

M. MLYNARIK^a, A. MAKATSORI^a, I. DICKO^a,
H.G. HINGHOFER-SZALKAY^b, D. JEZOVA^{a,*}

POSTURAL CHANGES ASSOCIATED WITH PUBLIC SPEECH TESTS LEAD TO MILD AND SELECTIVE ACTIVATION OF STRESS HORMONE RELEASE

^aLaboratory of Pharmacological Neuroendocrinology, Institute of Experimental Endocrinology,
Slovak Academy of Sciences, Bratislava, Slovakia

^b Institute for Adaptive and Spaceflight Physiology, Graz, Austria

We tested whether simulation of postural changes, which occur during public speech test procedures, activates cardiovascular system and stress hormone release that could interfere with the effect of psychosocial stress load. Young healthy male volunteers (n=8) underwent procedure imitating exactly all postural changes present in the psychosocial stress model based on public speech used in this laboratory (namely changes from sitting to standing and repeated sitting). Postural changes were associated with increases in heart rate, blood pressure, plasma concentrations of noradrenaline and aldosterone and elevation in plasma renin activity. In contrast to cardiovascular parameters, adrenocorticotrophic hormone, cortisol and adrenaline, the main characteristics of hormonal response during mental stress, were not significantly influenced. The overall magnitude of all observed alterations was much smaller than that seen following mental stress procedures in our previous studies. This study provides evidence that changes in body posture during public speech test procedure influence hemodynamics and endocrine responses in a mild manner. Though this influence may represent a source of unspecific variance, substantial confounding effects on responses to the psychosocial component of the procedure are unlikely. In any case, models combining mental stressors and changes in body posture must be interpreted as complex stress stimuli.

Keywords: orthostasis, body posture, blood pressure, cortisol, hormones, mental stress, public speech test

INTRODUCTION

Social stress has frequently been considered a relevant risk factor for psychosomatic (1) and psychiatric disorders (2). Simulation of distressing

psychological stimuli in laboratory settings in humans is difficult. The large interindividual variability of responses is a prominent and well-documented phenomenon in psychoendocrine studies (3), and most models (e.g. mental arithmetic tests, Stroop test, videogame playing) induce only a partial response. Often, cardiovascular activation is achieved without any significant rise in hormone release (4-8).

Among the models of mental stress, the most intensive neuroendocrine activation has been observed in tests based on public speech, such as the Trier Social Stress Test (9). Public speech in front of an audience has been shown to induce significant increases in cortisol, adrenocorticotrophic hormone (ACTH), catecholamines and other stress hormones (9,10). In our investigations, a modified version (11) of the Trier Social Stress Test is being applied. The duration of anticipation and speech is longer and the subject is standing on a stool in front of an unknown, mixed gender jury and watching himself on a monitor under bright light. We have demonstrated that this model leads to increased cardiovascular and endocrine activation (11-13). The speech task is done standing in upright position following a period of sitting during which the subject has been preparing his performance.

It is generally known that changes in body posture may induce complex cardiovascular and hormonal activation (14-19). It is therefore possible that changes in body posture, which are present in psychosocial stress tests, can induce hormone release. Hennig and colleagues (20) reported that salivary cortisol levels were higher following 20 min of standing compared to those following 20 min of sitting or supine positions. These authors assume that change in body posture may result in unspecific variance in psychosocial stress tests and propose further studies investigating the question whether or not shorter than 20 min of time in standing position would increase cortisol concentrations. Besides, subjects undergoing public speech task procedure are changing posture of the body several times in a quite specific manner.

In the present work we investigated whether simulation of short-term postural changes, namely from sitting to standing position and subsequent standing, which occur during public speech tests, would induce activation of cardiovascular system, release of cortisol and other stress hormones and thus might interfere with the effect of the psychosocial component of the procedure.

