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Tyrosine, a large neutral amino acid normally present in protein foods, is the precursor of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine. Animal studies indicate that systemic administration of tyrosine in pharmacologic quantities can reduce physiological and behavioral decrements induced by highly stressful conditions. The current study was designed to test the effects of tyrosine on humans exposed to cardiovascular stress. Twenty participants were exposed to two LBNP sessions (-50 mmHg for a maximum of 30 minutes) during each testing session of a repeated measures double-blind placebo-control study. Physiological (HR, BP, AER, EOG, & EMG) and Behavioral (Rt, Mood, & Vigilance) indices were monitored during testing. Comparisons between measures taken while ingesting tyrosine

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11. Performance in Stressful Environments: The Effects of Tyrosine Pre-Treatment on Lower Body Negative Pressure Stress.

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19. and placebo indicate that the effects of tyrosine ingestion include: 1) overall increase in pulse pressure (LBNP typically reduces pulse pressure). 2) an increase in P300 amplitude (indicating increased cognitive activity) when participating in the odd-ball task. 3) a non-significant increase (22%) in LBNP tolerance among subjects who could not withstand LBNP for the full 30 minute period. Results of this study indicate that elevated blood plasma tyrosine levels reduce physiological decrements caused by LBNP stress.

Report AFOSR-87-0402

Strategies to Sustain and Enhance Performance in Stressful Environments

The Effects of Tyrosine Pre-Treatment on Lower Body Negative Pressure Stress

Harris R. Lieberman
Andrew B. Dollins
Richard J Wurtman
Massachusetts Institute of Technology
Department of Brain and Cognitive Sciences
Bldg. E25-615, 77 Massachusetts Ave.
Cambridge, MA 02139

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INTRODUCTION

Tyrosine, a large neutral amino acid (LNAA) normally present in protein foods, is the precursor of the catecholamine (CA) neurotransmitters dopamine (DA), norepinephrine (NE), and epinephrine. When it is systematically administered in pharmacologic quantities, it can, under conditions such as stress, increase brain catecholamine concentration and turnover (Wurtman et al, 1974; Gibson and Wurtman, 1978; Wurtman et al, 1981). There are no known adverse effects of tyrosine administration. In fact, since it only exerts its effects when a localized deficiency state exists, the effects they appear to be system-specific and only present when needed (i.e., when local catecholamine stores are expended). Therefore, while more "specific" in its actions than most drugs, tyrosine's effects are likely to be less potent than a drug with precisely the same properties (assuming one existed). Tyrosine availability is rate-limiting for the synthesis of its neurotransmitter products in the brain only when a higher than normal level of transmitter release by catecholaminergic neurons is occurring. When CA neurons are firing frequently and therefore releasing more transmitter (dopamine or norepinephrine), they may require more of the component - tyrosine - that is the substrate for transmitter synthesis. Frequent neuronal firing may enhance the kinetic properties of tyrosine hydroxylase causing this rate-limiting, catecholamine-synthesizing enzyme to be more susceptible to control by this amino acid, or may deplete the tyrosine pools within nerve terminals (Lovenberg et al, 1975; Mandel, 1978; Wiener et al, 1978).

A number of animal studies have demonstrated that tyrosine, given either acutely (in a single dose) or chronically in the diet, reduces adverse physiological and behavioral concomitants of acute stress. In several of these studies (Lehnert 1984a,b) tail-shock was used to produce acute stress in rats. Following 60 minutes of such shocks, animals were permitted to recover for 15 minutes then placed in an open field/hole poke apparatus. After observing their spontaneous behavior for the next 10 minutes, the animals were sacrificed and their brains removed. Exposure to this experimental stress paradigm significantly decreased (by approximately 80%) open-field locomotor activity and several other spontaneous behaviors, such as rearing and hole poking, compared to unstressed control animals. However, in another group of animals who were given a diet supplemented with tyrosine and stressed, the frequency of these spontaneous

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behaviors did not differ significantly from the unstressed control animals. Increased dietary tyrosine apparently protected these animals from the behavioral inhibition produced by the stressor, presumably by augmenting noradrenergic, but also perhaps dopaminergic neurotransmission (Lehnert et al, 1984a). When a similar study was conducted with rats given single intraperitoneal doses of tyrosine (200 mg/kg) or placebo immediately prior to tail-shock, tyrosine also protected the animals from the acute behavioral depression induced by the stress (Lehnert et al, 1984b).

In both of these studies brain tyrosine levels increased, as expected, and in stressed but untreated animals declines of 30% - 40% in NE levels were noted in specific brain regions, for example hypothalamus, locus coeruleus and hippocampus. Tyrosine administration blocked this depletion in both studies. Tyrosine also increased NE turnover in specific brain regions of stressed animals as measured by regional differences in brain MHPG-SO4.

Tyrosine has also been shown to facilitate several other types of animal behavior under stressful conditions. For example, it has been found to restore normal levels of aggressive behavior in animals that have been subjected to cold-water stress (Brady et al, 1980). Additionally, in a stressful behavioral procedure sometimes considered to be a learned helplessness paradigm and used to screen drugs for anti-depressant activity (e.g. the Porsolt swim test; Porsolt et al, 1978), significant dose-related potentiation of escape behavior following tyrosine administration has been observed (Gibson et al, 1982). Specifically, animals pretreated with tyrosine and also phenylalanine (which is metabolized to tyrosine) continued to swim significantly longer than placebo-treated controls.

Tyrosine also has been shown to have other potentially beneficial effects. For example, its acute administration can lower blood pressure in spontaneously hypertensive rats that are subjected to stressful testing conditions (Sved et al, 1979) and raise blood-pressure in hypotensive animals (Conlay et al, 1981; 1985). It has also been found, in a preliminary clinical study, to be an effective treatment for essential hypertension in some individuals (Mauron, 1986). Tyrosine, in a dose-dependent manner, also decreases the vulnerability of the canine heart to ventricular fibrillation and may therefore prevent sudden, stress-induced cardiac arrest (Scott et al, 1981). Tyrosine may also have beneficial effects on the neuroendocrine response to stress since it blocks the rise in plasma corticosterone that occurs after unavoidable stress (Reinstein et al, 1985). It has been reported that animals who successfully "cope" with avoidable stress by escaping, have lower levels of plasma corticosterone than animals that fail to cope (Swenson and Vogel, 1983).

