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Differential effects of active versus passive coping on secretory immunity

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Abstract

This study examined the acute immunological effects of two laboratory stressors, expected to evoke distinct patterns of cardiac autonomic activity; namely an "active coping" time-paced memory test, and a "passive coping" stressful video showing surgical operations. We measured salivary S-IgA, IgA-subclasses (IgA1, IgA2), and secretory component (SC). SC is responsible for the transport of S-IgA across the epithelium, and thus a rate-determining step in S-IgA secretion. Thirty-two male undergraduates were subjected to both stressors and a control video (a didactic television program). The memory test induced a typical "fight-flight" response, characterized by increases in heart rate and blood pressure in association with a decrease in cardiac preejection period (PEP) and vagal tone. The surgical video produced a "conservation-withdrawal"-like response, characterized by an enhanced vagal tone, a decrease in heart rate, and a moderate sympathetic coactivation (as indicated by a shortened PEP and an increased systolic pressure). The memory test induced an increase in the concentration and, to a lesser extent, in the output of S-IgA, IgA1, and SC. The output of IgA2 was not significantly affected. For the surgical video, a different pattern emerged: During stressor exposure S-IgA remained unaffected, against the background of a small increase in SC output. However, 10 min after the surgical video S-IgA levels had decreased. This decrease in S-IgA was paralleled by a decrease in IgA1, but not IgA2. We conclude that acute stress can have both enhancing and suppressive effects on secretory immunity, the IgA1 subclass in particular. The mechanisms that underlie these divergent responses may include stressor-specific patterns of autonomic activation.

Descriptors: Autonomic balance, Cardiovascular reactivity, Mucosal immunity, Parasympathetic coactivation, Psychoneuroimmunology

There exists convincing evidence linking psychological stress to increased susceptibility to infectious diseases (Cohen & Herbert, 1996). Potential mediators of these effects are the secretory proteins, such as mucins, cystatins, lactoferrin, and secretory immunoglobulin A (S-IgA), which protect the epithelial layers of our bodies against the invasion of microorganisms and toxins (Henskens, Veerman, & Nieuw Amerongen, 1996; Lamm, 1997; Nieuw Amerongen, Bolscher, Bloemena, & Veerman, 1998; Schenkels, Veerman, & Nieuw Amerongen, 1995). S-IgA has already been suggested to have direct relevance in upper respiratory tract pathogenesis (Gleeson, 2000; Jemmott & McClelland, 1989), and can be measured fairly easy and noninvasively by collecting saliva.

Several studies have reported an association between chronic stress and reduced levels of salivary S-IgA (Valdimarsdottir & Stone, 1997). Conversely, acute stressful conditions have mostly been reported to enhance salivary S-IgA levels. For example, S-IgA levels are enhanced immediately before, during, and after academic examinations (Bosch et al., 1996, 1998; Huwe, Hennig, & Netter, 1998; McClelland, Ross, & Patel, 1985; Spangler, 1997), and in response to brief laboratory challenges (Ring et al., 1999; Willemsen et al., 1998; Winzer et al., 1999). The short time frame in which these increases occur suggests mediation by the autonomic nervous system. This is corroborated by animal studies

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showing that electrical stimulation of the autonomic nerves produces a rapid increase in S-IgA secretion (Carpenter, Garrett, Hartley, & Proctor, 1998; Carpenter, Proctor, Anderson, Zhang, & Garrett, 2000). However, in spite of the overall finding of enhanced S-IgA levels during acute stress, rapid and substantial decreases have been reported during cold pressor (Ring et al., 2000) and in response to dental surgery (Kugler, Breitfeld, Tewes, & Schedlowski, 1996).

The brief stressor manipulations used in psychoneuroimmunology have mostly been confined to a relatively standard array of active coping tasks; typically laboratory challenges that require some sort of mental effort (e.g., stroop tasks, mental arithmetic; Uchino, Berntson, Holt-Lunstad, & Cacioppo, 2001). Such manipulations reliably induce a "fight-flight" cardiovascular response pattern, characterized by increases in heart rate, blood pressure, and other indices of cardiac sympathetic drive, in association with a vagal withdrawal. However, stressful situations may not invariantly be associated with this prototypical response pattern. Indeed, particularly in the nonhuman research literature, important distinctions have been made between "active" and "passive" coping responses (Bohus et al., 1988; Fisher, 1990). The latter is typically characterized by a vagally induced decrease in heart rate in association with signs of an increased sympathetic activity (Carruthers & Taggart, 1973; McCabe & Schneiderman, 1985; Schneiderman & McCabe, 1989).

Recently we presented evidence suggesting that this passive coping response may also be relevant for understanding psychoneuroimmunological processes in humans (Bosch et al., 2000). In this laboratory study, we induced anxiety by having participants view an 11-min-long video showing surgical procedures. The stressor clearly generated the expected increase in anxiety reporting and exhibited a robust effect on secretory immunity; for example, it induced a near threefold increase in saliva-mediated bacterial adherence. However, on a cardiovascular level, only a modest bradycardia was observed (Bosch et al., 2000). On basis of these results we suggested that results of laboratory studies might have been inadequately subsumed under the broad label of "acute stress." A more differentiated perspective on acute stress, by differentiating between stressors with distinct autonomic nervous system effects, may yield a better understanding of the physiological mechanisms involved in stress-induced immunomodulation.

We propose two additional improvements that are pertinent to the psychophysiological investigation of secretory immunity. First, previous studies measured total levels S-IgA only, and ignored the fact that humans secrete two classes of S-IgA. These subclasses, denoted IgA1 and IgA2, are found in saliva in a ratio of 3:2, respectively (Brandtzaeg et al., 1999). Differentiating between these subclasses may be relevant as Gleeson and coworkers showed that decreased IgA1 levels in particular are associated with an increased risk of upper respiratory tract infections (Gleeson, Hall, McDonald, Flanagan, & Clancy, 1999). Interestingly, Gregory and coworkers reported that 10 min of maximal exercise decreased the concentrations of IgA1, but not IgA2, in breast milk of lactating women (Gregory, Wallace, Gfell, Marks, & King, 1997). This suggests that the secretions of the two subclasses are under different controls, and, hence, might also be differentially affected by psychological stress.

