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Time course of cardiovascular responses induced by mental and orthostatic challenges

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Cardiovascular responses to single stressors diminish over time. Interaction of different stressors influencing hemodynamic variables, indicative of stress-induced reactivity and physiological responses are, however, poorly understood. We investigated time course of mental (using mental arithmetic, MA) and orthostatic (using head up tilt, HUT) challenges induced responses in 16 males. Three protocols were used: HUT, MA and MA + HUT, with sessions randomized and two weeks apart. Hemodynamic responses were compared for 30 s epochs of stress application ($stress_{T1}$, $stress_{T2}$…). Compared to baseline, HUT, HUT + MA and MA applications affected heart rate (HR) (+15.1 ± 8.0 bpm, +20.0 ± 9.2 bpm, +11.9 ± 7.2 bpm, all p's < .001, respectively) and stroke volume (SV) (−22.3 ± 8.1 ml, −22.0 ± 10.4 ml, −7.6 ± 8.7 ml, all p's < .001, respectively). HUT and MA + HUT induced HR increases were higher in $stress_{T2}$ compared to $stress_{T1}$ ($p < .05$) and reached maximum at $stress_{T2}$. HUT and MA + HUT further reduced SV in $stress_{T2}$ as compared to $stress_{T1}$ ($p < .001$); lowest SV was in $stress_{T2}$. Mean arterial pressure reached its minimum in $stress_{T1}$ during HUT and MA + HUT (−6.0 ± 8.5 mm Hg, $p < .001$, −4.4 ± 9.7 mm Hg, $p < .01$, respectively) but increased in MA (+4.3 ± 3.7 mm Hg, $p < .01$). Combination of MA + HUT resulted in different time courses of blood pressure responses as compared to HUT alone. We conclude that application of single or combined stress challenges lead to stressor- and time dependent-initial changes in cardiovascular responses. Our findings provide novel insights regarding the duration a stressor must be applied to elicit maximal cardiovascular responses.

1. Introduction

A common physical stress for the human body is standing, which leads to dizziness in a significant number of persons. When a healthy person stands, 10–15% (approximately 650–700 ml in a person weighing 70–80 kg) of blood is pooled in the legs. This leads to decreases in venous return (cardiac pre-load), cardiac filling pressure and output. With normal regulatory capability, arterial pressure remains unaltered or even can be slightly increased. Passive head up tilt (HUT) is regularly used to provide orthostatic challenge.

The neurovascular responses to mental stress (Lackner et al., 2009) include activation of the sympathetic system, increases in heart rate, cardiac output and blood pressure and leads to vasoconstriction in the splanchnic and renal regions but vasodilatation in skeletal muscles (Anderson et al., 1987; Jezova et al., 2004; Lurie and Benditt, 1996; Papousek et al., in press).

Cardiovascular responses to single stressors are known to diminish over time. Interaction of different stressors and their effects on hemodynamic variables, indicative of stress-induced reactivity and physiological responses, have, however, received less attention. Previously, a time dependent decrease in the magnitude of hemodynamic responses to mental (Kelsey et al., 2000; Sant'Anna et al., 2003; Sinyor et al., 1983) and orthostatic challenges (Sheriff et al., 2007; Toska and Walloe, 2002) has also been reported. However, the initial responses – as well as the exact duration required to elicit maximal responses – to mental challenge (MA), orthostatic challenge (HUT) and particularly combinations of both (MA + HUT) are poorly understood. As the mechanisms of cardiovascular regulation have been reported to be different in the two (orthostatic and mental) forms of stress, we hypothesized that maximal effects in the responses would differ between these stressors, when done singly or in combination.

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2. Methodology

2.1. Participants

We focused on healthy men whose age and physical characteristics were homogeneous because gender and age may affect orthostatic stress responses (Goswami et al., 2008). The study was carried out in healthy, non-obese, non-smoking, non-medicated men who were free from any somatic or mental condition. The study criteria were met by 19 participants of age 25 ± 3 years, weight 73 ± 7 kg, height 180 ± 5 cm, and a heart rate of 60 ± 6 bpm during supine rest (mean ± SD). Data from these participants have been used in the companion study of Goswami et al. (in press, submitted for peer review in Int J Psychophysiol CCD545R1). The present study focused on reactivity but the companion study investigated the recovery of cardiovascular responses.

Participants were familiarized with the test protocol and gave written informed consent to participate in the study. The study was approved by the Graz University Ethics Board and the study performed in accordance with the 1989 WMA Declaration of Helsinki.

2.2. Study design and administration

We used a symmetric, crossover design with an online randomizer allocating the participants to each protocol. The participants served as their own control. Furthermore, we asked participants to abstain from vigorous exercise, coffee or any stimulants for 2 days before the test sessions.