We are aware of only a few studies where tyrosine has been administered to normal human subjects. Possible beneficial effects on the mood-state of certain subgroups of depressed

patients have been reported (Gelenberg et al, 1983). In other studies, with normal men (Glaeser et al, 1979; Lieberman et al, 1983), no adverse effects of tyrosine (100 or 150 mg/kg) were noted. In fact, we observed a small improvement in responsiveness as measured by auditory reaction time. However, subjects in these studies did not experience experimental stressors, and it is under stressful conditions that tyrosine would be expected to have its positive effects on behavior.

We are aware of only one study with tyrosine where human volunteers were subjected to psychologically and physiologically stressful environmental conditions. The study was conducted at the United States Army Institute of Environmental Medicine (USARIEM) (Banderet and Lieberman, 1989). The treatment employed was acute exposure (4 hr) to a combination of hypobaric hypoxia (13,800 or 15,500 ft) and cold (60° F). Tyrosine appeared to have robust effects when individuals who responded most adversely to the stressors on each behavioral task were selected from the group. Many of the decrements in performance, mood and symptoms induced by these treatments, including functions believed to be regulated by catecholaminergic neurons, were mitigated by tyrosine treatment.

For example, performance on a vigilance test (Dual-task information processing) was significantly better ($p<.05$) at the most stressful altitude (15,500 ft) and self-reported Tension-Anxiety, as measured by the Profile of Mood States (POMS), significantly declined ($p<.01$) following tyrosine administration (100 mg/kg).

The current study was undertaken to examine the physiological, psychological, and behavioral effects of acute tyrosine administration on normal healthy males exposed to physiological and behavioral stress. As mentioned above, tyrosine administration has consistently produced beneficial effects in animals subjected to various types of cardiovascular stress. It seems likely that tyrosine would prove effective in reducing the effects of cardiovascular stress in humans. The cardiovascular stressor, Lower Body Negative Pressure (LBNP), was thus chosen as our experimental stressor. LBNP is a technique used to simulate gravitational stress (orthostasis) by exposing the lower body to subatmospheric pressures. This causes blood and interstitial fluids to pool in the lower extremities which results in decreased venous return and increased sympathetic drive (Bonde-Petersen, et al 1984). Studies have shown that LBNP induces a variety of hemodynamic changes including increases in heart rate; narrowing of pulse pressure; and decreases in cardiac output, stroke volume, left ventricular ejection time and venous pressure (Stevens and Lamb, 1965; Graboys, Forlini et al, 1974). Brief exposures to LBNP (-50 mm Hg) have been shown to reduce plasma catecholamine levels (Graboys, Lille et al, 1974). Subjects exposed to LBNP typically respond initially with decreased blood pressure and increased heart rate. These changes continue until the cardiovascular system is no longer able to maintain

homeostatis. At this point, blood pressure and heart rate rapidly fall and consciousness is lost if exposure to LBNP is not halted.

The LBNP technique provides several advantages over other techniques requiring subject exertion. No subject training is required and the subject is relaxed and lying supine throughout testing making him accessible for artifact free physiological monitoring and electrophysiological recording. Because the subject is relaxed, he is able to complete behavioral tasks as he experiences LBNP. The experimenter and medical personnel have constant access to the subject and can thus monitor his condition as the stress progresses. In addition, LBNP offers the advantage of almost instantaneous release for subject safety.

METHODS

Subjects. Twenty-two healthy adult males (mean age = 28.13 ± 4.71 years) participated in this study. After a 1 h training session, each subject was tested on two separate mornings separated by at least seven days. All subjects met the USAFSAM medical requirements for human subjects as specified by the USAFSAM Advisory Committee on Human Experimentation (ACHE) and signed an informed consent form [in accordance with AFR 169-3 and the MIT Committee on the Use of Humans as Experimental Subjects (COUHES)] prior to testing. The subjects were paid $150.00 for participating in the study.

Apparatus

LBNP. The horizontal LBNP chamber was a 34.3 x 47.6 x 133.4 cm (height x width x length) wooden box that was sealed to withstand vacuum pressure. Subjects were supine, face up, with the lower half of their body (from the illiac crest to the feet) in the LBNP chamber throughout testing. A surgical rubber (3 mm thick) seal was placed around the subject at the level of illiac crest to maintain vacuum pressure. An adjustable wooden seat padded with two SPENCO(TM) pads (Spenco Medical Corporation, Waco, TX) was used to prevent the subject from sliding into the chamber when the vacuum was on. A removable plexiglas panel was mounted on top of the chamber to allow visual monitoring of the subject's legs and convenient access for electrode placement. A Hoover PowerMAX II 2 H.P. WET/DRY Vac (Model C2079, Hoover Company, North Canton, OH) mounted on a 55 gallon holding tank was used to generate vacuum pressure. Vacuum was pulled through a 3.8 cm flexible hose connecting the vacuum source to the bottom on the LBNP chamber. The vacuum source was located in a nearby electrically shielded and sound deadened chamber to prevent extraneous noise and electrical signals from interfering with subject testing. Pressure was regulated by opening and closing a valve (5.08 cm aperture) mounted on the side of the LBNP chamber. As a safety precaution, the subject held a Positive Pressure Switch in his left hand which controlled power to the vacuum source which was only active when the subject was pressing on the

positive pressure switch. Internal LBNP chamber vacuum was calibrated with a Wallace & Tierman 1500 Hi-Performance Gauge (Model 61A-1D-0800, Wallace & Tierman, Belleville, NJ) and monitored via a digital display driven by a pressure transducer.

