Secretory immunoglobulin A and cardiovascular activity during mental arithmetic and paced breathing

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Abstract

The role of the autonomic nervous system in secretory immunoglobulin A (sIgA) responses to laboratory challenge was explored in a study in which sIgA and cardiovascular activity were recorded at rest and during mental arithmetic and paced breathing. These tasks were selected to preferentially engage the sympathetic and parasympathetic nervous systems, respectively. Mental arithmetic elicited a mixed pattern of increased alpha- and beta-adrenergic activity and a reduction in parasympathetic activity; diastolic blood pressure, total peripheral resistance, and systolic blood pressure increased, preejection period shortened, and heart rate variability decreased. In contrast, paced breathing primarily elicited an increase in parasympathetic activity; heart rate variability increased. Mental arithmetic also provoked an increase in sIgA concentration but no change in saliva volume, whereas paced breathing affected neither sIgA concentration nor saliva volume. These data suggest that sIgA responses to laboratory challenges are mediated by sympathetic rather than parasympathetic processes.

Descriptors: Autonomic nervous system, Mental arithmetic, Paced breathing, Secretory immunoglobulin A

A number of studies in humans have now shown that the concentration and/or secretion rate of secretory immunoglobulin A (sIgA) in saliva increases in response to acute naturalistic challenges (Bristow, Hucklebridge, Clow, & Evans, 1997; Chan & Spinks, 1996; Evans, Bristow, Hucklebridge, Clow, & Pang, 1994; McClelland, Ross, & Patel, 1985; Zeier, Brauchli, & Joller-Jemelka, 1996) and laboratory stress tasks (Carroll et al., 1996; Willemsen et al., 1998). However, the mechanism(s) underlying these short-term increases in sIgA has yet to be determined. One way of elucidating mechanisms is to simultaneously record sIgA and cardiovascular variables during laboratory tasks known to elicit profiles of cardiovascular activity indicative of different patterns of autonomic neural activation (c.f., Cacioppo, 1994).

In a recent study in which this strategy was adopted, Willemsen et al. (1998) found increases in sIgA in response to a cold pressor test, which elicited mainly alpha-adrenergic cardiovascular activity, and to mental arithmetic, which elicited a mixed alpha- and beta-adrenergic cardiovascular response. Although these data implicate alpha-adrenergic activity in the sIgA increase, possible parasympathetic nervous system involvement cannot be discounted, particularly given preliminary evidence that formal relaxation elicits an increase in sIgA. In the first of two studies, Green and Green (1987) allocated participants to one of four 20-min relaxation protocols: (a) Benson's relaxation response, based on transcendental meditation (Benson, 1975), (b) guided visualization of their bodies

becoming more relaxed, (c) back massage, and (d) lying quietly with eyes closed. A fifth group was assigned to a control condition in which they were touched gently at designated points on the back by a masseur. The sIgA concentration increased significantly from pre- to immediately posttreatment for all of the relaxation protocols except lying quietly but did not change following the touching control condition. In the second study (Green, Green, & Santoro, 1988), participants were allocated either to 3 weeks of daily sessions of the relaxation response or guided visualisation or to a waiting list control group. Overall, sIgA secretion rate increased immediately following the first 20-min session, but more so for those undertaking relaxation than for those in the waiting list control group. Green et al. also reported a training effect; sIgA increased more from pre- to postsession for the relaxation groups than for the waiting list control group now undertaking their first relaxation sessions. Jasnoski and Kugler (1987) compared progressive relaxation and an auditory discrimination control task and found that relaxation resulted in higher sIgA concentrations than did the control condition.

Given that increased parasympathetic activity characterizes relaxation protocols such as these (Greenberg, 1990), there are prima facie grounds for suspecting general parasympathetic involvement in acute increases in sIgA. An important aspect of many relaxation protocols is controlled, regular, slow breathing (Taylor, 1995). Accordingly, the present study examined sIgA and cardiovascular activity during mental arithmetic and during paced breathing, tasks expected to elicit sympathetic and parasympathetic excitation, respectively (Grossman, Karemaker, & Wieling, 1991). It was hypothesized that increased autonomic activation, whether sympathetic or parasympathetic, would produce increases in sIgA.

