Innate Secretory Immunity in Response to Laboratory Stressors That Evoke Distinct Patterns of Cardiac Autonomic Activity

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Objective: Most infections begin at mucosal surfaces. These surfaces are covered by the secretory proteins of the exocrine glands (eg, the salivary, respiratory, and gastrointestinal glands), which provide a first line of innate defense. The release of these secretory proteins is under neuroendocrine control and thus, in theory, sensitive to modulation by psychosocial stress. This was empirically tested by measuring the salivary secretion of cystatin S, lactoferrin, α-amylase, the mucins MUC5B and MUC7, and total salivary protein in response to stressors known to evoke distinct patterns of cardiac autonomic activity. **Methods:** Thirty-two undergraduate volunteers were each subjected to two laboratory stressors and a control condition. Stressors were an active coping memory test and a passive coping video presentation showing surgical procedures. In the control condition participants viewed a didactic video presentation. **Results:** The stressors evoked the expected distinct patterns of cardiac autonomic activity. The memory test produced a strong increase in sympathetic activity (evidenced by a shortened preejection period), and a decrease in cardiac parasympathetic activity (evidenced by a decrease in heart rate variability). This active coping response was associated with an enhanced secretion (μg/min, controlling for salivary flow rate) of MUC7, lactoferrin, α-amylase, and total salivary protein. Conversely, the surgical video produced an increase in cardiac vagal tone and a modest increase in sympathetic activity. This passive coping response was associated with an enhanced secretion of all proteins studied. These secretory responses were generally larger than the secretory responses during the active coping memory test. Correlation analyses indicated that for both stressors autonomic and cardiovascular reactivity was positively associated with an enhanced and prolonged secretory activity. **Conclusions:** Stress-induced modulation of innate secretory immunity may be a contributing factor in the observed relationship between stress and susceptibility to infectious diseases. We further propose a more differentiated approach to acute stress by distinguishing among stressors with distinct autonomic nervous system effects. **Key words:** autonomic space, laboratory stress, nonspecific immunity, oral health, psychoneuroimmunology.

AMQ = Amsterdam Mood Questionnaire; DBP = diastolic blood pressure; ECG = electrocardiograph; ELISA = enzyme-linked immunosorbent assay; GLM = general linear model; ICG = impedance cardiograph; LVET = left ventricular ejection time; PBS = phosphate-buffered saline; PEP = preejection period; RMSSD = root mean square of successive differences; SBP = systolic blood pressure; SEM = standard error of mean; STAI = Spielberger State-Trait Anxiety Inventory.

INTRODUCTION

Both human and animal studies have provided convincing evidence that psychosocial stress is associated with an increased susceptibility to infectious diseases (1, 2). Much research is dedicated to identifying the exact pathways responsible for this association. An estimated 90% to 95% of all infections are initiated at mucosal surfaces, which form an interface with the outside world. These surfaces are covered and protected by the secretions of various exocrine glands, including the lacrimal glands (3, 4), the salivary glands (4–7), and the glands of the respiratory (6, 8–11) and gastrointestinal tract (4, 6, 12–14). The antimicrobial proteins (eg, mucins, cystatins, histatins, and lactoferrin) secreted by these glands constitute a first line of defense, preventing infection and disease by interfering with microbial entry and multiplication (3, 4, 6–8, 11–15). These secretory proteins are part of the innate immune system, because their secretion is not regulated by virtue of an immunological memory. Instead, the secretion of these protective proteins is under strong neurohormonal control (autonomic nervous system activity in particular) (9, 10, 15–18), presenting a possible psychoneuroimmunological pathway linking psychosocial stress with increased susceptibility to infectious disease.

The study presented here investigated the effects of acute stressors on innate secretory immunity. Specifically we measured the secretion into saliva of MUC5B, MUC7, cystatin S, lactoferrin, α-amylase, and total salivary protein (see Table 1 for detailed information on these measures). The salivary glands, being strategically

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TABLE 1. The Innate Secretory Immune Measures Used in This Study

Secretory Measure	Where Found	Functions	Additional Resources
MUC5B (also denoted as MG1)	Saliva, nasal fluid, bronchial mucus, middle ear secretions, gall bladder epithelia.	The major constituent of the mucous gels covering the mucosa. Protects the epithelial tissue against noxious substances and desiccation. Binds to a selected number of microorganisms, including *H. pylori*. Host-defense functions of MUC5B are enhanced by the formation of complexes with other protective secretory proteins.	(4–6, 64)
MUC7 (also denoted as MG2)	Saliva, bronchial mucus.	Binds and aggregates a large number of bacteria and fungi. Has been implicated in the inactivation of viruses.	(4, 5, 64)
Lactoferrin	Milk, nasal fluid, bronchial mucus, saliva, ocular fluid, and gastrointestinal secretions. Also secreted by neutrophils.	Inhibits microbial growth by binding iron, thereby depleting microorganisms from this essential substrate. Also exhibits anti-inflammatory effects and induces killing of a number of bacteria and fungi.	(64, 65)
α-Amylase	Saliva and pancreatic secretion. Low concentrations have also been demonstrated in other bodily fluids, including blood plasma, bronchial secretions, and tears.	Starch-degrading enzyme. Also affects the growth and adherence of streptococcal bacteria. Salivary α-amylase has been proposed as a measure of adrenergic activity, although this claim is still controversial (see Ref. 64).	(4, 64, 66, 67)
Cystatin S	Saliva, bronchial mucus.	Cystatins inhibit the activity of cysteine proteinases, a specific class of enzymes that, among other things, are involved in virus replication and tissue invasion by bacteria.	(64, 68)

located at the portal of entry to the respiratory and gastrointestinal tract, provide the host with a first line of defense against microorganisms that may develop their pathogenic potential elsewhere in the body. In addition, salivary host defense plays a crucial role in the maintenance of oral health. This aspect is particularly relevant from a psychosomatic perspective, because several pathologic conditions of the oral cavity have been associated with stress. These include periodontal disease (19–21), acute necrotizing gingivitis (20, 21), dental caries (21–23), herpes labialis (1, 21), recurrent aphthous ulcerations (21), and impaired oral wound healing (24). The relevance of these observations may not be limited to the dental sciences, because impaired oral health is a risk factor in cardiovascular disease and respiratory infection (25–28). Last but not least, saliva contains many of the antimicrobial proteins also found in other mucosal secretions (4), which makes saliva a representative model for studying secretory immunity.