Electrophysiological Measures. ECG, EMG, EOG, and EEG signals were monitored on a Biophysical Oscilloscope (Model 51-2681-00, GOULD Inc., Plantsville, CT) and Dynograph Recorder (Model R711, Beckman Instruments, Fullerton, CA) throughout the two LBNP periods of each testing session. Data for the initial (baseline through three minutes of LBNP at -50mmHg) and final portions of LBNP session 1, all of LBNP session 2, and EEG sampling were recorded on FM magnetic tape (Honeywell Model 101, Denver, CO). ECG, EMG, EOG, and EEG signals were amplified using Data Inc. differential amplifiers (Model 2124, Ft. Collins, CO). Electrode impedances were held below 5 Kohms at all sites.

Electrocardiography. ECG was recorded using silver/silver chloride electrodes (Cleartrace Model 1700-030, Medtronic Andover Medical, Haverhill, MA) placed at standard lead II and V configurations. Amplifier gains were set to 2 K with high and low pass filters set to 0.5 and 50 Hz. Beat-to-beat heart rates were digitized using a device developed in-house (USAFSAM JOCUS # 79301450). Average heart rates were calculated for the baseline prior to LBNP and for every 3 minutes throughout data acquisition. Because ECG data were recorded for only the first 15 minutes of LBNP session 1, only the first 15 minutes of data for both LBNP sessions were subjected to statistical analysis. Eleven data points from the Dynograph recorded ECG record were substituted for missing digitized data.

Pulse Pressure. The subjects' blood pressure was monitored using the auscultatory cuff technique prior to the beginning of LBNP and at least once every 3 minutes throughout each LBNP session continuing until the subject was again at baseline level. Pulse pressure was later calculated by subtracting the diastolic from the systolic blood pressure.

Electromyography. Surface EMG activity was monitored over the lower left abdomen (rectus abdominus), left thigh (rectus femoris), and right calf (intersection of the gastrocnemius and soleus) to prevent tensing of lower muscle groups which could reduce LBNP induced blood and interstitial fluid pooling. Amplifier gains were set to 10 K and high and low pass filters set to 0.1 and 1000 Hz respectively (60 Hz notch filters active). The subject was asked to relax appropriate portions of his lower body if an increase in EMG activity was observed.

Evoked Potentials. Auditory evoked potentials were recorded from Fz and Pz (10-20 system), referenced to linked ears (A1 & A2). The Electro-Cap IX system (Electro-Cap Inc., Dallas, TX) was used to attach electrodes to the scalp. Electrode impedances were checked prior to each evoked potential recording period. Amplifier gains were set to 10 K and the low and high pass

filters were set to 50 and 0.5 Hz respectively. The EOG was recorded from above and below the right eye using Grass E-5 electrodes (Grass Instruments Inc., Quincy, MA). The EOG amplifier high and low pass filters were set to 1000 and 0.5 Hz respectively (60 Hz notch filter active) and the gain was set to 10 K.

EEG was recorded during the auditory oddball task for later off-line analysis. Subjects were asked to count the number of infrequent tones (1000 Hz) occurring in a series of more frequent tones (2000 Hz) while resting with eyes closed. A total of 330 stimuli were played at the rate of 1.01 tone/s. The infrequent tones (20%) were randomly distributed throughout the 330 tones and the subjects were not told the correct number of infrequent tones until all testing was complete. The tones were generated by modifying a Z-200 microcomputer so the speaker output could be amplified with a SA-150 integrated stereo amplifier (Radio Shack, Ft. Worth, TX). Subjects heard the tones (90 dB SPL) over Sony button earphones which were covered with sound suppressers to prevent the impingement of extraneous sounds. A 50 ms marker was written to magnetic tape prior to the onset of each stimulus, simultaneously with the EEG and EOG signals. There were two evoked potential sessions during each testing session.

EEG, EOG, and ECG data for each trial were sampled at a rate of 512 samples per second for 600 msec. Averaging was time locked relative to the marker written to magnetic tape at the time of data recording. Trials with peak-to-peak EOG activity of greater than 45 uV were omitted from averaging. Many EEG trials contained an artifact corresponding to the QRS complex of the ECG. The data acquisition software was thus revised to detect the onset of the ECG-QRS complex and to omit averaging of EEG data for 117 msec following the onset of the QRS complex. EEG data for the frequent and rare stimuli were averaged separately. The data for subjects with an average of fewer than 30 trials were omitted from further processing. The P1, N1, P2, N2, P3, and N3 peaks and troughs were identified by moving a cursor along each average waveform as it was displayed on a CRT. The sequence of average waveforms displayed was randomized over treatment condition and subject throughout this process to prevent experimenter bias during peak and trough identification. Amplitudes for each subjects' P1-N1, P2-N2, and P3-N3 peaks and troughs were calculated for analysis.

Performance Tasks and Mood Scale. The subjects were required to complete three microcomputer based performance tasks and a mood inventory at specific times during each testing session. The software for the tasks was coded in house and administered using a stock Zenith Z-200 microcomputer (Zenith Data Systems, St. Joseph, MI) equipped with a Gravis Mk VI joystick (Advanced Gravis Computer Technology Ltd. Bellingham, WA) that was connected via a Magnitronic B107 game I/O card (Magnitronic, Taiwan). The computer CRT was mounted in a movable stand which was tilted to adjust the display for the subjects' maximum

viewing comfort. Subjects responded with their right hand which was positioned along the outside of the LBNP chamber. The Performance tests administered were: Dual task information processing, Four-choice Visual Reaction Time, and Simple Reaction Time.

