Improving the methodology for non-invasive autonomic nervous system recording and its implementation in behavioral research

van Lien, R.

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CHAPTER 5

The role of sympathetic and parasympathetic activation in sAA secretion during exercise

René van Lien, Petra A. M. van den Keijbus, Enno C.I. Veerman, and Eco J.C. de Geus

This chapter wil be merged with chapter 4 and submitted to PNEC.

Abstract

Salivary alpha amylase (sAA) secretion has gained interest as a potential non-invasive biomarker for activity of the sympathetic nervous system (SNS). However, the parasympathetic nervous system (PNS) can also affect sAA secretion through its synergistic enhancement of SNS effects. In the current study 28 participants underwent exercise testing with continuous recording of cardiac SNS and PNS activity. Saliva was collected before and after exercise using the passive drooling method. Exercise-induced changes in enzymatic sAA activity, the most often used sAA measure, were assessed as well as changes in the actual sAA protein concentration and the ratio of sAA protein to total salivary protein. sAA activity and sAA protein concentration were converted to reflect true changes in sAA output by multiplying by flow rate. SNS reactivity was measured as decreased preejection period (PEP) and PNS reactivity as decreased respiratory sinus arrhythmia (RSA). Participants that paired exercise-induced cardiac SNS activation to a relatively small loss of PNS activation showed the strongest increases in enzymatic sAA activity ($r_{RSA\text{-}sAA\ activity}$ = .36), but changes in the true sAA output were not significantly related to SNS or PNS reactivity. Overall, the findings indicate that neither true sAA output nor its easily obtained proxy, enzymatic sAA activity, should be used as a selective indicator of SNS activation.

Introduction

Salivary alpha amylase (sAA) is a digestive enzyme that is synthesized and secreted by the acinar cells (i.e., the main secretory cells) of the salivary glands, in particular those of the two parotid glands. A main function of sAA is to break down insoluble starch into soluble maltose and dextrin, and the salivary concentration of this enzyme can be measured relatively cheap and easy by assessing this enzymatic activity. Enzymatic sAA activity, expressed as Units per milliliter (U/ml), is widely used as a proxy for the amount of sAA protein produced per unit saliva (mg/ml), although the correlation between amylase concentration and activity is only moderate (r = 0.61, (Goedhart et al., 2007; Mandel, Peyrot des, Plank, Alarcon, & Breslin, 2010; Schwartz et al., 1992). This is likely due to the fact that the protein is secreted in several isoforms which differ in enzymatic activity.

Between-individual variation in sAA secretion is strongly determined by individual copy number variation of the *AMY1* gene on chromosome 1p21, with a reported range of anywhere from 2 to 15 diploid copies (Mandel et al., 2010). In the absence of eating/chewing, within-individual variation in sAA secretion is largely caused by changes in the activity of the autonomic nervous system (ANS). Over the past 15 years, enzymatic sAA activity has gained interest as a potential non-invasive biomarker for activity of the sympathetic nervous system (SNS) specifically, which is backed by various compelling findings (Nater et al., 2009; Rohleder et al., 2009). For example, significant elevations in enzymatic sAA activity have been found after exposure to stressors known to increase SNS activity, like a stressful academic examination (Bosch et al., 1996) parachute jump (Chatterton, Jr. et al., 1997) stressful computer games (Chatterton, Jr. et al., 1997; Skosnik et al., 2000; Takai et al., 2004), watching a stressful video (Bosch et al., 2003a) effortful arithmetic or memory test (Bosch et al., 2003a; Noto et al., 2005), and the Trier Social Stress Test (TSST) (Nater et al., 2006; Nater et al., 2005; Rohleder et al., 2006). Moreover, the stress-induced enzymatic sAA activity increase can be blocked pharmacologically by the β-adrenergic blocker propanolol (Van Stegeren et al., 2006), and a significant increase in enzymatic sAA activity was found after administration of yohimbine, which increases sympathetic drive by blocking the inhibitory α1-adrenergic autoreceptor (Ehlert, Erni, Hebisch, & Nater, 2006).