SUBJECTS AND METHODS

Participants

Eight healthy male volunteers, aged 22-29, participated in this study. They all had normal body weight (body mass index 20-25), took no medication and avoided alcohol and excessive physical activity for at least 24 h before the test. They were free of serious somatic or mental diseases and claimed to have no family history of psychiatric disorders. Their control blood pressure, taken with

automatic monitoring in a sitting relaxed situation for ≥ 15 minutes, was $<140/90$ mm Hg. The subjects gave their written consent to participate. The study was performed in accordance to the Declaration of Helsinki. The Ethical Committee of the Slovak Academy of Sciences, Bratislava, Slovakia, approved the protocol.

Model of Body Posture Changes

This model was designed to imitate all postural changes present in the psychosocial stress model based on public speech used in our laboratory. In brief, following an initial resting period in sitting position and basal sampling, the subject is asked to walk to another room. There he is seated, introduced to the topic and left alone to prepare his upcoming speech (anticipation period). After 15 minutes, the subject moves to a public corridor where, standing on a small elevated podium, he makes a 15 minutes speech (speech period). The procedure has been described in detail elsewhere (11).

Subjects in this study underwent the same procedure as described above but without any topic given, speech to be delivered, or audience present. To minimize possible effects of stress from anticipation, all subjects were well informed about the whole procedure and become familiar with all experimental rooms and experimenters before the procedure was started. In contrast to the mental stress model, the speech period was carried out in the same quiet room as the anticipation period, to avoid any possible disturbance of the subject.

Experimental procedure

The investigations started early in the afternoon after a ≥ 2 hours rest without eating. In groups of 2 or 3 with the subjects in a seated position, indwelling catheters were inserted into an antecubital vein in a quiet waiting room, followed by a ≥ 30 minute sitting period, during which the subjects were allowed to talk and read. They were also allowed to drink water ad libitum throughout the study (from a cap placed close to them to avoid any change of body posture during drinking). The first blood sample for measurements of basal hormone levels was taken at least 30 min after catheter insertion (time 0). Blood pressure was recorded (Dinamap, Critikon, Tampa, FL, USA) during each blood sampling. Saliva was collected simultaneously with the blood collection. After initial blood and saliva sampling, each subject walked to an adjacent room, was seated at a table and left alone for 15 minutes. Subsequently, he was asked to stand at the small elevated podium (exactly the same as used in the mental stress procedure) and to stay upright for 15 minutes. Slight movements of trunk, hands or head were allowed. Compliance of the subject was guaranteed by experimenter supervision. Sampling was done at the end of the sitting period (time 15 min) and of the upright period while the subject was still standing on the podium (time 30 min). At the end of the procedure each subject returned to the waiting room and got seated; the final sampling was done 15 min after the third sampling (time 45 min). Each volunteer was assigned to two experimenters who were responsible to carry out all the procedures.

Hormone measurements

Blood was collected into two polyethylene tubes using heparin (for catecholamines) or EDTA (for adrenocorticotrophic hormone, cortisol, plasma renin activity, and aldosterone) as anticoagulants. After centrifugation at 4°C , aliquots of plasma were stored frozen at -20°C until analyzed. A radioenzymatic method (21) was used to analyze plasma catecholamines. Concentrations of adrenocorticotrophic hormone (ACTH) were measured in unextracted plasma by radioimmunoassay (RIA) described previously (22). Commercial kits (Immunotech, Prague, Czech Republic) were used for RIA of aldosterone and measurement of plasma renin activity. Saliva samples were collected in saliva/sputum collecting tubes (Salivette® No./REF 51.1534, Sarstedt,

Germany), which were capped and frozen at -20°C until analysis. Salivary and plasma cortisol concentrations were measured by RIA (7,23).

Data analysis

Statistical evaluation of the cardiovascular and hormonal results was performed using analysis of variance (ANOVA) for repeated measures with protocol time as within-subjects factor. Calculations were made using STATISTICA 6 (StatSoft, USA). The probability level was set to 95% as a limit to verify the null hypothesis. Calculations were made from absolute values. Increments above baselines were used in graphical interpretation of the results. Exact basal values are stated in the figure legends. All data are presented as means \pm SEM.