The laboratory stressors utilized in this study were an 'active coping' time-paced memory test, and a 'passive coping' gruesome video presentation showing surgical procedures. The distinction between passive and active coping refers not only to specific task characteristics (ie, requiring effort or not), but also, particularly in the animal literature, to distinct modes of physiological adaptation to stress (29–31). Characteristic features of the active coping response are an increase in heart rate, an enhanced sympathetic activation, and a vagal withdrawal. This constellation of responses, typically evoked during conventional laboratory stressors such as mental arithmetic and public speaking, is also denoted as a "fight-flight" or "defense" response. Conversely, the passive coping response is characterized by a parasympathetic-induced bradycardia, often in association with a sympathetic coactivation (29–31). This "vagotonic" alarm response (also denoted as "conservation-withdrawal" (32) or "aversive vigilance" (31)), is less well studied in humans. Previously we showed that the selected laboratory stressors not only exhibit the aforementioned distinct patterns of cardiac autonomic activation, but also exhibit clearly different immunomodulatory effects (33). The present study examined the effects of these stressors on the salivary secretion of MUC5B, MUC7, cystatin S, lactoferrin, α-amylase, and total salivary protein. Because the salivary glands are largely under autonomic control, we also investigated the correlation between cardiac autonomic reactivity and the reactivity and recovery of these innate secretory immune measures.

MATERIALS AND METHODS

Participants

Thirty-two male university undergraduates (mean age, 23 years) volunteered to participate in this study. Participants gave written

informed consent and received 40 Dutch guilders for their participation. None of the participants were taking medication, and all reported to be in good health. In preparation for the study, participants were instructed to refrain from using alcohol or nonprescription drugs 24 hours before the experimental sessions. 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, and eating for 1 hour before the experimental session.

Manipulations and Design

All participants were subjected to three experimental conditions: 1) an 11-minute computerized memory test, 2) an 11-minute video presentation showing various surgical procedures, and 3) an 11-minute didactic video presentation on birds, which functioned as a control condition. These conditions were administered in counterbalanced order, each experimental condition on a separate day, approximately 1 week apart.

For the memory task, participants had up to 2 seconds to detect the presence of memorized characters in a six-character display and to press the corresponding "yes" or "no" button with the right-hand index and middle finger, respectively. Task difficulty (criterion reaction time) was automatically adjusted so that sustained effort was needed to maintain performance. This stressor is found to induce a combined sympathetic activation and a vagal diminution (34). The surgery video showed various forms of oral surgery. This stressor elicits a vagally mediated bradycardia (33, 35).

Procedures

Measurements were recorded between 1:30 PM and 4:00 PM. On arrival, the experimental procedure was explained to the participant, and electrodes for electrocardiography and impedance cardiography were attached. After rinsing their mouth with tap water, participants filled out several questionnaires and were allowed to read self-selected magazines with neutral content for 25 minutes. Subsequently, while continuing this quiet reading, "baseline" saliva was collected. This baseline measurement was followed by one of the three experimental manipulations. Saliva was collected again during the final part of each manipulation ("stress") and 9 minutes after the end of the manipulation ("recovery"), when subjects again were engaged in quiet reading. Immediately after each saliva collection, participants filled out a mood questionnaire. Blood pressure, an electrocardiogram (ECG), and an impedance cardiogram (ICG) were recorded continuously throughout each condition.

Saliva Collection

Saliva was collected by means of the "spitting method," according to the directions given by Navazesh (36). This method is recommended for unstimulated whole saliva collection on the basis of a comparative study (37). The method of saliva collection was practiced before the start of the first experiment to familiarize the participants with the procedure. The collection trial started with the instruction to void the mouth of saliva by swallowing. Subsequently, saliva was allowed to accumulate in the floor of the mouth without stimulation of saliva secretion by means of orofacial movements. The participant spit into a preweighed, ice-chilled polypropylene test tube every 60 seconds. Saliva was collected for 4 minutes. After collection saliva was homogenized by vigorous shaking using a vortex mixer and clarified by centrifugation (10,000*g*, 4 minutes) 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 (38, 39). Systolic (SBP) and diastolic blood pressure (DBP) were measured every 2 minutes. Indices of sympathetic and parasympathetic drive were obtained by analysis of ECG and thoracic impedance (ICG) signals (38, 40). The ECG and ICG complexes were ensemble averaged with reference to the ECG R wave across 1-minute periods. From these 1-minute 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 device have been reported elsewhere (38, 39). Changes in PEP were used to index changes in cardiac sympathetic drive (41), and RMSSD was used to index changes in cardiac vagal tone.

Biochemical Assays

Amylase activity was determined by using the quantitative kinetic determination kit (no. 577) from Sigma Diagnostics (Dordrecht, The Netherlands), as described by Bosch et al. (42, 43). In short, saliva (10 μl, starting dilution 1:60 in PBS) was mixed with 190 μl of amylase reagent and incubated for 2 minutes at 37°C. The increase in absorption (at 410 nm) over the subsequent 2 minutes was measured and compared with the activity of a multienzyme standard (Lintrol, Sigma Diagnostics). Amylase activity was expressed in units per milliliter (U/ml). All samples were assayed in quadruplicate, the samples of each participant in the same assay run.

For the determination of cystatin S, a sandwich ELISA was used, as described by Henskens et al. (44). Rabbit anti-human cystatin S polyclonal antiserum (purified immunoglobulin fraction) was used as a capture antibody, whereas a monoclonal antibody raised against cystatin S was used for detection (for details on the development of these antisera, see Refs. 45 and 46).

Lactoferrin was quantified using a sandwich ELISA, as described by Groenink et al. (47). Anti-human lactoferrin polyclonal rabbit antiserum (Sigma, Dordrecht, The Netherlands) was used to capture salivary lactoferrin. For detection we used horseradish peroxidase–conjugated anti-human polyclonal antiserum, which was a gift of J. Koopman, PhD (Pharming BV, Leiden, The Netherlands). (For details on the production of this antibody, see Ref. 48).

MUC5B and MUC7 were determined by ELISA, in which the antigen is directly coated to the microplate. Both the method and antisera for quantification of salivary MUC5B and MUC7 are described in detail in References 49 and 50, respectively. The monoclonal used for quantification of MUC5B specifically recognizes the terminal part of the carbohydrate moiety sulfo-Lewis^a, being SO_3-3Galβ1-3GlcNAc. This structure is present on a subpopulation of MUC5B that is mainly secreted by the palatal and sublingual salivary glands (51). Total protein was determined using the bicinchoninic acid method, as described by Bosch et al. (42, 43). The intraassay variability of each assay was below 5%.