Secondly, measuring both S-IgA and its transporter molecule (secretory component, SC) will provide information on the physiological mechanisms that underlie the reported effects of stress on salivary S-IgA levels, that is, whether they result from changes in IgA secretion by the *immune cells*, or from changes in IgA translocation by the *glandular cells*. It should be noted that S-IgA is formed and secreted in a two-step process (for a comprehensive review on this topic see Norderhaug, Johansen, Schjerven, & Brandtzaeg, 1999). First, a polymeric form of immunoglobulin A is synthesized and secreted by plasma cells (transformed B-lymphocytes) that are present in the glandular tissues. Subsequently, the secreted IgA molecule locks on to a receptor molecule (referred to as the poly-immunoglobulin receptor, pIgR) present on the basolateral, "backside," of a secretory glandular cell. The pIgR, with or without an immunoglobulin actually attached to it, is translocated from the backside to the apical front of the glandular cell. Here the receptor, with or without an immunoglobulin attached to it, becomes cleaved off and is secreted into saliva as secretory IgA or secretory component, respectively. Thus, *both* IgA secretion by the plasma cells and the availability of pIgR for translocation by the glandular cells are rate-determining steps in the secretion of S-IgA. As both the plasma cells and the glandular cells express receptors for various hormones and transmitters, each cell type may independently contribute to the levels of salivary S-IgA under stress.

Aim and Design of This Study

The study presented here examined the effects of two brief laboratory stressors, expected to evoke distinct patterns of cardiac autonomic activity, on secretory immunity. These manipulations were denoted as active or passive coping stressors on the basis of both task characteristics (requiring effort or not) and the profile of cardiac autonomic responses. A time-paced memory test was used as an active coping task, whereas a gruesome video showing surgical procedures was utilized as a passive coping condition (Bosch et al., 2000; de Geus, van Doornen, & Orlebeke, 1993). A nonstressing didactic television program was included as a control condition. Our main hypothesis was that different acute stressors may show different immunomodulatory effects. In addition to total S-IgA, we measured the two IgA subclasses (IgA1 and IgA2). If these subclasses are differentially affected, this will result in a shift in the IgA1/IgA2 ratio, which was therefore computed as well. We measured salivary SC to examine the involvement of the epithelial cells in S-IgA secretion under stress. Cardiovascular measures were recorded to verify whether the expected distinct patterns of cardiac autonomic activity were indeed found. In an exploratory analysis, we also examined the association between cardiovascular reactivity and concomitant immune reactivity.

Materials and Methods

Participants

Study participants were 34 male undergraduate volunteers, ranging in age from 18 to 31 (mean age 23.4). Participants gave written informed consent and received 40 Dutch guilders or study credits for their participation. The inclusion criteria for participation were that the participant (a) had no current health complaints, (b) did not receive medical treatment or used prescribed medication, (c) had no recent history of signs of upper respiratory infection or other inflammatory disease, (d) was not needle or blood phobic, and (e) was not a student of medicine, dentistry, or biology.

Procedure

In preparation for the study, participants were instructed to refrain from using alcohol or nonprescription drugs during the 24 hr preceding testing. In addition, participants were instructed not to engage in physical exercise on the day of the experiment, and to

abstain from smoking, drinking caffeinated beverages, eating, and brushing their teeth (to prevent gingival bleeding) 1 hr prior to the experiment.

Participants were subjected to three experimental conditions. Each condition was on a separate day, administered in counterbalanced order. The experimental conditions were (1) a time-paced memory test, (2) a video showing various surgical procedures, and (3) a didactic television program on the wing movements of birds, which functioned as a control condition. Most experimental sessions were scheduled seven days apart, but participants were allowed some flexibility. No experiments were conducted in the same week a class exam was scheduled. Measurements took place between 1:30 p.m. and 4:00 p.m.; for each participant the time of measurement was held constant.

On arrival, the experimental procedure was explained to the participant, electrodes for electrocardiogram (ECG) and impedance cardiogram (ICG) were attached, and participants rinsed their mouths with tap water. At the start of the first experimental session, the method of saliva collection was rehearsed (see section on saliva collection). Participants then filled out a self-report questionnaire and were allowed to read self-selected magazines with neutral content for 28 min. During the final 4 min of this quiet reading, baseline saliva was collected. The baseline measurement was followed by one of three experimental manipulations (memory test, surgical video, or control). Each manipulation lasted 11 min. The second saliva sample was collected during the final 4 min of each experimental manipulation (stress) and also 9 min after the end of the manipulation (recovery), when subjects were again quietly reading. Immediately after each saliva collection participants filled out a state anxiety questionnaire. The ECG and ICG were recorded continuously.

Saliva Collection

Saliva was collected by means of the "spitting-method," according to the directions given by Navazesh (1993). This method has been shown to have an adequate retest reliability, and is recommended for unstimulated whole saliva collection on basis of a comparative study (Navazesh & Christensen, 1982). The method of saliva collection was practiced before the start of the first experimental session to familiarize the participants with the procedure. The collection trial started with the instruction to void the mouth of saliva by swallowing. Subsequently, saliva is allowed to accumulate in the floor of the mouth, without stimulation of saliva secretion by means of oro-facial movements. The participant spitted out into a preweighed, ice-chilled polypropylene cup every 60 s. Saliva was collected for 4 min, and secretion was determined by weighting the cups and subtracting the preweighed values. After collection, saliva was homogenized by vigorous shaking by using a vortex and clarified by centrifugation (10,000 $\times$ g, 4 min.) to eliminate buccal cells and oral microorganisms. The clear supernatant was divided into 500 μl aliquots and stored at -20°C until use.

Cardiovascular Assessment

Assessment of cardiovascular response focused on blood pressure and cardiac autonomic balance. Blood pressure was measured with a Dinamap Vital Signs Monitor (Critikon model 845 XT). The ICG and ECG signals were recorded from six Ag/AgCl spot-electrodes (AMI type 1650-005, Medtronic) using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) device (de Geus & van Doornen, 1996; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996). Systolic (SBP) and diastolic blood pressure (DBP) were measured every 2 min. Indices of sympathetic and parasympathetic drive were obtained by analysis of ECG and thoracic impedance (ICG) signals (Berntson et al., 1997; de Geus & van Doornen, 1996). The ECG and ICG complexes were ensemble averaged with reference to the ECG R-wave across 1-min periods. From these 1-min ensembles, average levels were computed for interbeat interval (IBI), root mean square of successive difference (RMSSD), preejection period (PEP) and left ventricular ejection time (LVET). Reliability and validity of the VU-AMS have been reported elsewhere (de Geus & van Doornen, 1996; Willemsen et al., 1996). Changes in PEP are used to index changes in cardiac sympathetic drive (Sherwood et al., 1990), whereas changes in RMSSD are used to index changes in cardiac vagal tone.

