Salivary MUC5B-Mediated Adherence (Ex Vivo) of *Helicobacter pylori* During Acute Stress

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Objective: Biochemical host defenses at mucosal sites, such as the oral cavity, play a key role in the regulation of microbial ecology and the prevention of infectious disease. These biochemical factors have distinct features, some of which benefit the host and some that benefit bacteria. We investigated the effects of acute stress on the salivary levels of the carbohydrate structure sulfo-Lewis^a (sulfo-Le^a), which is linked to the mucosal glycoprotein MUC5B. Sulfo-Le^a was recently identified as an adhesion molecule for *Helicobacter pylori*; therefore, we also measured saliva-mediated adherence (ex vivo) of *H. pylori*. The oral cavity is suspected to be involved in the transmission of *H. pylori*. **Methods:** Saliva was collected from 17 undergraduates before (baseline), during (stress), and after (recovery) exposure to a video showing surgical procedures. In addition, blood pressure, an impedance cardiogram, and an electrocardiogram were recorded. **Results:** During stressor exposure, participants reported increased state anxiety. In addition, stroke volume increased and heart rate decreased. The stressor induced a strong increase in salivary sulfo-Le^a concentration (U/ml), sulfo-Le^a output (U/min), sulfo-Le^a/total protein ratio (U/mg protein), and saliva-mediated adherence (ex vivo) of *H. pylori*. As expected, sulfo-Le^a concentration correlated with the adherence of *H. pylori* ($r = 0.72$, $p < .05$). It was demonstrated that the observed adherence was induced by MUC5B and that the carbohydrate structure sulfo-Le^a contributed to this process. **Conclusions:** Our study demonstrated a direct link between stress-mediated biochemical changes and altered host-microbe interactions in humans. Increased bacterial adherence may be a contributing factor in the observed relationship between stress and susceptibility to infectious disease. **Key words:** sulfo-mucin, MG1, psychoneuroimmunology, innate immunity, laboratory stress, microbiology.

DBP = diastolic blood pressure; ECG = electrocardiogram; ELISA = enzyme-linked immunosorbent assay; HR = heart rate; ICG = impedance cardiogram; MANOVA = multivariate analysis of variance; PBS = phosphate-buffered saline; PEP = preinjection period; RSA = respiratory sinus arrhythmia; SBP = systolic blood pressure; S-IgA = secretory immunoglobulin A; sulfo-Le^a = sulfo-Lewis^a; SV = stroke volume; U = ELISA units.

INTRODUCTION

For many years, investigators have documented the impact of behavioral factors on immune functioning as a possible determinant of the observed relationship between stress and susceptibility to infectious disease (1, 2). Infections are often initiated at mucosal surfaces, which form a boundary with the outside world. Host defense at mucosal surfaces is mediated by both nonspecific and immunologically specific mechanisms (3–5). Several studies in psychoimmunology have utilized S-IgA as an index of specific mucosal immune function (6). However, the effects of behavioral factors on nonspecific mucosal defenses remain, with a few exceptions (7–9), unexplored.

Nonspecific mucosal defense is established by a multitude of proteins, most of which are not produced by cells of the immune system but by secretory gland cells (4, 10). These secretory proteins protect the body against invading bacteria, viruses, yeasts, and toxins, by means of many biochemically different mechanisms. Examples of such antimicrobial mechanisms are killing, growth inhibition, inactivation of proteolytic enzymes, and prevention of adherence to host tissue (11–16). In this regard, the adjective "nonspecific" is a misnomer, considering the enormous functional diversity and specificity of this heterogeneous population of proteins.

The study described here addressed "nonspecific" host defense in saliva. Saliva plays a crucial role in the maintenance of oral health, which becomes dramatically clear in patients with xerostomia, or dry mouth syndrome. One of the symptoms of this syndrome is a rapid increase in dental caries and other oral infections, accompanied by an increase in pathogenic bacteria (17). However, the functions of saliva are not limited to the protection of the oral cavity because the mouth also serves as a reservoir and portal of entry for microorganisms that are active elsewhere in the body. These include several *Streptococcus* species (18), *Hemophilus influenzae* (19), Epstein-Barr virus, and her-

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pes simplex virus (20). Here, the role of saliva is not necessarily a protective one. For example, whereas HIV is inactivated by saliva (21), pathogens such as Epstein-Barr virus and herpes simplex virus are, in fact, transmitted by saliva (20). It is for this reason that the pathological condition that results from Epstein-Barr virus infection is nicknamed "the kissing disease."

