Norepinephrine transporter inhibition alters the hemodynamic response to hypergravitation

Sebastian Strempel,1* Christoph Schroeder,1* Ruth Hemmersbach,2 Andrea Boese,2 Jens Tank,1 André Diedrich,3 Martina Heer,2 Friedrich C. Luft,1 and Jens Jordan1

1Franz-Volhard Clinical Research Center, Medical University Charité and Max-Delbrueck-Centrum and HELIOS Klinikum, Berlin; 2German Aerospace Center, Department of Aerospace Medicine, Cologne, Germany; and 3Department of Medicine, Division of Clinical Pharmacology, Autonomic Dysfunction Center, Vanderbilt University School of Medicine, Vanderbilt University, Nashville, Tennessee

Submitted 22 October 2007; accepted in final form 4 January 2008

Strempel S, Schroeder C, Hemmersbach R, Boese A, Tank J, Diedrich A, Heer M, Luft FC, Jordan J. Norepinephrine transporter inhibition alters the hemodynamic response to hypergravitation. J Appl Physiol 104: 756–760, 2008. First published January 10, 2008; doi:10.1152/japplphysiol.01128.2007.—Sympathetically mediated tachycardia and vasoconstriction maintain blood pressure during hypergravitational stress, thereby preventing gravitation-induced loss of consciousness. Norepinephrine transporter (NET) inhibition prevents neurally mediated (pre)syncope during gravitational stress imposed by head-up tilt testing. Thus it seems reasonable that NET inhibition could increase tolerance to hypergravitational stress. We performed a double-blind, randomized, placebo-controlled crossover study in 11 healthy men (26 ± 1 yr, body mass index 24 ± 1 kg/m²), who ingested the selective NET inhibitor reboxetine (4 mg) or matching placebo 25, 13, and 1 h before testing on separate days. We monitored heart rate, blood pressure, and thoracic impedance in three different body positions (supine, seated, standing) and during a graded centripetal run (incremental steps of 0.5 g for 3 min each, up to a maximal vertical acceleration load of 3 g). NET inhibition increased supine blood pressure and heart rate. With placebo, blood pressure increased in the seated position and was well maintained during standing. However, with NET inhibition, blood pressure increased in the seated position and was well maintained during standing.

norepinephrine reuptake transporter; cardiovascular regulation; autonomic nervous system; human

HYPERGRAVITATION INDUCED BY rapid flight maneuvers or centrifugation challenges the cardiovascular system through increased venous pooling and plasma extravasation into the dependent parts of the body. In addition, hypergravitation elicits considerable psychological stress and engages the vestibular system (1, 23). In this setting, the ability of the cardiovascular system to maintain cerebral perfusion pressure determines gravity (G) tolerance. Once the physiological challenge imposed by hypergravitation overwhels the adjustment mecha-

nisms, gravitation-induced loss of consciousness (G-LOC) ensues (19). Sympathetic nervous system activation, which is modulated by the vestibular system (23), is a crucial mechanism to maintain cerebral perfusion pressure (3, 24). Sympathetic cardiovascular responses are mediated by norepinephrine acting on adrenergic receptors (6). The synaptic norepinephrine concentration is regulated through norepinephrine release and norepinephrine re-uptake by the neuronal norepinephrine transporter (NET) (5). Recent studies suggest that NET function profoundly affects the cardiovascular response to the gravitational stimulus during standing. Impaired NET function due to a dysfunctional mutation of the NET gene causes hyperadrenergic orthostatic tachycardia (20). Similarly, orthostatic tachycardia is observed with pharmacological NET inhibition (2, 18). NET inhibition prevents neurally mediated presyncpe during head-up tilt testing, which is characterized by sympathetic withdrawal (17). In contrast, G-LOC occurs when the hydrostatic pressure gradient overwhelms sympathetically mediated adaptations in blood pressure control (19).

Given the importance of NET for blood pressure control at rest and during orthostatic stress on one hand, and the differences between orthostatic and hypergravitational stress on the other, we used selective pharmacological inhibition of NET to further elucidate the role of NET for hemodynamic adaptations to hypergravitational stress elicited by centrifugation.

METHODS

Subjects. We included 11 healthy men (26 ± 1 yr, body mass index 24 ± 1 kg/m²) in this study. A detailed history, medical exam, 12-lead ECG, blood pressure recordings, and routine venous blood sample ruled out preexisting diseases. Written, informed consent was obtained before inclusion. The study was approved by the local institutional review board and conducted in accordance with the Declaration of Helsinki and national regulations.