An enhancing effect of SNS activity on sAA secretion is also biologically plausible. The cellular vesicles containing sAA, and other salivary proteins, are released upon sympathetic activation and the amount of amylase that is secreted per unit of time is directly related to the extent of sympathetic activity (Proctor & Carpenter, 2007). However, to equate changes in salivary sAA concentration or enzymatic activity with changes in SNS activity remains contentious because the parasympathetic glandular nerves potently affect sAA secretion. First, changes in salivary sAA concentration/activity reflect the combined effect of changes in salivary fluid secretion which are considered to be largely regulated by parasympathetic nerves, and changes in protein secretion which are largely sympathetic. Changes in actual sAA output, which take into account changes in flow rate, yield a more meaningful measure of changes in secretion. Second, amylase-rich glands such as the palatine glands are almost exclusively innervated by parasympathetic nerves and protein release from these glands would be without sympathetic involvement (Proctor et al., 2007; Veerman et al., 1996). The third and potentially most important factor is that the sympathetic and parasympathetic nerves interactively modulate sAA secretion. For example, SNS activation in the presence of parasympathetic co-activation synergistically enhances sAA secretion (Asking, 1985). This autonomic synergism is termed 'augmented secretion'. Taken together, the interpretation of enzymatic sAA activity as a 'pure' marker of sympathetic activation remains uncertain (Bosch et al., 2011).

The current study examined the specific role of sympathetic and parasympathetic activation in sAA secretion during exposure to a manipulation that elicits a clear pattern of autonomic activation (exercise). We used passive drooling to collect saliva which avoids many of the problems identified with the more often employed Salivette method (Bosch et al., 2011; Nater et al., 2009). Furthermore, in light of the preceding discussion, a number of novel methodological approaches were implemented. First, in addition to determining the association of sAA reactivity with established measures of cardiac parasympathetic and sympathetic reactivity (respiratory sinus arrhythmia and preejection period, respectively) we also determined possible effects of their co-activation, quantified by cardiac autonomic regulation (CAR) (Berntson et al., 1994a; Berntson et al., 2008). Participants with large exercise-induced sympathetic activation paired to small parasympathetic inactivation, i.e. a state of relative high co-activation, were predicted to yield high sAA secretion, whereas participants with small exercise-induced sympathetic activation paired to a relatively strong parasympathetic inactivation, i.e. a state of relative low co-activation, were predicted to yield low sAA secretion. Second, since the assumption of increased enzymatic sAA activity as a proxy measure for increases in the amount of sAA protein has thus far remained untested, we sought to determine if analyses using sAA protein measured by ELISA would yield similar results as the standard enzymatic method. Third, we tested the effects on sAA output which adjusts for confounding effects of changes in salivary flow rate. Fourth, to determine the specificity of sAA secretion compared to other salivary protein secretion, we also tested the exercise effects on relative changes in sAA protein to total salivary protein.

Methods

Participants

In total 28 undergraduates (6 male, 23 female) with an average age of 27 years (SD=9) and an average body mass index (BMI) of 23 (SD=3) volunteered to participate in the laboratory study. Participants did not report any psychiatric diseases or cardiovascular problems and none were using prescribed medication. The Medical Ethical Committee of the VU University Medical Centre Amsterdam approved of the study protocol and all participants provided written consent prior to participation and received study credits or a €10 gift voucher for participating.