Method

Participants

Twenty-four male undergraduate students with no history of cardiovascular or pulmonary disease participated in the study. All participants were nonsmokers, and none reported or displayed symptoms of any upper respiratory tract infection. The data from 1 participant were lost because of equipment failure. The mean age of the remaining 23 participants was 21.30 ($SD = 1.79$) years, mean weight was 77.57 ($SD = 8.27$) kg, and mean height was 1.81 ($SD = 0.08$) m. Participants gave informed written consent and were advised that the best score on the mental arithmetic task would receive a £15 book token and that another £10 book token would be awarded at random to one of the others. None of the participants reported taking any medication within 4 weeks of testing, and all were urged to abstain from alcohol and exercise from the evening prior to testing and from caffeine during the hour before testing.

Cardiovascular Measures

Impedance cardiography (ICG) and electrocardiography (ECG) signals were recorded using a VU-AMD system (Vrije Universiteit, Amsterdam; see Willemsen, de Geus, Klaver, van Doornen, & Carroll, 1996). The ICG and ECG signals were sampled at 250 Hz and 1000 Hz, respectively, and recorded using six disposable pregelled Ag/AgCl spot electrodes (3M Healthcare). One electrode was a combined ECG/ICG electrode placed 4 cm above the jugular notch of the sternum. The other recording ECG electrode was placed at the apex of the heart over the ninth rib, and the ground ECG electrode was placed above the right iliac crest. The second ICG recording electrode was placed directly over the tip of the xiphoid process of the sternum. The two ICG current electrodes were placed on the back, at the base of the neck and over the T8–T9 vertebrae, at least 3 cm higher and lower, respectively, than the recording electrodes on the front.

Ensemble averages of the ICG were based on 60-s epochs that were scored and edited off line using an interactive software program. The first derivative (dZ/dt) of the change in transthoracic impedance was used to identify the opening of the aortic valve (B-point), the maximum rate of change of blood flow (dZ/dt_{max}), and the closing of the aortic valve (X-point). The onset of left ventricular contraction (R-wave) was obtained from the ECG; the timing of the onset of the Q-wave was estimated as 48 ms prior to the R-wave. The 60-s ensemble averages of the ICG and ECG signals were used to determine heart rate (HR), preejection period (PEP), defined as the time between the Q-wave onset and the B-point, left ventricular ejection time, and dZ/dt_{max} relative to the B-point. Stroke volume (SV) was determined using Kubicek et al.'s (1974) formula, and cardiac output (CO) was calculated as the product of HR and SV. Total peripheral resistance (TPR) was calculated using the formula TPR $= $ (MAP/CO) $\times$ 80, where MAP $=$ mean arterial pressure.

Heart rate variability (HRV) was obtained by sampling the ECG over 60-s epochs. Each R-R interbeat interval (IBI) within the epoch was used to calculate the square root of the mean of sum of the squared successive differences (RMSSD), using the formula RMSSD (ms) $= \sqrt{1/n \sum (IBI_i - IBI_{i-1})^2}$, where $i =$ current IBI, and $n =$ number of IBIs in the epoch (Ewing, Borsey, Bellavere, & Clarke, 1981; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded using an auscultatory monitor (Accutraker II, Sun-

tech, Model 104). The monitor also recorded ECG using a spot electrode configuration. The first recording electrode was placed above the right clavicle bone. The second recording electrode was placed adjacent to the ECG electrode of the ICG system at the apex of the heart. A ground electrode was placed over the ninth rib on the right side directly opposite the second ECG electrode. A microphone was centered over the brachial artery on the nondominant arm, and the cuff was attached over the microphone. The monitor operated in a dynamic pressure inflation mode: the cuff was inflated to 30 mmHg more than the previous SBP, and then deflated in steps of 3 mmHg. MAP was calculated using the formula MAP $=$ DBP $+ [(SBP - DBP)/3]$.

sIgA in Saliva Measurements

Using Salivettes (Sarstedt Ltd.), unstimulated saliva samples were obtained to determine saliva volume and sIgA concentration. Before each sample, the participant was asked to swallow to dry the mouth and then to place a cotton swab underneath the tongue for 2 min. The participant then placed the swab in a Salivette tube, which was sealed and frozen at $-20\,°C$ for later analysis.