Quantification of Secretory IgA, IgA1, IgA2 and Total Secretory Component

The concentrations of total IgA, IgA subclasses, and total (free and bound) secretory component were measured by using a sandwich enzyme-linked immunosorbent assay (ELISA). The ELISA for total S-IgA was a modified version of the ELISA described by Bosch et al. (1996). In this adapted version, an antibody directed against secretory component (Clone GA-1, Sigma diagnostic, Dordrecht, The Netherlands) was used to capture S-IgA. The high specificity and quality of this antibody has been established in a multicenter WHO/NIUIS study (Mestecky et al., 1996), and corroborated in our laboratory by Western blot and ELISA. For detection, an HRP-conjugated polyclonal rabbit antiserum was used, directed against human IgA heavy chain (Sigma diagnostics, Dordrecht, The Netherlands). At working dilutions, the S-IgA ELISA did not respond to both the secretions (saliva, breast milk) of IgA-deficient patients and normal serum. Together this indicates that test values are not affected by any of the components present in exocrine fluids or serum other than S-IgA.

The ELISA for SC was developed in-house. For capture of salivary SC, the same monoclonal antibody (GA-1) was used as in the ELISA for S-IgA (see above). For detection we used HRP-conjugated rabbit antihuman polyclonal antiserum directed against secretory component (DAKO, Glostrup, Denmark).

The ELISA for the IgA subclasses was performed as described by de Fijter, van den Wall Bake, Braam, van Es, and Daha (1995). The monoclonal capture antibodies for this assay were obtained from Nordic (Tilburg, The Netherlands), and have been found to be of high quality and specificity in several comparative studies (de Fijter et al., 1995; Mestecky et al., 1996; Reimer, Phillips, Aloisio, Black, & Wells, 1989; Valentijn et al., 1984). In addition, we validated the specificity of these antibodies in our laboratory by Western blot and ELISA.

The standards for S-IgA, IgA1, IgA2, and SC were obtained from Nordic (Tilburg, The Netherlands). The intra-assay variability of the assays was $<4\%$.

Questionnaires

Participants were administered the Dutch translation of the Spielberger State/Trait-anxiety Inventory (van der Ploeg, 1988). The state-anxiety part (20 items) of this questionnaire was administered immediately after each saliva collection. This instrument is developed to evaluate feelings of tension, nervousness, apprehension, and worry, and was used in this study as an indicator of distress. The instruction after the second saliva collection was adapted to report on the anxiety the subject felt during the experimental manipulation. Participants also filled out a self-report questionnaire on health (perceived health, use of medication or other medical treatment), life-habits, and behavior during the 24 hr

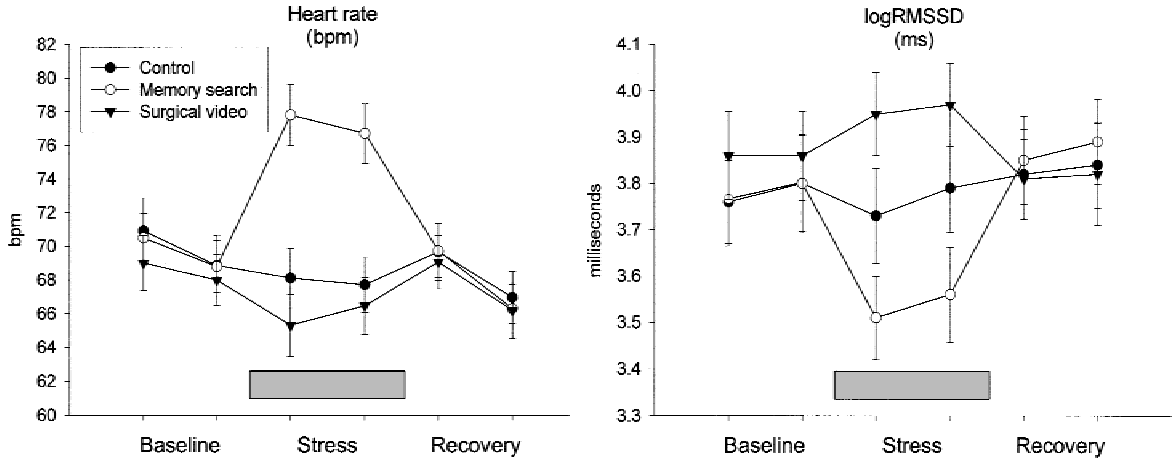

Figure 1. Heart rate and logRMSSD (a measure of cardiac vagal tone) during the control condition, memory test, and surgical video. Each measurement (baseline, stress, recovery) is divided in two time points, which, respectively, represent the 4 min immediately before and the 4 min during saliva sampling. Dots indicate means, vertical bars indicate SEM.

preceding the experiment (smoking, alcohol, tea and coffee consumption, physical exercise, and sleep duration and quality).

Data Analysis

Each experimental condition (memory test, surgical video, control) consisted of three measurement periods (baseline, stress, and recovery). For the cardiovascular parameters, two values were determined per measurement period, representing the averages of the 4 min preceding and during saliva collection. This procedure allowed testing of potential effects of saliva sampling on cardiovascular parameters. Statistical analysis of cardiovascular parameters was performed by repeated measurements ANOVA with Condition (3), Measurement Period (3) and Saliva Collection (2) as repeated factors. Two separate analyses were performed: contrasting the memory test with the control condition, and contrasting the surgical video with control condition. The main effects for each experimental condition are reported also. For the immunological data, the same procedure was followed, with now only one value (the saliva sample) per measurement period. Data were analyzed using SPSS for windows 9.0.

Results

Missing Values

Two participants terminated the experiment while viewing the surgical video. In both cases the participants reported feeling too uncomfortable with the gruesome content of the video. Their data were excluded from further analysis. After laboratory analyses, the immunoglobulin data of two more participants were excluded. For one participant, this was because IgA values consistently deviated more than 2.5 standard deviations from the group mean, whereas the other participant appeared IgA deficient. Subsequently, for salivary immunoglobulin, a full set of data was available for 30 study participants. Due to a technical problem there were three occasions on which the ICG data of a participant was not complete, therefore the *df* of the analyses of PEP show some variation.

Cardiovascular Measures

Figures 1, 2, and 3 present the summary data for heart rate, RMSSD, PEP, diastolic pressure, and systolic pressure.

Heart rate. The heart rate increased during the memory search task, whereas both the control condition and surgical video induced a moderate decrease in heart rate (Figure 1). Time × Condition analysis, contrasting responses within each stressor with the control condition, yielded a significant increase in heart rate increase during the memory test, $F(5,155) = 41.74$, $\epsilon = .456$, $p < .001$, and showed that the decrease during the surgical video was marginally larger than the decrease observed during the control condition, $F(5,155) = 2.18$, $\epsilon = .612$, $p < .06$.