General Procedures

Participants were asked to refrain from smoking and alcohol- or caffeine-containing beverages the evening before the test day and in the morning before coming to the laboratory. The experimental sessions took place between 10 a.m. and 4 p.m. and lasted approximately 90 minutes. After the participants had given informed consent, a short standardized interview was taken to obtain demographic information, health behaviors (smoking, exercise, alcohol), medication use, and to verify that they had no current anxious or depressive complaints or were receiving medication or treatment for cardiovascular disease. Height and weight were measured using standard procedures. Electrodes and ECG and ICG leads for cardiac assessments were attached to the participants, after which they were seated on an ergometer bicycle in a dimly lighted, electrically-shielded, sound-attenuated room. The experimental session commenced with general task instructions, saliva sampling practice, and a brief period of rest in which optimal signal quality was ensured. A first saliva sample was obtained at the end of a 4 minute baseline while seated on the bicycle. Then 4 minutes

of biking ensued at a resistance of 100 Watt at 60 rotations per minute. A second saliva sample was obtained immediately after the exercise task.

Saliva sampling

Saliva was obtained by the spitting method (Navazesh, 1993), directly following baseline and exercise. At the start of each saliva collection participants were asked to void the mouth by swallowing and subsequently let saliva accumulate at the floor of the mouth, without oral facial movements, and to expectorate each 30 seconds for 2 minutes in pre-weighed vials. Saliva volume was determined by weighing the vials before and after the saliva collection, assuming the density of saliva to be 1.0 g/ml, (Chicharro, Lucia, Perez, Vaquero, & Urena, 1998). Saliva secretion rate was expressed in ml/min. The vials were kept on ice until the end of the experiment, after which they were vortexed for one minute and centrifuged at 10.000xg for 10 minutes to remove buccal celles and oral micro organisms. The clear supernatant was divided over several aliquots and stored at -20^{o} C until analysis.

sAA determination

sAA enyzymatic activity (U/ml) was determined using The EnzChek® Ultra Amylase Assay by Molecular Probes adapted for a 96-well format™. This enzymatic assay uses a fluochrome-labeled starch derivate, which, upon enzymatic break down by amylase, releases its fluorescent probe. The accompanying increase in fluorescence over time is a measure of the amylase activity. The assay was performed according the instructions of the manufacturer. In short, 50 µl of 1: 20,000 diluted saliva was added to the wells of a 96-wells microtiterplate. Then 50 µl of a 10-fold diluted substrate solution was added and ithe ncrease in fluorescence was monitored at 2 min intervals during 30 minutes in a Fluostar Galaxy microplate fluorimeter (BMFG Labtechnologies, Offenburg, Germany). As reference was used a standard amylase solution (Meridian Life Sciences Inc, Memphis USA) at excitation and emission wavelenghts of 505 and 512 nm, respectively. The intra assay CV was 6% and the between assay CV was 11%.

sAA protein concentration was determined by a direct ELISA. Diluted saliva (1:10,000) was added in duplicate to a 96-well micro-titer plate (Microlon, Greiner bio-one), and incubated overnight at 4^{o} C. After rinsing with phosphate-buffered saline containing 0.1% (v/v) Tween-20 (PBS-T), the plates were incubated at 37°C with a monclonal antibody directed against amylase (clone G-8, Santa Cruz), diluted 1:3,000 in PBS-T supplemented with 2% BSA (w/v), at 100 ul per well. After 2 hours the plates were rinsed 3 times with PBS-T and incubated for 1 hour with an HRP-labelled rabbit anti-mouse polyclonal antibody (P-260, DakoCytomation), in PBS-T supplemented with 1% BSA. After 3 times rinsing with PBS-T the enzymatic colour reaction was initiated using OPD and H2O2, and the plates were read at 450nm using an ELISA reader. The concentration of sAA protein is expressed as a percentage of the amount of sAA protein found in a sample of standard pooled saliva.