RESULTS

Mild but significant elevations were observed in most of the parameters measured. Repeated measures ANOVA of absolute values revealed significant time related changes for systolic ($F_{3,21}=4.5$, $p<0.05$) and diastolic ($F_{3,21}=3.9$, $p<0.05$) blood pressure, heart rate ($F_{3,21}=3.8$, $p<0.05$), plasma noradrenaline ($F_{3,18}=3.5$, $p<0.05$), plasma renin activity ($F_{3,18}=3.2$, $p<0.05$) and plasma aldosterone ($F_{3,21}=3.6$, $p<0.05$). Highest values were reached immediately after the end of the standing period (*Fig. 1*). No statistically significant changes were observed in case of plasma ACTH, plasma or salivary cortisol and plasma adrenaline (*Fig. 2*). The overall pattern of changes in absolute values and in increments was the same.

DISCUSSION

Postural changes that were applied to simulate conditions occurring in the course of public speech stress tests increased heart rate, blood pressure, plasma noradrenaline, aldosterone and plasma renin activity. Thus, changes in body posture, namely from sitting to standing position and subsequent standing, may interfere with cardiovascular and certain hormonal effects of the psychosocial component of public speech tasks.

In contrast to cardiovascular parameters, hormones of the hypothalamic-pituitary-adrenocortical axis (ACTH, cortisol) and adrenaline were not significantly influenced by postural changes. As these hormones represent the main characteristics of hormonal response during mental stress, this is an important finding for mental stress research.

These results extend the knowledge obtained by Hennig et al (20). In a carefully performed study, concentrations of salivary cortisol were found to be higher following 20 minutes standing than following 20 minutes of sitting or lying. Present study shows that in contrast to 20 minutes spent in controlled upright position (20), complex changes in body posture occurring during the