On thawing, saliva was extracted from the cotton by centrifugation at 5×10^3 rpm for 5 min. Saliva volume was determined by weight and by assuming a specific gravity of 1. The concentration of sIgA in saliva was determined by a radial immunodiffusion (RID) assay (Bind A Rid, The Binding Site). The assay was principally derived from the work of Fahey and McKelvey (1965) and Mancini, Carbonara, and Heremans (1965). The method involved antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate concentration of antibody. Aliquots of neat saliva were applied to the wells in a volume of 10 μl. The antibody in the agarose gel was polyclonal sheep IgG directed against epitopes specific to human IgA. These junctional epitopes arise from the physical combination of the J chain/IgA dimer and secretory piece. The antibody does not react with IgA monomer, IgA dimer, free secretory piece, free J chain, or J chain bound to IgM. Antigen–antibody complexes are formed, producing a precipitin ring. The ring size increases until equilibrium is reached between the formation and breakdown of these complexes (completed within 3 days). At this stage, a linear relationship exists between the square of the ring diameter and the antigen concentration. The assay is calibrated using three concentrations of purified sIgA. These were obtained by column chromatography from human milk, and purity was established by demonstration of one line by immunodiffusion techniques using antibodies against whole serum, IgA, secretory piece, and sIgA and by analysis on sodium dodecyl sulfate polyacrylamide gel electrophoresis. The concentration of the three calibration solutions was determined by optical density. Within-plate coefficient of variation and interbatch coefficient of variation were low, $<3\%$ and $<5\%$, respectively. Each 2-min sample provided a measure of the saliva sIgA concentration (μg/ml) and saliva volume (ml/min). The sIgA secretion rate (μg/min) was calculated from the formula secretion rate $=$ concentration $\times$ volume.

Mental Arithmetic and Paced Breathing Tasks

In the 8-min paced auditory serial arithmetic test (PASAT), participants were required to add two sequentially presented single-digit numbers and to retain the latter of the two numbers in memory for subsequent addition to the next number presented (Willemsen et al., 1998). Numbers (1–9) were delivered using a tape player. Participants were instructed to add each number they heard to the immediately preceding number and to write out the answer. The

task consisted of four 2-min series of 50, 60, 75, and 100 digits at presentation rates of 2.4, 2.0, 1.6, and 1.2 s, respectively.

In the 8-min paced breathing task, participants were required to breathe nasally at the rate of 6 breaths/min (Angelone & Coulter, 1964; de Geus, Willemsen, Klaver, & van Doornen, 1995), inhaling for 6 s and exhaling for 4 s. Prompts to inhale and exhale were delivered using a tape player.

Procedure

The electrodes and brachial cuff were attached, and the participant sat for 5 min to accommodate to the laboratory setting. The session consisted of two 8-min tasks (PASAT and paced breathing), each preceded by an 8-min rest period. Task order was counterbalanced across participants. ICG was recorded continuously during the session. During each task and rest period, blood pressure was recorded during minutes 2, 5, and 8, and a saliva sample was obtained during minutes 7–8.

Data Reduction and Analysis

The 60-s averages for HR, CO, PEP, and HRV were used to calculate a series of 2-min interval means: the last minutes (7–8) of each rest period and minutes 1–2, 3–4, 5–6, and 7–8 of each task. For SBP, DBP, and MAP, the following measurements were used for analysis: recordings during minute 8 of each rest period and during minutes 2, 5, and 8 of each task. TPR was calculated using the data from the minutes associated with the blood pressure recordings. These data were treated to a series of repeated measures multivariate analyses of variance (MANOVAs) applied separately to the measurement intervals for each of the two tasks plus their respective rest periods. Wilks's lambda (λ), the associated F value, and degrees of freedom are reported. Significant effects were followed by post hoc comparisons using the Newman–Keuls method.

Saliva volume, sIgA concentration, and sIgA secretion rate were obtained from the samples taken during each rest and subsequent task. A series of repeated measures analyses of variance (ANOVAs) were applied separately to data from each of the two tasks plus their associated rest periods. Because of the large interindividual differences in sIgA concentration and sIgA secretion rate, a square root transformation was performed to increase homogeneity of variance (Myers, 1972). A 5% significance level was adopted in all tests.

Results

Cardiovascular Activity

PASAT. As shown in Figure 1, SBP, DBP, TPR, and HR increased at task onset and remained elevated throughout the task, although both SBP and DBP declined during the last 2 min of the task. Furthermore, PEP and HRV were lower during the task than during rest. A MANOVA for four intervals yielded significant effects for SBP, $\lambda = .231$, $F(3,20) = 22.20$, $p < .05$, DBP, $\lambda = .339$, $F(3,20) = 12.98$, $p < .05$, and TPR, $\lambda = .294$, $F(3,20) = 16.03$, $p < .05$. A MANOVA for five intervals also revealed significant effects for HR, $\lambda = .361$, $F(4,19) = 8.42$, $p < .05$, PEP, $\lambda = .554$, $F(4,19) = 3.83$, $p < .05$, and HRV, $\lambda = .381$, $F(4,19) = 7.71$, $p < .05$. In all cases, post hoc comparisons indicated that the task values differed from the rest values. There were no significant differences among the task values for TPR, HR, PEP, and HRV. However, the pressor reactions had declined significantly by the end of the task.