Concentration of salivary protein (mg/ml) was measured by the bicinchoninic acid method (Pierce® BCA Protein Assay (Rockford, UK), which provides an accurate determination of total protein concentration in saliva (Bosch et al., 1996). Bovine serum albumin (BSA) was used as a standard (2 mg/ml standard solution in 0.9% NaCl). Samples of the same person were assayed in one assay to eliminate interassay error of measurement. Within-analyses CV=4.5%, between analyses CV=7% (Bosch et al., 1996).

sAA output (u/min) was calculated by multiplying sAA activity (u/ml) with flow rate (ml/min). In the same way sAA protein output (protein in %pooled/min) was calculated. Specific output of amylase was calculated as sAA protein divided by total salivary protein

Cardiac autonomic reactivity

Changes in the preejection period (PEP) are regarded as the gold standard of cardiac SNS reactivity and can be obtained non-invasively by thoracic impedance cardiography (Berntson et al., 1994a; Mezzacappa et al., 1999; Richter et al., 2009; Schachinger et al., 2001; Sherwood et al., 1986). Likewise, respiratory sinus arrhythmia (RSA) is the preferred measure of cardiac parasympathetic reactivity (de Geus et al., 1995; Frazer et al., 2007). RSA was quantified using the peak-valley method (Grossman, 1983; Katona et al., 1975; van Lien et al., 2011).

The ECG and ICG signals in the laboratory study were continuously recorded at a sample rate of 1000HZ with use of the ECG100C and NICO1000C modules of the BioPac data-acquisition system (BioPac systems INC, SAAnta Barbara, CA). Cleaning of the skin with alcohol before electrode application ensured that electrode resistance was kept low. Cardiac signals were recorded using a 6 Ag/Cl electrodes (Ultratrace, Cosmed, USA). ICG and ECG signals were imported into the VU-AMS Data Management and Analysis software (downloadable from www.vu-ams.nl) to score the inter beat interval IBI (ms), PEP (ms) and RSA (ms). For each experimental condition the interbeat interval IBI was scored from the R-wave peaks in the ECG. The IBI time series was visually inspected and missed or incorrect R-peaks were interactively corrected while bad ECG signal fragments were removed before averaging to obtain an experimental condition mean for the IBI (van Lien et al., 2011).

PEP was calculated using the R-wave-locked ensemble averaged ICG signal, using all valid beats within the basline and exercise condition. PEP was defined as the interval from the onset of electrical activity in the ECG (Q-onset) to the B-point in the ICG signal, and automated scoring of both points was confirmed by visual inspection of all ensemble averages. RSA was obtained through the peak-valley method (Bosch et al., 1996; Grossman, 1983; Katona et al., 1975). Breathing cycles that showed irregularities like gasps, breath holding, coughing etc., were not considered valid and were removed from further processing, as were the shortest and longest breaths that deviated more than 3SD. When no respiratory phase-related acceleration or deceleration was found or when the shortest beat in inspiration was longer than the longest beat during expiration, the breath was assigned an RSA value of zero. Mean respiration rate (RR) and RSA were computed across all valid breaths to a single mean RSA for each of the experimental conditions.

Cardiac autonomic regulation

We calculated the CAR as a measure of co-activation/co-inhibition of the cardiac sympathetic and parasympathetic branches of the autonomic nervous system (Berntson et al., 2008). CAR reactivity was derived as the sum of the normalized values of RSA reactivity and PEP reactivity (formula: $\Delta CAR = Z\Delta RSA + (-Z\Delta PEP)$), with low values representing co-inhibition and high values co-activation of SNS and PNS.

Statistical Analyses

Pre-planned paired t-tests contrasted the ergometer baseline level to the bicycle ergometer test (100W at 60 cpm). Reactivity scores reflecting this contrast were created for IBI, PEP, RSA, and the sAA measures and Pearson correlations were calculated to explore the association between the reactivity of the various sAA measures amongst themselves and between cardiac autonomic and sAA reactivity. Significance level was liberally set to 0.05 for all correlations.