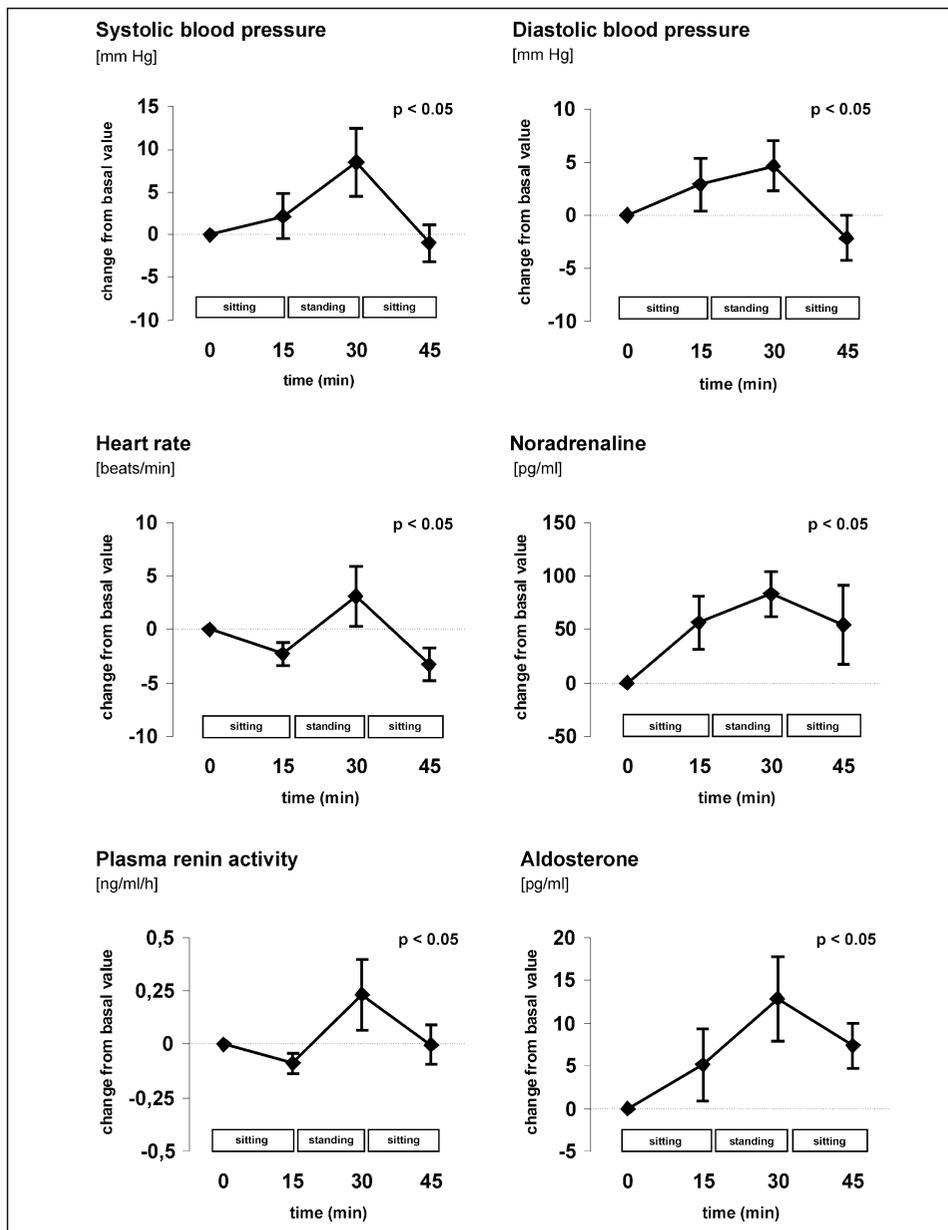


Fig. 1. Variables affected by postural changes in the model used

Data in the figure represent changes (increments) from baselines sampled at time 0 min after 30 minutes of quiet sitting. Absolute basal values were 121.1 ± 4.2 mmHg and 70.6 ± 2.5 mmHg for systolic/diastolic blood pressure, 66.4 ± 3.1 beats/min for heart rate, 227.9 ± 47.9 pg/ml for noradrenalin, 0.447 ± 0.080 ng/ml/h for plasma-renin activity and 53.0 ± 5.0 pg/ml for aldosterone. All values are shown as means \pm SEM. Expressed p-values represent statistical significance revealed by repeated measures ANOVA of absolute values with *time* as within-subjects factor.