Dual task information processing. Subjects' simultaneously performed two tasks in this test: a modified version of the Bakan vigilance test (Jones et al, 1979) and the "estimation of two classes of events in a signal stream" (PROP) test (Smith, 1984). The Bakan task presents a three-digit number on the CRT screen every 1.5 seconds. Each successive number usually differed from the previous number by one digit. However, occasionally (11% of the time) all three digits were repeated. The position of the repeated number was random with the constraint that the same value could not be repeated on three consecutive displays. The subjects' task was to detect and respond to the occurrence of the repeated values by pressing the joystick button. The PROP test consisted of single letters or digits presented simultaneously with the Bakan stimuli but to the right of them on the CRT screen. The letters "I" and "O" were omitted to prevent confusion with "1" and "Ø". Every 2ØØ trials the presentation of all stimuli were halted and the subject was instructed to estimate the proportion of letters in the last 2ØØ PROP stimuli. This was done by manipulating the joystick to select a proportion from the menu displayed (Ø.Ø to 1.Ø in increments of Ø.1) on the CRT and pressing the joystick button to make a selection. The actual proportion was randomly varied between Ø.2 and Ø.8, in increments of Ø.1, for each block of 2ØØ stimuli. The computer recorded the number of correct and incorrect responses as well as the proportion of letters guessed and the number that actually appeared.

Subjects were required to perform the dual task three times during each testing session. The task was composed of six blocks of 2ØØ trials (1,2ØØ total trials) the first two times the subjects performed the task and only two blocks of 2ØØ trials the third time.

Four-choice Visual Reaction Time. This test resembles the Wilkinson four-choice RT task and is a measure of visual vigilance (Wilkinson and Houghton, 1975). A row of four empty squares was displayed on the CRT screen. The fingers of the subjects right hand were positioned over a row of four adjacent keys on the computer keyboard. The subjects were instructed to respond when one of the empty squares became a filled square by pressing a key in the corresponding location on the computer keyboard. There was a 3ØØ ms pause between the subjects response and the appearance of the next stimulus. Premature responses caused an error message to appear as did waiting more than three seconds before responding. Five hundred stimuli were presented during each test. The subjects completed this task four times during each testing session. The computer recorded the number of

correct and incorrect responses, the latencies of each, and the number of premature and time-out errors.

Simple Reaction Time. Simple reaction time to the onset of a visual stimulus was measured by this task. The CRT displayed a 1.5 by 3.5 cm rectangle which served as a visual fixation point throughout the task. When the task was begun, the word READY would appear in the rectangle for 300 ms and then disappear. A random delay of 100 to 900 ms then occurred, followed by the appearance of a bar within the rectangle. The subjects were instructed to respond to the appearance of the bar as quickly as possible by pressing a response key with the index finger of their right hand. The subjects' index finger remained poised over the response key throughout the task. The response latency appeared (in ms) on the screen immediately after each response. After a 300 ms delay the word READY would appear again and the next trial would begin. The sequence would continue until two hundred trials were completed. Premature responses caused an error message to appear as did waiting more than three seconds to respond. The subjects completed this task three times during each testing session. Response latencies and number of premature and time-out errors were retained for statistical analysis.

Profile of Mood States (POMS). A computerized version of the POMS was administered five times during each testing session. The POMS is a self-report mood questionnaire which yields 6 factors when analyzed: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. The test consists of 65 adjectives, each of which is rated on a 5-point scale (McNair et al, 1971). The adjectives 'Flushed', 'Light-headed', 'Heaviness in legs', and 'Sweaty' were added to the usual 65 questions to monitor specific reactions to LBNP induced stress. The additional questions were scored and referred to as the LBNP-stress factor. Subjects responded to the adjectives and phrases by moving a joystick to manipulate a cursor and pressing a button on the joystick to select a choice. The computer program retained the subjects responses to each question and calculated a score for each of the six factors.

Blood Catecholamine, LNAA and Cortisol Levels. Blood was drawn from an antecubital vein of the right arm using an indwelling I.V. catheter (18 g, 3.2 cm, Becton Dickinson & Company, Sandy, UT) capped with a Luer Lock PRN Adapter (Park-Davis & Co, Sandy, UT). Saline (Bacteriostatic Sodium Chloride, USP 0.9%) was injected after each sample was removed to prevent coagulation within the catheter. This technique permitted consistent sampling without the use of a heparin lock. Five 20 cc blood samples were drawn throughout each testing session. The catheter was inserted at least 20 minutes prior to drawing the first blood sample to allow catecholamine levels to stabilize prior to sampling.

After the blood samples were drawn they were immediately transferred to chilled centrifuge tubes (containing 1 ml of EDTA solution) and placed on ice. The plasma and serum components of

the blood were then separated by centrifugation and divided. Three 2 ml aliquots of plasma were mixed with 40 ul of glutathione and stored at -80°C for later HPLC catecholamine analysis. Two 1 ml aliquots of plasma were stored (-80°C) for HPLC amino acid assay. Two 0.5 ml aliquots of the serum were stored (-80°C) for cortisol analysis, which was performed using a commercially available radioimmunoassay kit.

Procedure. All testing occurred in the USAFSAM/VNB Psychophysiology laboratory (Building 170, Brooks AFB). The study employed a double-blind placebo-control crossover design to eliminate subject and experimenter bias. Subjects participated in a 1 h training session to familiarize them with the laboratory, procedures, and tasks prior to actual testing. Each subject was then tested on two occasions separated by at least seven days. Each test session lasted 4.5 h and consisted of two 39 minute periods of LBNP. Subjects were not tested on Mondays or following holidays to reduce the effects of irregular schedules. The subjects received tyrosine or placebo (100 mg/kg in 300 mg capsules) in a divided dose during each of the testing sessions. Table 1 provides specific times for events occurring during each testing session. A detailed description follows.

Each subject fasted (water only) from 0000 until arrival at the laboratory (0725) for testing. Upon arrival, the subject was asked to read and sign an informed consent form (first session only), then to complete the POMS. The subject was then given a breakfast of cereal granola bars (maximum 4) and his choice of orange juice or decaffinated coffee. Baseline blood pressure and heart rate were recorded and the subject was given Treatment dose 1. Electrodes were then attached as follows: 7 electrodes (right calf (2), left thigh (2), abdomen (2), and ground (1)) for EMG monitoring; 4 electrodes (lead configurations II and V) for ECG; 6 electrodes (right foot to right shoulder) for impedance plethysmography; and Electro-Cap (Fz and Pz) and reference (A1 and A2) for evoked potentials. The subject then put the waist seal on and was helped into the LBNP chamber. The I.V. catheter was then inserted and electrode leads were attached to appropriate amplifiers. The subject was then asked to relax until 0850 when he began the first four choice reaction time task. When the task was completed, the first blood sample was drawn and the subject's baseline blood pressure and heart rate were recorded.