Vagal tone and preejection period. Due to the skewedness of the data, RMSSD was logarithmically transformed (ln + 1) to yield a logRMSSD, which is subsequently used for statistical analysis and also presented in Figure 1.

Contrasting each of the two stressors with the control condition revealed a significant decrease in logRMSSD in response to the

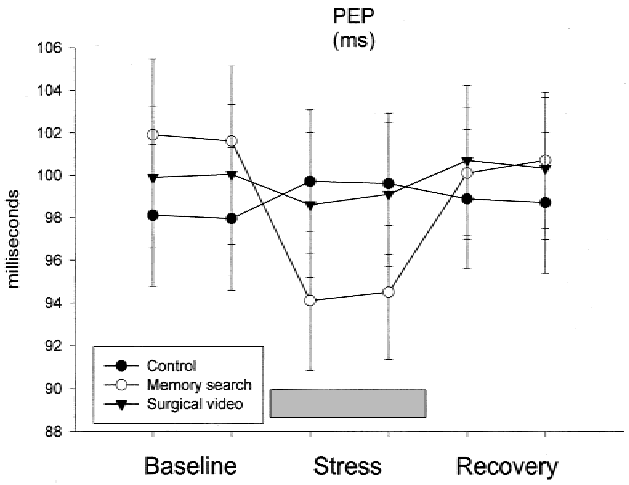

Figure 2. PEP (a measure of cardiac sympathetic drive) during the control condition, memory test, and surgical video. Each measurement (baseline, stress, recovery) is divided in two time points, which, respectively, represent the 4 min immediately before and the 4 min during saliva sampling. Dots indicate means, vertical bars indicate SEM.

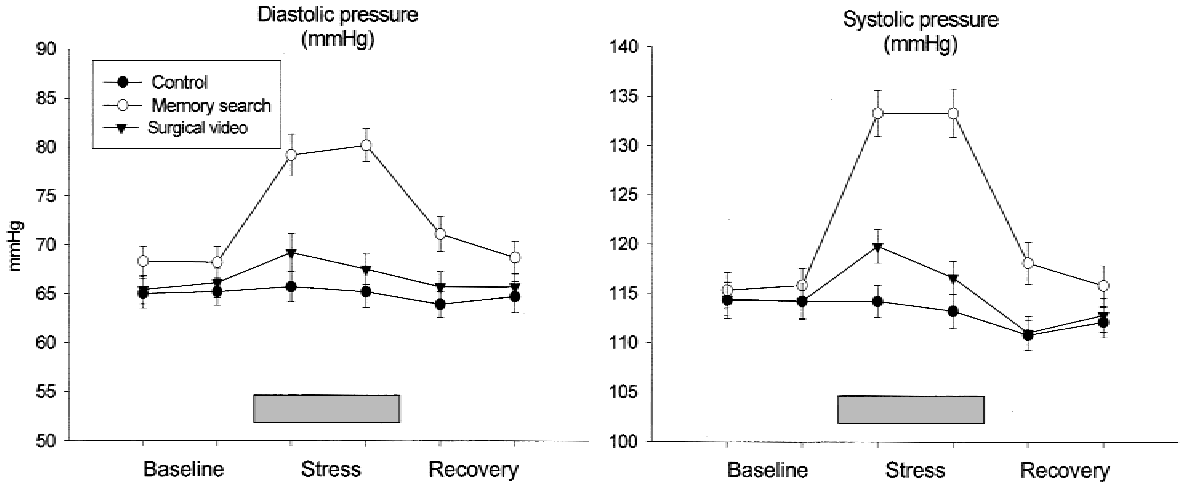

Figure 3. Diastolic and systolic blood pressure during the control condition, memory test, and surgical video. Each measurement (baseline, stress, recovery) is divided in two time points, which, respectively, represent the 4 min immediately before and the 4 min during saliva sampling. Dots indicate means, vertical bars indicate SEM.

memory test, $F(5,155) = 6.77$, $\epsilon = .552$, $p < .001$, whereas the logRMSSD response during the surgical video was increased in comparison to the control condition, $F(5,155) = 9.01$, $\epsilon = .715$, $p < .001$. The latter interaction effect appeared to be driven, at least in part, by a significant increase in logRMSSD during the surgical video, $F(5,155) = 4.13$, $\epsilon = .615$, $p < .001$. In contrast, PEP shortened, in comparison to the control, during the memory test, $F(5,145) = 40.00$, $\epsilon = .474$, $p < .001$, as well as during the surgical video, $F(5,150) = 6.95$, $\epsilon = .536$, $p < .001$ (Figure 2). A significant time main effect for the surgical video condition further confirmed that PEP shortened during this stressor, $F(5,150) = 3.65$, $\epsilon = .544$, $p < .01$.

Blood pressure. Contrasting each of the two stressors with the control condition revealed a significant increase in diastolic blood pressure in response to the memory test, $F(5,155) = 20.89$, $\epsilon = .563$, $p < .001$, whereas the diastolic response during the surgical video did not differ significantly from the control condition, $F(5,155) = 1.53$, $\epsilon = .829$, $p > .1$ (Figure 3). The same analysis applied to systolic pressure showed a significant effect for both the Control × Memory Test interaction, $F(5,155) = 39.16$, $\epsilon = .673$, $p < .001$, and the Control × Surgical Video interaction, $F(5,155) = 5.51$, $\epsilon = .697$, $p < .001$. The latter interaction effect appeared to be driven by a moderate increase in systolic pressure during the surgical video, $F(5,155) = 15.13$, $\epsilon = .818$, $p < .001$ (Figure 3).

Anxiety Reporting

Table 1 gives the summary data for the state anxiety inventory. Both the memory test and the surgical video generated a substantial increase in anxiety; $p < .001$, $F(2,56) = 117.45$, $\epsilon = .687$, and $p < .001$, $F(2,62) = 53.67$, $\epsilon = .790$, respectively. During the control condition feelings of anxiety slightly decreased, $F(2,62) = 4.27$, $\epsilon = .915$, $p < .05$.