Questionnaires

Participants were administered the Dutch translation of the Spielberger State-Trait Anxiety Inventory (STAI) (52) and a short version of the Amsterdam Mood Questionnaire (AMQ). The STAI measures feelings of nervousness, tension, apprehension, and worry, and was used as an indicator of distress. The short version of the AMQ consists of five 6-item mood scales, measuring depression (eg, sad, desperate, depressed), fear (eg, afraid, scared, anxious), anger (eg, irritated, angry, indignant), arousal (eg, aroused, excited, activated), and fatigue (eg, tired, sleepy) on a five-point Likert scale. The state anxiety part of the STAI was administered immediately after each saliva collection, whereas the AMQ was administered only after the second saliva collection (ie, immediately after the experimental manipulation). Participants also filled out a self-report questionnaire on health (perceived health, use of medication or other medical treatment), general health behavior, and health behaviors in the 24 hours preceding the experiment (smoking; alcohol, tea, and coffee consumption; physical exercise, sleep duration and quality).

Statistical Analysis

Each experimental condition (memory test, surgical video, control) consisted of three measurement periods (baseline, stress, and recovery). The experimental conditions ("condition") and the measurement periods ("time") are both within-subject variables. The effect of each stressor was examined by analysis of variance (ANOVA) for repeated-measures, analyzing the condition-by-time interaction, that is, contrasting the responses within each stress condition with the control condition. For each condition, the results of the repeated-measures ANOVA for the variable time will be reported as well.

Extreme values (>2 SD from the mean) were removed. On three occasions ICG data were incomplete because of a computer failure during recording. Consequently, for PEP the numbers fluctuated between 32 and 29. RMSSD data were logarithmically transformed (ln + 1) for statistical analyses; the untransformed values are presented in the tables. Data were analyzed using SPSS for Windows 10.0.

RESULTS

Mood Questionnaires

Figure 1 presents the summary data for the STAI. State anxiety increased during both the memory task and the surgical video, whereas state anxiety during the control condition was virtually unaffected (see Fig. 1). ANOVA for repeated-measures yielded a significant time-by-condition interaction ($F(2,62) = 42.13$, $p < .001$), which is clearly driven by the increases in anxiety during the stressors.

Compared with the control condition, both stressors were rated higher on the dimensions of depression, anger, arousal, and fear (all paired t tests yielded $p < .001$). Compared with the surgical video, the memory test was considered more arousing ($t(32) = 4.08$, $p < .001$) and anger-inducing ($t(32) = 2.89$, $p < .01$). The two stressors did not differ on reported fear and depression ($p > .4$).

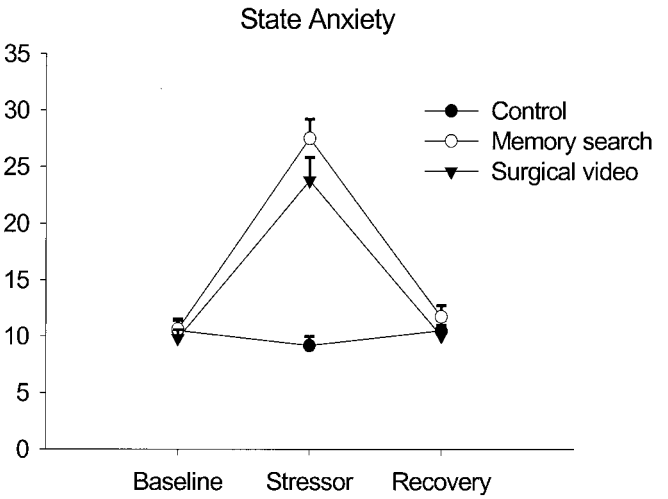

Fig. 1. State anxiety (STAI) during the control condition, memory test, and surgical video. Points indicate means; vertical bars indicate SEMs.

Cardiovascular Measures

Table 2 presents the summary data and results of statistical testing for heart rate, RMSSD, PEP, SBP, and DBP. Results of the repeated-measures ANOVA, analyzing the time-by-condition interaction, by contrasting the control condition with either the memory task or the surgical video, showed that the stressors evoked cardiovascular responses that were significantly different from those seen during the control video (results presented in Table 2). The memory search task produced an increase in heart rate, a diminution of both RMSSD and PEP (indicating a reduced cardiac parasympathetic drive and an increased sympathetic drive, respectively), and elevated blood pressure (see Table 2). Conversely, the repeated-measures ANOVA indicated that the surgical video significantly reduced heart rate ($F(2,62) = 11.13$, $p < .001$), although this decrease did not significantly differ from the decrease seen during the control condition (time-by-condition interaction, $p > .10$; see Table 2). However, during the surgical video the decrease in heart rate was associated with an increase in RMSSD (repeated measures ANOVA for time: $F(2,62) = 7.04$, $p < .01$), reflecting an increased vagal tone. As becomes clear from Table 2, this decrease in vagal tone was absent in the control condition. Analysis of the time-by-condition interaction, contrasting the responses during the surgical video with the control video, further confirmed that the increase in RMSSD induced by the stressful video differed significantly from the response during the control condition (see Table 2). The surgical video also induced a small shortening of PEP ($F(2,62) = 5.35$, $p < .01$), reflecting an increased cardiac sympathetic drive,

TABLE 2. Mean (SEM) Values of Cardiovascular Measures and Results of Analyses for Condition-by-Time Interactions[a]