The subject then began the dual task as the pressure inside the LBNP chamber was reduced. Prior to lowering the chamber pressure, a USAFSAM physician was alerted and remained available throughout the session as stipulated by the USAFSAM human use committee. LBNP chamber pressure was initially reduced to -20 mm Hg and held at that point for 3 minutes. Pressure was then reduced an additional 10 mm Hg every 3 minutes until the internal chamber pressure was -50 mm Hg. The chamber pressure was held at -50 mm Hg for the next 30 minutes or until the subject could no longer tolerate the stress. The subject's ECG and general

Table 1

Approximate Tyrosine / LBNP Study Time Line *

Start Time	Event
0725	Arrive Laboratory and Sign Consent Form (Session 1)
0728	Profile of Mood States 1
0735	Eat Breakfast
0748	TREATMENT dose 1
0750	Attach Electrodes and waist seal
0820	Enter LBNP chamber
0822	Insert IV Catheter and Attach Electrode Leads
0850	Four Choice RT Task 1
0857	Blood Draw 1
0900	TREATMENT dose 2
0902	Begin Lower Body Negative Pressure Session 1
0907	Dual Vigilance Task 1
0937	Simple RT 1
0940	Profile of Mood States 2
0943	Blood Draw 2
0944	Lower Body Negative Pressure off
0950	Evoked Potential task 1
1009	Four Choice RT Task 2
1019	Simple RT 2
1025	Profile of Mood States 3
1030	Dual Vigilance Task 2
1042	Blood Draw 3
1045	Begin Lower Body Negative Pressure Session 2
1107	Four Choice RT Task 3
1118	Evoked Potential task 2
1124	Profile of Mood States 4
1125	Blood Draw 4
1125	Lower Body Negative Pressure off
1129	Simple RT 3
1138	Dual Vigilance Task 3
1150	Four Choice RT Task 4
1158	Blood Draw 5
1158	Profile of Mood States 5
1200	Exit LBNP chamber, remove electrodes,
1215	Session
END	

* Task and LBNP start times are actual average times (n=22).

condition were monitored continuously throughout the LBNP session. Heart rate and blood pressure were recorded a minimum of once every 3 minutes (more frequently if irregular shifts were observed) throughout the LBNP session and until physiological indices returned to initial baseline levels. A second blood sample was drawn prior to pressure release or as quickly as possible after pressure release when the subject released the positive pressure switch without warning. Behavioral testing continued regardless of when LBNP was returned to the ambient level.

When the dual task was complete, the subject proceeded with a second simple reaction time task and the POMS. EOG electrodes were then attached and EEG electrode impedances checked prior to beginning the EEG oddball task. This task was was followed by the four choice reaction time task, the simple choice reaction time task, the POMS and the dual task.

The subject was asked to pause when the second block of the dual task was complete. A third blood sample was then drawn and baseline heart rate and blood pressure were recorded. LBNP began as the subject completed the dual task. The second LBNP session followed the same sequence described above, beginning at -20 mm Hg and dropping an additional -10 mm Hg every 3 minutes to -50 mm Hg which was maintained for 30 minutes or until the subject had reached maximum tolerance. The dual task was followed by the four choice reaction time task, the oddball (EEG) task, and the POMS. Blood was sampled for the fourth time prior to, or as soon as possible after, LBNP release. The fourth blood draw was followed by a simple choice reaction time task. The subject then completed the dual task (2 blocks, 400 trials) and the four choice reaction time task. A final blood sample was drawn as the subject completed the POMS for the fifth time. The subject was then released from the LBNP chamber and debriefed as electrodes and the I.V. catheter were removed. The next testing date was then confirmed or the subject was paid (second session) and released.

Analyses. Except where noted, all analyses of variance were calculated using the SAS GLM procedure for repeated measures.

RESULTS

LBNP Tolerance. Of the 22 subjects tested, 9 subjects tolerated LBNP at -50 mm Hg for the full 30 minutes throughout testing. Two of the remaining 13 subjects did not tolerate LNBP to -50 mm Hg in at least one of the four LBNP sessions and were dropped from further analyses. A Treatment (Tyrosine vs. Placebo) x Stressor (2 LBNP Sessions per testing session) x Order (Treatment Order) ANOVA was used to test the LBNP tolerance levels of the remaining 11 subjects. A significant [$F(1,9)=12.91$, $p<0.005$] Treatment by Order interaction was found. Examination of individual tolerance durations reveals that subjects consistently tolerated longer periods of LBNP stress on the second day of

testing, regardless of the treatment received. No other significant effects or interactions were found in the overall ANOVA. Simple effects indicated that Tyrosine treatment significantly [$F(1,5)=6.46$, $p<.05$] increased LBNP tolerance in subjects receiving Placebo followed by Tyrosine and decreased [$F(1,4)=13.50$, $p<.02$] LBNP tolerance in subjects receiving Tyrosine followed by Placebo. Paired t-tests were used to examine within order treatment effects. Tyrosine treatment increased [$t(4)=4.20$, $p<.0085$] the LBNP tolerance of subjects receiving Placebo followed by Tyrosine from 18.61 minutes to 32.53 minutes during LBNP session 1, but had no effect during LBNP session 2. Tyrosine treatment decreased [$t(4)=3.77$, $p<.02$] the LBNP session 2 tolerance (an average of 9.40 minutes) of subjects receiving Tyrosine on the first day of testing, but had no effect on LBNP session 1 tolerances for this group.