All the participants underwent the three protocols: a) HUT alone, b) MA in supine position and c) combined MA and HUT. The protocols were randomized, open and separated by two weeks. On a given day, any two participants were investigated (at 9–11AM; 11AM–1PM). Stress application was for 10 min in all the protocols.

The test was carried out in a semi-dark and quiet room, maintained at 24 °C and humidity at 55%, using an electronically controlled tilt table.

2.3. Orthostatic challenge (protocol HUT)

The orthostatic challenge was provided by a 10 min HUT protocol. A 30 min supine rest preceded each experiment. At minute zero, the tilt table was brought to 70° head up position and after 10 min the table was returned to supine position. During the test the participants were supported by an adjustable footrest and were instructed to avoid undue movements of the lower limbs and to breathe normally.

Since the aim of the experiment was to induce orthostatic stress without inducing syncope, the protocol was terminated if *any* of the following occurred (Goswami et al., 2009): a) presyncope defined in hemodynamic terms – blood pressure falling below systolic 80 mm Hg, or a rapid drop in pressure (systolic by ≥20 mm Hg/min, diastolic by ≥10 mm Hg/min), or a heart rate drop by ≥15 bpm; b) Lightheadedness, dizziness, visual disturbances, nausea, stomach awareness, clammy skin, excessive sweating, or skin pallor. All the participants went through all the protocols with no problems.

2.4. Mental challenge (protocol MA)

MA represents mental challenge induced by mental arithmetic. Participants were asked to add or subtract continuously the numbers 6 or 7, randomly, from a 2 or 3 digit number and to state the correct answer while lying supine. A new number combination was provided every 5 s on a computer screen fixed at level of the eye of the participant.

At the beginning of selection process, participants were informed about the three protocols; however, participants were not notified in advance which protocol they would encounter on a given test day. During their first visit, the participants were familiarized with the laboratory, personnel and equipment. They received standardized verbal instructions about the protocol, tasks, and computer administered mental arithmetic at the beginning of the first session. Participants were told that they should solve the tasks as accurately and as fast as possible and that the answers were recorded. A timer applied additional pressure. Halfway through the mental arithmetic, participants were asked to answer more correctly, irrespective of their correct answers. These procedures were designed to help reduce adaptation to the stress condition. No external feedback regarding performance during the mental arithmetic was provided during the study.

2.5. Combined orthostatic and mental challenge (protocol MA + HUT)

MA was started immediately upon assumption of the upright posture (HUT), and was ended when the participant returned to supine position.

2.6. Self reported measures

Emotional status was assessed on arrival at the laboratory using the State-Trait anxiety inventory (STAI) (Laux et al., 1981) and the General Depression scale (ADS) (Hautzinger and Bailer, 1993).

Performance (mistakes made) on the mental arithmetic task was assessed and ratings of perception of stress (PSS) were made, shortly before commencing the stresses and also retrospectively at the end of the stress application using a 5 point Likert scale (1: not stressful; 5: extremely stressful).

2.7. Recording physiological stress responses

The baseline data were collected for 30 min with the participants in supine position. During baseline the participants were requested to relax without falling asleep. After the stress period, physiological data were recorded for 45 min.

2.8. Hemodynamic monitoring

Continuous hemodynamic monitoring of blood pressure (sampling rate, sr = 100 Hz, BP_{range} = 50–250 mm Hg, ±5 mm Hg), heart rate (3–lead ECG, sr = 1 kHz, $f_{cut\text{-}off}$ = 0.08–150 Hz) and thoracic impedance (sr = 50 Hz, $Z_{0,range}$ = 10–75 Ω, $dZ/dt = \pm 10$ Ω/s) were carried out with the Task Force Monitor® (TFM; CNSystems, Graz, Austria).

For the variables related to impedance cardiography, beat to beat values computed by the TFM® were used. Thoracic impedance $Z_0(t)$ and impedance variation $dZ(t)/dt$ were used to calculate beat-to-beat stroke volume based on an improved Kubicek approach and cardiac output. Total peripheral resistance (TPR) was calculated as 80× mean arterial blood pressure/cardiac output (Gratze et al., 1998).

TFM® ECG/impedance electrodes were positioned at the neck and thoracic regions, the latter at the midclavicular line at the xiphoid process level (Fortin et al., 2006).

2.9. Sample size and data analysis

Using typical cardiovascular changes during orthostatic loading from previous studies (Gao et al., 2008; Grasser et al., 2009; Hinghofer-Szalkay et al., 2008), error probability (α) of 0.05 and power ($1-\beta$) of 0.80 we estimated the number of participants required to be 15.

