Hemodynamic and Autonomic Adjustments to Real Life Stress Conditions in Humans

Daniela Lucini, Guido Norbiato, Mario Clerici, Massimo Pagani

Abstract—Psychological stress represents a risk factor for hypertension, but mechanisms are not known in detail. In this investigation we tested the hypothesis that real-life stress conditions produce changes in autonomic cardiac and vascular regulation that might differ in magnitude. University students, a well-established model of mild real-life stress, were examined shortly before a university examination, and a second time 3 months afterward, during holiday. Autonomic cardiovascular regulation was assessed by a noninvasive approach, based on autoregressive analysis of RR interval variability (V) and of systolic arterial pressure (SAP) V. The overall level of stress in the two sessions was gauged from the elevated salivary cortisol (5.6±0.5 versus 2.4±0.2 ng/mL, P<0.05) and altered cytokine profile (P<0.05). During the stress day, the RR interval was reduced and arterial pressure increased significantly; simultaneously, the normalized low frequency component of RRV (a marker of sympathetic modulation of the sinoatrial node) was increased and the index α (a measure of baroreflex gain) reduced. Concomitantly, the autonomic response to the sympathetic excitation produced by standing was altered: cardiac response was impaired and vascular responsiveness increased. Markers of autonomic regulation of the sinoatrial node correlated significantly with cortisol levels, both at rest and also considering standing induced changes, suggesting a gradual range of effects. The data support the concept that mild real-life stress increases arterial pressure and impairs cardiovascular homeostasis. These changes, assessable with spectral analysis of cardiovascular variability, might contribute, in susceptible individuals, to the link between psychological stress and increased cardiovascular risk of hypertension. (Hypertension. 2002;39:184-188.)

Key Words: autonomic nervous system ■ baroreceptors ■ stress hormones ■ risk factors

The importance and nature of the complex, multifarious mechanisms linking psychological stress to arterial hypertension1,2 have been extensively explored in simulated conditions. In laboratory models, mental stress leads to a rise in arterial pressure and heart rate, by way of altered neural cardiovascular regulation, typically consisting of increased sympathetic activity3,4 and reduced baroreflex gain,5,6 coupled to a prolonged endothelial dysfunction.7,8 Sympathetic activation is noninvasively recognized by spectral analysis of RR variability,9,10 indicating a predominant low frequency (LF) component of RR variability, in conditions of acute laboratory stress.7 Data from laboratory experiments, however, are limited by their intrinsic artificiality and by the fact that some of the more efficacious models, such as public speaking and mental arithmetic, might involve changes in respiration that could obscure the interpretation of results, particularly when autonomic regulation is inferred solely by spectral analysis of RR variability.12 Conversely, the effects of real-life psychological stressors on cardiovascular regulations, although more elusive,13 might be more clinically relevant and occur in the absence of the bias produced by immediate changes in respiratory pattern. In this context the possibility offered by heart rate variability (HRV) analysis to study autonomic dysregulation also in essential hypertension3,14 might offer new clues to the link between psychosocial factors and cardiovascular conditions.13-18

The principal aim of the present investigation was, accordingly, to test the hypothesis that a common real-life stressor of moderate intensity, as represented by the preparation for a major university examination, might alter basal hemodynamic indices of resting autonomic cardiac and vascular regulations, as assessed by computer analysis of RR and systolic arterial pressure (SAP) variability,10,11 in the absence of the bias of changes in respiratory pattern induced by talking. We also assessed possible changes in autonomic cardiac and vascular responsiveness14 to the sympathetic excitation produced by active orthostatism.

Methods

We enrolled 30 healthy, nonsmokers, medical students (age 22±1 years) of either gender. They were studied twice: the first time (stress day) 30 to 60 minutes before an university examination and a second time (control day) 3 months afterward, in a period away from study activities.
TABLE 1. Descriptive Statistics of RR Interval and SAP Variabilities in Healthy Subjects During Stress and Control Days, Both at Rest and Standing