Paced breathing task. Figure 2 shows that SBP and TPR decreased substantially whereas HRV almost doubled from rest to task. The pattern for the other variables was more complex; DBP and PEP decreased initially but returned to resting levels, whereas HR increased marginally midway through the task. A MANOVA for four intervals yielded significant effects for SBP, $\lambda = .359$, $F(3,20) = 11.91$, $p < .05$, and TPR, $\lambda = .360$, $F(3,20) = 11.84$, $p < .05$, but not for DBP, $\lambda = .784$, $F(3,20) = 1.84$, $p > .05$. A MANOVA for five intervals showed significant effects for HRV, $\lambda = .301$, $F(4,19) = 11.05$, $p < .05$, HR, $\lambda = .210$, $F(4,19) = 17.87$, $p < .05$, and PEP, $\lambda = .380$, $F(4,19) = 7.76$, $p < .05$. Post hoc comparisons revealed that for SBP, TPR, and HRV, task values differed from the rest values, but there were no significant differences among the task values. Further, PEP was significantly shorter during the first half of the task than during rest or the second half of the task. Although the absolute differences in HR were slight (ca. 2 beats/min), post hoc tests nonetheless indicated that HR was faster during minutes 3–8 of the task than during rest and minutes 1–2 of the task.

Salivary Measures

PASAT. Figure 3 shows that the PASAT was associated with a substantial rise in sIgA concentration but little change in either saliva volume or sIgA secretion rate. An ANOVA (two intervals) revealed a significant increase in sIgA concentration, $F(1,19) = 10.24$, $p < .05$, but no significant changes in saliva volume, $F(1,19) = 0.75$, $p > .05$. The rise in sIgA secretion rate was also not significant, $F(1,19) = 0.41$, $p > .05$.

Paced breathing task. As depicted in Figure 4, saliva volume, sIgA concentration, and sIgA secretion rate were largely unaffected by paced breathing. An ANOVA (two intervals) revealed that the following variables were not significantly different between rest and paced breathing: saliva volume, $F(1,18) = 3.04$, $p > .05$, sIgA concentration, $F(1,18) = 0.29$, $p > .05$, and sIgA secretion rate, $F(1,18) = 0.03$, $p > .05$.

Relationship Between Changes in sIgA Concentration and Sympathetic and Parasympathetic Indices

Correlation coefficients were computed, using change scores, between sIgA concentration and TPR, PEP, and HRV, as indices of alpha-adrenergic activity, beta-adrenergic activity, and parasympathetic activity, respectively (Cacioppo, 1994). None of the coefficients were statistically significant.