Because the mouth functions as the portal of entry to the gastrointestinal system, the possible role of the oral cavity in the transmission of *Helicobacter pylori* has been debated in recent years (22–24). *H. pylori* plays a causative role in the pathogenesis of gastritis, gastric atrophy, and peptic and duodenal ulcers (25, 26). Infection with this bacterium is also associated with an increased risk of gastric adenocarcinoma and the occurrence of mucosa-associated lymphoid tissue lymphoma (27, 28). Consequently, *H. pylori* is classified as a type I human carcinogen (29). Although the human stomach is the only well-established "reservoir" of *H. pylori*, in patients suffering from gastric infections, the microorganism has been detected at various sites of the oral cavity, such as the palate, cheeks, gingival pockets, dental plaque, and saliva (30–36). Therefore, the oral-oral route has been suggested as a modality of transmission for this infection. Although the debate on the oral-oral transmission of *H. pylori* is as yet undecided, alternative modalities of transmission (eg, fecal-oral and gastric-oral) also involve the oral cavity (22, 25, 37, 38). In addition, it has been suggested that the oral cavity is a possible source of reinfection in cases where *H. pylori* has been eradicated from the stomach (32, 39).

The carbohydrate structures that are linked to glycoproteins often function as attachment sites for bacteria and viruses (40, 41). Recently, the carbohydrate structure sulfo-Le^a was identified as an adhesion molecule for *H. pylori* (42, 43). In saliva, sulfo-Le^a is uniquely present on the glycoprotein MUC5B[1], terminating the carbohydrate chains linked to this glycoprotein (10). MUC5B, secreted by the sublingual, submandibular, and palatine salivary glands, is a very large mucosal glycoprotein, or mucin, present in a mucous protein layer that covers and protects the tissues of the oral cavity (44, 45). As a main constituent of this mucous layer, MUC5B is a potential site of attachment for organisms that colonize the oral cavity. MUC5B is also present in the secretions of the respiratory tract, eyes, and gastrointestinal tract (10). Although the exact physiological significance of the attachment of *H. pylori* to salivary MUC5B is still unclear, possible functions may be facilitation of transport to the stomach or colonization of the oral epithelium.

Aim of Study

There exists convincing evidence of links between stress and susceptibility to infectious disease in humans, and much research in behavioral medicine is dedicated to specifying the exact physiological pathways responsible for this link. However, most research in this field is concentrated on humoral and cellular immunity and almost completely ignores the possible role of innate biochemical defenses. In this study, we investigated the effects of acute stress on the secretion in saliva of the carbohydrate structure sulfo-Le^a, which is linked to the glycoprotein MUC5B. In addition, we quantified the saliva-mediated adherence (ex vivo) of *H. pylori*. Adhesion is a process by which microbes gain a stable foothold at the portal of entry. Microbial adhesion forms a first and essential step in the course of an infection (46). The primary aim of this study was to establish whether stress-mediated biochemical alterations in mucosal secretions (salivary levels of sulfo-Le^a) could be linked directly to an altered host-microbe interaction (saliva-mediated adherence of *H. pylori*).

MATERIALS AND METHODS

Participants and Procedure

Study participants were 18 male undergraduate volunteers ranging in age from 18 to 29 years (mean, 23 years). Participants received 15 Dutch guilders for their participation. None of the students were on medication, and all stated that they were in good health. Participants were instructed to abstain from smoking, drinking caffeine-containing beverages, and engaging in physical exercise for 1 hour before the experiment. All participants gave written informed consent on the aim and procedure of the study.

Measurements were recorded between 1:30 and 4:00 PM. On arrival, the experimental procedure was explained to the subject, electrodes for electrocardiography and impedance cardiography were attached, and the method of saliva collection was rehearsed. Participants then filled out a self-report questionnaire and were allowed to read self-selected magazines with neutral content for 20 minutes. The experiment started with a baseline measurement lasting 8 minutes. The experimental manipulation in this study was an 11-minute video showing bloody dental procedures, such as the extraction of front teeth and removal of a molar with a pick. This video was selected from among several videos on the basis of results of a separate pilot study ($N = 20$). Pilot study participants typically characterized the selected video as "horrible" or "gruesome" and reported increased anxiety during viewing.