<table>
<thead>
<tr>
<th>Day of Recording</th>
<th>Condition</th>
<th>RR (ms)</th>
<th>VARRR (ms²)</th>
<th>LFRR (nu)</th>
<th>HFR (nu)</th>
<th>HFRR (nu)</th>
<th>LF/HF</th>
<th>Respiratory Frequency (Hz)</th>
<th>SAP (mm Hg)</th>
<th>DAP (mm Hg)</th>
<th>LF SAVP (mm Hg²)</th>
<th>α (ms/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control day</td>
<td>At rest</td>
<td>861±10</td>
<td>3764±68</td>
<td>1057±150</td>
<td>50.8±3.4</td>
<td>1113±320</td>
<td>38.6±3.0</td>
<td>2.6±0.8</td>
<td>0.28±0.02</td>
<td>104±2</td>
<td>74±1</td>
<td>28±1.0</td>
</tr>
<tr>
<td></td>
<td>Standing</td>
<td>673±13†</td>
<td>2271±224</td>
<td>1018±143</td>
<td>83.6±2.2</td>
<td>107±16†</td>
<td>13.5±2.5</td>
<td>12.7±1.9†</td>
<td>0.29±0.01</td>
<td>106±1</td>
<td>75±1</td>
<td>8.9±1.7†</td>
</tr>
<tr>
<td>Stress day</td>
<td>At rest</td>
<td>714±19*</td>
<td>3129±565</td>
<td>1250±235</td>
<td>64.1±3.4</td>
<td>539±236</td>
<td>24.3±3.1*</td>
<td>5.8±1.6*</td>
<td>0.28±0.01</td>
<td>114±2*</td>
<td>77±1*</td>
<td>4.4±0.9</td>
</tr>
<tr>
<td></td>
<td>Standing</td>
<td>575.13†</td>
<td>2768±349</td>
<td>1599±273</td>
<td>87.9±1.1†</td>
<td>137±32†</td>
<td>7.6±0.8*</td>
<td>17.9±3.0†</td>
<td>0.26±0.01</td>
<td>113±3*</td>
<td>79±2*</td>
<td>28.0±7.4†</td>
</tr>
</tbody>
</table>

Data are presented as mean± SE.

*P < 0.05 Stress day vs Control day.
†P < 0.05 standing vs at rest.

routines. In both instances, we assessed endocrine, immunological, psychological, and autonomic involvement. The protocol of the study was approved by our Institutional Review Board.

Assessment of Overall Stress Level

We used a battery of noninvasive tests. Endocrine involvement was assessed in all 30 subjects by measuring by radioimmunoassay the free salivary cortisol level that reflects the concentration of free hormone in plasma. Saliva samples (2 mL), from both the stress day and the control day, were obtained at approximately the same hour (10:30 AM ±1). Immunological involvement was estimated in a random subgroup of 14 subjects whose salivary sample was technically adequate by also assessing the salivary levels of selected cytokines (interleukin [IL]1, IL2, tumor necrosis factor [TNFα] with commercially available ELISA assays. Prolonged exposure to stressors is signaled by alterations in cytokine profile, such as increasing IL1 and TNFα, and decreasing IL2.

Psychological involvement was gauged by a battery of questionnaires providing self-rated scales that, in line with previous and more recent studies, focuses on appraisal, coping, and health. In brief, we assessed the following factors:

• the appraisal of stress, tiredness, or activation by a global scoring index (0–30)
• coping by a graphic questionnaire (scores 0–10), exploring in particular the dimension of control
• somatic complaints by a symptoms’ list that furnishes a global scoring index (scores 0–50).

Study of Autonomic Regulation

In both days, all subjects were studied in resting conditions (10 minutes) and also during active orthostatism (7 minutes).

Recorded Variables

Using standard Ag-AgCl electrodes and a piezoelectric respiratory belt, both the ECG (CMS) and the respiratory signal were monitored with a 2-way radiotelemetry system. The arterial pressure waveform was continuously estimated noninvasively with a plethysmographic device (Finapres, Ohmeda). Data were recorded on a computer for subsequent analysis.

Data Analysis

From the ECG-derived tachogram, an autoregressive approach provides, in both absolute (ie, ms²) and normalized units (ie, nu), spectral powers of low (LF) and high frequency (HF) components as previously described. Spectral analysis was also performed on the systolic arterial pressure and the respiratory signals using a similar procedure. This latter assessed the main respiratory frequency, which was always higher than 0.15 Hz. From the simultaneous analysis of arterial pressure and RR interval variabilities, a frequency domain index (α) can be derived, which is a measure of the overall gain of the arterial pressure-heart period relationship.