All calculations were made with Matlab R2007 (The MathWorks Inc.) and SPSS version 16. Each protocol lasted 90 min. For statistical analysis the data were analyzed in 30 s frames. Data reported in this paper are from the period 30 s before stress application till the end of stress application (depicted as $baseline_{T0}$, $stress_{T1}$, $stress_{T2}$, …, $stress_{T20}$;

in total 21 frames of 30 s, representing 10.5 min of the protocol) at which we denote the period of 30 s before stress application as baseline.

Artifact handling was done semi-automatically by a visible check of every signal in combination with a Matlab-function which identifies artifacts by using the following criteria: 1) physiological limits and 2) maximal percentage of change in relationship to standard deviation of the signal, using the time series with equidistant time steps after resampling beat to beat values with 4 Hz (piecewise cubic spline interpolation). Single artifacts were replaced by linear interpolation. Due to the strict artifact handling – only 30 s frames with 1) single artifacts and 2) 90% valid data were accepted – 16 out of 19 participants were used for further analysis. In total, artifact corrections for the remaining 16 participants were done in 10.7% of the 30 s frames (total number of 30 s frames = 7056).

To evaluate the differences in initial responses induced by mental and orthostatic stressors, 6×3 analyses of variance (ANOVAs) were conducted, with phase (baseline, $stress_{T1}$, $stress_{T2}$, $stress_{T3}$, $stress_{T4}$, $stress_{T5}$) and protocol (HUT only, HUT + MA, or MA alone) as within-subjects factors, and the hemodynamic measures as the dependent variables, followed by post hoc tests (Tukey's Honestly Significant Difference, HSD). The multivariate approach to repeated measures analyses was used in case of violation of the sphericity assumption, which allows valid tests under nonsphericity conditions (Vasey and Thayer, 1987). For ANOVAs, estimates of effect size are reported using partial eta-squared (η_p^2), which gives the proportion of variance a factor or interaction explains of the overall (effect + error) variance.

Additional 5×3 analyses of variance (ANOVAs), with phase ($stress_{T1–T5}$, $stress_{T6–T10}$, $stress_{T11–15}$ and $stress_{T16–20}$) and protocol as within-subjects factors were conducted to see if the effect remained in the second, third and fourth quarters of the stress application.

For comparing emotional stress (ADS, STAI) between the protocols analyses of variance for repeated measurements were used. The non-parametric Friedman test was used to analyze perception of stress (PSS) between the baselines of the protocols. Similarly, the difference in PSS between during- and beginning of the protocols was compared. Wilcoxon signed-ranks test was used to analyze mistakes made.

3. Results

Data presented here are from 16 Caucasian male participants of age 25.5 ± 2.9 years, weight 73.9 ± 7.8 kg and height 179.4 ± 4.3 cm. Means and SD of hemodynamic variables as well as the statistics are shown in Table 1.

The analysis revealed a significant main effect of protocol on heart rate (HR), stroke volume (SV) cardiac output (CO) and systolic blood pressure (SBP) but not for mean arterial pressure (MAP), diastolic blood pressure (DBP) and total peripheral resistance (TPR).

The analysis revealed a significant main effect of phase for all hemodynamic measures (HR, SV, SBP, MAP, DBP and TPR).

The interaction phase × protocol was significant for all hemodynamic measures, too (see Table 1).

The performed post hoc tests indicated no differences in the baseline of all cardiovascular variables.

The post hoc tests for the main effect phase showed, that the HR increased significantly from baseline to $stress_{T1}$ in protocol HUT (15.1 ± 8.0 bpm, $p<.001$), for MA + HUT (20.0 ± 9.2 bpm, $p<.001$) and MA

Table 1
Hemodynamic variables (mean ± SD) of participants and results of the 6×3 ANOVA, corresponding to the grey shaded areas in the respective figures, across the three protocols.