Saliva was collected before the video was shown (baseline), during the final part of the video (stressor), and 10 minutes after the end of the video (recovery). Immediately after each saliva collection,

[1] **Note added at proofs:** In this paper, the term MUC5B denotes the whole high-molecular weight salivary mucin fraction, for which the term MG1 is in use also. This high-molecular weight mucin fraction may contain some other mucin gene products besides MUC5B.

participants also filled out a state anxiety questionnaire. The ECG and ICG were recorded continuously.

Saliva Collection

The method of saliva collection was practiced before the start of the experiment to ensure standardization. Saliva was collected by means of the spitting method according to the directions given by Navazesh (47). This method was shown to have adequate retest reliability ($r = 0.79$), which was confirmed by our data. The collection trial starts 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 orofacial movements (unstimulated saliva). The participant spits into a preweighed, ice-chilled test tube every 60 seconds. Each saliva collection period was 4 minutes. After collection, saliva was homogenized by vigorous shaking using a vortex and clarified by centrifugation (10,000*g*, 5 minutes) to eliminate buccal cells and oral microorganisms. The clear supernatant was divided into 500-μl aliquots and stored at −20°C until use.

Materials

Monoclonal antibody against sulfo-Le^a was prepared in our laboratory as described by Veerman et al. (48, 49). A panel of polyclonal sera against *H. pylori* was kindly provided by Ben J. Appelmelk and Theo Verboom (Department of Medical Microbiology, Vrije Universiteit, Amsterdam). Details on the production of these antisera are described by Appelmelk et al. (50). The polyclonal antiserum used in this study was selected after testing a panel of anti-*H. pylori* polyclonal antisera for cross-reactivity with saliva. *H. pylori* strain NCTC 11637 was obtained from the National Collection of Type Cultures (Public Health Laboratory Service, London, UK). Bicinchoninic acid (BCA) protein assay reagent was obtained from Pierce (Rockford, UK), and microtiter plates were from Greiner (Recklinghausen, Germany) and Dynex (Chantilly, VA). Polymeric neoglycoconjugates for the blocking assay were obtained from Synstone (Munich, Germany). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). Blood pressure was measured with a Dinamap Vital Signs Monitor (model 845 XT, Critikon, Tampa, FL). ICG and ECG signals were recorded using six Ag/AgCl spot electrodes (AMI type 1650–005, Medtronic, Haverhill, MA) and the Vrije Universiteit Ambulatory Monitoring Device (Amsterdam, The Netherlands) (51).

Cardiovascular Assessment

Assessment of cardiovascular response focused on blood pressure and cardiac autonomic balance. SBP and DBP were measured every 2 minutes during each of the three separate conditions. Indices of sympathetic and parasympathetic drive were obtained by analysis of the ECG and thoracic impedance (ICG) signals (52, 53). The ECG and ICG complexes were ensemble-averaged with reference to the ECG R wave across 1-minute periods (54). From these ensembles, condition averages were extracted for interbeat interval, PEP, SV, and left ventricular ejection time. Spectral power of HR was derived by frequency domain analysis of the continuously recorded interbeat interval time series using the CARSPAN program (55). Two frequency bands were deemed of interest in the integrated power density spectra: the frequency band around the intrinsic blood pressure oscillations (0.07–0.14, BLOOD) and the high-frequency band (0.15–0.40) that reflects RSA. In the time domain, the root mean square of successive differences was used as an alternative index of RSA. Changes in PEP and BLOOD have been used to index changes in cardiac sympathetic drive (56, 57), whereas changes in RSA and root mean square of successive differences are used to index changes in cardiac vagal tone.

Determination of MUC5B-Linked Sulfo-Le^a and Total Salivary Protein

For the quantification of sulfo-Le^a, an ELISA was used as described in detail by Veerman et al. (48). Protein was measured by the bicinchoninic acid method (58). This assay is described in detail by Bosch et al. (7). All assays have a split-half reliability of >0.98.