Pulse Pressures. The mean pulse pressures for which full data were available (i.e., from resting until 6 minutes of LBNP at -50 mmHg) are plotted in Figure 1. Pulse pressures decreased significantly [$F(5,90)=28.59$, $p<.001$] from baseline to the end of measurement. The subjects' pulse pressures were significantly higher [$F(1,18)=8.22$, $p<.01$] when ingesting Tyrosine than when ingesting Placebo throughout both LBNP sessions. In addition, pulse pressures during LBNP session 1 were significantly higher [$F(5,90)=23.23$, $p<.001$] than during LBNP session 2. No significant order effects or interactions were found. The data for LBNP sessions 1 and 2 were analyzed separately to test for a consistent treatment effect. The subjects exhibited significant increases in pulse pressure when ingesting tyrosine during both LBNP sessions.

The data for 10 subjects who tolerated LBNP for the full duration throughout testing were subjected to a separate analysis (Note: one of these subjects tolerated only 37 minutes of LBNP during one session). Significant baseline to end of LBNP [$F(8,13)=12.07$, $p<.001$] decreases in pulse pressure and significant [$F(1,8)=11.62$, $p<.01$] decreases in pulse pressure between LBNP sessions 1 and 2 were again found. The increase in pulse pressure when taking Tyrosine approached significance ($p<.06$) further supporting the above Tyrosine effect.

Heart Rate. Heart rate analysis indicated that there was a significant [$F(4,72)=61.30$, $p<.0001$] increase in heart rate throughout the first 15 minutes of LBNP. The mean heart rate during LBNP session 1 was significantly faster [$F(1,18)=26.87$, $p<.0001$] than that during LBNP session 2. A significant [$F(1,18)=8.28$, $p<.01$] Treatment by LBNP session effect was also found. No other effects were significant. The Treatment by LBNP session interaction indicates that heart rates were increased by tyrosine treatment during LBNP session 1, but not session 2. Separate analyses for the two LBNP sessions indicate that there was a significant increase in heart rate during both LBNP sessions. A significant Treatment by heart rate interaction was found during LBNP session 1 but not during LBNP session 2. The

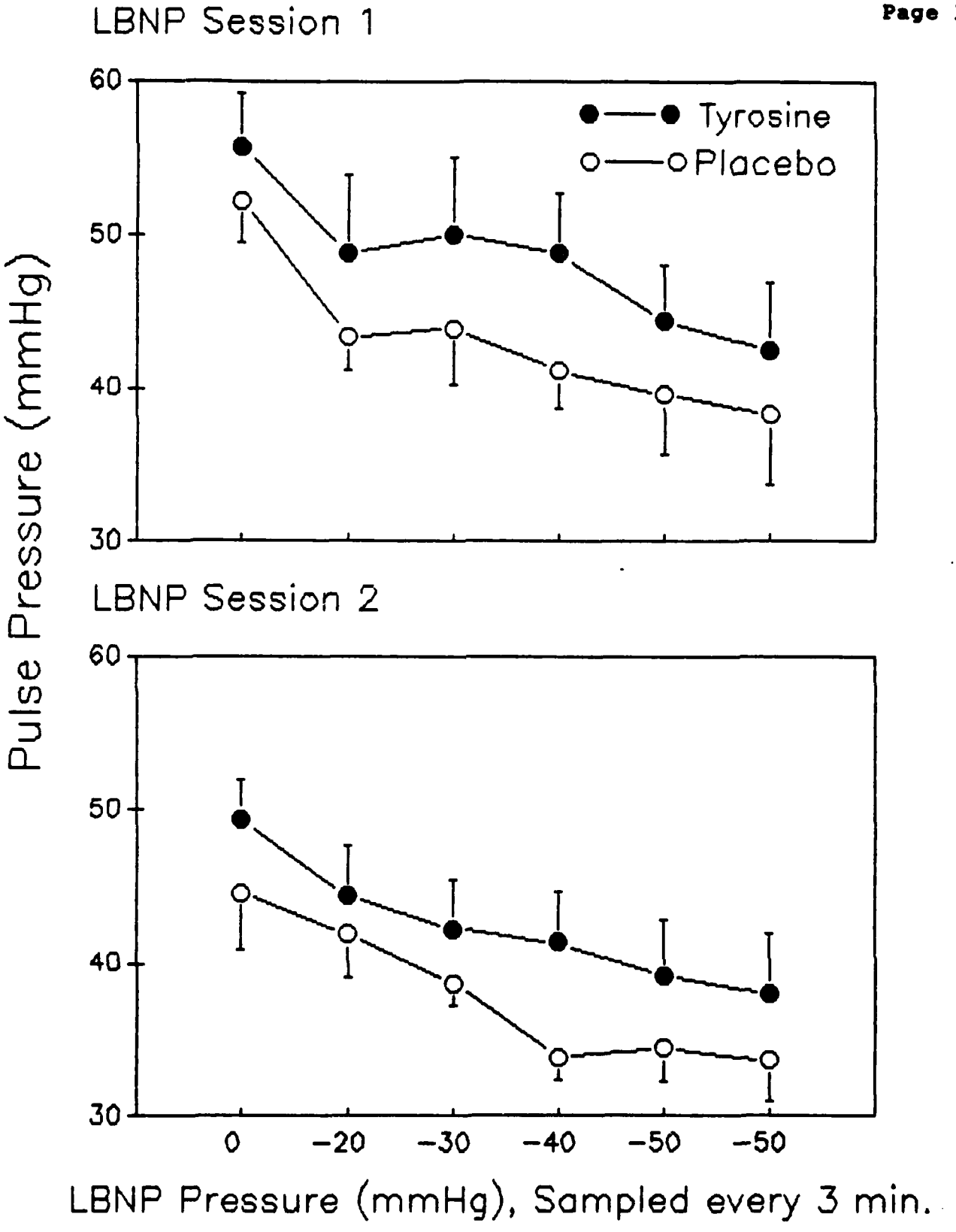

Figure 1. Average pulse pressures from Baseline through 6 minutes of LBNP at -50mmHg (vertical bars are SEM, N=20).

significant interaction (Figure 2) indicates that the heart rates of tyrosine treated subjects were initially faster and increased more slowly than placebo treated subjects.