Table 1. *Mean (SEM) State-Anxiety Reporting*

	Baseline	Stressor	Recovery
Control video	10.47 (0.92)	9.15 (0.81)	10.47 (0.91)
Memory-search task	10.59 (0.95)	27.47 (1.69)	11.71 (1.00)
Surgical video	9.78 (0.77)	22.75 (2.03)	10.00 (0.94)

Salivary Immune Measures

S-IgA concentration and output. Figure 4 presents the summary data for total S-IgA concentration (in micrograms per milliliter) and S-IgA output (in micrograms per minute, controlling for the effects of salivary flow rate). S-IgA concentration did significantly increase during the memory test when compared to the control condition (Time × Condition, $F(2,58) = 13.69$, $\epsilon = .848$, $p < .001$). S-IgA output also increased over baseline during the memory test, $F(2,58) = 3.30$, $\epsilon = .827$, $p < .05$, but this increase did not significantly differ from the control condition (Time × Condition, $F(2,56) = 0.47$, $\epsilon = .927$, $p > .60$). Conversely, the surgical video caused a significant decrease in S-IgA concentration (Time × Condition, $F(2,58) = 5.16$, $\epsilon = .977$, $p < .01$) as well as output (Time × Condition, $F(2,58) = 4.05$, $\epsilon = .814$, $p < .05$). As Figure 4 suggests, these effects are largely driven by the reduced concentration and output of S-IgA during the recovery period after the surgical video (for both main effects $p < .001$).

IgA1 concentration and output. Figure 5 present the summary data for IgA1 concentration (in micrograms per milliliter) and output (in micrograms per minute). Time × Condition analysis showed that the memory test, $F(2,58) = 17.86$, $\epsilon = .824$, $p < .001$, induced a significant increase in IgA1 concentration relative to the control condition. The memory test also induced a significant increase in IgA1 output (in micrograms per minute), $F(2,58) = 4.97$, $\epsilon = .830$, $p = .01$. However, analyses of Time × Condition effects indicated this increase did not differ from the response within the control condition, $F(2,58) = 0.469$, $\epsilon = .992$, $p > .60$.

Conversely, Time × Condition analysis indicated that during the surgical video both IgA1 concentration, $F(2,58) = 9.35$, $\epsilon = .910$, $p < .001$, and output, $F(2,58) = 5.77$, $\epsilon = .815$, $p < .01$, were significantly decreased relative to the control condition. These effects were mainly driven by a lowering IgA1 concentration and output during the recovery period (for both time main effects $p < .001$).

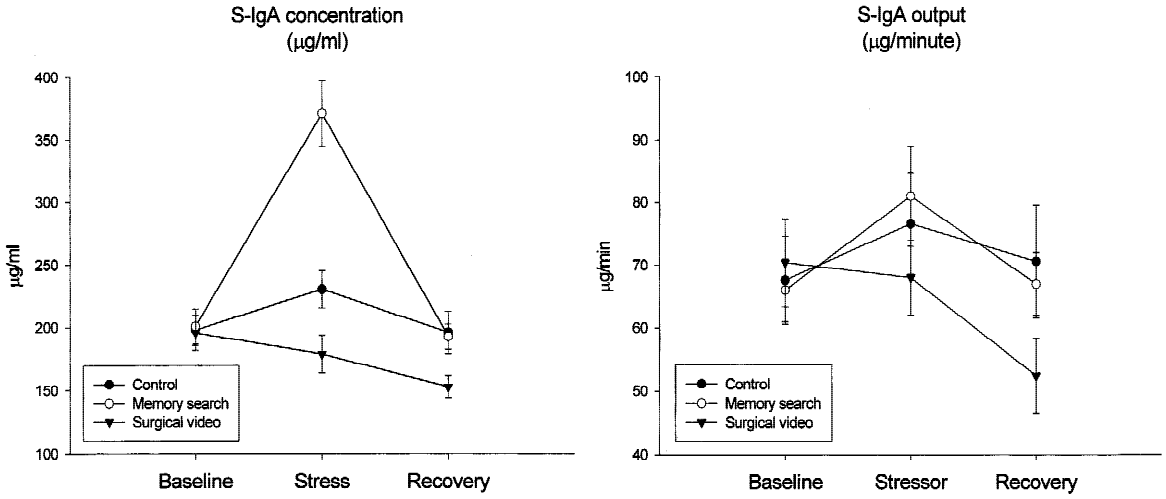

Figure 4. S-IgA concentration and output during the control condition, memory test, and surgical video. Dots indicate means, vertical bars indicate SEM.

IgA2 concentration and output. Figure 6 present the summary data for IgA2 concentration and output. Time × Condition analysis showed that both the memory test condition, $F(2,58) = 22.31$, $\epsilon = .903$, $p < .001$, and the surgical video condition, $F(2,58) = 3.46$, $\epsilon = .856$, $p < .05$, were differentially affected when contrasted with the control condition. However, repeated measures analysis showed that there was no main effect for the surgical video on IgA2 concentration, $p > .2$.

GLM repeated measures applied to IgA2 output (in micrograms per minute) yielded no "overall" Time × Condition effect, $F(4,116) = 1.37$, $\epsilon = .952$, $p > .2$. Moreover, although there was a significant "overall" main effect for measurement period, $F(2,58) = 3.20$, $\epsilon = .952$, $p < .05$, none of the separate analyses of time main effects within each experimental condition yielded a significant result (all $p > .10$). This indicates that IgA2 output did not differ in comparison to the other conditions (reflected by the nonsignificant Time × Condition interaction) and within each condition (reflected by the nonsignificant time main effects).

IgA1/IgA2 ratio. The statistical analyses presented above suggested that during each condition salivary IgA1 levels were more substantially affected than IgA2 levels. To examine this further, the IgA1/IgA2 ratio was computed [ratio = IgA1/(IgA1 + IgA2)], as described by de Fijter et al. (1995). IgA1/IgA2 ratio is presented in Figure 7. Repeated measures applied to IgA1/IgA2 ratio indeed yielded a significant overall time main effect, $F(2,58) = 22.14$, $\epsilon = .918$, $p < .001$. Subsequent post hoc analyses of main effects indicated that during each condition, IgA1/IgA2 ratio was significantly affected (all $p < .001$). However, Time × Condition analyses provided little support for the idea that the transient variations in IgA1/IgA2 ratio differed between the three experimental conditions; contrasting the change during the memory test with the control condition did not yield a significant effect, $F(2,58) = 0.31$, $\epsilon = .700$, $p > .6$, whereas Time × Condition analysis contrasting the surgical video with the control condition yielded a nonsignificant trend, $F(2,58) = 2.62$, $\epsilon = .775$, $p < .08$.