Bacteria

A strain of *H. pylori* NCTC 11637 (also designated ATCC 34504, American Type Culture Collection, Manassas, VA) was grown for 4 days under microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2) at 37°C on Dent agar plates (59) supplemented with 2,3,5-triphenyltetrazolium chloride (40 mg/ml). Cultures were harvested by centrifugation (4000*g*, 4°C), washed in sodium acetate buffer (100 mM, pH 5.0), and resuspended in sodium acetate buffer (with 0.5% Tween-20 added) to an optical density of 0.1 at 700 nm.

Adherence Assay

Adherence of *H. pylori* was measured by means of an ELISA. In 96-well microtiter plates (Immulon 1B, medium affinity), 100 μl of saliva was threefold serially diluted in 0.1 M $NaHCO_3$ buffer (pH 9.6) with a starting solution of 1:400. Microtiter plates were subsequently incubated for 3 hours at 37°C. After incubation, the wells were rinsed three times with PBS (50 mM potassium phosphate, 0.15 M sodium chloride, pH 7.4) containing 0.1% (v/v) Tween-20, followed by two rinses with demineralized water. This rinsing was done between each incubation step. Then, 100 μl of a freshly prepared bacterial suspension was added. After incubation (1 hour, 37°C) and subsequent washing, 100 μl of rabbit-anti-*H. pylori* polyclonal antibody (diluted 1:500 in PBS with 0.1% Tween-20 plus 3% bovine serum albumin) was added. After 1 hour of incubation and subsequent washing, horseradish peroxidase–conjugated goat anti-rabbit immunoglobulin (100 μl, diluted 1:6000 in PBS with 0.1% Tween-20 plus 3% bovine serum albumin) was added. After incubation (1 hour, 37°C), the microtiter wells were again rinsed, and 90 μl of the substrate-containing solution was added (1,1′ orthophenylene diamine, 0.03% H_2O_2 in 50 mM citrate, 0.1 M disodium phosphate, pH 5.0). Color was allowed to develop until sufficient intensity was reached. The reaction was stopped by adding 50 μl of a 4M H_2SO_4 solution, and absorbance was measured at 492 nm using a microtiter plate reader (Dynatech MR 7000). Adherence of *H. pylori* was quantified by calibration against the dose-response curve obtained in the same assay of a pooled saliva sample. The level of adherence of this pooled sample was indexed on 100. The assay was performed in triplicate, and the average was used. The intraassay variability of the assay is 8.1%.

Purification of MUC5B

MUC5B was isolated from saliva by ultracentrifugation (100,000*g*, 24 hours) under dissociative conditions, followed by gel filtration (Sephacryl HR-500, Pharmacia, Uppsala, Sweden) in 50 mM Tris/HCl/4 M guanidine chloride, pH 7.4 (60). The mucin-containing fractions were pooled, dialyzed against double-distilled water, and stored as aliquots at −20°C.

Inhibition Assays

To test whether the adherence in our assay was mediated by MUC5B, purified MUC5B was added to the *H. pylori* suspension and incubated for 1 hour at 37°C. The suspensions contained three concentrations of MUC5B: 100, 400, and 1600 μg/ml. A bacterial suspension without MUC5B was used as a control. Subsequently, adherence assays were performed.

In a second experiment, the bacterial suspension was incubated with an excess (30 μg/ml) of synthetic sulfo-3-galactose, the terminal part of sulfo-Le^a, bound to a polyacrylamide carrier (61). This synthetic oligosaccharide is known to compete with MUC5B for binding to *H. pylori* by occupying an adhesion molecule (designated neutrophil-activating protein) that specifically binds to sulfated carbohydrate (43). The bacterial suspension was incubated for 1 hour at 37°C. Subsequently, adherence assays were performed. Controls involved adherence of *H. pylori* after preincubation with buffer and with a nonrelevant carbohydrate-polyacrylamide conjugate (β-galactose-polyacrylamide).

Questionnaires

Participants were administered the Dutch translation of the Spielberger State/Trait-Anxiety Inventory (62). The state anxiety part (20 items) of this questionnaire was administered immediately after each saliva collection. The instruction after the second saliva collection was adapted to report on the anxiety the subject felt while viewing the video.

Participants also filled out a self-report questionnaire on health (perceived health and use of medication or other medical treatment), life habits, and behavior on the day of the experiment (smoking, alcohol, tea and coffee consumption, physical exercise, and sleep duration and quality).