Study design and protocol. After completion of the screening tests, we conducted a human centrifuge test run to familiarize subjects with the procedure. The protocol of the centrifuge test run matched the actual centrifuge protocol, as indicated below. Afterward, subjects were studied twice in a double-blind, randomized, crossover fashion. Before both study days, subjects were admitted to the metabolic ward of the German Aerospace Center, Cologne. Subjects were given a balanced diet with constant fluid ingestion (50 ml water·kg body wt⁻¹·day⁻¹), sodium content (2.5 mmol·kg body wt⁻¹·day⁻¹), and...
caloric intake, according to their energy expenditure for 48 h before testing. Energy expenditure was calculated as the basal metabolic rate, according to the World Health Organization equation, plus 40% of basal metabolic rate for light physical activity in the ward, plus 10% for associated thermogenesis from food and beverages. The diet was devoid of substances that interfere with catecholamine turnover. Subjects ingested 4 mg of the selective NET inhibitor reboxetine (14) or matching placebo 25, 13, and 1 h before the physiological evaluation (12 mg total dose of reboxetine over 24 h). Orthostatic vital sign measurements and centrifuge runs were conducted after an overnight fast between 8:00 and 12:00 AM. The washout period between both treatments was at least 5 days.

Heart rate was monitored continuously by electrocardiography (Cardioscreen, Medis, Germany). Brachial blood pressure was measured at regular intervals with an automated oscillometric device (Dinamap, Critikon). Beat-to-beat blood pressure was continuously monitored by a finger servo-plethysmomanometer (2300 Finapres, Ohmeda) that was kept at heart level throughout the experiments. Impedance cardiography (Cardioscreen, Medis) was used to measure changes in stroke volume. An antecubital intravenous line was placed for repeated blood sampling. Special attention was paid to ensure identical positions of electrodes, pressure cuffs, and intravenous lines on both testing days.

For baseline recordings, subjects were supine in a quiet laboratory at an ambient temperature of 22–23°C. Subjects stood up for 3 min to record orthostatic blood pressure and heart rate responses. Then subjects were seated in a centrifuge facing forward in the direction of motion. The centrifuge was equipped with a fully closed swing-out cabin at 5-m arm length. Thus, G load was directed caudally [vertical acceleration (Gz)] at all times throughout centrifugation. A sufficient resting period was allowed to obtain stable baseline readings for all parameters before centrifuge runs. Centrifuge runs followed a preset protocol, during which Gz load was increased stepwise by 0.5 g every 3 min, up to a maximal Gz load of 3 g (Fig. 1). Acceleration between respective Gz load steps as well as deceleration at the end of centrifuge runs was 0.1 g/s.

Data acquisition and analysis. ECG, finger blood pressure, and thoracic impedance signals were analog-to-digital converted at 500 Hz using the Windaq pro+ software (Dataq Instruments). RR intervals (time between subsequent R waves in the electrocardiogram), blood pressure, and bioimpedance signals were defined off-line using a program written by Andre Diedrich (Vanderbilt University, Nashville, TN) based on PV-Wave software (Visual Numerics). Cardiac stroke volume was calculated according to Sramek’s formula (21). Cardiac output was calculated as stroke volume × heart rate. Systemic vascular resistance was calculated as mean finger blood pressure divided by cardiac output. We report relative changes in stroke volume, cardiac output, and systemic vascular resistance. Blood samples were drawn from intravenous lines after 20 min of supine rest and again immediately after centrifuge runs. Samples were stored on ice, centrifuged (10 min, 3,000 U/min, 4°C), and deep-frozen (= −80°C). Plasma concentrations of reboxetine and catecholamines were measured with high-performance liquid chromatography (4, 11).

Statistics. All data are expressed as means ± SE. We compared variances between the groups by using the F-test. Intraindividual and interindividual differences in parametric data were compared by paired t-tests. Nonparametric data were analyzed by Wilcoxon matched-pairs test. ANOVA testing for repeated measures was used for multiple comparisons. A value for P < 0.05 was considered significant.

RESULTS

Blood pressure, heart rate, and plasma reboxetine and catecholamine concentrations in the supine position with placebo and with NET inhibition are given in Table 1. NET inhibition substantially increased supine blood pressure and heart rate, as shown in Fig. 2. With placebo, systolic blood pressure during standing was maintained (0 ± 3 mmHg), whereas diastolic blood pressure increased slightly (5 ± 1 mmHg). With NET inhibition, blood pressure decreased 19 ± 4 mmHg/7 ± 3 mmHg with standing (P < 0.001 compared with placebo). With standing, heart rate increased 22 ± 3 beats/min with placebo and 58 ± 6 beats/min with NET inhibition (P < 0.001). Hemodynamic responses in the seated position recapitulated the response to standing, albeit to a lesser degree.