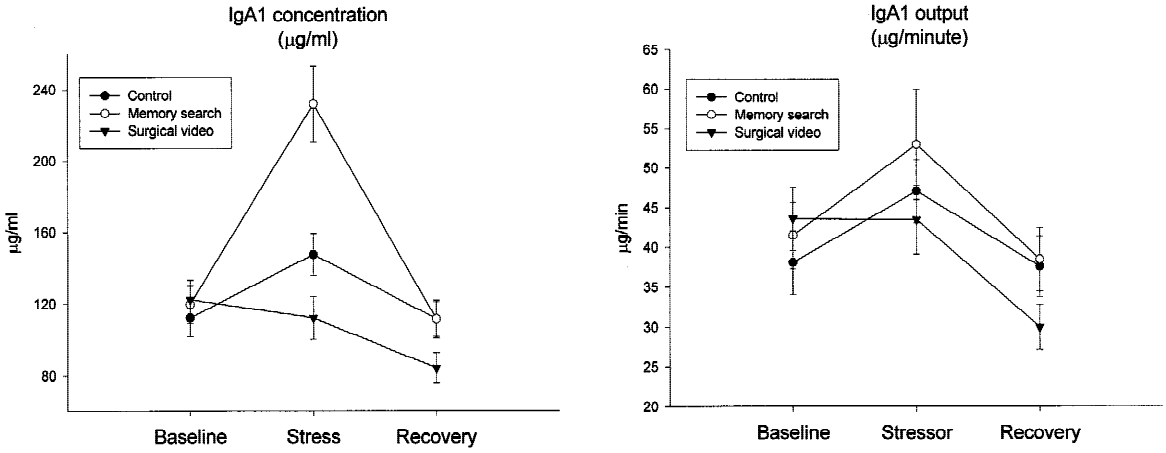

Figure 5. IgA1 concentration and output during the control condition, memory test, and surgical video. Dots indicate means, vertical bars indicate SEM.

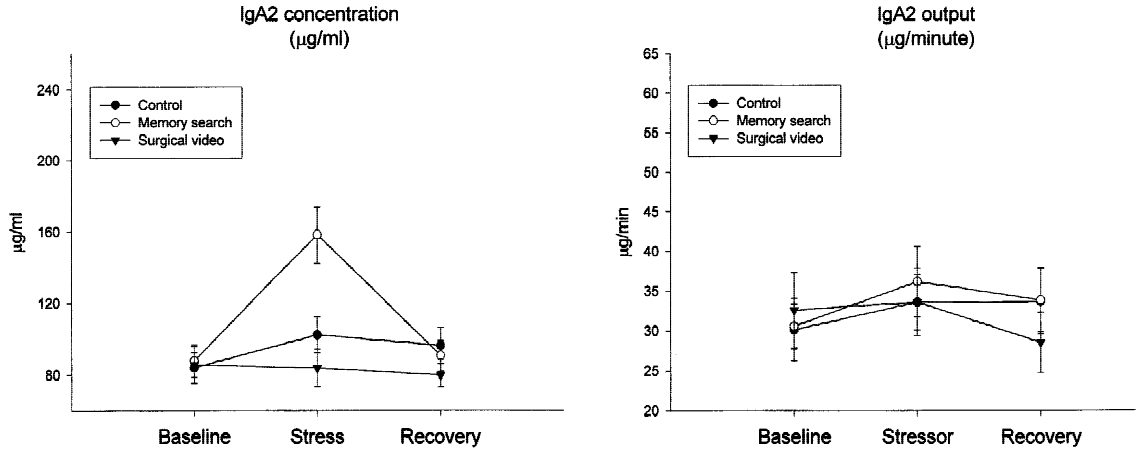

Figure 6. IgA2 concentration and output during the control condition, memory test and surgical video. Dots indicate means, vertical bars indicate SEM.

Secretory component concentration and output. Figure 8 presents the summary data for total SC concentration and output. Analyses of Time × Condition effects indicated that, relative to the control condition, the memory test increased SC concentration, $F(2,56) = 14.55$, $\epsilon = .798$, $p < .001$, whereas during the surgical video SC concentration remained low, $F(2,58) = 3.15$, $\epsilon = .804$, $p < .05$.

Analyses of Time × Condition effects of SC output showed that the three experimental conditions did not differ in terms of variation in SC output ($p > .5$). Repeated-measures analyses of the separate conditions revealed a significant increase during both the control condition, $F(2,58) = 6.27$, $\epsilon = .887$, $p < .01$, and the memory test, $F(2,58) = 4.39$, $\epsilon = .807$, $p < .05$, whereas the surgical video condition only marginally increased SC output, $F(2,58) = 3.04$, $\epsilon = .786$, $p < .06$.

Test-Retest Reliability of S-IgA, IgA Subclasses, IgA1/IgA2 Ratio, and SC

Spearman rank-order correlations were computed, correlating the baseline values of the first, second, and third experimental session, which were scheduled 1 week apart. In general, no systematic differences were observed when comparing the temporal stability of the 1-week periods (Sessions 1 vs. 2, and 2 vs. 3) with the retest stability over 2 weeks (Sessions 1 vs. 3). The range of the test-retest correlations are presented in Table 2.

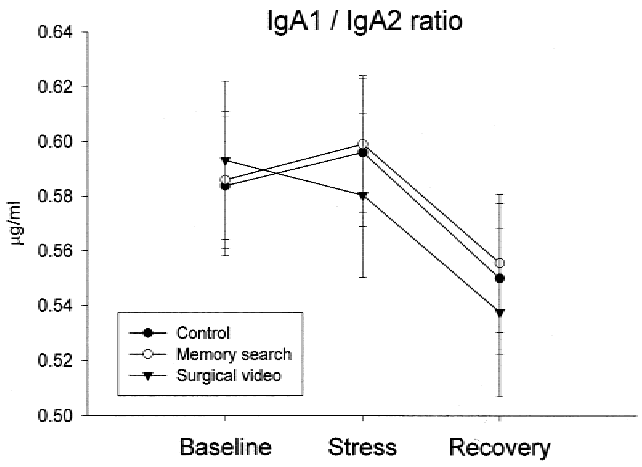

Figure 7. IgA1/IgA2 ratio [ratio = IgA1/(IgA1 + IgA2)] during the control condition, memory test, and surgical video. Dots indicate means, vertical bars indicate SEM.

Association Between Cardiovascular and Immunological Reactivity

To explore the association between change in autonomic activation and secretion of S-IgA during the experimental tasks, we correlated the change in cardiovascular measures during the 4 min of saliva collection with concurrent immune changes (reactivity = task values − baseline values). There appeared to be little evidence for a significant association between cardiovascular reactivity and reactivity of the salivary immune measures during the active coping memory test, either computed as absolute differences or relative differences, with or without correction for basal values. Only the correlation between change (absolute differences) in S-IgA output and systolic pressure, both measured during the task, was significant, .36, $p < .05$.