Data Analysis

Data were analyzed with MANOVA (SPSSWIN7.5) using the three conditions (baseline, film, recovery) as within-subject factors. Because of their skewed distribution, salivary parameters and spectral data were logarithmically transformed before statistical analyses. For readability, the original (untransformed) values are used in the figures.

RESULTS

One participant terminated his participation in the experiment because he feared fainting. The analyses are confined to the remaining 17 subjects.

State Anxiety

Mean state anxiety increased from 11.8 (SEM = 2.4) at baseline to 19.3 (SEM = 2.6) during the stressor ($t(16) = 2.7$, $p = .01$). After recovery, mean state anxiety returned to just below the baseline value (mean = 9.9, SEM = 2.1; $t(16) = 3.6$, $p = .002$).

MUC5B-Linked Sulfo-Le^a and Adherence of *H. pylori*

Figure 1 shows the median, mean, and standard error of salivary concentration (U/ml), output (U/min), and total protein ratio (U/mg protein) of sulfo-Le^a measured at baseline, during the stressor, and during recovery. Sulfo-Le^a was quantified as ELISA units (U) using a pooled saliva sample as a standard. A measure of sulfo-Le^a output (U/min) was computed to control for salivary flow rate and is obtained by multiplying concentration (U/ml) by flow rate (ml/min). The sulfo-Le^a/protein ratio was computed to obtain a measure of specificity: An increased ratio indicates that sulfo-Le^a output occurs at a higher rate than the output of other secretory proteins. Figure 2 shows the median, mean, and standard error of saliva-mediated adherence of *H. pylori* (ex vivo).

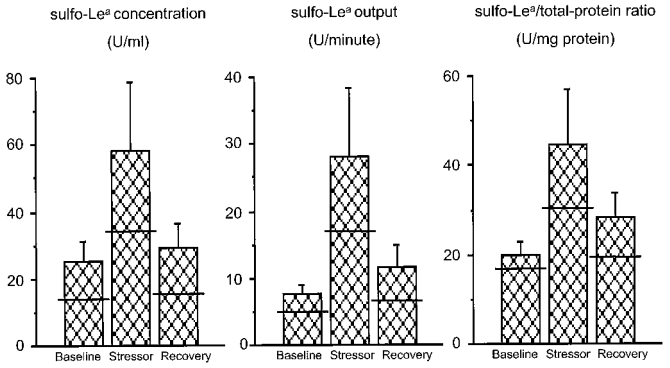

Fig. 1. Values of sulfo-Le^a concentration (U/ml), sulfo-Le^a output (U/min), and sulfo-Le^a/total protein ratio (U/mg protein). Values are expressed in ELISA units (U). Bars and error bars indicate mean values and standard errors, respectively. Horizontal lines indicate median values. For statistical analysis, log-transformed values were used.

MANOVA indicated a significant effect of the manipulation on sulfo-Le^a levels and adherence ($F(8,9) = 4.59$, $p = .018$). Subsequent multivariate analyses showed that baseline and stressor values differed significantly ($F(4,13) = 9.68$, $p = .001$) and that recovery and stressor values also differed significantly ($F(4,13) = 4.61$, $p = .015$), whereas baseline and recovery values did not differ significantly($F(4,13) = 1.44$, $p = .275$).

Post hoc analysis, using paired *t* tests, indicated that sulfo-Le^a concentration differed both between baseline and stressor ($t(16) = 3.73$, $p = .002$) and between stressor and recovery ($t(16) = 3.14$, $p = .006$) (Figure 1). Sulfo-Le^a output (U/ml) also showed an increase during stressor exposure, as compared with both baseline ($t(16) = 4.53$, $p < .001$) and recovery ($t(16) = 3.69$, $p = .002$) (Figure 1), indicating that the observed increase in sulfo-Le^a concentration is not due to alterations in flow rate. The sulfo-Le^a/protein ratio showed the same pattern as the two previous parameters: a significant increase during the stressor, as compared with both baseline ($t(16) = 2.90$, $p = .01$) and recovery