Results

sAA and cardiac autonomic reactivity to exercise

Table 1 shows the mean levels of salivary and cardiovascular parameters during baseline and the bicycle ergometer test as well as their reactivity scores to exercise. Exercise increased sympathetic activity (lower PEP, $t(27)=14.27$, $p<.001$) and decreased parasympathetic activity (lower RSA, $t(27)=7.8$, $p<.001$), which led to a substantial decrease in IBI ($t(27)=18.1$, $p<.001$). Exercise also increased enzymatic sAA activity ($t(27)=-2.58$, $p=.016$), total salivary protein concentration($t(27)=-6.29$, $p<.001$), and flow rate ($t(27)=-3.28$, $p=.003$) but increases in sAA protein concentration did not reach significance ($t(26)=-1.89$, $p = .07$). When flow rate was used to create sAA output measures, the effects of exercise remained significant for sAA secretion based on enzymatic activity ($t(27)= -2.254$, $p= .033$) whereas sAA secretion based on the increase in protein concentration relative to the pooled standard again showed a trend only ($t(26)=-1.84$, $p = .077$). The specific amylase protein concentration (sAA divided by total salivary protein) did not reach significance reflecting the parallel increase in total salivary protein induced by exercise.

Table 1. *Means, standard deviations of IBI, RSA, PEP, flow rate, total salivary protein, sAA activity , sAA protein, total salivary protein output, sAA output based on activity, sAA protein output, and sAA protein/total salivary protein ratio during the bicycle ergometer test.*

	Bicycle Ergometer Baseline	**Bicycle Ergometer Exercise**	**Bicycle Ergometer Reactivity**
IBI (msec)	866 (132)	537 (92)	*-339
RSA (msec)	66 (25)	18 (19)	*-47
PEP (msec)	122 (17)	72 (22)	*-50
Flow rate (ml/min)	.41 (.25)	.46 (.27)	*.05
Total salivary protein (mg/ml)	1.39 (4.34)	1.72 (4.83)	*3.33
sAA activity (U/ml)	97.7 (61.8)	113.8 (74.3)	*16.1
sAA protein (% of pooled sample)	67 (58)	88 (70)	21
Total salivary protein output (mg/min)	.58 (.39)	.77 (.45)	*.18
sAA output based on activity (U/min)	45.6 (44.5)	52.8 (47.0)	*7.2
sAA protein output (% of pooled sample/min)	33 (39)	40 (36)	7
sAA protein / total salivary protein ratio	46 (34)	47 (30)	1

**= significantly different compared to the baseline ($p < 0.05$).*

Enzymatic sAA activity compared to sAA output

Table 2 shows the full pattern of correlations of changes in enzymatic sAA activity, the most often used proxy of sAA secretion, to the exercise induced changes in all other sAA measures assessed. Reactivity of enzymatic sAA activity and sAA protein concentration were significantly but modestly correlated (r=0.60). Exercise induced changes in flow rate were not significantly related to changes in enzymatic sAA activity which caused the correlation between enzymatic sAA activity and sAA output measures to be modest only. Finally, changes in sAA enzymatic activity were significantly correlated to changes in the ratio of sAA protein to total salivary protein.

Table 2. *Spearman rank correlations between the sAA activity reactivity and the other sAA measures to the exercise test.*

	Δ sAA activity
Δ sAA protein	*.60
Δ Flow rate	-.32
Δ sAA output based on activity	*.68
Δ sAA protein output	*.59
Δ Total salivary protein output	*.44
Δ sAA protein / Δ total salivary protein ratio	*.51

** =significant at p<.05.*

The association between autonomic and sAA reactivity to the exercise test.