Discussion

As expected, the mental arithmetic task elicited an increase in indices of alpha- and beta-adrenergic cardiovascular function. During mental arithmetic, DBP, TPR, SBP, and HR increased but PEP shortened. In addition, HRV, a marker for parasympathetic activity, decreased with mental arithmetic. Broadly in line with our previous finding (Willemsen et al., 1998), sIgA concentration increased during mental arithmetic. However, neither saliva volume nor sIgA secretion rate changed significantly, a result at odds with those of Willemsen et al. (1998). This discrepancy may stem from procedural differences between the two studies. Whereas Willemsen et al. (1998) collected the saliva sample immediately following the task, in the present study saliva collection took place during the final 2 min of the task. Current investigations in our laboratory should resolve this issue. Nonetheless, the increase in sIgA in response to mental arithmetic is a robust phenomenon that is independent of the assay method. Our earlier study used a non-
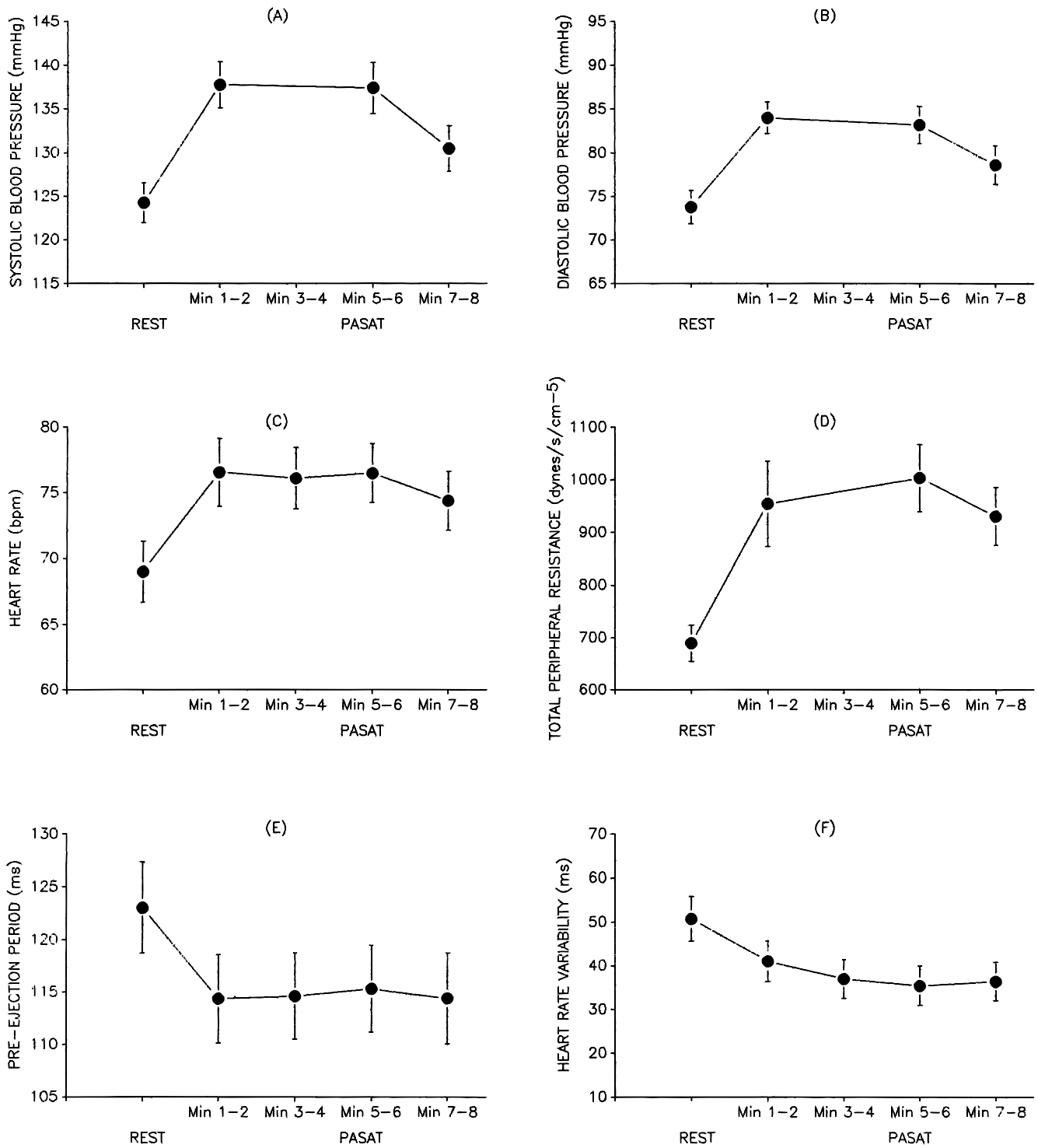
Figure 1. Mean (*SE*) levels of cardiovascular activity during rest and PASAT: SBP (panel A), DBP (panel B), HR (panel C), TPR (panel D), PEP (panel E), and HRV (panel F).

specific enzyme-linked immunosorbent assay (Willemsen et al., 1998), whereas the present study employed a new RID assay, which specifically binds junctional epitopes (J chain and secretory piece) on sIgA and not IgA monomer, IgA dimer, free secretory piece, free J chain, or J chain bound to IgM. All of these molecules coexist with secretory IgA in bodily fluids.