With placebo, all subjects completed centrifuge runs without symptoms. With NET inhibition, three centrifuge runs had to be aborted prematurely. In two subjects, heart rate exceeded the predefined abortion criterion of 160 beats/min. Both individuals were asymptomatic. In the third subject, the centrifuge run was aborted prematurely after 3 min at 2.5 g because of visual symptoms (tunnel vision, gray out) that indicated imminent syncope.

Figure 1 shows original tracings of finger blood pressure and heart rate during hypergravitation in a representative individual. With placebo, finger blood pressure increased with gravitational load in a graded fashion and reached 145 ± 5 mmHg/98 ± 3 mmHg at 3 g (P < 0.01/ <0.001 vs. baseline), as shown in Fig. 3. Heart rate increased 38 ± 5 beats/min during the centrifuge run (P < 0.001 vs. baseline). NET inhibition attenuated the pressor response to gravitational stress (P < 0.001 for systolic and P < 0.01 for diastolic blood...
pressure). In contrast, the hypergravitation-induced increase in heart rate was augmented with NET inhibition (+42 ± 5 beats/min at 3 g, \( P < 0.01 \) compared with placebo).

Figure 4 illustrates relative changes in cardiac stroke volume, cardiac output, and total peripheral resistance during hypergravitation. Cardiac stroke volume decreased in a graded fashion with increasing \( G_z \) load, both with placebo and with NET inhibition. Cardiac output and total peripheral resistance were better maintained on placebo than with NET inhibition.

Supine epinephrine and norepinephrine plasma concentrations were similar with placebo and with NET inhibition (Table 1). Hypergravitation increased both epinephrine (0.6 ± 0.1 pmol/ml) and norepinephrine plasma concentrations (2.7 ± 0.3 pmol/ml) on the placebo day (\( P < 0.001 \) vs. baseline for both). Equally, epinephrine and norepinephrine plasma concentrations increased with hypergravitation under NET inhibition (0.4 ± 0.2 and 3.1 ± 0.6 pmol/ml, respectively, not significant compared with placebo).

**DISCUSSION**

We investigated the influence of short-term pharmacological NET inhibition on cardiovascular regulation during graded hypergravitation in human subjects. NET inhibition profoundly changed the hemodynamic response to centrifugation. NET inhibition attenuated the pressor response to centrifugation. The failure to raise blood pressure resulted from blunted systemic vasoconstriction. In contrast, the tachycardic response to hypergravitation was substantially augmented with NET inhibition. This mismatch between cardiac and vascular responses to NET inhibition was observed earlier during orthostatic stimulation (2, 16, 18). Systemic norepinephrine concentrations did not change with NET inhibition, which further supports the idea of a differential sympathetic response between tissues.

Given its selectivity for NET, reboxetine has been proven a useful tool for physiological studies. We determined reboxetine plasma concentrations to confirm sufficient dosing in our subjects. A particular strength of our study is the double-blind, randomized, placebo-controlled, and crossover design. Furthermore, we conducted our study under carefully controlled conditions on a metabolic ward. Therefore, we are confident that our study provides valid data on human NET physiology.

With centrifugation, central blood volume, as indicated by thoracic impedance, did not change significantly with either placebo or NET inhibition. Similarly, NET inhibition did not affect thoracic impedance responses during head-up tilt testing (18). This observation suggests that differences in the response to gravitational stress between placebo and NET inhibition are explained by altered autonomic reflex adjustment rather than different hemodynamic stimulation.

Changes in the cardiovascular response to gravitational stress with NET inhibition are explained by complex actions of NET in the brain and in peripheral tissues. In animals, NET inhibition in the brain decreases sympathetic activity through \( \alpha_2 \)-adrenoceptor stimulation (9). In human subjects, systemic NET inhibition decreases muscle sympathetic nerve activity and resets the sympathetic baroreflex to higher blood pressure values (7, 12, 22). Central sympathetic inhibition is opposed by sympathetic stimulation through NET inhibition in peripheral tissues (7). Thus the net result of NET inhibition on regional hemodynamics differs between tissues. In the heart, NET inhibition increases sympathetic responses. In the vasculature and in the kidney, sympathetic responses are reduced with NET inhibition.
Predominant cardiac sympathetic stimulation with NET inhibition may be explained by ultrastructural or functional differences in cardiac adrenergic nerve terminals (6). We propose that redistribution of sympathetic activity to the heart explains excessive tachycardia with gravitational stress in subjects on NET inhibition.