	$baseline_{T0}$	$stress_{T1}$	$stress_{T2}$	$stress_{T3}$	$stress_{T4}$	$stress_{T5}$	*F* statistics	
Heart rate (bpm)								
HUT	62.4 ± 10.5	77.5 ± 11.5	77.5 ± 11.5	78.2 ± 10.9	79.8 ± 12.6	81.0 ± 11.8	*protocol* $F(2,30) = 12.5^{***}$	$\eta_p^2 = 0.454$
MA	65.0 ± 8.5	76.9 ± 12.5	75.9 ± 14.3	73.9 ± 12.9	73.8 ± 12.2	73.6 ± 12.1	*phase* $F(5,11) = 18.6^{***}$	$\eta_p^2 = 0.792$
MA + HUT	65.6 ± 7.1	86.6 ± 8.9	91.0 ± 11.7	89.5 ± 10.5	89.0 ± 9.5	90.4 ± 8.9	*interaction* $F(10,6) = 6.0^{*}$	$\eta_p^2 = 0.356$
Stroke volume (ml)								
HUT	105.9 ± 14.1	83.7 ± 11.5	72.7 ± 10.4	72.2 ± 11.1	71.1 ± 9.9	71.1 ± 9.5	*protocol* $F(2,14) = 22.7^{***}$	$\eta_p^2 = 0.711$
MA	102.5 ± 16.5	94.9 ± 16.5	97.2 ± 17.7	99.3 ± 17.8	99.4 ± 18.3	100.0 ± 17.4	*phase* $F(5,11) = 26.5^{***}$	$\eta_p^2 = 0.850$
MA + HUT	101.1 ± 14.6	79.1 ± 8.6	70.6 ± 7.3	69.4 ± 7.3	69.8 ± 7.7	69.2 ± 7.4	*interaction* $F(10,6) = 9.8^{**}$	$\eta_p^2 = 0.664$
Cardiac output (l/min)								
HUT	6.6 ± 1.4	6.4 ± 1.0	5.6 ± 0.9	5.6 ± 0.8	5.6 ± 0.9	5.7 ± 0.9	*protocol* $F(2,14) = 20.9^{***}$	$\eta_p^2 = 0.412$
MA	6.7 ± 1.5	7.2 ± 1.5	7.3 ± 1.7	7.3 ± 1.7	7.3 ± 1.7	7.3 ± 1.6	*phase* $F(5,11) = 6.3^{**}$	$\eta_p^2 = 0.212$
MA + HUT	6.6 ± 1.0	6.7 ± 0.7	6.4 ± 1.1	6.2 ± 1.0	6.2 ± 0.9	6.3 ± 0.9	*interaction* $F(10,6) = 9.9^{**}$	$\eta_p^2 = 0.408$
Systolic blood pressure (mm Hg)								
HUT	120.7 ± 11.5	112.1 ± 16.3	114.4 ± 17.3	115.7 ± 18.3	115.9 ± 16.6	116.7 ± 17.0	*protocol* $F(2,30) = 3.5^{*}$	$\eta_p^2 = 0.189$
MA	120.1 ± 12.9	123.5 ± 15.4	125.4 ± 15.5	127.3 ± 15.7	128.4 ± 14.8	128.2 ± 15.5	*phase* $F(5,11) = 6.4^{**}$	$\eta_p^2 = 0.381$
MA + HUT	123.8 ± 11.0	115.9 ± 16.4	121.6 ± 20.6	126.1 ± 20.2	128.5 ± 18.9	128.3 ± 18.9	*interaction* $F(10,6) = 4.6^{**}$	$\eta_p^2 = 0.256$
Mean arterial pressure (mm Hg)								
HUT	90.4 ± 12.9	84.4 ± 16.1	89.2 ± 16.8	91.1 ± 17.3	91.8 ± 16.6	92.6 ± 17.1	*protocol* $F(2,30) = 2.8$	
MA	90.1 ± 11.3	94.4 ± 13.6	95.2 ± 13.7	96.8 ± 13.9	98.2 ± 13.7	98.5 ± 14.0	*phase* $F(5,11) = 21.8^{***}$	$\eta_p^2 = 0.543$
MA + HUT	93.4 ± 9.3	89.0 ± 14.0	96.6 ± 17.3	100.7 ± 17.2	102.2 ± 15.6	102.4 ± 15.0	*interaction* $F(10,6) = 11.7^{**}$	$\eta_p^2 = 0.238$
Diastolic blood pressure (mm Hg)								
HUT	75.4 ± 12.4	70.9 ± 14.7	76.4 ± 15.6	78.6 ± 15.6	79.1 ± 15.8	80.3 ± 16.3	*protocol* $F(2,30) = 2.7$	
MA	75.8 ± 10.9	79.8 ± 13.0	80.3 ± 13.3	81.6 ± 13.8	83.1 ± 13.4	83.6 ± 13.4	*phase* $F(5,11) = 29.6^{***}$	$\eta_p^2 = 0.577$
MA + HUT	78.7 ± 8.6	75.9 ± 12.6	84.0 ± 16.3	88.1 ± 16.4	89.1 ± 14.5	89.4 ± 13.9	*interaction* $F(10,6) = 12.0^{**}$	$\eta_p^2 = 0.232$
*Total peripheral resistance (dyne*s/cm^5)*								
HUT	1118 ± 317	1061 ± 271	1281 ± 338	1304 ± 326	1320 ± 374	1307 ± 368	*protocol* $F(2,30) = 2.9$	
MA	1108 ± 323	1069 ± 303	1178 ± 349	1101 ± 350	1115 ± 347	1107 ± 318	*phase* $F(5,11) = 4.0^{*}$	$\eta_p^2 = 0.209$
MA + HUT	1135 ± 232	1129 ± 229	1039 ± 226	1074 ± 254	1077 ± 236	1097 ± 229	*interaction* $F(10,6) = 4.8^{*}$	$\eta_p^2 = 0.386$

MA: Mental arithmetic; HUT: Head up tilt; MA + HUT: combined protocol. $Baseline_{T0}$: last 30 s before stress application; $stress_{T1}$: first 30 s of stress; $stress_{T2}$: 30–60 s of stress; $stress_{T3}$: 60–90 s; $stress_{T4}$: 90–120 s; $stress_{T5}$: 120–150 s.