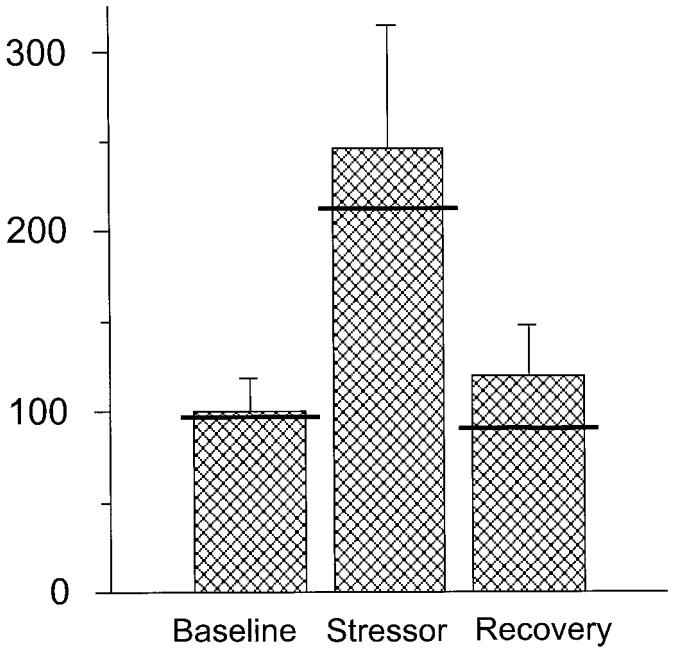

Fig. 2. Saliva-mediated adherence (ex vivo) of *H. pylori*. Bars and error bars indicate mean values and standard errors, respectively. Horizontal lines indicate median values. Adherence is quantified as an index value based on a pooled saliva sample as a standard, indexed on 100.

($t(16) = 2.47$, $p = .03$) (Figure 1), indicating that the observed increase in sulfo-Le^a output is specific and cannot be attributed to a nonspecifically increased protein secretion during stress. Finally, saliva-mediated adherence of *H. pylori* was significantly increased during the stressor, as compared with both baseline ($t(16) = 4.31$, $p = .001$) and recovery ($t(16) = 4.05$, $p = .001$) (Figure 2). Because of the relatively small sample size and skewness of our data, analyses were repeated using nonparametric tests (Wilcoxon and sign tests), yielding virtually the same results, with most significant p values well below the .01 criterion.

Salivary flow rate showed a nonsignificant increase during stressor exposure ($F(2,15) = 1.50$, $p = .24$). Variations in sulfo-Le^a at baseline and during the stressor and recovery were consistently and significantly correlated with variations in adherence of *H. pylori* (with Spearman rank correlation coefficients between 0.62 and 0.84, $.05 > p > .001$). Variations in salivary flow rate did not correlate with adherence of *H. pylori* or sulfo-Le^a ($p > .2$), indicating that the increased adherence of *H. pylori* was not a concentration artifact due to an altered salivary flow rate.

Inhibition Studies

To test whether the observed correlation between sulfo-Le^a and adherence of *H. pylori* underlies a causal relationship, two additional assays were performed as described in Materials and Methods. Preincubation of *H. pylori* with purified MUC5B reduced adherence in a dose-dependent manner, completely blocking adherence at a level of 1600 μg MUC5B/ml suspension (Figure 3). This result indicates that MUC5B is responsible for the observed adherence of *H. pylori* to the saliva-coated microtiter plate.

Preincubation of the *H. pylori* suspension with galactose-3-sulfate resulted in an approximately 4-fold decrease in adherence, as compared with the controls, preincubation with buffer, or preincubation with galactose-polyacrylamide (not shown). However, adherence was not blocked completely, indicating that besides sulfated glycans, other domains on the MUC5B molecule are involved in the binding to *H. pylori*.

Cardiovascular Response

Significant effects of condition emerged only for HR ($F(2,15) = 4.40$, $p = .031$) and SV ($F(2,15) = 6.38$, $p = .019$). Post hoc analysis indicated that HR decreased during the film in comparison with the baseline value, whereas baseline and recovery HR did not differ. The decrease in HR during the film was paralleled by an increase in SV. No other effects of condition emerged. Values of selected cardiovascular parameters are presented in Table 1.