Although the exercise stressor generally decreased both PEP and RSA, there were substantial individual differences in the patterning of ΔPEP and ΔRSA reactivity (illustrated in figure 1). Table 3 shows the correlation between these cardiac ANS reactivity scores and the concurrent changes in the salivary measures. Changes in enzymatic sAA activity correlated positively with changes in RSA, such that smaller increases in enzymatic sAA activity were associated with larger decrements in RSA ($r_{RSA\text{-}sAA}$=.36, p=.032), suggesting that parasympathetic withdrawal attenuates the exercise-induced increase in enzymatic sAA activity. Changes in enzymatic sAA activity were not correlated with PEP reactivity. When flow rate was taken into account, neither the sAA output measure based on enzymatic activity nor the reactivity of the sAA output measure based on the ELISA were significantly correlated to either PEP or RSA reactivity. Changes in flow rate, however, were very sensitive to changes in SNS activation ($R_{PEP\text{-}flowrate}$ = .46, p=0.01) with participants showing the largest increase in SNS activation having the largest increase in flow rate.

The CAR parameter combines the effects of SNS and PNS activation and, based on the concept of augmented secretion, participants with high CAR values that show an above-average increase in SNS activity with a below-average decrease in PNS should be expected to have the highest sAA output. We found, however, no significant correlation between sAA output measures and CAR. The three parameters which did show a significant correlation with CAR were enzymatic sAA activity ($r_{CAR\text{-}sAA}$= .32, p=.049), sAA protein ($r_{CAR\text{-}sAA}$= .36, p=.032) and the specific amylase concentration (sAA protein concentration/ total salivary protein concentration, $r_{CAR\text{-}ratio}$ = .36, p=.032).

Table 3. *Correlations of IBI, PEP, RSA and CAR reactivity with parallel reactivity of sAA activity, sAA protein, flow rate, sAA secretion based on activity and protein, and sAA protein / total salivary protein ratio.*

	Δ sAA activity	Δ sAA protein	Δ Flow rate	Δ sAA output (based on activity)	Δ sAA output (based on protein)	Δ sAA protein / Δtotal salivary protein ratio
Δ IBI	-.11	-.08	-.06	-.05	-.10	-.10
Δ PEP	-.02	-.19	*.46	.28	-.02	-.13
Δ RSA	*.36	.24	.01	.18	.20	.30
Δ CAR	*.32	*.36	*-.38	-.08	.18	*.36

