



Affective and inflammatory responses among orchestra musicians in performance situation [☆]



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ABSTRACT

A number of studies have shown that mental challenge under controlled experimental conditions is associated with elevations in inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP). However, relatively little work has been done on the effects of 'naturalistic' stressors on acute changes in inflammatory markers. The present study examined whether perceived arousal, valence and dominance in musicians are associated with pro-inflammatory and oxidative responses to a concert situation. Blood and salivary samples obtained from 48 members of a symphony orchestra on the day of rehearsal (i.e. control situation) and on the following day of premiere concert (i.e. test situation) were used to determine changes in salivary cortisol, pro-inflammatory markers (plasma myeloperoxidase, serum CRP, plasma IL-6), oxidative stress markers (paraoxonase1 activity and malondialdehyde), and homocysteine, a risk factor for vascular disease. Results of regression analyses showed a significant trend to increased myeloperoxidase (MPO) response in individuals with low valence score. Both affective states, valence and arousal, were identified as significant predictors of cortisol response during concert. In addition, control levels of plasma malondialdehyde were positively correlated with differences in IL-6 levels between premiere and rehearsal ($r = .38, p = .012$), pointing to higher oxidative stress in individuals with pronounced IL-6 response. Our results indicate that stress of public performance leads to increased concentrations of plasma MPO (20%), IL-6 (27%) and salivary cortisol (44%) in musicians. The decreasing effect of pleasantness on the MPO response was highly pronounced in non-smokers ($r = -.60, p < .001$), suggesting a significant role of emotional valence in stress-induced secretion of MPO. Additional studies are needed to assess the generalizability of these findings to other 'naturalistic' stress situations.

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1. Introduction

There is now good evidence that emotions can influence immune functions and immune-mediated disease (Kemeny and Shestyuk, 2008; Marsland et al., 2007). In addition to the extensive work on the relationship between depression and inflammation (Blume et al., 2011; Miller et al., 2009; Vaccarino et al., 2008; Irwin and Miller, 2007) there is a growing interest in understanding the

effects of acute psychological stress on inflammatory factors (Steptoe et al., 2007). Acute mental stress in healthy subjects was found to result in significant increases in interleukin-6 (IL-6) and interleukin-1 receptor antagonist whereas no effect on the level of TNF- α could be detected (Steptoe et al., 2001). There is also indication that people of low socioeconomic status have an impaired post-stress recovery in IL-6 (Brydon et al., 2004), and that acute stress-induced increases in IL-6 and fibrinogen are predictive of future ambulatory blood pressure (Brydon and Steptoe, 2005). In a study including 183 participants from a large university, greater fear in response to stress was found to be associated with higher levels of the proinflammatory cytokine IL-6 in oral mucosal transudate, whereas anger was not (Moons et al., 2010). Recently, angry and anxious responses to a simulated public speaking task

[☆] Please see Brief Commentary by J. Felger found on page 21 of this issue.

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have been reported to be positively correlated with changes in circulating levels of IL-6 (Carroll et al., 2011). These correlations were independent of age, race, body mass index, menopausal status for women and baseline levels of IL-6. Beside the effects on proinflammatory cytokine secretion, acute psychological stressors have also been found to induce short-term elevation of plasma homocysteine (Stoney, 1999).

While most studies to date have focused on affective responses to controlled experimental conditions and their relations to variations of the immune system and the level of cortisol, relatively little work has been done on the effects of real-life tasks on acute changes in inflammatory markers and cortisol. This may be due to the fact that 'naturalistic' stressors (such as academic examinations) are complex which makes comparisons with results from controlled experimental studies somewhat difficult. The present study, however, was undertaken to investigate proinflammatory responses under a 'naturalistic' setting that involves a substantial number of subjects at the same time and place. For this purpose, we examined whether stress of public performance is associated with increases in inflammatory markers in members of a symphony orchestra. Orchestra musicians have been described as a highly selected group with respect to work demands and stress exposure (Middlestadt and Fishbein, 1988; Halleland et al., 2009; Holst et al., 2012). To address questions of inflammatory alterations and affective states in these professionally trained subjects our approach included two different types of situation: (i) a final rehearsal with no audience present (i.e. control situation) and (ii) the following public concert. All study participants underwent blood sampling for determination of the enzyme myeloperoxidase (MPO), a well-known leukocyte-derived protagonist in inflammation (Nussbaum et al., 2013), along with the more commonly used markers C-reactive protein (CRP) and IL-6. Since inflammation and homocysteine have been suggested to be linked to oxidative processes (Ventura et al., 2009; Perna et al., 2003) we added two established parameters of oxidative stress, paraoxonase-1 activity (Aviram et al., 1998) and plasma malondialdehyde (Nielsen et al., 1997) to our study. A main objective of this work was to examine whether perceived arousal, valence and dominance in musicians might predict their potential inflammatory, oxidative and/or cortisol responses to a concert situation.