	Condition	Time			Condition-By-Time Interaction	
		Baseline	Manipulation	Recovery		
Heart rate (beats/min)	Control	69.1 (1.8)	67.0 (1.7)	67.7 (1.6)		
	Memory test	69.6 (1.5)	77.6 (1.8)	68.7 (1.6)	$F(2,62) = 50.08$	$p<.001$
	Surgical video	68.6 (1.6)	65.8 (1.7)	67.6 (1.6)	$F(2,62) = 1.67$	NS
RMSSD (ms)	Control	50.8 (5.1)	49.3 (5.1)	54.3 (5.3)		
	Memory test	50.9 (5.9)	40.0 (5.9)	52.7 (6.2)	$F(2,62) = 6.20$	$p<.01$
	Surgical video	53.7 (5.6)	58.8 (5.2)	51.1 (4.6)	$F(2,62) = 16.44$	$p<.001$
PEP (ms)	Control	98.0 (3.3)	99.6 (3.3)	98.8 (3.3)		
	Memory test	101.8 (3.5)	94.3 (3.2)	100.4 (3.2)	$F(2,58) = 52.38$	$p<.001$
	Surgical video	100.0 (3.3)	98.2 (3.4)	100.3 (3.4)	$F(2,62) = 9.38$	$p<.001$
SBP (mm Hg)	Control	114.2 (1.8)	113.7 (1.7)	111.5 (1.4)		
	Memory test	115.2 (1.7)	133.1 (2.3)	116.8 (2.0)	$F(2,62) = 54.77$	$p<.001$
	Surgical video	114.3 (1.7)	118.3 (1.6)	111.8 (1.6)	$F(2,62) = 8.17$	$p=.001$
DBP (mm Hg)	Control	65.1 (1.4)	65.4 (1.6)	64.3 (1.4)		
	Memory test	68.2 (1.6)	79.7 (1.9)	69.9 (1.6)	$F(2,62) = 28.48$	$p<.001$
	Surgical video	65.7 (1.4)	68.3 (1.7)	65.7 (1.4)	$F(2,62) = 2.25$	NS

[a] In the analyses for condition-by-time interactions, the responses during each stressor condition were contrasted with responses during the control condition.

and small rises in DBP ($F(2,62) = 4.44$, $p < .05$) and SBP ($F(2,62) = 18.62$, $p < .001$). Except for DBP, these effects remained significant when contrasted with the responses within the control condition (see Table 2).

Figure 2 presents a scatterplot of PEP and RMSSD reactivity (percentage of change from baseline value), showing the characteristic differences in the position of the active and passive tasks in the autonomic space (53).

Salivary Flow Rate

Changes in salivary flow rate were examined before testing the effects of the two experimental conditions on the various salivary immune measures. Changes in flow rate would necessitate a control for mere dilution effects, because we were primarily interested in protein secretion by the glandular cells and not in effects secondary to altered fluid secre-

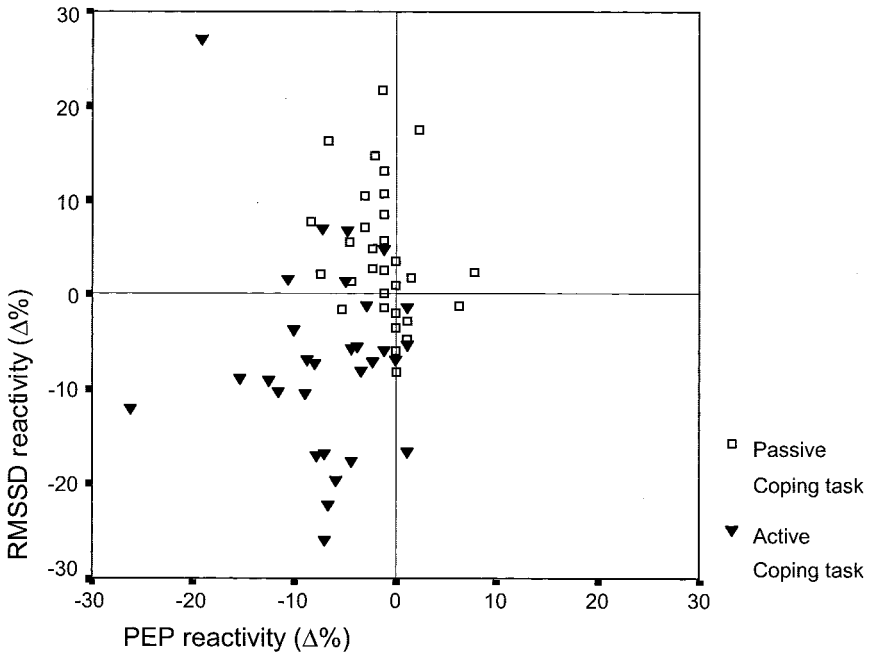

Fig. 2. Position of the active and passive tasks in autonomic space.

tion. Figure 3 shows that the two stressors had opposite effects on salivary flow rate; the memory test reduced flow rate ($p < .001$), whereas the surgical video enhanced flow rate ($p < .001$). These effects on salivary flow rate were controlled for by computing protein output, expressed in micrograms per minute, as follows: protein output (μg/min) = protein concentration (μg/ml) $\times$ flow rate (ml/min).

Salivary Immune Measures

Memory test. As indicated in Figures 4, 5, and 6, the ANOVA for repeated measures yielded significant effects for the increased output of MUC7, lactoferrin, and total salivary protein during the memory test. Cystatin S, α-amylase, and MUC5B output were not significantly affected (see Figs. 4, 5, and 6). Contrasting the responses during this stressor with the responses during the control condition yielded a significant time-by-condition interaction for MUC7 output ($F(2,58) = 4.99$, $p < .05$), α-amylase ($F(2,58) = 3.32$, $p < .05$), and total salivary protein ($F(2,56) = 3.91$, $p < .05$), but not for lactoferrin, cystatin S, or MUC5B (all p values $> .2$).

Surgical video. The surgical video increased the output of all secretory proteins, and the ANOVA for repeated measures (for the within-subject factor of time) yielded mostly p values well below the 0.001 criterion (see Figs. 4, 5, and 6). The largest effects were found for the output of the mucins MUC5B and MUC7, which showed increases of approximately 200% and 90%, respectively. Increases of approximately 50% were observed for lactoferrin and α-amylase output, whereas only a small increase (approximately 20%) was observed for cystatin S output. Contrasting these effects with the control condition yielded a significant time-by-condition interaction for MUC5B ($F(2,58) = 17.12$, $p < .001$), MUC7 output ($F(2,60) = 14.54$, $p < .001$), lactoferrin ($F(2,58) = 5.61$, $p < .01$), α-amylase output ($F(2,60) = 8.63$, $p < .001$), cystatin S ($F(2,58) = 4.02$, $p < .05$), and total salivary protein ($F(2,58) = 12.02$, $p < .001$).

