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Running header: Stress reactivity and tear SIgA response to stress.

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Abstract

Tear secretory immunoglobulin-A (SIgA) is a putative biomarker of common-cold risk with potential utility in non-invasive diagnostics. As SIgA secretion at the ocular surface is under strong autonomic control, we investigated the relationship between HR reactivity and tear SIgA responses to novel experiential stress. Thirty-two healthy participants undertook a 60-second zip-line ride to evoke acute stress and a seated-rest control trial in a randomised-crossover design. We recorded heart rate (HR) continuously and collected unstimulated tear samples 5-min-pre-, 2-min-post- and 20-min-post-stress/control. Stress increased HR and state anxiety whereas tear SIgA concentration decreased 44% post-stress vs. control. Higher peak HR values during stress uniquely explained 21% of the variance in tear SIgA reactivity to stress ($p < .01$); high HR reactors displayed greater decreases in tear SIgA concentration. We conclude that physiological arousal increases immune reactivity to acute stress and highlight tear SIgA as a minimally-invasive, physiologically relevant biomarker of immune reactivity.

Introduction

Mucosal secretions are an attractive medium for the repeated, non-invasive assessment of endocrine, immune and inflammatory responses to stress (Papacosta & Nassis, 2011; Slavish, Graham-Engeland, Smyth, & Engeland, 2015). Secretory immunoglobulin-A (SIgA) provides a direct measure of immune competence due to its antimicrobial actions at the mucosal epithelia (Brandtzaeg, 2013). Low salivary SIgA levels have been highlighted as a risk factor for upper respiratory illness in athletes (Gleeson et al., 2012; Neville, Gleeson, & Folland, 2008) and the general population (Jemmott & McClelland, 1989; Volkmann & Weekes, 2006).

Several previous studies of mucosal immune responses to acute stressors have utilised salivary SIgA as a biomarker of immune reactivity to acute laboratory stressors (Benham, 2007; Bosch et al., 2001; Bosch, de Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003; Campisi, Bravo, Cole, & Gobeil, 2012) and longer-term naturalistic stress (Engeland et al., 2016; Phillips et al., 2006; Volkmann & Weekes, 2006). However, the tear fluid offers an alternative, minimally-invasive medium to assess immune function. Transmission of upper respiratory tract infections (URTI) has been demonstrated at the ocular surface (Bischoff, Reid, Russell, & Peters, 2011) whereas oral transmission of URTI may be less common (Hendley & Gwaltney, 1988). It is likely that the tear fluid plays an important role in host defence and indeed recent evidence suggests that tear fluid SIgA can outperform salivary SIgA to assess URTI risk (Hanstock et al., 2016). Tear SIgA has been shown to decrease immediately after prolonged exercise (Hanstock et al., 2016), but the effect of acute stress on this putative immune biomarker remains unexplored.

Immune reactivity to acute experiential stress has been demonstrated in first-time skydivers (Schedlowski et al., 1993) and bungee jumpers (van Westerloo et al., 2011). These activities increase state anxiety (Hare, Wetherell, & Smith, 2013), activate sympathoadrenal-medullary and hypothalamic-pituitary-adrenal stress responses (Chatterton, Vogelsong, Lu, & Hudgens, 1997). Acute experiential stress may acutely activate cellular immune parameters, for example by mobilising NK cells (Schedlowski et al., 1993); a finding that has been mirrored in numerous studies employing acute laboratory-based stressors (Segerstrom & Miller, 2004), but may also inhibit innate immune function (van Westerloo et al., 2011). Individual differences in stress-induced sympathetic activation can predict the magnitude of cellular immune responses to acute laboratory stressors (Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991; Marsland, Bachen, Cohen, Rabin, & Manuck, 2002). Given that secretion of SIgA at the ocular surface is under strong autonomic control (Dartt, 2009) it is likely that tear

SIgA reactivity to stress will correlate with other autonomic responses such as the heart rate (HR) response to stress. Thus, our aim was to investigate the relationship between HR, state anxiety and tear SIgA responses to a novel experiential stressor.