* $p<.05$.
** $p<.01$.
*** $p<.001$.

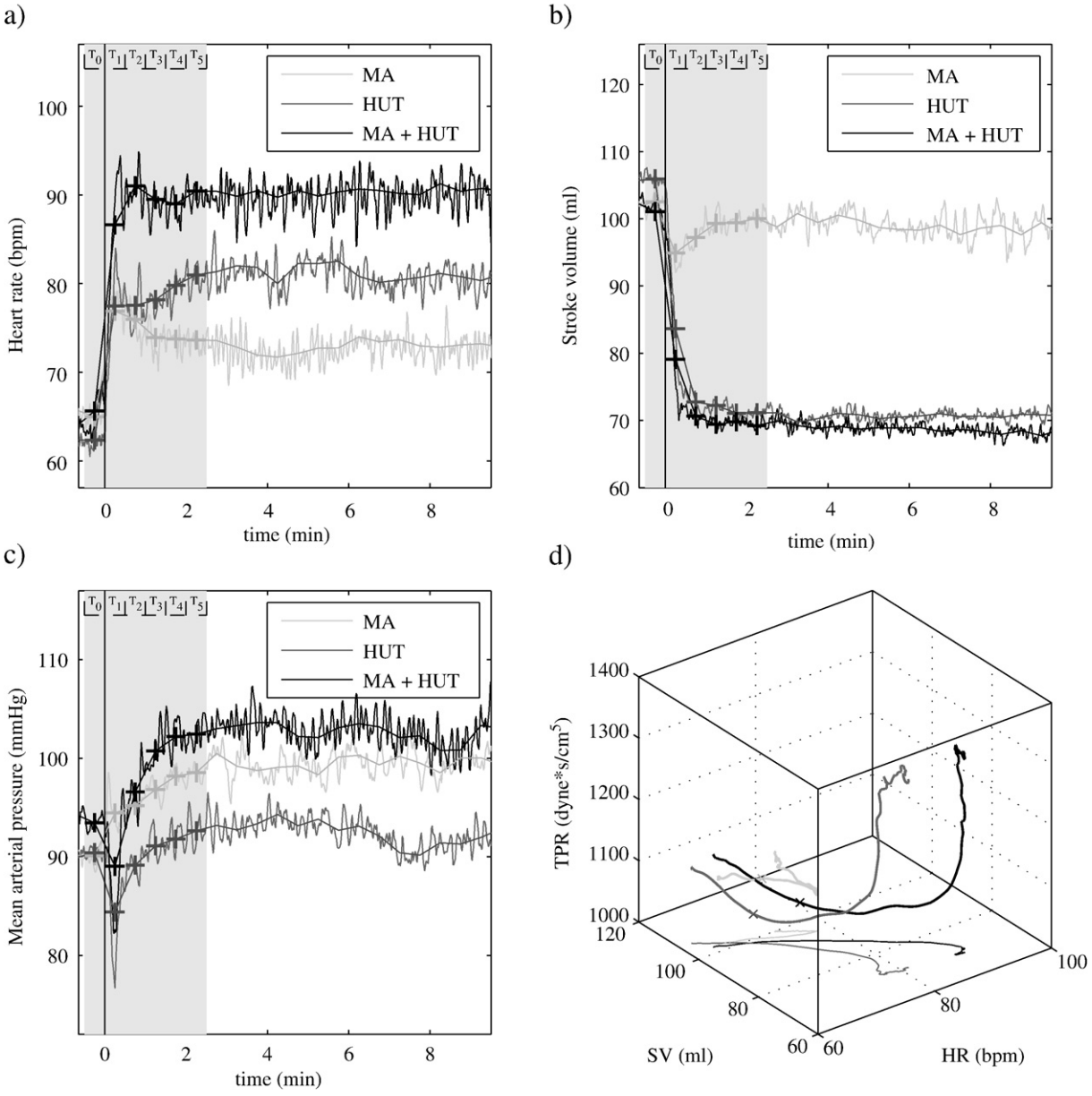

Fig. 1. Time course of a) heart rate (HR) b) stroke volume (SV) and c) mean arterial pressure (MAP) responses across mental challenge (MA), orthostatic challenge (HUT) and combined orthostatic and mental challenge (MA + HUT). T0: last 30 s before stress application ($baseline_{T0}$); T_1: first 30 s of stress ($stress_{T1}$); T_2: 30–60 s of stress ($stress_{T2}$); T_3: 60–90 s ($stress_{T3}$); T_4: 90–120 s ($stress_{T4}$); T_5: 120–150 s ($stress_{T5}$). Each + depicts the mean HR value over all the participants of each 30 s intervals. Thin lines connect these 30 s means. 1 d) shows cubical representation of the moving average of heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR) (behavior of these hemodynamic responses) from 30 s before stress application till 150 s of stress application. × depicts the beginning of stress application; commencements of experiment are shown on the left hand side. The shadowed area and hatch marks at the bottom of the figure panel represent stroke volume and heart rate, from which cardiac output can be calculated. When cardiac output is seen in relation to the other variable in the cube (total peripheral resistance) the mean arterial pressure can be obtained, according to the relationship: MAP ~ TPR × SV × HR.

(11.9 ± 7.2 bpm, $p<.001$) (see Fig. 1a). In protocol MA + HUT the HR was significantly higher in $stress_{T2}$ compared to $stress_{T1}$ ($p<.05$) and reached its maximum at $stress_{T2}$ with an increase of 25.4 ± 11.7 bpm compared to baseline. The post hoc tests for the interaction phase × protocol indicated, that the time response of HUT against MA + HUT as well as MA + HUT and MA are different but not for HUT against MA. The subsequently performed ANOVA for the periods $stress_{T1-5}$, $stress_{T6-10}$ and $stress_{T11-15}$ indicated no significant differences in the time course of the HR (interaction phase × protocol for HUT, MA + HUT and MA) of these periods.