2. Methods

2.1. Subjects and sampling

Forty-eight members of the Radio Symphony Orchestra of Vienna (47 musicians and the conductor) participated in this study. Characteristics of participants are shown in Table 1. Seven musicians had current medication: two male participants used antidepressants, one male participant used statins, one male participant

Table 1
Characteristics of study participants.

	Mean (SD)	Range	n	%
Age (yr)	39.2 (11.2)	22–59		
BMI (kg/m ²)	23.1 (2.8)	16.4–30.5		
Work ability index	39.9 (5.7)	25–49		
Gender				
Male			26	54
Female			22	46
Smoking				
Yes			17	35
No			31	65
Exercise regularly				
Yes			27	56
No			21	44

used valaciclovir for treatment of herpes zoster, one male participant used levothyroxine for treatment of hypothyroidism, one female participant used an ACE-inhibitor and one female participant used tamoxifen.

Together with the conductor and the orchestra organizers, the presumably most stressful concert of the year (that was broadcast over radio and included the premiere of Klaus Lang's "Seventeen Steps", Sibelius' concert for violin and orchestra op. 47 and Scho-stakowitschs' symphony No. 9 op. 70) was determined as the test situation for our investigation. The final rehearsal (with no audience present) on the day before concert was chosen as the control situation, in order to ensure similar conditions (e.g., work load, technical demands, level of training, etc.) between the test and control day. Blood sampling was done at noontime immediately after the rehearsal and during the concert intermission at noon on the day of premiere. Blood serum, EDTA-anticoagulated plasma as well as EDTA-anticoagulated whole blood (for isolation of nucleic acids) were collected by venipuncture. Blood samples were stored immediately after donation in vertical position below 4 °C until centrifugation. Plasma and serum were obtained by centrifugation at 3500g at 4 °C. Plasma and serum samples were aliquoted and immediately stored at –80 °C. Salivette[®] cortisol tubes (Sarstedt AG & Co., Nuembrecht, Germany) have been used for the collection of saliva samples. Each participant has been instructed to collect saliva samples on the day of rehearsal and on the day of premiere at defined time points (T1: at awakening, T2: 30 min after awakening, T3: 11:30 am–12:30 pm, T4: 3:00 pm–4:00 pm, T5: 6:00 pm–7:00 pm).

2.2. Determination of standard laboratory parameters

Cortisol levels were assessed from saliva by means of electrochemiluminescence assays (ECLIA) on a Roche Modular E170 platform (F. Hoffmann-La Roche AG, Basel, Switzerland). The area under the curve of cortisol levels from T1 to T5 (AUC) was used as measure of total cortisol concentration (Pruessner et al., 2003). Plasma homocysteine (Hcy) and serum folic acid concentrations were measured using chemiluminescent microparticle immunoassays (CLIA) on an Abbott Architect (Abbott Laboratories, Abbott Park, USA). The response of each parameter was defined as the difference of values between the day of premiere and the day of rehearsal. Quantification of serum C-reactive protein (CRP) was conducted by turbidimetry on Olympus AU5400/AU5800[®] Chemistry Analyzers (Beckman Coulter, Brea, USA).

2.3. Measurement of MPO and IL-6 in plasma

Plasma concentrations of MPO were measured using commercially available Quantikine[®] Human MPO Immunoassay kits (R&D Systems, Inc., Minneapolis, USA). The analyses were carried out according to the standard protocols provided by the manufacturer using frozen (–80 °C) EDTA-plasma. A 7-point standard curve was prepared from a MPO standard solution provided with the kit. Absorbance at 450 nm was measured on a Wallac 1420 Victor2[™] Multilabel Counter (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). IL-6 levels were assessed again using frozen (–80 °C) EDTA-plasma by means of electrochemiluminescence on a cobas[®] e 411 analyzer (Roche Diagnostik GmbH, Mannheim, Germany). All analyses were done at the Department of Laboratory Medicine, Medical University of Vienna, which is running a certified (ISO 9001:2008) and accredited (ISO 15189) quality management system.

MPO and IL-6 concentrations were given as ng/ml and pg/ml, respectively. The responses of these parameters were defined as the differences of values between the day of premiere and the day of rehearsal.

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