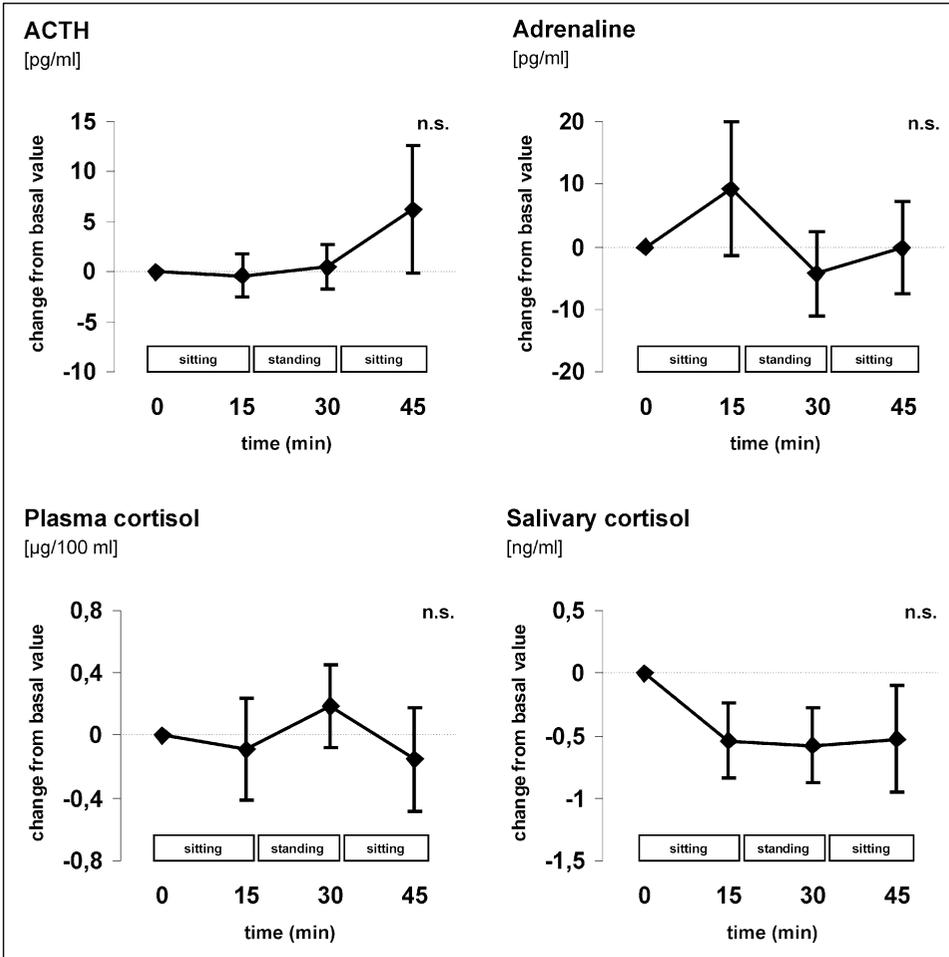


Fig. 2. Variables not modified by postural changes in the model used

Data in the figure represent changes (increments) from baselines sampled at time 0 min after 30 minutes of quiet sitting. Absolute basal values were 25.1 ± 4.3 pg/ml for ACTH, 47.0 ± 7.4 pg/ml for adrenaline and 4.5 ± 0.41 $\mu\text{g}/100\text{ ml}$ and 1.51 ± 0.62 ng/ml for plasma and salivary cortisol respectively. All values are showed as means \pm SEM. Statistical evaluation was performed from absolute values by repeated measures ANOVA with *time* as within-subjects factor.

course of public speech test do not influence neither salivary nor plasma cortisol concentrations. Next to details in experimental protocol, the main difference was that the standing in the present study lasted 15 min only.

Observed changes in cardiovascular function and related regulatory hormones are consistent with known effects of orthostatic stimuli, which occur in certain stress- and duration-dependent order (24-29). We have shown earlier that actively moving from a sitting to a quiet standing position decreases blood volume by an

average 6% within 20 minutes (17). This is very similar to the situation used in our investigations where subjects stood for 15 minutes after a sitting control period, which produces about 5% blood volume loss (17). Therefore, the activation of cardiovascular system and volume regulatory hormones observed in the present study is not surprising.

The magnitude of the rise in blood pressure and hormone release found in the present model of body posture changes was much smaller compared to that observed in our previous studies with public speech test (11-13). For instance, in our study investigating the influence of glutamate release-inhibiting drug lamotrigine on neuroendocrine response during psychosocial stress (11), blood pressure and heart rate responses in control placebo treated subjects culminated 20 to 40% above the basal value (increases of about 20-30 mmHg and 10 bpm). In the present study, increases were ranging from 4 to 7% (4-9 mmHg, 3 bpm). Similarly, peak levels of catecholamines, ACTH and cortisol during psychosocial stress were doubled compared to basal levels (120–190 % increases) in contrast to 20-40 % increases or insignificant changes (about 10 %) in the present investigation. Correspondingly, calculated p and F values in the present study, although showing statistical significance, are close to boundary levels ($p=0.05$), contrary to the highly significant effects of mental stress ($p<0.001$). We cannot conclude whether the changes in body posture do or do not contribute to the stress response during public speech, as it was not directly measured. Nevertheless, based on above mentioned data obtained in our previous studies (11-13) and the results of present investigations we assume that certain influences of postural changes on general outcome of public speech task are present but they are not substantial.