Method

Participants

Thirty-two healthy adults (17 males, 15 females) aged 23 years (SD = 4 years) provided informed consent to participate in the study. Participants had no previous experience of the stressor and avoided alcohol, caffeine, over-the-counter medication and heavy exercise for 24 h preceding experimental trials. No participants self-reported URTI symptoms during the 4 weeks prior to the study.

Experimental procedures

Participants completed two experimental trials on consecutive days in a randomised-crossover design. The stress trial involved a ride on a 1.6 km Zip-line (ZipWorld Velocity, Gwynedd, UK), lasting approximately 60 s. Participants wore a transparent plastic eye mask to prevent watering of the eyes during the ride. Trained instructors attached participants' safety harness to the line in a suspended prone position. Participant's movement was minimal in the suspended position and no physical effort was required to complete the task. During the control trial, participants sat quietly in the laboratory for 20 min. We recorded heart rate (HR) continuously in both trials (FT7, Polar Electro, Kempele, Finland) so that peak HR during stress (HR_{peak}) could be detected. Two participants' HR monitors recorded incomplete data and were excluded from HR-based analyses. To assess state anxiety, participants completed form Y1 of the State-Trait Anxiety Inventory (STAI-Y1; Spielberger, 1983) 5 min before each trial.

Sample collection, handling and analysis

We collected tear samples at 5-min-pre, 2-min-post and 20-min-post stress onset and at the same times of day during the control trial using methods previously described (Hanstock et al., 2016). Briefly, tear fluid collected from the inferior marginal tear strip via glass microcapillary pipette was transferred to a pre-weighed microcentrifuge tube and refrigerated. At 3 h post-collection, samples were weighed to 0.01 mg, diluted 1:99 in phosphate-buffered saline and frozen at -80°C. We demonstrated stability of SIgA-C in tear samples after 3 hours refrigeration in a pilot study (see Supplementary Material). After thawing, we used an enzyme-linked immunosorbent assay to determine tear SIgA-C in duplicate (Salimetrics, PA, USA; intra-assay CV = 1.6%). We calculated SIgA secretion rate (SIgA-SR) by multiplying tear flow rate (sample mass/collection time) by SIgA-C.

Statistical analyses

We performed statistical analyses using SPSS (v24, IBM, New York, USA) and GraphPad Prism (v5, San Diego, USA). With power 0.8 and alpha 0.05, we estimated a sample size of 32 participants for a model with three predictors to detect a large f^2 effect size of 0.4 (G*Power 3.1.9, Germany). Tear SIgA-C and SIgA-SR displayed log-normal distributions and were log-transformed before analysis. The efficacy of the zip-line ride to increase state anxiety and HR was assessed using paired t-tests; effect sizes are Cohen's *d*. Two-way repeated-measures ANOVA was used to explore the influence of stress on SIgA-C and SIgA-SR. Reactivity effects were explored using hierarchical linear regression. We defined tear SIgA reactivity as the difference in log-transformed values (log_2 fold-change) between the control condition and 2-min-post-stress to give equal weighting to increases and decreases from control values in the regression analysis.

Results

Physiological and psychological responses to stress.

Peak HR during the zip-line ride was higher than mean HR during seated rest (Table 1); we defined this difference as ΔHR. Prior to the zip-line ride state anxiety increased compared to control (Table 1); we defined this difference as ΔSTAI-Y1.

Table 1. Efficacy of zip-line protocol to increase HR and state anxiety.

	Stress Trial		**Control Trial**		**Statistics**			
	Mean Peak	SD	Mean	SD	*t*	*df*	*p*	*d*
Heart rate (bpm)	126	21	73	9	15.01	31	<.001	3.45
STAI-Y1 score	41	14	28	7	5.88	29	<.001	1.19

Effect of stress on tear SIgA-C and SIgA-SR.

Repeated-measures ANOVA revealed that tear SIgA-C decreased during the stress trial (time * trial interaction effect: $F(2,62) = 4.58$, $p = .01$; Fig 1a); Tukey's HSD revealed a reduction in SIgA-C at 2-min-post-stress compared to 5-min-pre-stress and lower SIgA-C during stress vs. control at all time points. At 2-min-post-stress, 28 of 32 participants' SIgA-C was lower than control, with a 44% mean decrease (SD = 36%, $d = 1.23$). There was a trend towards decreased SIgA-SR throughout the stress trial (main effect of trial: $F(1,31) = 3.37$, $p = .08$, Fig 1b).