In contrast, paced breathing was characterized by a substantial increase in parasympathetic tone; HRV almost doubled. Additionally, TPR decreased throughout the task, implying reduced alpha-adrenergic activity. During paced breathing, HR increased by less than 2 beats/min. Early in the task, PEP shortened; the reduction in PEP can be accounted for by heterometric autoregulation,
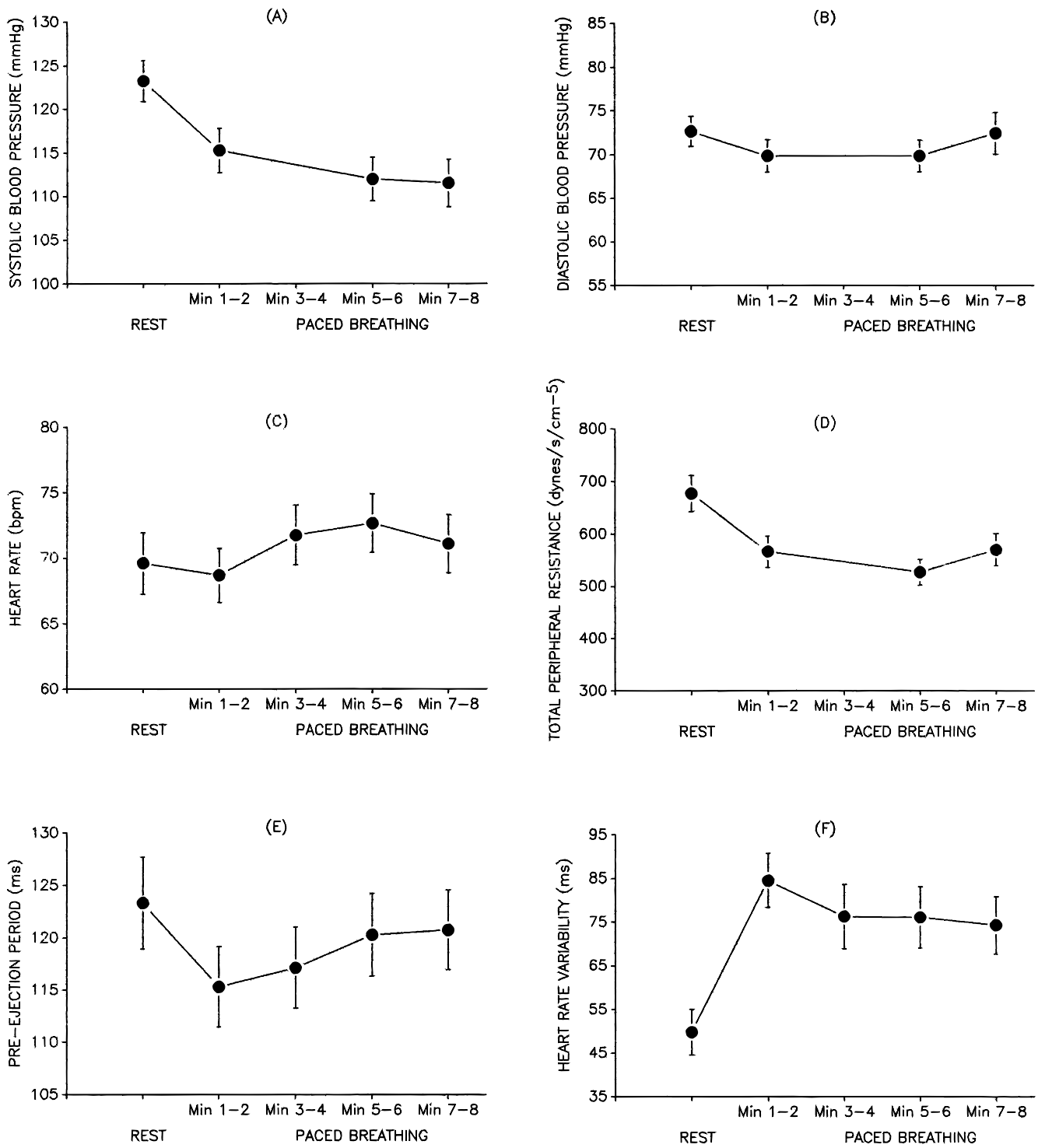
Figure 2. Mean (*SE*) levels of cardiovascular activity during rest and paced breathing: SBP (panel A), DBP (panel B), HR (panel C), TPR (panel D), PEP (panel E), and HRV (panel F).