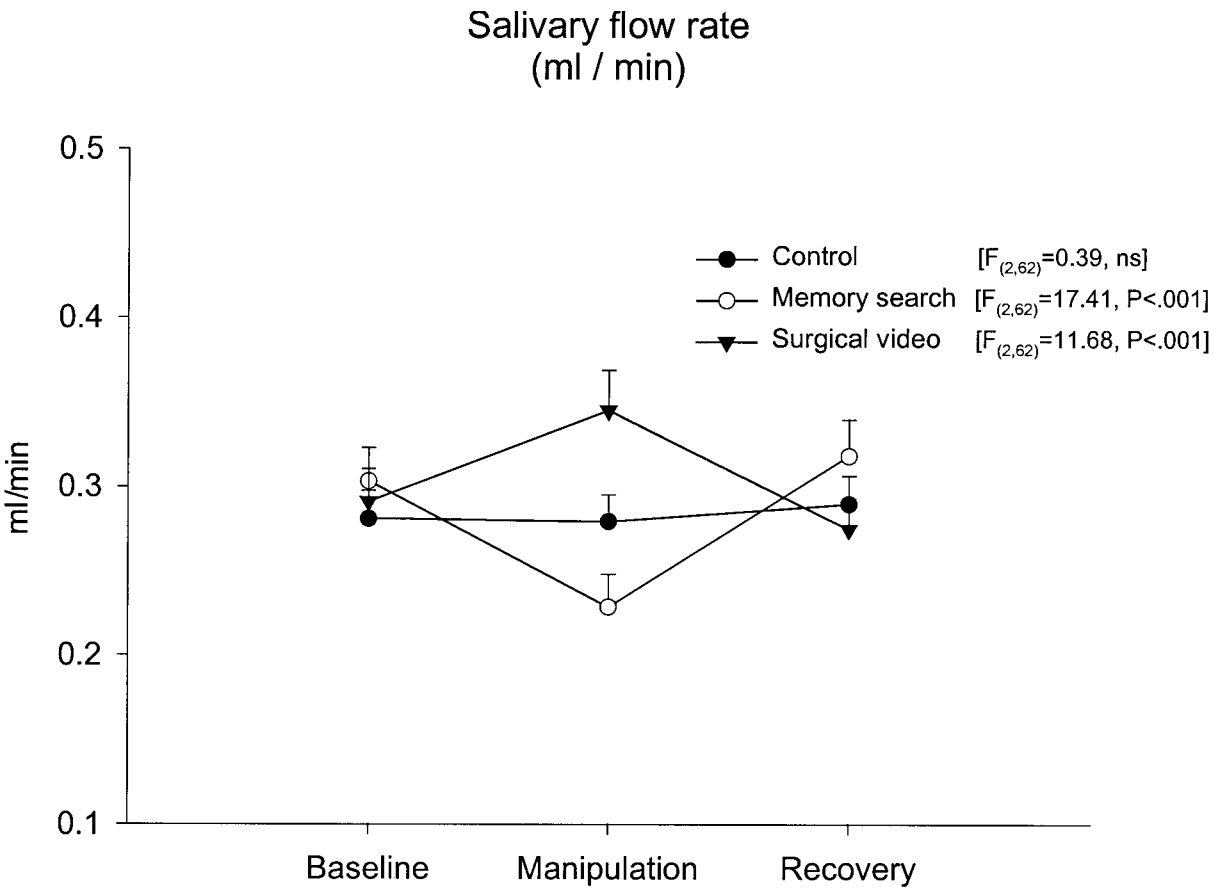

Fig. 3. Salivary secretion rate during the control condition, memory test, and surgical video. Points indicate means; vertical bars indicate SEMs. Results for repeated-measures analyses of time (F and p values) are presented in the figure.

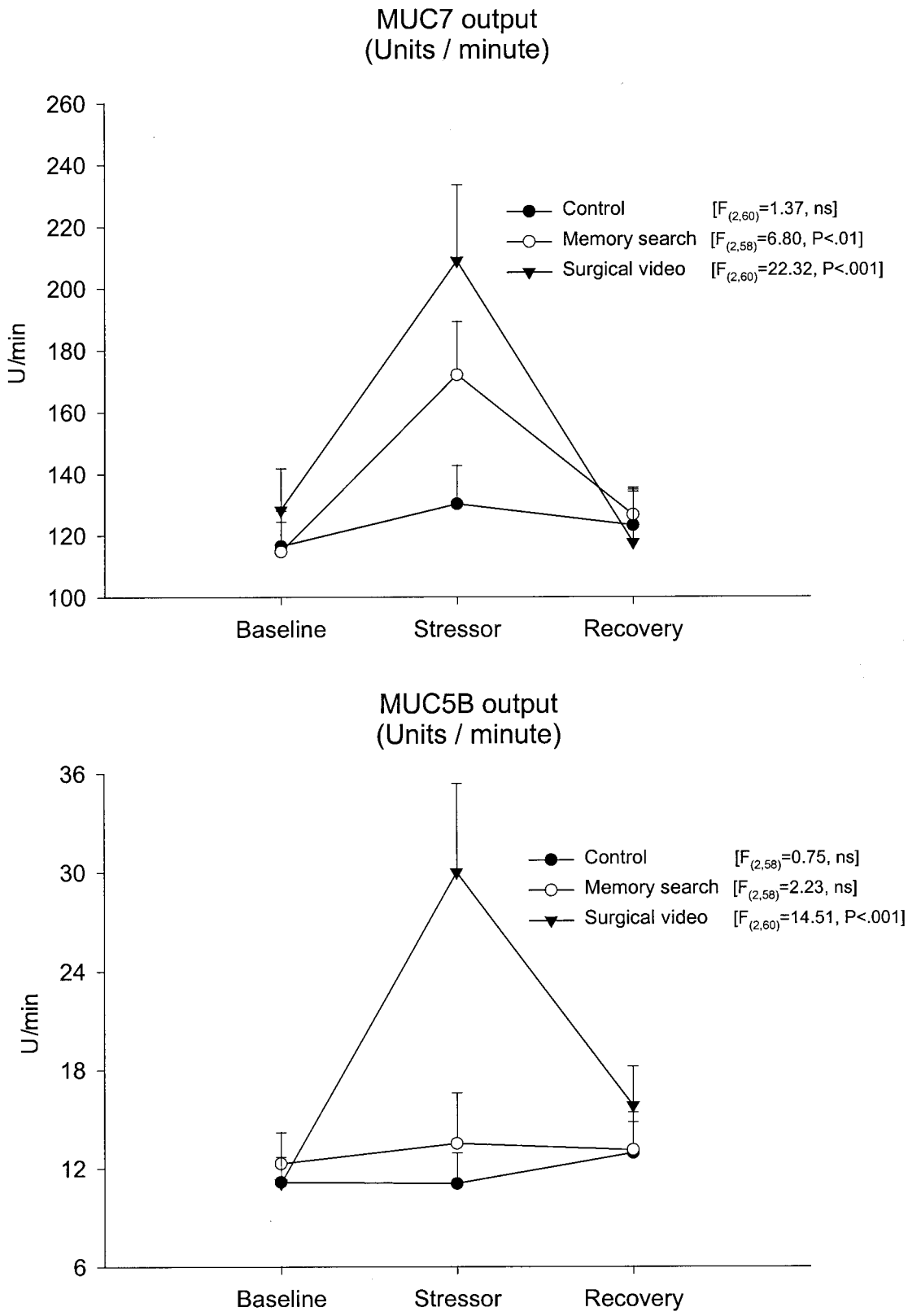

Fig. 4. Secretion of the mucins MUC7 (*top*) and MUC5B (*bottom*) during the control condition, memory test, and surgical video. Points indicate means; vertical bars indicate SEMs. Results for repeated-measures analyses of time (F and p values) are presented in the figure.