Central sympathetic inhibition may explain why NET inhibition reduced systemic vasoconstriction during hypergravitational stress. Similarly, NET inhibition attenuated vasoconstriction during the Valsalva maneuver and during head-up tilt (18). Moreover, NET inhibition reduced isometric handgrip and cold pressor responses (2, 18). In previous studies, NET inhibition increased plasma norepinephrine in the upright position (17). In the present study, norepinephrine responded similarly to hypergravitational stress with NET inhibition and with placebo. We cannot exclude that autonomic function testing or transfer of subjects into the centrifuge influenced catecholamine measurements after the centrifuge run. Furthermore, the relatively small number of subjects might have influenced our findings. Finally, vestibular stimulation or psychological stress during centrifugation might have modulated sympathetic responses, thus explaining discrepancies in catecholamine measurements between the present and earlier studies.

The excessive heart rate response to centrifugation on NET inhibition could be mediated through baroreflex mechanisms. Indeed, blood pressure decreased during centrifugation. However, in earlier studies, NET inhibition decreased baroreflex sensitivity during orthostatic stress (18), which would tend to reduce tachycardia during centrifugation. Yet NET inhibition resets the baroreflex to higher blood pressure values (22). Thus, at a given blood pressure, heart rate is increased during NET inhibition. A decrease in baroreflex vasomotor control (22) may have contributed to the blunted blood pressure response during centrifugation.

Cardiac autonomic responses may limit G tolerance in different ways. A “hypoadrenergic” response may predispose to neurally mediated syncope with hypotension and relative bradycardia. A “hyperadrenergic” response may result in symptomatic tachycardia. It appears that too much NET function predisposes to hypoadrenergic responses. Pharmacological NET inhibition has been shown to prevent neurally mediated (pre)syncope during head-up tilt testing in a placebo-controlled and crossover fashion (17). Conversely, too little NET function predisposes to orthostatic tachycardia, as evidenced by patients with NET gene mutations (20) and in pharmacological NET inhibition (18). This notion is further supported by patients with chronic orthostatic tachycardia, in whom cardiac sympathetic overactivity can be found (8).

Our study has several potential limitations. Due to safety concerns, the maximal Gz load used in this study was too small...
to induce G-LOC in our subjects. Thus it remains unclear whether or not NET inhibition affects the tolerance to G-LOC. However, both the failure of blood pressure to rise and the tachycardia that occurred during hypergravitation are indications that NET inhibition rather reduces tolerance to hypergravitation. The observation contrasts with head-up tilt studies showing prevention of neurally mediated presyncope with NET inhibition (17). The discrepancy may be explained by fundamental pathophysiologic differences between neurally mediated syncope and G-LOC (19). Neurally mediated syncope is characterized by sudden sympathetic withdrawal, G-LOC occurs when hydrostatic pressure overrules sympathetically mediated hemodynamic adaptations. The dosage regimen of NET inhibition in this study differed from earlier studies (17) in regard to both single doses and application duration. Despite comparable reboxetine plasma concentrations, we, therefore, cannot exclude that adaptive changes to repeated NET inhibition might have influenced our findings. Also, our results may not exactly model the long-term changes in NET function through genetic variability in the NET gene (10). Noteworthy, the resting heart rate in our volunteers was slightly lower than in our earlier studies, possibly due to differences in sex distribution and physical fitness. Finally, we did not directly assess sympathetic tone in different vascular beds. Thus our conclusions in regard to distribution of sympathetic tone warrant further verification with more direct methods.

We conclude that variability in NET function profoundly alters the hemodynamic response to hypergravitational stress. The phenomenon is explained by combination of central nervous and peripheral mechanisms. Furthermore, it is likely that changes in sympathetic baroreflex responses contributed to hemodynamic effects of NET inhibition. The tachycardia limits the utility of conventional NET inhibitor doses in improving tolerance to hypergravitation. Possibly, individuals with excessive NET function could show a beneficial hemodynamic response to hypergravitational stress.

ACKNOWLEDGMENTS

Christoph Hiemke of the Department for Psychiatry of the University of Mainz, Germany, determined reboxetine plasma concentrations. Suzanna Loncé at the Catecholamine laboratory of the Vanderbilt University, TN, measured plasma catecholamine concentrations. Drs. Götz Kluge and Klaus Müller of the German Aerospace Center performed screening medical exams and provided medical safety during experiments, while technical support was granted by Hartmund Friedrich, Norbert Laks, and Gernot Plath. Furthermore, Natalie Bäcker, Melanie von der Wiesche, and Elfriede Huth of the German Aerospace Center all were involved in study implementation.

GRANTS

We thankfully appreciate funding by both the European Space Agency and the Germany Aerospace Center.

REFERENCES