The post hoc tests for the main effect phase showed that the SV decreased significantly from baseline to $stress_{T1}$ in protocol HUT (−22.3 ± 8.1 ml, $p<.001$), for MA + HUT (−22.0 ± 10.4 ml, $p<.001$) and MA (−7.6 ± 8.7 ml, $p<.001$) (Fig. 1b). The SV in protocol HUT and MA + HUT was significantly lower in $stress_{T2}$ compared to $stress_{T1}$ ($p<.001$) and reached its minimum at $stress_{T2}$ with a decrease of −33.2 ± 12.4 ml for HUT and −30.4 ± 14.8 ml for MA + HUT, respectively, compared to baseline. The post hoc tests for the interaction phase × protocol indicated, that the time response of HUT against MA as well as MA + HUT and MA are different but not for HUT against MA + HUT. The subsequently performed ANOVA for the periods $stress_{T1-5}$, $stress_{T6-10}$ and $stress_{T11-15}$ indicated significant differences in the time course of the SV for the period $stress_{T1-5}$ and $stress_{T6-T10}$ but not for $stress_{T11-15}$, (interaction phase × protocol for HUT, MA + HUT and MA) of these periods.

For CO the post hoc tests for the main effect phase indicated a significant increase from baseline to $stress_{T1}$ in protocol MA (0.6 ± 0.5 l/min, $p<.001$) and a delayed decrease from $stress_{T1}$ compared to $stress_{T2}$ ($p<.001$) of −1.0 ± 1.1 l/min obtained to baseline in HUT. The post hoc tests for the interaction phase × protocol indicated, that the time courses of HUT and MA are different against each other ($p<.001$).

The subsequently performed ANOVA for the periods $stress_{T6-10}$ and $stress_{T11-15}$, respectively indicated no significant differences in the time course of CO (interaction phase × protocol for HUT, MA + HUT and MA) of these periods.

The post hoc tests for the main effect phase showed, that the SBP decreased significantly from baseline to $stress_{T1}$ in protocol HUT (-8.7 ± 9.2 mm Hg, $p<.001$) and for MA + HUT (-7.9 ± 10.9 mm Hg, $p<.001$) and reached its minimum. In the protocol MA + HUT, systolic blood pressure returned to baseline within the next two periods ($stress_{T2}$ and $stress_{T3}$). The post hoc tests for the interaction phase × protocol indicated, that the time response of HUT against MA as well as MA + HUT and MA were different but not for HUT versus MA + HUT. The subsequently performed ANOVA for the periods $stress_{T6-10}$ and $stress_{T11-15}$, respectively indicated no significant differences in the time course of systolic blood pressure (interaction phase × protocol for HUT, MA + HUT and MA) of these periods.