DISCUSSION

To the best of our knowledge, this is the first study that has succeeded in demonstrating a direct link between stress-mediated biochemical changes and al-

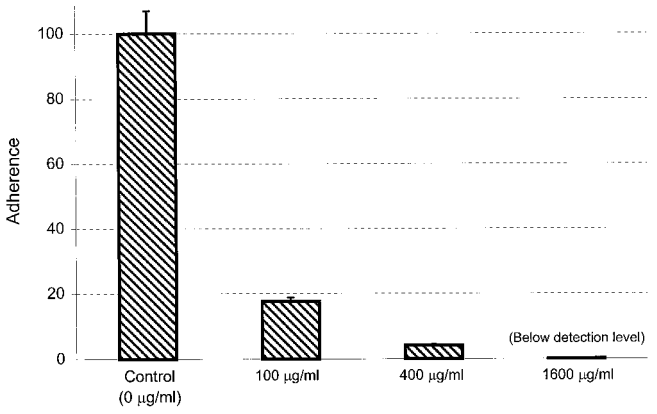

Fig. 3. Effects of preincubation of *H. pylori* suspension with 0, 100, 400, and 1600 μg/ml MUC5B. These results show that preincubation of *H. pylori* reduces adherence in a dose-dependent manner, indicating that the observed adherence is MUC5B dependent. Adherence was quantified as an index value based on a pooled saliva sample as a standard.

TABLE 1. Means and Standard Deviations of Selected Cardiovascular Parameters

	Baseline	Stressor	Recovery
HRate (bpm)	68.1 (10.4)	65.6 (9.8)	66.4 (9.2)
SV (cm^3)	116.2 (33.2)	123.4 (36.1)	114.0 (33.4)
RSA (ms^2)	2230 (2367)	2347 (2524)	2309 (2829)
BLOOD (ms^2)	1875 (1271)	2043 (2168)	1735 (1267)
PEP (ms)	113.1 (23.8)	113.9 (25.6)	115.4 (25.9)
SBP (mm Hg)	115.3 (10.7)	114.6 (10.7)	113.2 (7.8)
DBP (mm Hg)	69.1 (6.7)	68.4 (10.2)	68.6 (8.9)

tered host-microbe interactions in humans. Our results show enhanced saliva-mediated adherence (ex vivo) of *H. pylori* during an acute stressor. This increase was shown to correlate with an increase in sulfo-Le^a, a carbohydrate structure linked to the mucous glycoprotein MUC5B. Subsequent experiments, aimed at identifying the biochemical factors involved, demonstrated that MUC5B is responsible for the observed saliva-mediated adherence and that the oligosaccharide galactose-3-sulfate (the terminal part of the carbohydrate moiety of sulfo-Le^a) largely contributes to this process. These results are in agreement with those of recent studies showing that sulfated oligosaccharides on MUC5B function as adhesion molecules for *H. pylori* (42, 43).

The findings of our study resemble the results of animal studies investigating the effects of acute stressors (ie, water immersion and restraint stress) on the activity of secretory cells in the colon, stomach, and nasopharynx (63–71). These studies demonstrated robust effects of acute stress on mucin release, synthesis, and glycosylation (a process in which carbohydrate structures on proteins are formed). The relatively acute increase in the secretion of sulfo-Le^a observed in our study is most likely due to an increased release of MUC5B, not to an altered synthesis or glycosylation of MUC5B, because the time necessary for glycosylation and intracellular transport far exceeds 10 minutes (72).

The release of salivary proteins is mainly governed by the synergistic actions of both the sympathetic and parasympathetic branches of the autonomic nervous system (73, 74) and is also modulated by several neuropeptides that are released in conjunction with the conventional transmitters acetylcholine and noradrenaline (75, 76). Therefore, stress-induced autonomic activation is the most likely explanation for the observed release of MUC5B. Surprisingly, no consistent association was observed between our cardiovascular indices and salivary parameters. Possibly, cardiac vagal and sympathetic drive do not properly reflect activity in parasympathetic and sympathetic branches that innervate the MUC5B-secreting salivary glands. Future pharmacological blockade studies may elucidate the neural mechanisms involved.