by way of the Frank–Starling mechanism (Sarnoff, Mitchell, Gilmore, & Remensnyder, 1960; Starling, 1915), rather than extrinsic beta-adrenergic processes. A limitation of PEP, as an index of contractility and beta-adrenergic influence on the heart, is its susceptibility to changes in preload (Talley, Meyer, & McNay, 1971) and afterload (Cacioppo, 1994). Accordingly, the paced breathing task would seem to be primarily characterized by a marked increase in cardiac vagal tone and a reduction in alpha-adrenergic activity.
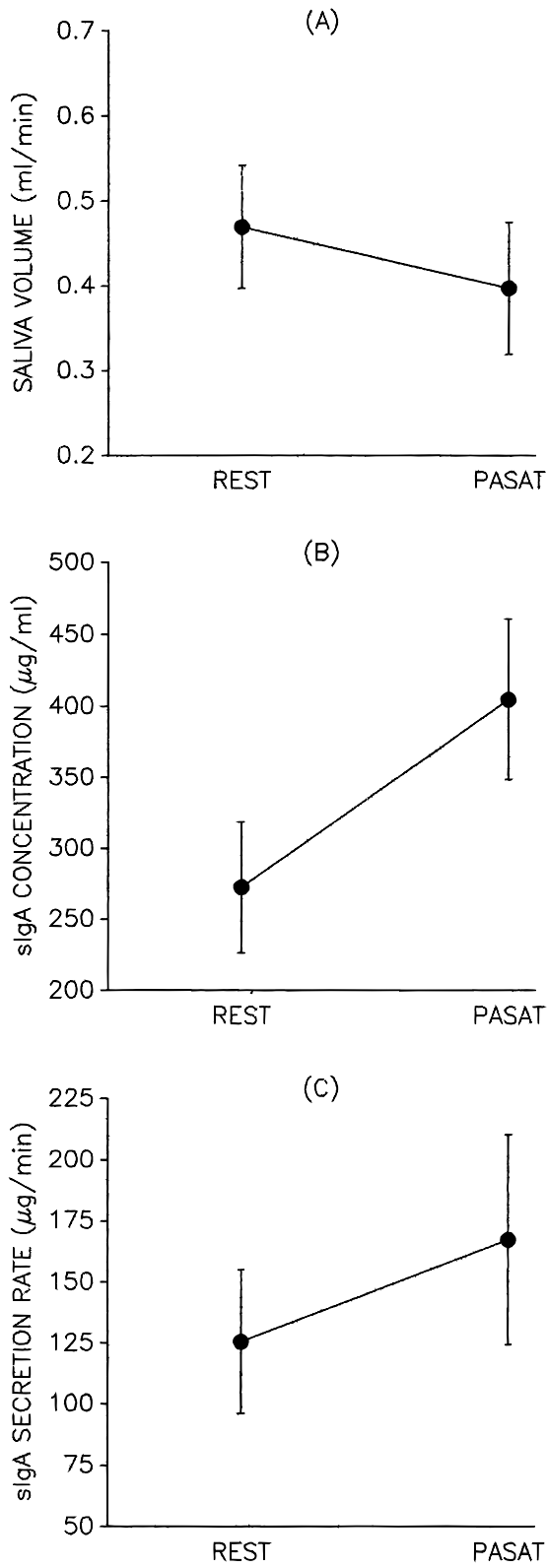
Figure 3. Mean (*SE*) levels of secretory activity during rest and PASAT: saliva volume (panel A), sIgA concentration (panel B), and sIgA secretion rate (panel C).
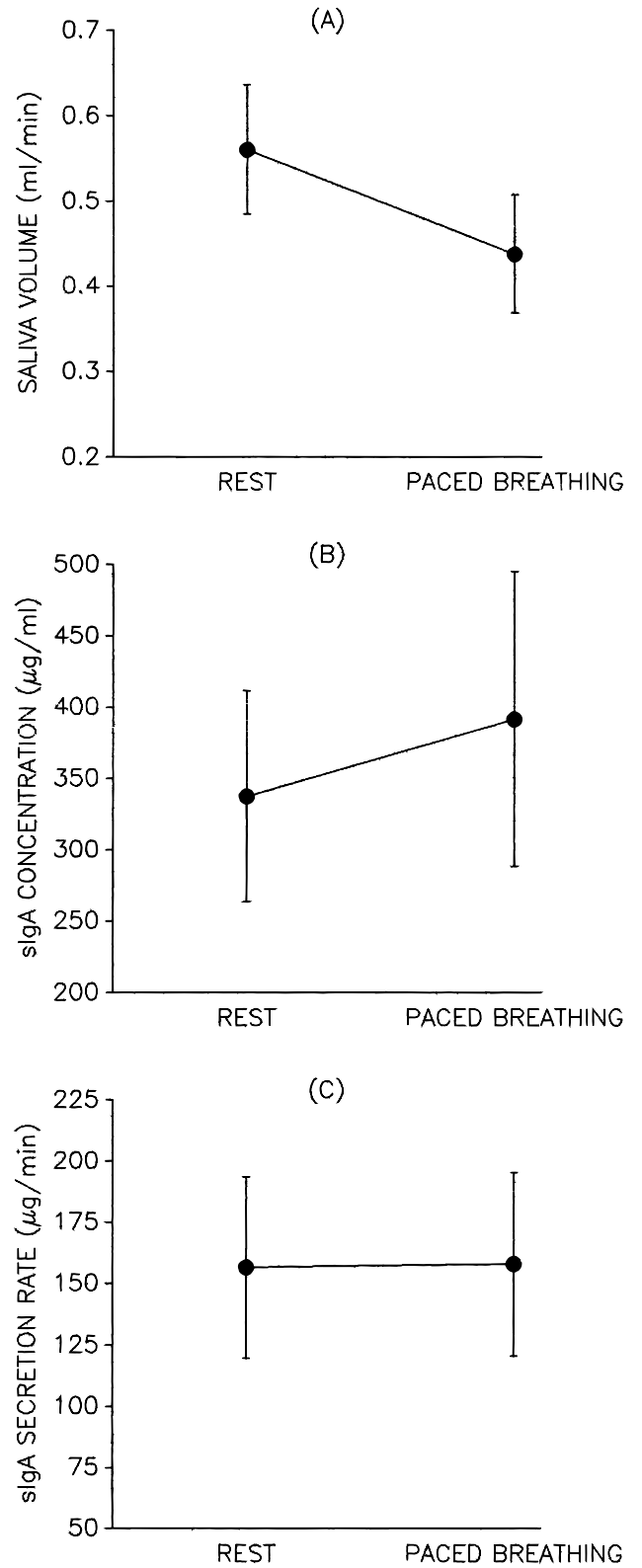
Figure 4. Mean (*SE*) levels of secretory activity during rest and paced breathing: saliva volume (panel A), sIgA concentration (panel B), and sIgA secretion rate (panel C).