The post hoc tests for the main effect phase indicated that in protocol HUT and MA + HUT, the MAP reached its minimum in $stress_{T1}$ (-6.0 ± 8.5 mm Hg, $p<.001$ and -4.4 ± 9.7 mm Hg, $p<.01$, respectively), whereas the MAP in MA increased compared to baseline (4.3 ± 3.7 mm Hg, $p<.01$) (Fig. 1c). In MA + HUT the mean arterial pressure increased from $stress_{T2}$ compared to $stress_{T1}$ ($p<.001$) and from $stress_{T3}$ to $stress_{T2}$ ($p<.01$), respectively. In HUT the MAP increased from $stress_{T2}$ compared to $stress_{T1}$ ($p<.01$). The post hoc tests for the interaction phase × protocol indicated, that the time response of HUT against MA as well as MA + HUT and MA are different but not for HUT against MA + HUT. The subsequently performed ANOVA for the periods $stress_{T6-10}$ and $stress_{T11-15}$, respectively indicated no significant differences in the time course of the MAP (interaction phase × protocol for HUT, MA + HUT and MA) of these periods.

The time course of diastolic blood pressure showed a decrease in HUT of -4.6 ± 8.6 mm Hg from baseline to $stress_{T1}$ ($p<.01$). Between $stress_{T1}$ to $stress_{T2}$, however, the DBP returned to baseline levels. In MA + HUT the DBP increased from $stress_{T1}$ to $stress_{T2}$ ($p<.01$) and from $stress_{T2}$ to $stress_{T3}$ ($p<.05$), whereas the decrease from baseline to $stress_{T1}$ was not significant. In MA the DBP increased from baseline to $stress_{T1}$ (4.0 ± 3.8 mm Hg, $p<.05$). The post hoc tests for the interaction phase × protocol indicated, that the time response of HUT against MA as well as MA + HUT and MA are different but not for HUT against MA + HUT. The subsequently performed ANOVA for the periods $stress_{T6-10}$ and $stress_{T11-15}$, respectively indicated no significant differences in the time course of the DBP (interaction phase x protocol for HUT, MA + HUT and MA) of these periods.

In HUT the TPR increased from $stress_{T1}$ to $stress_{T2}$ ($p<.001$, main effect phase), whereas the decrease from baseline to $stress_{T1}$ was not significant. In MA + HUT the TPR decreased by -85 ± 156 dyne*s/cm^5 from baseline to $stress_{T1}$ ($p<.01$). Following this the TPR increased significantly from $stress_{T2}$ compared to $stress_{T1}$ ($p<.001$) and from $stress_{T3}$ to $stress_{T2}$ ($p<.001$), respectively. In $stress_{T3}$ it increased by 176 ± 248 dyne*s/cm^5 compared to baseline. No differences were seen in protocol MA. The time courses were different between HUT and MA ($p<.001$, interaction phase × protocol) and between MA + HUT and MA ($p<.01$). The subsequently performed ANOVA for the periods $stress_{T6-10}$ and $stress_{T11-15}$, respectively indicated no significant differences in the time course of the TPR (interaction phase × protocol for HUT, MA + HUT and MA) during these periods.

Fig. 1d) depicts a cubical representation of the moving average of HR, SV and TPR from 30 s before stress application till 150 s of stress application. The × depicts the beginning of stress application; commencements of experiment are shown on the left hand side of the cube. The shadowed area and hatch marks at the bottom of the figure panel represent HR and SV, from which cardiac output (CO) can be calculated. The product of HR, SV (that is, the CO) when related to TPR provides mean arterial pressure changes, according to the relationship MAP ~ TPR × SV × HR.

Perception of stress (PSS) increased in response to mental, orthostatic challenges and the combination of mental and orthostatic stress (all p's<.01). Stress perception of the three protocols, however, was not different (PSS_{begin} $p>.20$, $PSS_{during-begin}$ $p=.09$). The self reported variables did not differ across the three stress conditions (STAI and ADS all p's>.30). Additionally, no difference mistakes made between HUT + MA and MA alone were seen ($p>.40$).

4. Discussion

Application of single or combined stress challenges led to stressor- and time dependent-initial changes in cardiovascular responses. Within the time course of 30 s before stress application till 150 s of stress application (that is $baseline_{T0}$ and $stress_{T1-T5}$), the heart rate, stroke volume, cardiac output, systolic, diastolic and mean arterial pressure as well as total peripheral resistance showed varying response patterns to the different stresses. In the period $stress_{T6-T10}$, only the stroke volume showed different time courses to the stressors. However, by $stress_{T11-T15}$ none of these showed significant differences in their responses. These findings suggest that the transient initial responses to the stressors are different but stabilize during sustained application of these stressors. Our findings provide novel insights regarding the time duration a stressor must be applied to elicit maximal cardiovascular responses, particularly as longer applications result in habituation and adaptation of responses.