** =significant at p<.05.*

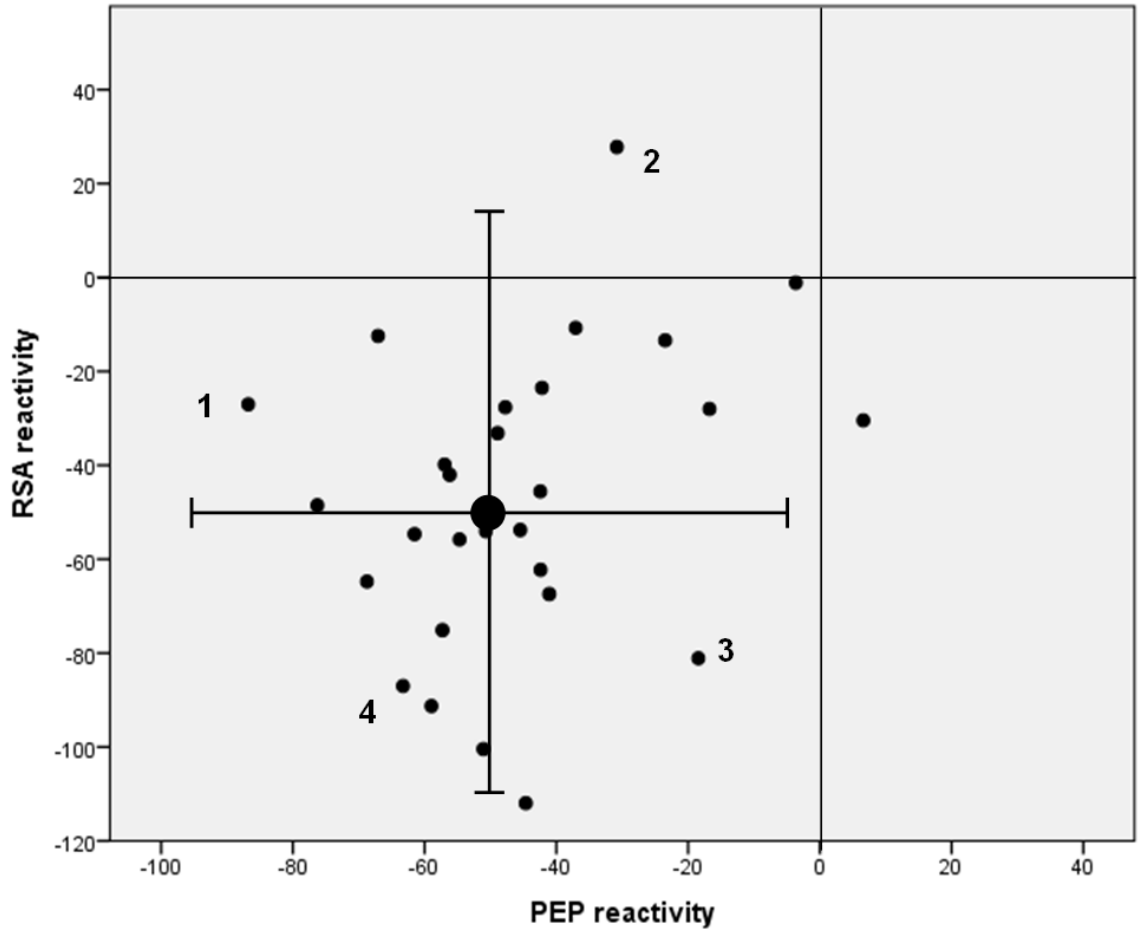

Figure 1. *Scatter plot of RSA and PEP reactivity to the bicycle ergometer test. Circles are the bivariate reactivity scores for the 28 participants. The large filled circle is the group average with 2 SD bars for both RSA and PEP. Four participants are highlighted for illustrative purposes. Participant 1 has above average cardiac sympathetic reactivity but below average parasympathetic reactivity. Participant 2 has below average cardiac sympathetic reactivity and below average parasympathetic reactivity. Participant 3 has below average cardiac sympathetic reactivity but above average parasympathetic reactivity. Participant 4 has above average cardiac sympathetic reactivity and above average parasympathetic reactivity.*

Discussion

The present study attempted to elucidate the role of sympathetic and parasympathetic activation, as well as their interaction, on the increase in enzymatic sAA activity induced by moderate intensity bicycle exercise. The results confirmed an effect of exercise on enzymatic sAA activity, and this effect remained intact when the increase in enzymatic sAA activity was multiplied by flow rate to obtain an indication of the actual increase in sAA secretion.

Exercise led to a significant increase in SNS activity, as evidenced by a decrease in the PEP, and a decrease in PNS activity, as evidenced by a decrease in RSA. Importantly, both cardiac autonomic and salivary responses to exercise showed substantial individual differences and these were used to probe the relative contribution of the autonomic branches to changes in sAA secretion. We found a significant association between the reactivity of enzymatic sAA activity and cardiac vagal reactivity such that a larger vagal withdrawal during exercise predicted a smaller exercise-induced increase in enzymatic sAA activity. In fact, visual inspection of the scatter plots showed that individuals with the largest vagal withdrawal during exercise even showed a net *decrease* in enzymatic sAA activity.

In contrast to our expectation, which we based on the previous use of sAA as an indicator of SNS activity (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Gordis, Granger, Susman, & Trickett, 2008; Vaughn, Bradley, Byrd-Craven, & Kennison, 2010), the association between increases in cardiac SNS activity and increases in enzymatic sAA activity was not significant. Only when the increase in SNS activity was combined with the decrease in PNS activity, using the CAR measure, enzymatic sAA activity changes showed a significant correlation in the expected direction such that the activation of SNS with a relatively small deactivation of the PNS yielded the largest increase in enzymatic sAA activity. The pattern of the data clearly suggests, however, that the PNS contribution to the CAR measure was the main driver of this effect.