During the passive coping surgical video, a more consistent pattern emerged, in that absolute change in logRMSSD showed a significant negative correlation with the absolute change in S-IgA output (−.49), S-IgA concentration (−.44), IgA1 concentration (−.41), IgA2 output (−.41), IgA2 concentration (−.47), and SC concentration (−.39), indicating that an enhanced logRMSSD during the surgical video was associated with lower levels of IgA and SC during that condition. In addition a significant positive correlation between change in heart rate and SC output was observed (.40). Computing relative differences (percent change) gave essentially the same pattern of results, with now also the association between RMSSD and SC output reaching statistical significance. No other significant associations between cardiovascular and salivary measures were observed.

Discussion

This study examined the salivary levels of S-IgA, IgA subclasses, and SC in response to two brief laboratory stressors that were expected to evoke distinct patterns of cardiovascular and autonomic activity. To the best of our knowledge, this is the first study

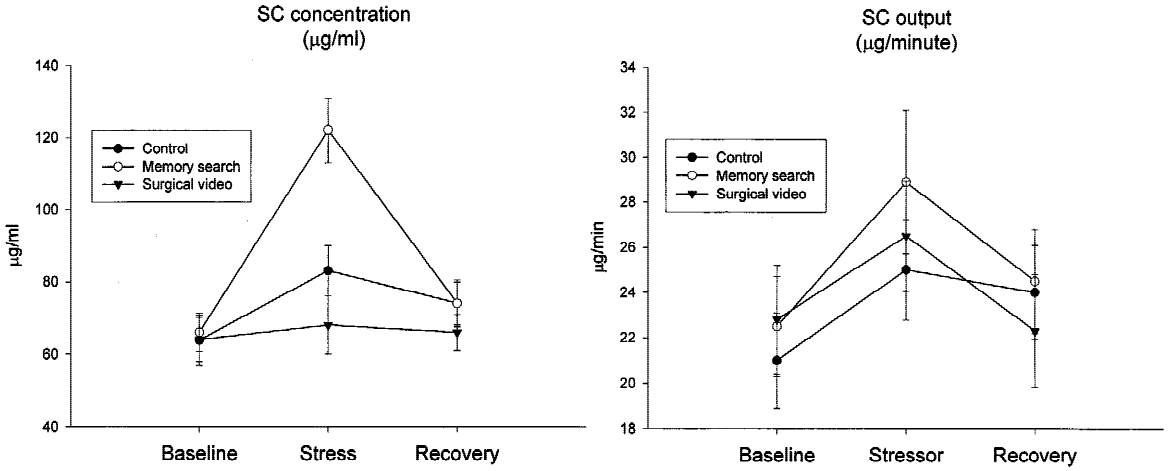

Figure 8. SC concentration and output during the control condition, memory test, and surgical video. Dots indicate means, vertical bars indicate SEM.

to report on the effects of psychological factors on IgA subclasses and SC. The two stressors displayed the expected distinct modes of autonomic cardiovascular control. The memory test produced a prototypical pattern of cardiovascular responses (essentially a sympathetic activation and a vagal withdrawal), variously referred to as an active coping, fight-flight, or defense response (Rosen & Schulkin, 1998; Schneiderman & McCabe, 1989). The constellation of responses induced by the surgical video (essentially a parasympathetic cardiac activation and a moderate sympathetic coactivation) has been labeled as a passive coping response (Bohus et al., 1988; Fisher, 1990; Schneiderman & McCabe, 1989). However, other labels are also used to characterize this response pattern, such as "conservation-withdrawal," "aversive vigilance," and "aversive coping" (Engel, 1977; Schneiderman & McCabe, 1989). Although frequently reported in the animal literature, only a few studies have examined this vagotonic stress response in humans (for examples, see Bosch et al., 2000; Carruthers & Taggart, 1973; Hubert & de Jong-Meyer, 1991; Vingerhoets, Ratliff-Crain, Jabaaij, Menges, & Baum, 1996). The different stressors were rated as equally anxiety provoking. Future studies might explore whether these distinct autonomic profiles relate to specific affective dimensions other than anxiety.

In support of our hypothesis, we found the distinct cardiovascular and autonomic responses to be coupled to distinct immunomodulatory effects. The memory test substantially enhanced total S-IgA concentration and, to a lesser extent, also enhanced S-IgA output. This corroborated the findings of other studies that utilized this type of brief active coping tasks (Ring et al., 1999; Willemsen et al., 1998; Winzer et al., 1999). Conversely, the surgical video induced a decrease in both the concentration and output of S-IgA. This decrease showed a time-lagged relationship with the stressor, becoming apparent between 9 and 13 min after the stressful video (i.e., between 20 and 24 min after stressor onset).

The decreased S-IgA output in response to the surgical video appeared to be largely driven by a decrease in output of salivary IgA1: This subclass showed both the largest absolute change (in terms of output and concentration) and relative change (reflected by the change in IgA1/IgA2-ratio), whereas the decrease in IgA2 did not reach statistical significance. Moreover, also during the control and memory-test condition, the output of IgA1 was more reactive than that of IgA2, which explains the variation in IgA1/IgA2 ratio that was observed during all three conditions. Statistical analysis provided little support for the idea that the effects on IgA1/IgA2 ratio differed between the three conditions. Only a nonsignificant trend, $p = .08$, was observed when contrasting the control condition with the surgical video. However, what is supported by our data is the conclusion that salivary IgA1 levels are more responsive under a variety of conditions, which include psychological stress, than salivary IgA2 levels.

The mechanisms that underlie the higher variability in salivary IgA1 levels remain elusive. The variability cannot be explained by a higher capacity or preference of the glandular cells to transport IgA1, because the transporter molecule SC binds and transports both subclasses equally well (Norderhaug et al., 1999). The higher responsivity of the IgA1 subclass may not be unique to the salivary glands, as IgA1 levels are also found to be more variable in breast milk in response to physical exercise (Gregory et al., 1997). Although it is tempting to speculate that the secretion of the IgA subclasses is under differential neuro-endocrine control, we are not

Table 2. *Range of Test-Retest Correlations (Spearman Rank-Order Correlation Coefficients) of Resting Values (Baseline) of Salivary Measures*

Measure	Range of retest correlations
Salivary flow rate	.68–.74
S-IgA concentration	.43–.67
S-IgA output	.49–.68
IgA1 concentration	.51–.71
IgA1 output	.48–.77
IgA2 concentration	.64–.69
IgA2 output	.70–.81
IgA1/IgA2 ratio	.70–.78
SC concentration	.65–.74
SC output	.62–.73

Note: The range represents the retest correlations over a 1- to 2-week period, as each experimental session was scheduled 1 week apart. $N = 30$

aware of any direct evidence that could either support or refute this notion.