Paced breathing affected neither saliva volume nor sIgA concentration.[1] These results are at odds with expectations based on earlier studies that have reported increases in sIgA following formal relaxation (Green & Green, 1987; Green et al., 1988; Jasnoski & Kugler, 1987). However, not all relaxation protocols emphasize controlled breathing. For example, the progressive relaxation procedure employed by Jasnoski and Kugler (1987) involves alternately tensing and relaxing muscle groups. Accordingly, the increase in sIgA they reported may be accounted for by increased muscle tension. However, the increases in sIgA observed by Green and associates cannot be explained in this way because the Benson relaxation protocol they used is derived from transcendental meditation and emphasizes respiratory control (Benson, 1975). The discrepant findings may reflect other methodological differences.

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[1]The underlying rationale for the approach adopted here is that the indices of cardiovascular autonomic function can also be used as proxies for sympathetic and parasympathetic influences on salivary function. However, the neuroanatomical organization of the heart and the salivary glands is different. Nevertheless, it is reasonable to presume that changes in cardiovascular and salivary function reflect generalized sympathetic and parasympathetic activation (Cannon, 1915; Stern & Sison, 1990).

For example, in the present study paced breathing was undertaken for just 8 min, whereas previous studies have employed 20-min or 60-min relaxation sessions. The increase in sIgA with relaxation may be apparent only after prolonged relaxation. However, another explanation of differences among studies relates to the timing of saliva collection. In the present study, saliva was collected while participants were engaged in paced breathing, whereas in all previous studies, saliva was sampled after relaxation. There may be a relative increase in physiological activation following protracted and deliberate relaxation, and increases in sIgA may reflect such activation.

Considered along with our previous findings (Carroll et al., 1996; Willemsen et al., 1998), the present data are consistent with the conclusion that acute rises in sIgA in saliva are mediated by increased adrenergic activity. In support, Carpenter, Garrett, Hartley, and Proctor (1998) reported that direct sympathetic nerve stimulation of the submandibular glands in rats resulted in a six-fold increase in sIgA secretion, more than double that elicited by parasympathetic stimulation. As a next step, however, selective pharmacological manipulations in humans should be undertaken to confirm that increased sympathetic activity is the primary determinant of acute sIgA reactions.

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