Although enzymatic sAA activity is an easily obtainable proxy for sAA secretion, it is now well recognized that it may not accurately measure concentration and that, in addition, flow rate should be taken into account to improve its correlation to true sAA secretion (Bosch et al., 2011; Nater et al., 2009; Rohleder et al., 2009). For instance, Mandel et al. (Mandel et al., 2010) showed that enzymatic sAA activity does not perfectly capture sAA protein concentration at rest (r=0.61) and we replicate this finding (r =0.60) for the changes in enzymatic sAA activity and sAA protein concentration during exercise. The imperfect association between changes in sAA amount obtained by using an ELISA based method and the more often used enzymatic sAA activity may be explained by the fact that saliva contains multiple sAA iso-forms that differ in enzymatic activity and which may be affected differentially by exercise.

Flow rate was also confirmed as an important confounder of the relationship between enzymatic sAA activity and sAA secretion during exercise. At a fixed level of secretion an increase in salivary fluid production would decrease sAA activity per volume of saliva, underestimating actual secretion. As flow rate appeared to be very sensitive to the degree of SNS activation during exercise it will attenuate any relationship between enzymatic sAA activity amd and actual sAA secretion. This can be resolved by quantifying actual sAA output. In this study we did so by multiplying the changes in enzymatic sAA activity and sAA protein concentration by flow rate. The resulting output measures theoretically best capture the phenomenon of interest: the changes in SNS and PNS activity to the secretory cells in the salivary glands. Exercise induced changes in these output measures did not

show a correlation to cardiac SNS reactivity. Furthermore, in contrast to enzymatic sAA activity, our secretion measure no longer showed a correlation to cardiac PNS reactivity.

Because changes in flow rate would affect sAA protein and total protein concentration to the same degree, an alternative measure of sAA secretion is the ratio of sAA protein to total protein. This measure additionally takes into account the potential specificity of increases in sAA compared to other salivary proteins. When the increases in sAA protein were expressed as a ratio of the total increase in salivary protein, the pattern of correlations to RSA and CAR seen for enzymatic sAA activity was recaptured. In the presence of increased SNS activity, a relatively small deactivation of the PNS during exercise yields the largest increase in sAA to total protein ratio. The sAA to total salivary protein ratio could therefore be a useful index of SNS and PNS co-activation.

Our results are consistent with experimental studies using psychological stressors characterized as "passive coping" tasks (Bosch et al., 2003a; Sanchez-Navarro, Maldonado, Martinez-Selva, Enguix, & Ortiz, 2012). Such tasks are known to engage different brain circuits than active coping tasks (Keay & Bandler, 2001) and increase rather than decrease PNS activity (Obrist et al., 1974). In keeping with a role for PNS activity, these tasks tend to cause augmented sAA output. For example, Bosch et al. (2003) found that secretory responses during a passive coping task, eliciting little change in PEP but an increase in RSA (a surgical video), were markedly higher than during an active coping task that elicited a decrease in PEP and a decrease in RSA. Sanchez-Navarro et al. (Sanchez-Navarro et al., 2012) found a marked increase in sAA activity after viewing unpleasant pictures like human mutilations which most likely elicit passive coping with PNS activation, but no or only a modest sAA response after pictures of human attack or erotic pictures which are likely to give rise to the more reciprocal SNS activation/PNS deactivation seen in active coping.

Conclusion

Analogous to the regulation of heart rate, and consistent with glandular biology, SNS-mediated sAA secretion is co-determined by concurrent PNS effects on sAA secretion, which may show substantial individual differences that are only partly correlated to individual differences in SNS reactivity. This means that sAA secretion, like heart rate, cannot be used to index either SNS or PNS activity selectively. Importantly, the findings indicate that neither true sAA output nor its easily obtained proxy, enzymatic sAA activity, should be used as a selective indicator of SNS activation.