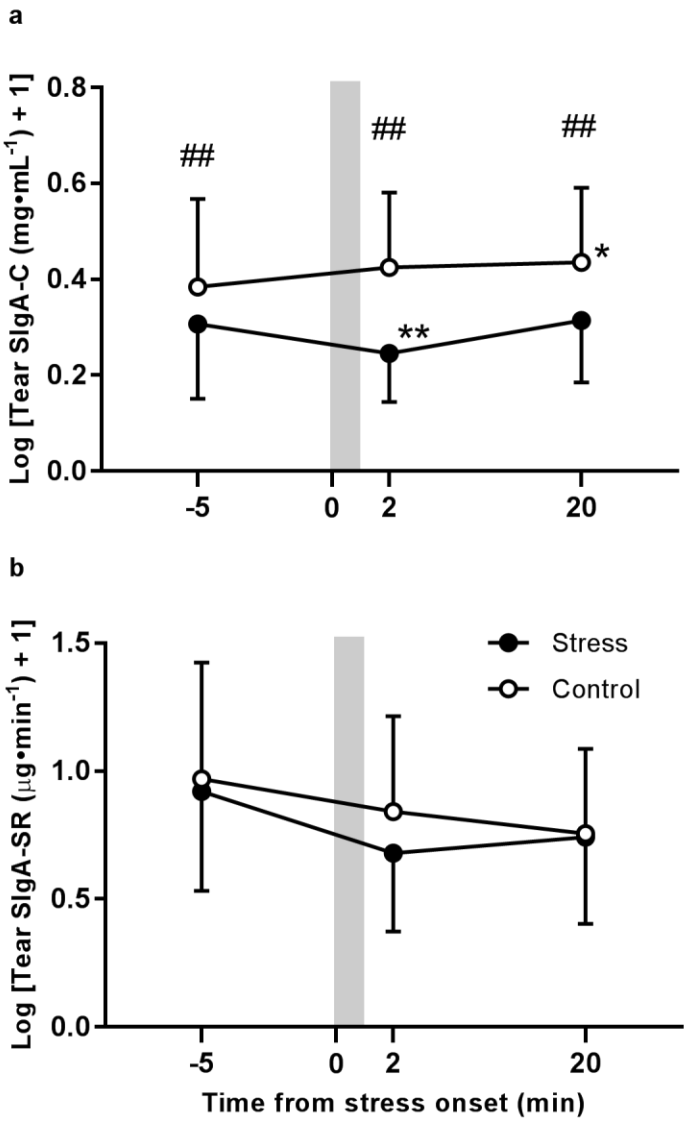

Figure 1. Tear SIgA-C and SIgA-SR responses to stress and control. Mean ± SD. Grey shade represents zip-line ride duration. Significant difference from 5-min-pre: *, $p < .05$, **, $p < .01$; ##, between trials, $p < .01$

Heart rate, state anxiety and tear SIgA reactivity to stress.

We used hierarchical linear regression to determine the relationship between stress reactivity and tear SIgA-C reactivity to stress. We entered participants' sex into the regression model first, followed by ΔHR at Step 2 and ΔSTAI-Y1 at Step 3. Collinearity statistics were within accepted ranges. At Step 2 addition of ΔHR was able to significantly explain SIgA-C reactivity ($F(2,27) = 5.67$, $p = .009$), but addition of ΔSTAI-Y1 at step 3 did not improve the model further (Table 2). No significant relationships were found between sex, ΔHR or ΔSTAI-Y1 and SIgA-SR reactivity to stress ($F(3,26) = .77$, $p = .52$).

Table 2. Hierarchical linear regression reveals ΔHR as a significant explanatory variable for the tear SIgA-C response to stress. **, $p < .01$.