Statistics

Data are presented as mean±SEM. Statistical tests included 2-way ANOVA for repeated measures, followed by individual contrasts, and the paired t test, as appropriate (Sigmatostat, Jandel). An α level ≤0.05 was considered significant. Correlation analysis was used to estimate the statistical link between selected parameters treating data from the 2 experimental days as independent observations.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Hemodynamics

The RR interval was significantly smaller, whereas SAP was significantly more elevated during the stress day compared with the control day, during both rest and active orthostatism. This maneuver, in addition, reduced the RR interval, but not SAP, compared with the baseline on both days. On the stress day, however, the standing induced reduction in the RR interval was smaller than observed from a higher baseline (Table 1) on the control day.

Autonomic Control of the Circulation

Rest

The stress day was characterized by significantly higher values of low frequency RR (LF RR ) (in nu) and of LF/HF, whereas, in contrast, high frequency RR (HF RR ) was significantly smaller. Low frequency SAP (LF SAP ) was slightly, but not significantly, greater during the stress day (Table 1 and Figure 1). The index α, marker of the overall gain of the arterial pressure-heart period baroreflex, appeared significantly reduced on the stress day.

Active Orthostatism (Standing)

On the control day, this maneuver, as expected, induced clear increases in LF (nu) RR variability and in LF/HF, and decreases in HF (nu) RR variability. On the stress day, standing induced changes in markers of autonomic modulation of the SA node were, however, less apparent (Figure 2). LF SAP increased significantly during both the control and the stress day (Table 1). However, on the stress day, LF SAP increased approximately 6-fold from the same-day baseline value. This increase was significantly greater than at the control (Figure 2). The index α was reduced by standing both on the control day and the stress day and reached, in this latter instance, minimal values (Table 1).
Respiration
Respiratory rate and pattern, as assessed with spectral analysis of respiratory movement, were not significantly modified in the group, in either the standing or the stressful situation (Table 1).

Overall Stress Level
Salivary cortisol levels (sampled at 10:30 AM) appeared elevated (P<0.001) during the stress day compared with the baseline, showing, respectively, values of 5.59±0.55 and 2.45±0.16 ng/mL. Notably, a significant correlation was found between salivary cortisol and markers of autonomic modulation of the sinoatrial (SA) node (Table 2, Column A), both at rest and considering changes induced by standing. Conversely, no correlation was found between salivary cortisol and markers of autonomic vascular modulation.

During the stress day, IL1 and TNFα were significantly greater (P<0.001) compared with baseline (respectively, IL1 2.94±0.37 during the stress day and 2.08±0.35 at control; TNFα 10.82±1.17 and 5.12±0.52 pg/mL), whereas IL2 was significantly (P<0.001) smaller (respectively, 2.09±0.72 and 6.50±1.14 pg/mL), suggesting a marked change in cytokine profile. Significant differences in stress and symptoms scores were found between the stress day and the control day (16.0±0.7 versus 6.9±0.6 arbitrary units for stress scores and 29.6±1.6 versus 15.4±2.2 arbitrary units for symptoms scores, both P<0.001). Subjective stress scores (Table 2, Column B) were significantly correlated with the resting values of RR, and SAP, and with indices of vascular autonomic modulation, particularly considering standing-induced changes. Similar results were obtained with the symptoms scores (data not shown). Conversely, the scores related to the dimension of control were similar (P=0.64) on the stress and control days. Notably, the performance of all subjects at the subsequent examination was excellent (median score 29, range 24–30 on a scale of 0–30).

Discussion
The main finding of this study on healthy young subjects is that a mild real life stressor, such as that produced by the preparation for a routine university examination, is capable of significantly altering the hypothalamic-pituitary-adrenal (HPA) axis and the cytokine profile, raises arterial pressure and produces complex changes in major cardiovascular regulatory mechanisms, without significantly affecting the respiratory pattern.

Real Life Stress
The subjects of the present study were exposed for several months to a routine of long study and work hours in
preparation for the anticipated challenge of the examination. The presence, on the stress day, of elevated psychological involvement was confirmed by increased stress and symptoms scores in all subjects. Conversely, scores relating to feelings of control were similar on both the rest and the stress days. This finding appears justifiable by the excellent grades that all students received on the subsequent examination. Therefore, the feelings of uncontrollability and hopelessness that might acutely disrupt individual responses to stressors were unlikely to represent a significant bias in the present study. The increase in salivary cortisol clearly pointed to a major engagement of the HPA axis with possible excitatory neurotransmitters that might acutely disrupt individual responses to stressful events.