A limitation of this study is the absence of a simultaneous control group reflecting hormonal changes related to daily variations. In spite of this limitation and a relatively low sample size, we suppose that the observed changes are relevant and reliable.

It appears that within the frame of psychological investigations, the considerable influence of postural changes onto the cardiovascular and endocrine systems is often overlooked. For instance, in the original publication describing the Trier Social Stress Test, there is a brief mentioning of the subjects “standing” during the public speech but no specification of body posture before that test, e.g. during venous cannulation (9). It is fair to assume that this is done in a sitting or even supine position. If so, postural changes inevitably influence the data.

In conclusion, results of the present study demonstrate that specific changes in body posture during public speech test influence hemodynamics and certain endocrine responses in a mild manner. Though this influence may represent a source of unspecific variance, substantial confounding effects on responses to the psychosocial component of the procedure are unlikely. Despite of that, changes of body position should be taken into account and minimized (or carefully controlled) whenever psychological effects onto cardiovascular and endocrine

variables are being monitored. In any case, models combining mental stressors and changes in body posture must be interpreted as complex stress stimuli.

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REFERENCES

1. Goldstein DS. Catecholamines and stress. *Endocr Regul* 2003; 37: 69-80.
2. Claes SJ. Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. *Ann Med* 2004; 36: 50-61.
3. Gerra G, Zaimovic A, Mascetti GG, et al. Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. *Psychoneuroendocrinology* 2001; 26: 91-107.
4. Kok FW, Westenberg HG, Thijssen JH, van Ree JM. Endocrine and cardiovascular responses to a series of graded physical and psychological stress stimuli in healthy volunteers. *Eur Neuropsychopharmacol* 1995; 5: 515-522.
5. McCleery JM, Bhagwagar Z, Smith KA, Goodwin GM, Cowen PJ. Modelling a loss event: effect of imagined bereavement on the hypothalamic-pituitary-adrenal axis. *Psychol Med* 2000; 30: 219-223.
6. Skosnik PD, Chatterton RT Jr, Swisher T, Park S. Modulation of attentional inhibition by noradrenaline and cortisol after psychological stress. *Int J Psychophysiol* 2000; 36:59-68.
7. Jezova D, Duncko R, Lassanova M, Kriska M, Moncek F. Reduction of rise in blood pressure and cortisol release during stress by Ginkgo biloba extract (EGb 761) in healthy volunteers. *J Physiol Pharmacol* 2002; 53: 337-348.
8. Duncko R, Novakova L, Notova P, Stepankova O, Jezova D. Behavioral and neuroendocrine changes during mental stress and repeated treatment with antidepressants in healthy men. *Ann N Y Acad Sci* 2004; 1018: 524-532.
9. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993; 28: 76-81.
10. Clow A, Patel S, Najafi M, Evans PD, Hucklebridge F. The cortisol response to psychological challenge is preceded by a transient rise in endogenous inhibitor of monoamine oxidase. *Life Sci* 1997; 61: 567-575.
11. Makatsori A, Duncko R, Moncek F, Loder I, Katina S, Jezova D. Modulation of neuroendocrine response and non-verbal behavior during psychosocial stress in healthy volunteers by the glutamate release-inhibiting drug Lamotrigine. *Neuroendocrinology* 2004; 79: 34-42.
12. Jezova D, Makatsori A, Duncko R, Moncek F, Jakubek M. High trait anxiety in healthy subjects: association with low neuroendocrine responses during psychosocial stress. *Prog Neuropsychopharmacol Biol Psychiatry* 2004; 28:1331-1336.
13. Jezova D, Makatsori A, Smriga M, Morinaga Y, Duncko R. Subchronic treatment with amino acid mixture of L-lysine and L-arginine modifies neuroendocrine activation during psychosocial stress in subjects with high trait anxiety. *Nutr Neurosci* 2005; 8:155-160.
14. Gauer OH, Thron HL. Postural changes in the circulation; in Shepherd JT, Abboud FM (eds): Circulation III. *Handbook of Physiology*. Bethesda, The American Physiological Society 1965, pp 2409-2039.