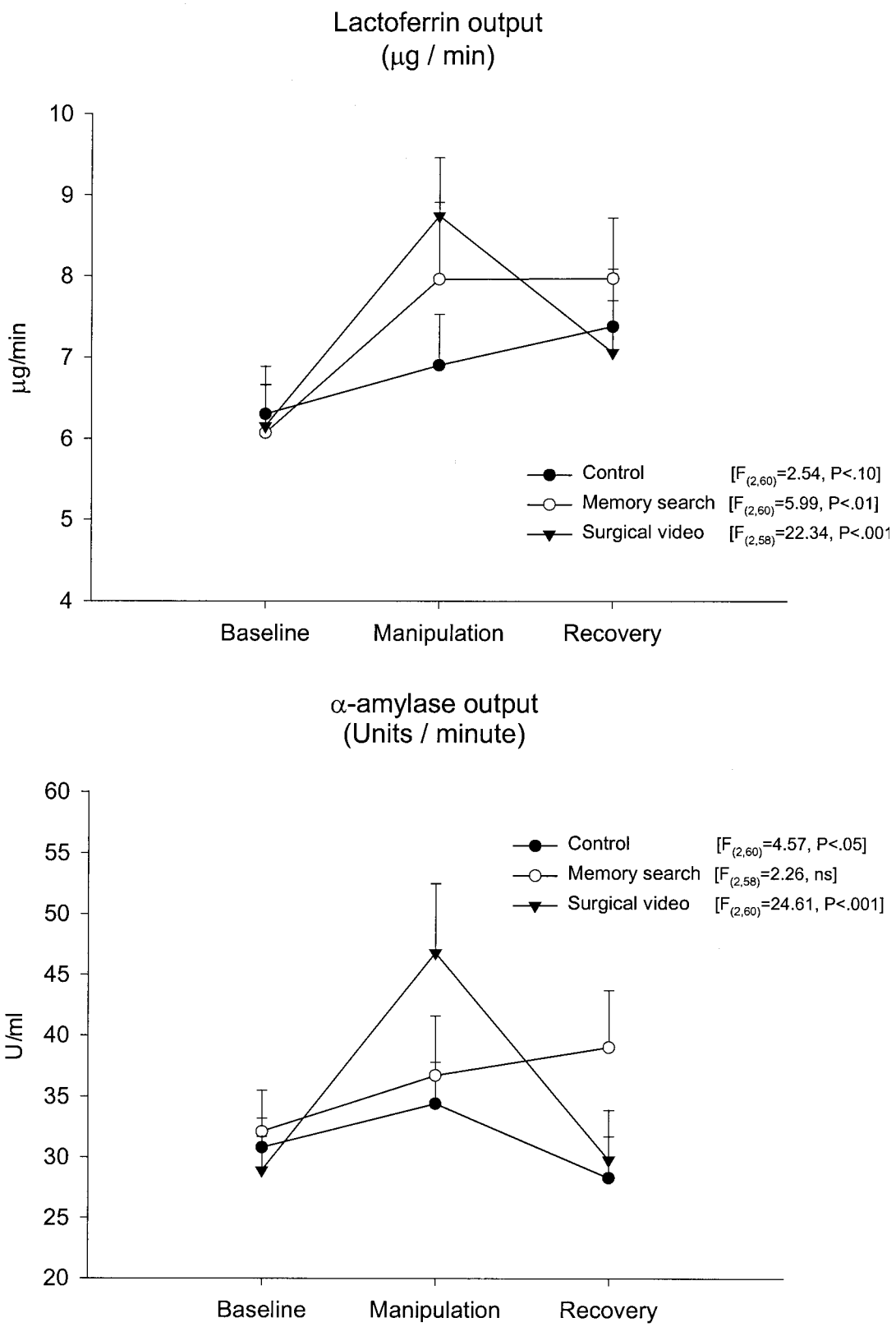

Fig. 5. Secretion of lactoferrin (*top*) and α-amylase (*bottom*) during the control condition, memory test, and surgical video. Points indicate means; vertical bars indicate SEMs. Results for repeated-measures analyses of time (*F* and *p* values) are presented in the figure.