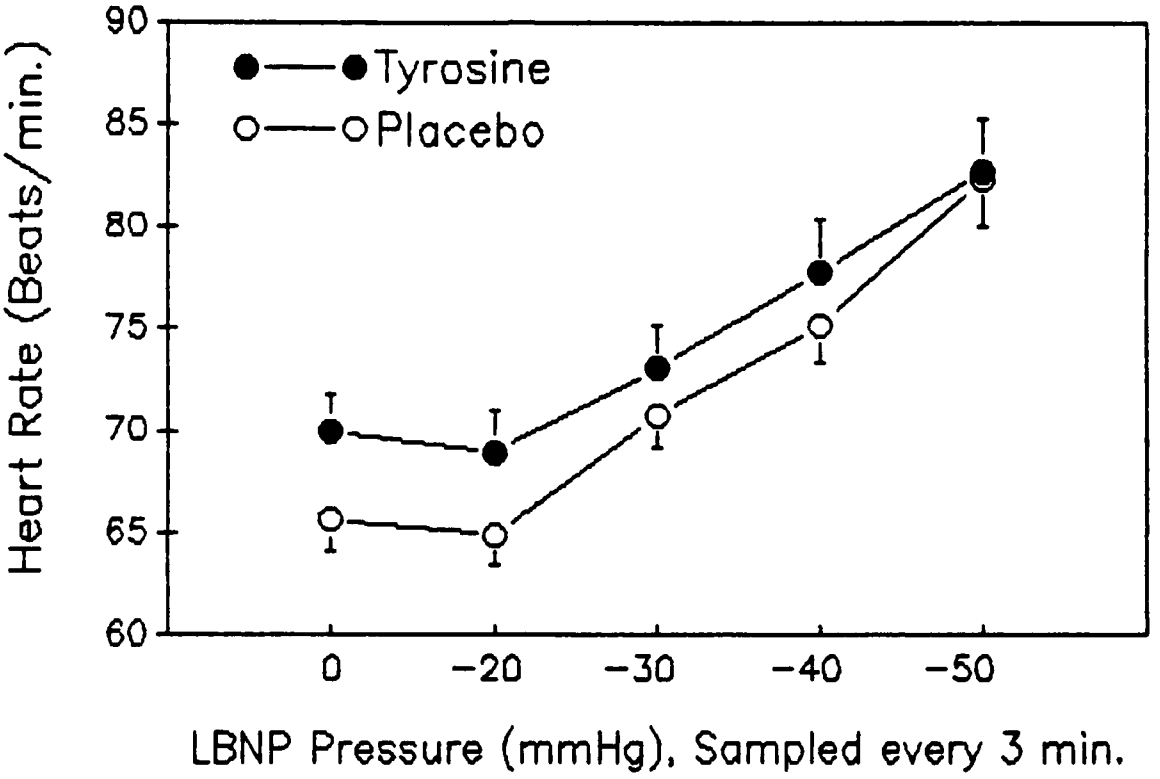

Figure 2. Treatment by Heart Rate interaction found in LBNP session 1 average heart rates from Baseline through 3 minutes at -50mmHg (vertical bars are SEM, N=20).

Evoked Potentials. Sixteen subjects had artifact free data for both treatment conditions during EEG session 1 while only eight subjects had complete data during EEG session 2. Paired t-tests were used to compare the treatment effects of the P1-N1, P2-N2, and P3-N3 amplitudes for Channels Fz and Pz. No significant treatment differences were found among the P1-N2 or P2-N2 amplitudes for frequent or rare trials of either EEG session or among the P3-N3 amplitudes for EEG session 2. A significant [$t(15)=2.13$, $p<.05$] increase in channel Pz P3-N3 EEG session 1 amplitude occurred when subjects ingested Tyrosine as illustrated in Figure 3. Similar though non-significant increases in P3-N3 amplitudes were also found in channel Fz of EEG session 1 and both channels of EEG session 2.