We observed that the decrease in salivary S-IgA after the surgical video was not paralleled by a decrease in SC, the molecule responsible for transporting S-IgA into the secretions. The most likely explanation for this is that the decreased output of S-IgA is due to a decreased availability of IgA for transepithelial transport. This implies that the decrease in S-IgA after the surgical video was caused by an effect of the stressor on the immunoglobulin-secreting plasma cells, rather than on the translocating epithelial cells. Conversely, the memory test increased the output of both SC and S-IgA (IgA1 in particular), which might reflect an effect on both the plasma cells and the epithelial cells. In vitro studies, however, showed that increasing IgA concentrations at the basolateral epithelial surface (thus before it is transported by the epithelia) does not affect the rate in which S-IgA is translocated (Norderhaug et al., 1999). This implies that even if the memory-test stressor caused an increase in IgA at the basolateral epithelial surface, it is unlikely that this would cause the observed increase in salivary S-IgA. Such increases should therefore primarily be ascribed to an increased transporting activity of the salivary glandular cells.

Methodological Issues

A large number of studies have combined cardiovascular measurements with the collection of saliva, mostly to measure salivary cortisol or S-IgA. It is therefore relevant to know to what extent the saliva collection itself may affect the cardiovascular outcomes. Not comforting in that regard are the results of Kistler, Mariauzouls, and von Berlepsch (1998), showing that collecting stimulated saliva by having the participants chew on cotton rolls, a method often used in cortisol studies, is a potent sympathetic stimulus, increasing heart rate and decreasing cardiac preejection period. It was therefore reassuring to find that the method of unstimulated saliva collection used in our study had little effect on most cardiovascular parameters. Only a minor, but statistically significant, decrease in heart rate between 1.8 and 0.9 bpm was observed. We therefore recommend unstimulated saliva collection as the method of choice in studies that additionally monitor the cardiovascular system.

No data was previously available on the temporal stability of the IgA subclasses and SC in saliva. Comparing the baseline values over a 1- to 2-week period, we found the retest reliability of S-IgA, and related parameters, to be moderately high. That is, although occasionally correlations in the .40–.50 range were found, most correlations fell in the .60 and the .70 range. In that regard the stability of the set of salivary immune measures presented here appears to be at least as good as that of more commonly used plasma immune markers (Marsland, Manuck, Fazzari, Stewart, & Rabin, 1995; Mills, Haeri, & Dimsdale, 1995).

A final methodological issue concerns the lack of a control condition in most reactivity studies. Our results showed that viewing an didactic television program, which had little effect on cardiovascular functioning and psychological state, affected S-IgA output to the same extent as a typical laboratory stressor, which conversely had a robust effect on both cardiovascular functioning and anxiety reporting. Harrison et al. (2000) reported similar findings when comparing the effects of video clips that were either distressing, humorous, or didactic. Together these data suggest that the frequently reported increases in S-IgA output in response to acute stressors might not be all that specific to distress or arousal. To solve this issue, future studies may pay better attention to the inclusion of relevant controls.

S-IgA and Cardiac Autonomic Drive

Consistent with the reports of other laboratory studies (e.g., Ring et al., 1999; Willemsen et al., 1998), we found little evidence for an association between cardiac autonomic reactions and reactivity of S-IgA, IgA subclasses, or SC during the active coping stressor. These findings are in contrast with the results of laboratory studies investigating serum immune measures, for which correlations with cardiovascular measures have been reported frequently (Uchino et al., 2001). However, when correlating measures of cardiac and autonomic reactivity during the passive coping surgical video with concurrent S-IgA changes, we observed a fairly consistent negative correlation between vagal tone and salivary levels of S-IgA, IgA subclasses, and SC. That is, although mean values were not affected, an enhanced vagal tone during the surgical video was associated with lower concomitant levels of S-IgA, IgA subclasses, and SC. The direction of this association was somewhat unexpected because neurophysiological studies show that stimulation of the parasympathetic nerves causes an increase in S-IgA secretion (Carpenter et al., 1998, 2000). Enhancing effects on S-IgA secretion are also reported for various parasympathetic peptide transmitters (e.g., substance P, vasointestinal peptide), whereas the results for the parasympathetic transmitter acetylcholine are mixed (Freier, Eran, & Alon, 1989; Kelleher, Hann, Edwards, & Sullivan, 1991; McGee, Eran, McGhee, & Freier, 1995; Schmidt, Eriksen, Loftager, Rasmussen, & Holst, 1999; Wilson, Soltis, Olson, & Erlandsen, 1982). Future pharmacological blockade studies might further elucidate the exact neurophysiological mechanisms underlying the stress-induced changes in S-IgA.

We may add that there are many factors that may obscure a potential association between "overall" release of S-IgA and cardiac autonomic drive, as salivary S-IgA derives from many different glandular sources that are under differential neuronal control. Salivary S-IgA is the joint product of three pairs of major glands (parotid, sublingual, and submandibular glands) and hundreds of minor glands in the tongue, lip and palate (Brandtzaeg, 1998). Large differences exist in the innervation of these glands (Garrett, 1999a). For example, whereas most glands receive dual input from the ANS, the sublingual glands and some of the minor glands (together responsible for secreting the larger part of salivary S-IgA) appear to receive predominantly parasympathetic inputs (Garrett, 1998; Rossoni, Machado, & Machado, 1979). Also, the presence of various autonomic transmitter substances (e.g., peptides, nitric oxide, purines) and receptors types varies both between and within glands (Garrett, 1999a, 1999b; Kusakabe et al., 1997). Consequently, individual glands may respond quite differently to the same stressor (Bosch et al., submitted). Another complicating factor is that the secretion of S-IgA does not increase proportionally to an increasing stimulation (i.e., frequency of electrical pulses) of the autonomic nerves innervating the salivary glands (Carpenter et al., 2000), and, likewise, S-IgA secretion may not correlate with a measure of autonomic drive. Interestingly, in contrast to S-IgA, total salivary protein secretion does increase in parallel with increasing nerve stimulation (Carpenter et al., 2000). We therefore tentatively conclude that if a correlation between cardiac autonomic measures and total salivary protein secretion could be established in future studies, this would support the hypothesis that the lack of a clear association between S-IgA and cardiac autonomic control lies in the idiosyncratic dynamics of nerve-induced S-IgA secretion. Moreover, such a finding would refute the hypothesis that correlations between S-IgA and autonomic measures are attenuated because S-IgA derives from multiple glandular sources (see above), because total salivary protein also derives from multiple glandular sources.

In conclusion, we have presented data showing that acute stress can have both enhancing and suppressing effects on secretory immunity. These diverging effects were associated with stressors that evoked distinct patterns of cardiac autonomic activity. Future research is needed to determine the specific mechanisms that underlie these divergent immunological responses, as well as clarifying what accounts for the observed higher variability of the IgA1 subclass.

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