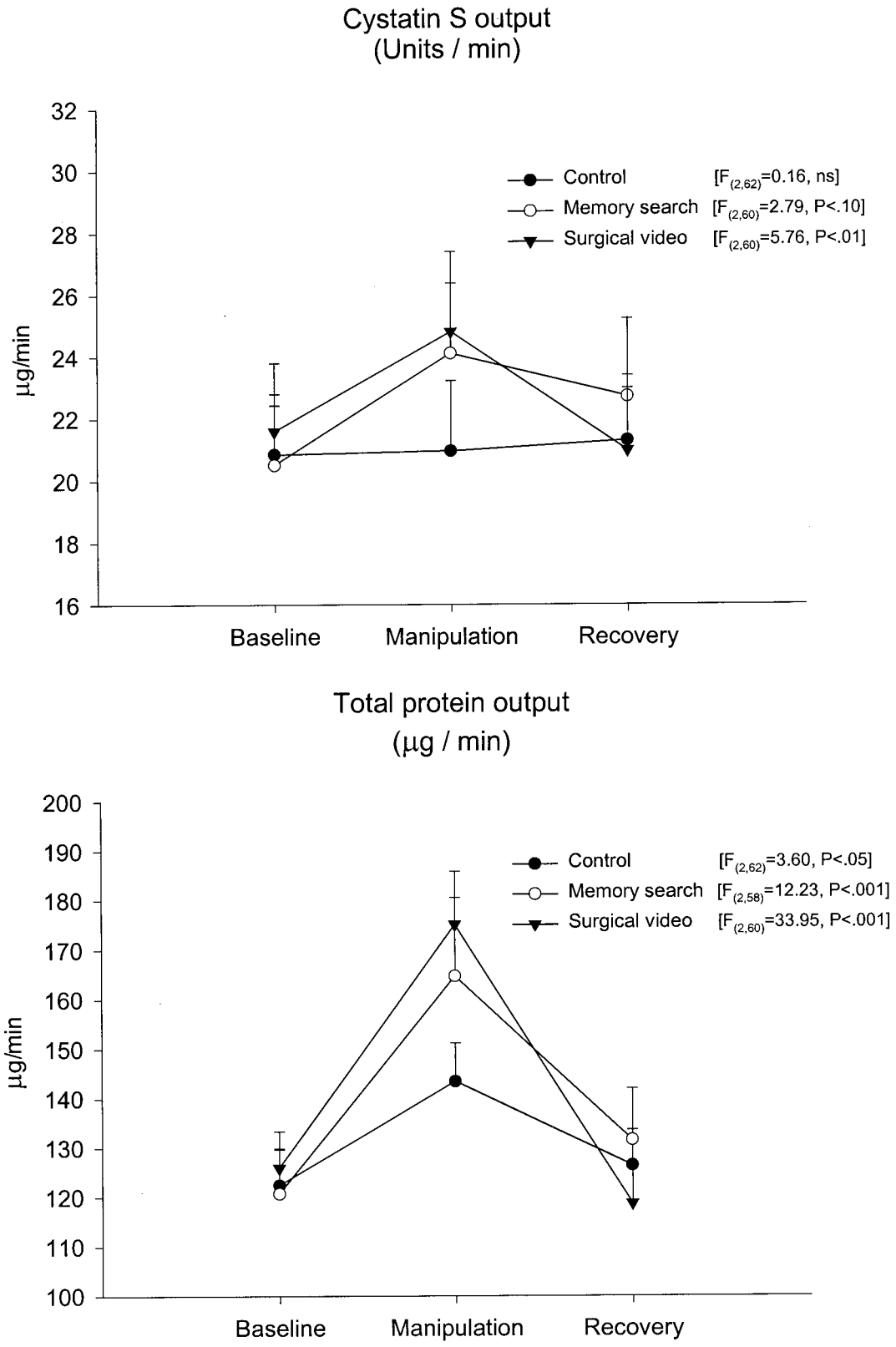

Fig. 6. Secretion of cystatin S (*top*) and total salivary protein (*bottom*) during the control condition, memory test, and surgical video. Points indicate means; vertical bars indicate SEMs. Results for repeated-measures analyses of time (*F* and *p* values) are presented in the figure.

Associations Between Cardiovascular and Secretory Measures

Associations between cardiovascular reactivity and secretory reactivity and recovery were computed as rank-order correlations of absolute differences. Computing relative differences (%) yielded comparable results. Table 3 presents the associations between cardiovascular reactivity and secretory reactivity and recovery during the memory test. The results show numerous significant correlations (21 of 60) between the protein measures and measures of autonomic and cardiovascular activation (heart rate, PEP, LVET, RMSSD, SBP, DBP).

Table 4 presents the association between cardiovascular reactivity, secretory reactivity, and secretory recovery during the surgical video. In this analysis significant correlations between salivary measures and measures of cardiac autonomic drive were less frequent but of the same direction. The most frequent significant correlations were observed with LVET, which showed a negative correlation with three measures of secretory reactivity (lactoferrin, cystatin S, α-amylase) and three recovery measures (MUC7, MUC5B, α-amylase).

DISCUSSION

This study examined the effects of acute stress on innate secretory immunity, providing the first comprehensive analysis of this aspect of immunity within the realm of psychoneuroimmunology. In general we found an enhancement of this type of immune defense in response to acute stressors that exhibited distinct patterns of autonomic cardiac control. These cardiac autonomic responses were denoted as an active coping response (31) (evoked by the memory search test) or a passive coping (29–31) response (evoked by the surgical video).

Considering the pivotal role of innate factors in mucosal defense (ie, before a microorganism can establish an infection, it must somehow manage to circumvent this first line of defense), as well as the strong involvement of neuroendocrine systems in their regulation, the paucity of psychoneuroimmunological research into this aspect of host defense is difficult to understand. Interestingly, available data on the susceptibility to infectious disease in stressed humans suggests a role for this type of immune factors that are important in *preventing* infection, rather than a role for immune factors that are important in *responding* to infection. For example, in a seminal study by Cohen et al. (54), in which volunteers were exposed to cold viruses after a stress assessment, a strong relationship between stress reporting and infection was observed (odds ratio, 5.81). However, the association between stress and actual disease occurrence was much weaker (odds ratio, 2.16). The authors concluded that, ". . .the relation between stress and colds was primarily attributable to an increased rate of *infections* among subjects with higher stress-index scores, rather than to an increase in *clinical colds* among infected persons. . ." (54) [emphasis added]. Preventing infection is the primary function of the secretory immune defenses, and the role of this type of immunity in mediating the association between stress and infectious disease clearly deserves further research. Such future research may extend our findings by studying other mucosal secretions, such as bronchial and nasal lavage, assess-

TABLE 3. Concordance Between Cardiovascular Reactivity and Secretory Reactivity, and Secretory Recovery During the Active Coping Memory Test[a]

Cardiovascular Reactivity	MUC7	MUC5B	Lactoferrin	Cystatin S	α-Amylase	Total Protein
Reactivity of salivary measures						
Heart rate				0.43	0.56	0.33
PEP	−0.34					
LVET					−0.35	
RMSSD			−0.40		−0.36	
SBP						
DBP			0.43	0.31	0.34	0.33
Recovery of salivary measures						
Heart rate			0.40		0.47	0.54
PEP	−0.31		−0.38			−0.31
LVET			−0.34		−0.47	−0.59
RMSSD						
SBP		−0.57	0.31			0.44
DBP						

[a] $N = 29–32$; rank-order correlations are listed if $p < .05$.

TABLE 4. Concordance Between Cardiovascular Reactivity and Secretory Reactivity and Secretory Recovery During the Passive Coping Surgical Video[a]

Cardiovascular reactivity	MUC7	MUC5B	Lactoferrin	Cystatin S	α-Amylase	Total Protein
Reactivity of salivary measures						
Heart rate			0.56			0.42
PEP		−0.41		−0.37		
LVET			−0.34	−0.30	−0.30	
RMSSD						
SBP						
DBP						
Recovery of salivary measures						
Heart rate						
PEP						
LVET	−0.44	−0.37			−0.37	
RMSSD	−0.40		−0.39			
SBP						
DBP						

[a] $N = 29–32$; rank-order correlations are listed if $p < .05$.

ing immune competence directly at the sites where infections are initiated.