The presence, on the stress day, of elevated psychological stress and increased vascular sympathetic responsiveness is a consequence of the permissive action of an enhanced cortisol level on adrenergic responses. In this context, a stress-induced impairment of endothelial function might play an additional role. The extent to which prolonged psychological stress and increased vascular sympathetic responsiveness might have a role in clinical cardiology, possibly underpinning the link between life events and acute triggers, remains to be elucidated. Although at the moment we cannot provide definitive information on the central, peripheral, or humoral mechanisms of the observed changes in cardiovascular regulation, the finding of a significant correlation between cortisol levels and indices of autonomic modulation of the SA node, suggests that common mechanisms underlay gradual and individualized neural and humoral responses to stressors. At the periphery, autonomic adrenomedullary innervation could be implicated, whereas additional changes in central autonomic oscillation might also play a role.

**Hemodynamic and Autonomic Effects**

A marked elevation in systolic and diastolic arterial pressure and in heart rate was observed in all subjects on the stress day, corresponding to a rise in the double product of about 36%, which implies a greater oxygen demand at rest. Regarding RR variability, no changes were observed in resting RR variance, a simple time domain measure of HRV, which may, therefore, be ineffective in assessing the influence of this type of mild stressor on autonomic cardiac regulations.

However, a clear shift in the resting spectral profile was observed, being characterized by higher LF/HR (in nu) and LF/HF, accompanied by a diminished HF/HR (in nu). This finding appears to confirm that time and frequency domain measures of HRV have a different capacity to signal changes in underlying neural modulation and that spectral measures and normalized units might be more informative also in the case of the autonomic changes produced by mild real-life stressors. Although the indirect nature of spectral derived parameters calls for caution in inferring autonomic regulation, the simultaneous tachycardia and enhanced arterial pressure, together with a reduced baroreflex gain, as evidenced by the smaller index point to a major rearrangement of resting autonomic balance toward sympathetic predominance and vagal withdrawal on the stress day, suggesting a reset of major cardiac regulatory mechanisms to a different resting level. Data from the present study, in addition, seem to confirm that mental stress induces a marked increase in the LF component of RR variability, well evidenced by autoregressive spectral analysis, independently of significant respiratory changes. Moreover, the findings obtained in response to the short-term physical stress imposed by active orthostatism merit some comment, particularly in view of the different magnitude of changes observed in markers of vascular, as opposed to cardiac, autonomic responsiveness. In fact, on the stress day, active standing induced lesser tachycardia and smaller increases in LF/HR, whereas it induced a 6-fold rise in the power of LF/HF, suggesting greater vascular, as opposed to cardiac, responses to standing-induced sympathetic excitation. We can hypothesize that such an increased sympathetic vasomotor responsiveness is a consequence of the permissive action of an enhanced cortisol level on adrenergic responses. In this context, a stress-induced impairment of endothelial function might play an additional role. The extent to which prolonged psychological stress and increased vascular sympathetic responsiveness might have a role in clinical cardiology, possibly underpinning the link between life events and acute triggers, remains to be elucidated. Although at the moment we cannot provide definitive information on the central, peripheral, or humoral mechanisms of the observed changes in cardiovascular regulation, the finding of a significant correlation between cortisol levels and indices of autonomic modulation of the SA node, suggests that common mechanisms underlay gradual and individualized neural and humoral responses to stressors. At the periphery, autonomic adrenomedullary innervation could be implicated, whereas additional changes in central autonomic oscillation might also play a role.

**Study Limitations**

We did not measure directly sympathetic nerve activity, which requires invasive techniques, but only inferred rest autonomic balance and responses to an excitatory stimulus indirectly with spectral analysis of RR interval and SAP variability. We also examined baroreflex gain and sympathetic vasoconstrictor modulation, thus addressing simultaneously multiple autonomic functional mechanisms to better capture the information about cardiovascular regulation, which is likely to be spread across different variables. Salivary cortisol and cytokine levels might not perfectly correspond to plasma values but were obtained without needles, thus avoiding the attendant possible emotional bias.

**Conclusions**

In this longitudinal study, we have observed that, in healthy medical students, the preparation to undergo the expected challenge of a university examination, which represents a widely employed model of a moderate real-life stressor, raises resting arterial pressure and, in addition, induces important humoral changes and impairs autonomic regulation. Overall, the clear activation of the HPA axis and altered cytokine profile is accompanied by a shift toward sympathetic activation and vagal withdrawal at rest and by enhanced vasomotor and reduced cardiac sympathetic responses to standing. These alterations of humoral and autonomic homeostasis suggest a treatable mechanistic link between real-life stress and components of increased cardiovascular risk, such as increased arterial pressure and reduced baroreflex gain.

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