changes. Both the measurement start point and the duration of saliva samples are affected by this issue. In light of the current findings, it is possible that a 4-min sample collected after completion of the acute stress task may show lower levels of S-IgA than a 2-min sample collected during the last 2 min of the task itself; which may, in turn, influence the effect size of the stress manipulation. Based on S-IgA levels in the present study, the average of the first two recovery samples (representing a timed 4-min sample post-stress) is significantly lower than the measure obtained from the last 2 min of the stress task ($t(22) = 2.15$, $p = 0.022$).

While the results of this study provide some new information on the immune response to acute stress, there are a number of limitations that need to be acknowledged. The study was conducted using a purposefully homogenous sample of females under 30 years of age, with all sessions conducted during the afternoon, thus the generalizability of results is uncertain. It is important to also note that the method of frequent sampling of saliva samples is novel and there is the potential for the procedure of ongoing measurements itself to have some influence on S-IgA levels. However, in this particular study, baseline levels remained relatively stable across five repeated measures, the onset of changes in S-IgA concentration were closely tied to the various experimenter manipulated stages, and the standardized mental stress task produced increases in S-IgA levels similar to those seen in prior studies.

One potential limitation of this study is its use of S-IgA concentration levels rather than S-IgA secretion rate. While some have supported the use of S-IgA concentration as an appropriate measure (Jemmott & McClelland, 1989), and while it continues to be reported in S-IgA articles, a number of researchers have cautioned that the use of S-IgA concentration does not take into account variations in saliva flow rate across sampled time periods and thus introduces a potential confound. If the effects of acute stress are to decrease saliva flow rate, increases in S-IgA concentration may simply be the result of a decrease in the volume of saliva being produced. However, in examining prior studies, there is little evidence to support this concern. The trend in studies of acute mental stress is that subjects demonstrate no significant change in saliva secretion rate (e.g. Kimura et al., 2005; Ring, Drayson, Walkey, Dale, & Carroll, 2002; Winzer et al., 1999) or produce an *increase* in saliva secretion rate (e.g. Isowa et al., 2004; Wetherell et al., 2004; Willemsen et al., 1998). While the method of frequent sampling differs from prior studies and the procedure itself could arguably cause a decrease in saliva flow rate, no changes in S-IgA concentration were observed across five repeated baseline measures, suggesting that this is not a valid concern.

The results of this study suggest that the effects of acute stress were maximized approximately 7–8 min into the task. It is not clear how long the increased S-IgA levels may have maintained if the stressor continued. Prior research has demonstrated that an extended 33-min PASAT session does not increase S-IgA levels above those obtained 14 min into the task, although the elevated levels are maintained during the extended stressor (Ring et al., 2002). Given that the PASAT increases in difficulty, it is possible that the increases in S-IgA are tied to the difficulty of the task, but at a certain level of difficulty participants have reached maximum levels of S-IgA secretion or have simply begun to mentally disengage from the stress task. Research regarding the effects of task difficulty is limited; while some research has shown no effect for task difficulty on S-IgA (Willemsen et al., 2000), more recent research suggests that medium levels of task difficulty may evoke the biggest increases in S-IgA whereas tasks that are too difficult or that are appraised as 'impossible' may be less effective (Wetherell & Sidgreaves, 2005). It would be informative to see if a similar pattern of S-IgA increases would be demonstrated using a different acute stress task such as the Trier social stress test (Kirschbaum, Pirke, & Hellhammer, 1993).

It is proposed that the methodology of frequent sampling of salivary biomarkers can be readily applied to a number of areas of inquiry. While frequent sampling increases the cost of sample analysis, it provides distinct advantages over simple pre- and post-measures. The procedure generates higher temporal resolution, allowing researchers to investigate the pattern of task-

induced changes and subsequent recovery. The technique could also be used to more thoroughly investigate individual differences in response to stress, particularly in terms of the rate of recovery of S-IgA to baseline levels.

The present study provides the first attempt to apply a frequent sampling technique to the study of changes in S-IgA. While there are some limitations to its applicability, the apparent viability of the method and the additional data that it provides on the pattern of changes indicate that it is an effective approach for certain S-IgA research. In addition, further research using multiple salivary biomarkers may assist in identifying informative time lags between physiological stress markers.

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