A striking feature of our results is that the passive coping surgical video produced a more robust secretory response than the active coping memory test, although the latter evoked much larger changes in cardiac autonomic activity. This observation is in line with the well-established notion of a synergistic effect of the two branches of the autonomic nervous system on the activity of the secretory glands (55, 56). That is, although sympathetic activation is the main stimulus for glandular protein secretion, these sympathetic effects are strongly augmented by concurrent parasympathetic activity. Thus, the effects of a moderate sympathetic drive during the passive stressor were likely enhanced by a concurrent parasympathetic coactivation.

It is not known to what extent such autonomic interactions also shape the responses of the more commonly used blood-based immune measures. This lack of knowledge may derive from the fact that the acute stressor manipulations used in psychoneuroimmunology have been confined to a relatively standard array of active coping tasks, typically challenging tasks that demand mental effort (eg, mental arithmetic, speech tasks) and that elicit a characteristic pattern of sympathetic activation and vagal withdrawal (57). Clearly, both psychologically and physiologically such laboratory manipulations may provide only a limited perspective on the stresses encountered in everyday life. The present findings, and other findings of our group (33, 35), suggest that stress-immune studies may broaden the scope by differentiating among stressors with distinct autonomic nervous system effects, for example, by examining the responses to passive coping stressors in addition to the conventional active coping laboratory challenges.

Correlation analyses showed several associations between secretory reactivity and reactivity of cardiovascular measures (PEP, LVET, heart rate, DBP) that may be interpreted as reflecting, at least in part, sympathetic nervous system activation of the secretory glands. This interpretation would be consistent with the fact that protein secretion is largely under sympathetic control and evoked by β-adrenergic stimulation in particular (17). Correlation analyses further indicated that enhanced autonomic and cardiovascular responsiveness during acute stress are also related to a prolonged activation of this type of immunity. Various significant correlations were observed, particularly within the active coping condition, between cardiovascular reactivity and elevated protein levels during the recovery period (ie, 9 to 14 minutes after the stressor). These laboratory findings suggest that the effects of everyday stressors on secretory immunity may endure well beyond the actual stressful encounter, depending on autonomic and cardiovascular responsiveness to the stressor.

Another notable feature of our data is the strong heterogeneity in the responses of the various secretory proteins. Heterogeneous responses were observed both within (eg, cystatin S vs MUC7) and between (eg, MUC5B) stressors. Because the different secretory proteins derive from different salivary glands (ie, the three pairs of major glands and the numerous minor glands in lip, tongue, cheek, and palate) and from different cell populations within these glands (eg, mucous cells, serous cells, ductal cells) (56, 58), this heterogeneity of responses indicates that the stressful manipulations

activated the various glandular sources differently. Such differentiated secretory responses are made possible by gland-specific patterns of autonomic innervation (eg, density of innervations and local differences in the corelease of peptide transmitters) and cell-specific carriage of neuroendocrine receptors (56, 58). Thus, rather than being suited mainly for generalized adjustments, the salivary glands form a sophisticated end point in the central nervous system control of local immune defenses, capable of responding instantly and with a high level of specificity to potential sources of harm (eg, food, stress, inflammation). This remarkable ability, together with their strategic location, make these glands ideally suited to provide the host with a first line of innate defenses.

The largest secretory responses were seen for the mucins MUC7 and, during the passive coping stressor, MUC5B. The latter finding confirms an earlier report of our group (35). Although the effects of stress on mucin secretion have received scant attention in the human literature, this topic has been extensively studied in animal research. These studies similarly report that acute stressors (eg, restraint stress, water immersion) increase mucin secretion and synthesis in the gastrointestinal and respiratory tracts (discussed in Refs. 35 and 59). Although such increases are generally interpreted as reflecting an enhanced mucosal barrier function (59), it should be noted that the exact immunological meaning of an increased mucin secretion depends on various factors, such as the specific microorganism under consideration. For instance, *Helicobacter pylori* specifically binds certain mucin types, which may in fact contribute to the potential of this bacterium to colonize the host (6, 60). We found that stress-induced increases in secretion of MUC5B enhance the adherence (ex vivo) of *H. pylori* (35). Thus, increases in salivary mucin may have distinct consequences, some that benefit the host and some that benefit the microorganism. This "amphifunctionality" (61) is a characteristic of many other secretory proteins as well. Therefore, to gain insight into the implications of stress-induced changes in secretory immunity, future studies might also use functional assays assessing the effects on specific interactions between microbes and secretory defenses factors (eg, microbial adherence, growth inhibition, and killing; see Refs. 35, 42, 43 for examples).

At the end of this section we wish to add a note on what is perhaps the most ancient psychophysiological parameter, salivary flow rate. Although the inhibitory effects of anxiety on salivation may seem common knowledge, the various authors reviewing this topic were unanimously puzzled by the fact that salivary volume is found to decrease in some studies and to increase in others (23, 62, 63). The results of our study may provide a key to this puzzle, since we found that anxiety may both increase and decrease salivary flow rate depending on the type of stressor. These changes in flow rate were paralleled by changes in cardiac parasympathetic drive; decreasing during the active coping stressor and increasing during the passive coping stressor. Because the parasympathetic nerves are mainly responsible for regulating salivary flow rate (17) (and not the sympathetic nerves as is sometimes assumed), we propose that the mechanism behind these flow changes is a stressor-specific change in parasympathetic activity.

Concluding Remarks

Infections are typically initiated at mucosal surfaces. Previous research on secretory immunoglobulin A (S-IgA), a measure of adaptive secretory immunity, showed that protection of the mucosal surfaces is under neuroendocrine control and influenced by stress (reviewed in Ref. 64). However, S-IgA is just one of many protective secretory proteins. This study showed that innate secretory immunity is also affected in humans under various types of acute psychological stress. Coactivation of the sympathetic and parasympathetic nervous systems, as seen during the passive coping stressor, resulted in the largest immunosecretory effects. Analyses of the interactions between cardiovascular, autonomic, and secretory immune responses supported the notion that immune changes during stress are part of a coordinated response that involves all of these systems. Further research is needed to determine the specific mechanisms underlying these neuroimmunological associations as well as to assess the impact of more protracted forms of psychological stress.

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