The initial effects of mental challenge during orthostatic challenge (Table 1 and Fig. 1a) include greater increases in heart rate than with MA or HUT alone. As shown in Fig. 1d), mental challenge alone increased the heart rate with minimal decreases in stroke volume, thus resulting in increases in cardiac output. On the other hand, orthostatic stress decreased the stroke volume but the accompanying increases in heart rate were not able to sustain the cardiac output. Combination of both these stressors led to greater increases in heart rate, despite similar reductions in stroke volume such as those during orthostatic stress alone, which was able to maintain the cardiac output. Overall, in the first 2.5 min of stress application, the cardiac output (representative of global tissue perfusion in healthy populations) decreases were less with combinations of mental and orthostatic challenges in comparison to orthostasis alone. This would suggest that maximal cardiovascular effects of these stressors occur in the first 2.5 min of stress application.

When comparing effects of the three stressors, we observed no differences in the mean and diastolic blood pressures. This confirms previous observations that arterial blood pressure is the primary regulated variable during stress applications (Julius, 1988). However, stress applications resulted in different initial hemodynamic effects. This indicates that mean values of responses provide only a rough insight into the behavior of the cardiovascular system under stress and highlights the importance of time course analysis as a more reliable indicator to assess the changes imposed by the stressors.

Orthostatic and mental stresses induce different physiological responses (Kamiya et al., 2000). The effects of mental stress in combination with orthostatic stress are not surprising, as baroreflex function can be modulated by behavior/mental challenge at relay sites in the medulla, pons and hypothalamus (Stephenson, 1984). Indeed, the central-autonomic regulation mechanisms fundamentally differ during psychological vs. physical stressors (Lovallo, 2005; Sawchenko and Li, 2000). Accordingly, it has been reported that cardiopulmonary baroreceptor unloading due to central hypovolemia occurs with orthostatic stress while an increase in central command and arterial baroreceptor loading is noticed under mental stress (Sweene et al., 1995). It is plausible that during a combination of the two, the observed increases in heart rate are attributed to an increase in arterial baroreceptor unloading.

Contradictory responses of total peripheral resistance to mental stress have been reported (Jain et al., 1998). However, we observed no significant changes in total peripheral resistance in response to the

mental arithmetic. During orthostatic stress, total peripheral resistance showed an initial drastic reduction, followed by an over compensatory rise, which was then sustained throughout. The increased peripheral resistance contributes to the maintenance of blood pressure during standing (Goswami et al., 2009b). Combinations of both the stressors resulted in similar, but less drastic, time course in peripheral resistance responses as with orthostatic stress alone. This less gradual increase in peripheral resistance could be partially attributed to the mental component of the challenge, as the baselines were not different. This probably explains why the decrease in total peripheral resistance, from baseline to $stress_{T1}$ is significant for the chosen 30 s periods for combined stressors as compared to orthostatic challenge alone (see Limitations).

5. Limitations

Our observations of the initial hemodynamic effects are based on single applications of these stressors. It is possible that these effects might be different with repeated applications of these stressors. Preliminary results from our laboratory, however, suggest that repeated mental challenge applications result in increases in cardiovascular responses every time they are applied (Lackner et al., 2009). Finally, we could not discriminate the effects of mental arithmetic from the known effects of speaking on hemodynamic responses.

6. Conclusions and future directions

As has been pointed out by others (Lovallo, 2005), stress reactivity has become an important area of examination for the study of specific disease risks, and could help to determine exactly which response components are engaged in particular subgroups. We could demonstrate that stressor- and time dependent-initial changes in cardiovascular responses occur when orthostatic and mental stressors are applied singly or in combination. These results suggest that the peak cardiovascular effects of these stressors occur within the first few minutes (2.5 min). Our findings provide novel insights regarding the duration a stressor must be applied to elicit maximal cardiovascular responses, particularly as longer applications result in habituation and adaptation of responses. Furthermore, our findings raise the question which different stressors and combinations thereof could be useful as an additional tool to investigate which response components are engaged in those particular subgroups.

The results of this study have applications in the interpretation of responses induced by mental stressors as well as orthostatic challenge tasks that are commonly used in psychology, cardiology as well as epidemiological studies. Orthostatic stress directly challenges homeostatic regulation and employs hypothalamic or peripheral reactivity in the absence of psychological engagement, thus, comparisons between mental and orthostatic challenges may be particularly telling (Lovallo, 2005).

Having identified the different initial responses to varying stress stimuli, future studies should examine if there are specific hemodynamic response patterns (cardiac versus vascular), possibly reflecting differences in sympathoadrenal activation (Gramer and Berner, 2005; McCaffery et al., 2000), and whether these responses differ between mental challenge, orthostatic challenge, and combinations of both.

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