		Coefficients			***Model***		***Change statistics***		
		B	SE	β	R^2	*F*	*df*	ΔR^2	*p*
1					.090	2.78	1, 28	-	.106
	(Constant)	-.294	.565	-					
	Sex	-.622	.373	-.301					
2					**.296**	**5.27**	**1, 27**	**.205**	**.009****
	(Constant)	.655	.609	-					
	Sex	-.341	.349	-.165					
	ΔHR	-.025	.009	-.473**					
3					.317	0.28	1, 26	.022	.372
	(Constant)	.683	.612	-					
	Sex	-.330	.350	-.160					
	ΔHR	-.030	.010	-.553**					
	ΔSTAI-Y1	.015	.016	.167					

Discussion

This study is the first to explore the effect of acute psychological stress on ocular immune parameters, and provides preliminary validation of tear SIgA-C as a biomarker of immune reactivity to acute stress. We observed that the zip-line protocol produced marked elevations of HR and state anxiety, and decreased tear SIgA-C throughout the duration of the stress trial. Participants with the greatest HR responses to the stressor tended to exhibit

greater decreases in tear SIgA post-stress. These observations support a role for physiological arousal in determining tear SIgA-C reactivity to stress.

During the stress trial, SIgA-C was lowest immediately post-stress, but was lower than control throughout, from 5-min before to 20-min after the zip line ride. That we did not blind participants to the stressor in advance likely caused anticipatory stress accounting for the lower tear SIgA-C at 5-min-pre; together with the lower tear SIgA-C at 20-min-post indicates that the salient influence of the stressor extends beyond 60 s duration of the zip line ride. The magnitude of the decrease in tear SIgA-C post-stress was a little smaller than previously reported decreases in tear SIgA-C following 2 h moderate-intensity exercise (-44% vs. -57%; Hanstock et al., 2016). These observations further support a role for physiological arousal, as occurs during exercise, in mediating the tear SIgA response to stress. Since the lacrimal gland secretions are primarily under parasympathetic control (Dartt, 2009), we speculate that the decrease in tear SIgA-C may arise as a result of the parasympathetic withdrawal that typically occurs during acute stress (Brindle, Ginty, Phillips, & Carroll, 2014). A limitation of this study was that we did not assess autonomic balance, but future studies could explore the relationship between autonomic activity and tear SIgA secretion in humans.

Tear SIgA-C has been previously highlighted as a potential biomarker of common cold risk (Hanstock et al., 2016). As the decrease in tear SIgA-C post-stress in the present study (-44%) was of greater magnitude than the 34% decrease in tear SIgA-C reported during the week before upper respiratory illness (Hanstock et al., 2016), the SIgA-C response to stress in the present study may have been of sufficient magnitude to compromise host defence in some of the higher reactors. These observations are consistent with the reactivity hypothesis which proposes that extremely high or low stress reactivity could exacerbate day-to-day fluctuations in immune function, increase susceptibility to opportunistic infections

(Cacioppo et al., 1998) and indicate poor states of long-term health (Lovallo, 2011). It has also been suggested that stress reactivity is a trainable trait and that lifestyle interventions such as exercise training (Forcier et al., 2006; Klaperski, von Dawans, Heinrichs, & Fuchs, 2014; von Haaren et al., 2016) and mindfulness meditation (Hoge et al., 2013) could attenuate stress reactivity, thus may have potential to improve health-related outcomes. Thus, future work is warranted to explore the influence of repeated daily hassles and subsequently lifestyle interventions on tear immunological responses to stress.

Here we demonstrate in a field-based study that tear SIgA-C is responsive to acute stress and that participants with higher HR reactivity display greater decreases in tear SIgA-C. This proof-of-concept study paves the way for future studies to examine tear SIgA responses to controlled laboratory stressors and naturalistic chronic stress. Characterising tear SIgA responses to acute and prolonged stress is warranted because the ocular surface is an important point of entry for pathogens that cause URTI (Bischoff et al., 2011) and because tear fluid is gaining interest as a medium from which to assess biomarkers (Farandos, Yetisen, Monteiro, Lowe, & Yun, 2015; Hagan, Martin, & Enríquez-de-Salamanca, 2016). If tear biomarkers are able to reliably predict health-related outcomes, wearable biosensors such as "smart" contact lenses could afford consumers the opportunity to self-monitor changes in immune status alongside other biomarkers of stress and health.

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