15. Blomqvist CG, Stone HL. Cardiovascular adjustments to gravitational stress; in Shepherd JT, Abboud FM (eds.): *The Cardiovascular System. Handbook of Physiology, Bethesda, The American Physiological Society*, 1983, pp 1025-1063.
16. Bie P, Secher NH, Astrup A, Warberg J. Cardiovascular and endocrine responses to head-up tilt and vasopressin infusion in humans. *Am J Physiol* 1986; 251: R735-741.
17. Hinghofer-Szalkay H, Greenleaf J. Continuous monitoring of blood volume changes in humans. *J Appl Physiol* 1987; 63:1003-1007.
18. Matzen S, Secher NH, Knigge U, Bach FW, Warberg J. Pituitary-adrenal responses to head-up tilt in humans: effect of H1- and H2-receptor blockade. *Am J Physiol* 1992; 263:R156-R163.
19. Pump B, Talleruphuus U, Christensen NJ, Warberg J, Norsk P. Effects of supine, prone, and lateral positions on cardiovascular and renal variables in humans. *Am J Physiol* 2002; 283: R174-180.
20. Hennig J, Friebe J, Ryl I, Kramer B, Bottcher J, Netter P. Upright posture influences salivary cortisol. *Psychoneuroendocrinology* 2000; 25:69-83.
21. Peuler JD, Johnson GA. Simultaneous single isotope radioenzymatic assay of plasma noradrenaline, adrenaline and dopamine. *Life Sci* 1977; 21: 625-636.
22. Jezova D, Kvetnansky R, Kovacs K, Oprsalova Z, Vigas M, Makara GB. Insulin-induced hypoglycemia activates the release of adrenocorticotropin predominantly via central and propranolol insensitive mechanisms. *Endocrinology* 1987; 120: 409-415.
23. Jezova D, Vigas M. Apomorphine injection stimulates beta-endorphin, adrenocorticotropin, and cortisol release in healthy man. *Psychoneuroendocrinology* 1988; 13: 479-485.
24. Brown JJ, Davies DL, Lever AF, McPherson D, Robertson JIS. Plasma renin concentration in relation to changes in posture. *Clin Sci* 1966; 30: 279-284.
25. Hinghofer-Szalkay HG, Vigas M, Sauseng-Fellegger G, Konig EM, Jezova D. Head-up tilt and lower body suction: comparison of hormone responses in healthy men. *Physiol Res* 1996; 45: 369-378.
26. Koska J, Ksinantova L, Kvetnansky R, et al. Effect of head-down bed rest on the neuroendocrine response to orthostatic stress in physically fit men. *Physiol Res* 2003; 52: 333-339.
27. Molzahn M, Dissmann T, Halim S, Lohmann FW, Oelkers W. Orthostatic changes of haemodynamics, renal function, plasma catecholamines and plasma renin concentration in normal and hypertensive man. *Clin Sci* 1972; 42: 209-222.
28. Norsk P. Gravitational stress and volume regulation. *Clin Physiol* 1992; 12: 505-526.
29. Radikova Z, Penesova A, Koska J, Kvetnansky R, Jezova D, Huckova M, Vigas M, Macho L. Does orthostatic stress influence the neuroendocrine response to subsequent hypoglycemia in humans? *Ann N Y Acad Sci* 2004; 1018: 576-581.

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Author's address: Dr. Daniela Jezova, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlarska 3, Bratislava 83306, Slovakia. Telephone: 421-2-54773800, FAX: 421-2-54774247; e-mail: ueenjezo@savba.sk