Most laboratory studies in psychoneuroimmunology make use of stressors that require mental effort and that are characterized by their potential to evoke a classic fight-flight cardiovascular response pattern (eg, mental arithmetic, Stroop task, and reaction tasks) (77–84). This clearly being the dominant paradigm, it may become overlooked that there is a body of literature showing that many stressful situations do not necessarily elicit a substantial cardiovascular response and may even be associated with a reduced HR (85–89). What becomes clear from our study is that such stressors are nevertheless capable of inducing robust biochemical changes with a clear effect on immunological functioning.

The binding of *H. pylori* to salivary mucins may play a role in the colonization of this pathogen through the oral route. Moreover, several pathogenic bacteria other than *H. pylori* bind to MUC5B and/or sulfo-Le^a, and our results may therefore be extrapolated to these microorganisms. Examples are *Escherichia coli* (90), *Staphylococcus aureus* (91), *Candida albicans* (92), *Bordetella pertussis* (93), *Hemophilus influenzae* (94), and *Fusobacterium nucleatum* (Groenink J, Veerman ECI, Nieuw Amerongen AV. 1999, unpublished data). The latter two pathogens, which are involved in opportunistic infections of the respiratory tract and in periodontal disease (chronic infection of the tooth-supporting tissues), respectively, are especially of interest within the context of this study because there is a growing body of research showing an association of both pathologies with stress (2, 95).

Because the mouth is the habitat of many microorganisms and is involved in the transmission of many microorganisms, an increased adherence of MUC5B- and/or sulfo-Le^a-binding organisms could affect susceptibility in three different ways. First, an increased opportunity for microorganisms to colonize the oral epithelium may imply an increased risk of being infected through the oral route. Second, the increased adherence to the tissues of the oral cavity might, in case of oral-oral contact with a carrier of the pathogen, cause exposure of another host to a relatively larger inoculating dose (the number of microorganisms transmitted). Because the inoculation dose is a crucial factor determining whether an infection will proceed (46), increased adherence may therefore imply a greater risk of infecting others. Third, a specifically increased release of MUC5B, as seen in our study, might favor the colonization of bacteria that preferentially bind to MUC5B and/or the carbohydrates linked to this glycoprotein. Likewise, microorganisms that do not bind MUC5B (among which are many streptococci

and staphylococci) are likely to be relatively unaffected by an increase in MUC5B (94, 96–98). Consequently, stress-mediated release of MUC5B might affect the microbial homeostasis of the mucosa; that is, when stress induces biochemical changes that are favorable for some species but not necessarily for others, these changes may lead to a microbial population shift (99–101).

CONCLUDING REMARKS

Research on the effects of psychosocial factors on mucosal defense has concentrated almost exclusively on S-IgA, the predominant immunoglobulin in mucosal secretions (6). However, the fact that S-IgA deficiency is the most common form of immunodeficiency and that individuals deficient in S-IgA show only a marginally increased susceptibility to infectious disease (5) indicates that S-IgA is not the only factor. Instead, mucosal defense is brought about by the combined activity of many proteins, which for the most part are produced not by cells of the immune system but by the secretory glandular cells. In our opinion, the relevance of the current study lies in the fact that it exemplifies how psychological factors, by altering the secretion of such proteins, can affect host-microbe interactions.

The mucosa, especially that of the oral cavity, is an extremely complex environment in which an enormous diversity in microorganisms and host factors interact. Therefore, some caution is needed in extrapolating our results to the in vivo situation. Future studies may extend the approach presented in our current study by examining different aspects of host-microbe interactions simultaneously. The present study and other studies carried out by our group (7, 8) indicate that the secretion of nonimmunological proteins is altered in subjects under stress. Moreover, our results show that these alterations directly affect the way in which microorganisms are dealt with. We therefore conclude that the inclusion of innate defense-system parameters in studies in the field of behavioral medicine may contribute to our understanding of the relationship between stress and susceptibility to infectious disease.

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ANNOUNCEMENT

The 12th International Congress on Psychonephrology will be held in Yokohama, Japan, June 2 and 3, 2000. Deadline for submission is April 15, 2000. Contact: Norman B. Levy, MD, Dept of Psychiatry, Coney Island Hospital, 2601 Ocean Parkway, Brooklyn, NY 11235; FAX: (718) 616-4371. For other countries, contact: Shingo Takesawa, PhD, Suzuka Univ of Medical Science, 10001-1 Kishiokacho, Suzuka City, Mie 5100293, Japan, FAX: 81-593-83-9666.