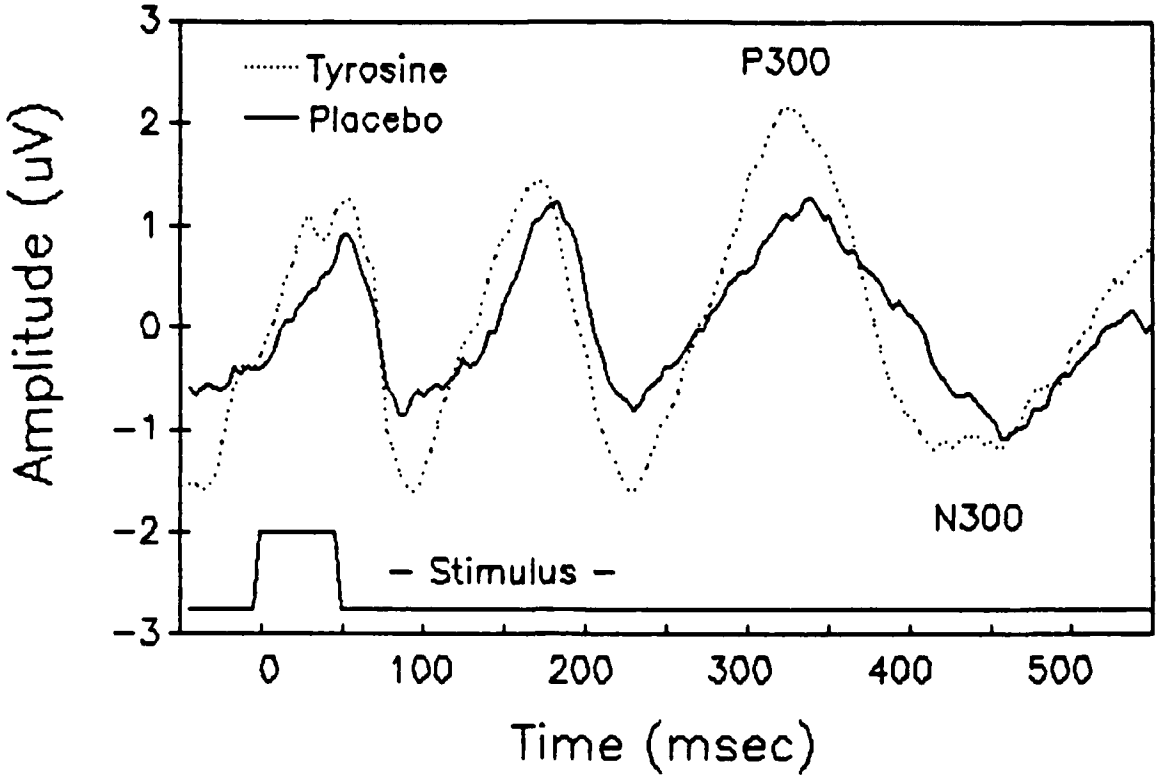

Figure 3. Grand Average Evoked Potential for Channel Pz (N=16).

Dual task information processing. The number of correctly identified repeated digits was not significantly effected by the subject testing order or treatment, but did vary significantly [F(2,36)=5.63, p<0.007, N=20] as a function of testing time. Out of 22 possible correct responses per 200 trials, subjects correctly identified an average of 15.22 and 13.52 repeated digits during LBNP sessions 1 and 2 respectively. They correctly identified an average of 14.62 repeated digits during the dual task presented near the end of testing. No significant differences were found among subject estimates of the proportion of letters presented or the number of digits erroneously identified as repeated.

Four-choice Visual Reaction Time. Significant treatment and treatment order by treatment effects were found in the latency of correct responses. Subjects responded significantly slower [F(1,16)=5.05, p<0.04] when ingesting tyrosine. The significant treatment by treatment order interaction indicates that all subjects had shorter reaction time latencies on the second day of testing, regardless of the treatment received. The number of incorrect responses increased significantly [F(3,48)=3.13, p<0.03] as a testing progressed. The latency of incorrect responses also changed significantly [F(3,48)=3.67, p<0.01] throughout a testing session with subjects responding more quickly during the first and last times the task was

quickly during the first and last times the task was administered. Subjects receiving tyrosine followed by placebo made significantly [$F(1,16)=8.19$, $p<0.01$] fewer premature response errors than those receiving treatment in the opposite order. There were no significant effects among the number of correct responses or the number of time-out responses.

Simple Reaction Time. The data for three of the 20 subjects tested were lost through experimenter error, thus only 17 subjects were included in this analysis. There were no significant reaction time latency treatment, treatment order, or task time effects. A significant [$F(1,15)=6.09$, $p<0.02$] treatment order by treatment interaction indicates that subject reaction times were shorter throughout the second day of testing, regardless of the treatment received. No differences were found in the number of premature response or time-out errors throughout testing.

Cortisol Levels. The cortisol levels of 10 subjects were analyzed. Five of the ten subjects received placebo followed by tyrosine and vice versa. Mean plasma cortisol levels increase significantly [$F(4,32)=3.48$, $p<0.01$] from a baseline of 7.11 to 11.29 ug/dl at the end testing. There were no significant treatment, treatment order, or interaction effects.

Profile of Mood States. The drug, treatment order, and interaction effects of all POMS factors were nonsignificant except for a significant [$F(4,72)=3.11$, $p<0.02$] Vigor/Activity scale drug by test time interaction. Subjects ingesting tyrosine had lower Vigor/Activity scores during the first half of a testing session but higher scores during during the second half. The subjects' responses on every POMS scale changed significantly as testing progressed. Response averages indicate that subjects felt the greatest Tension/Anxiety, Depression/Dejection, Anger/Hostility, Fatigue/Inertia, Confusion/Bewilderment, and LBNP-stress during LBNP session 2 and the least when testing began. Responses indicate that subjects felt the the greatest Vigor/Activity prior to LBNP session 1 and the least during LBNP session 2. These differences were significant at the $p<.05$ level on the Duncan's multiple range test. Responses on the Tension/Anxiety, Depression/Dejection, Confusion/Bewilderment, and LBNP-stress scales were measured (from second greatest to second least) during LBNP session 1, the end of the testing session, and the period between LBNP sessions. The second largest Anger/Hostility scores were measured at the end of the testing session, followed by LBNP session 1 and end of testing. Fatigue/Inertia scores were the second greatest during the period between LBNP sessions, then LBNP session 1 and end of testing. The next greatest Vigor/Activity scores were obtained at the end of testing, followed by LBNP session 1 and the rest period between LBNP sessions.

Blood Catecholamine, and LNAA. These assays have not been completed at this time.

DISCUSSION

Tyrosine treatment caused a significant increase in LBNP session 1 tolerance in subjects receiving placebo followed by tyrosine, but no difference in LBNP session 2. Subjects receiving tyrosine followed by placebo had lower LBNP session 2 tolerances when ingesting tyrosine but no tolerance differences during LBNP session 1. The average LBNP session 2 tolerances for all subjects differed by 0.47 min (N=11) while the mean LBNP session 1 tolerance was 5.5 min longer when taking tyrosine. As indicated above, all subjects had higher LBNP tolerances on the second day of testing, regardless of treatment. While this treatment order effect was not significant, it does suggest that the subjects experienced more stress on the first day of testing than on the second day.

We interpret these data as follows. LBNP session 2 was more stressful than LBNP session 1 because LBNP session 1 proceeded it. This is supported by the fact that LBNP session 1 pulse pressures and heart rates were significantly higher than those of session 2 as well as the pattern of POMS responses. All subjects experienced a "first day" stress which reduced tolerance on the first day of testing. Subjects receiving placebo on the first day of testing experienced "first day" plus LBNP stress. On the second day of testing they experienced LBNP stress, tyrosine treatment, and no "first day" stress. These subjects exhibited a significant increase in LBNP session 1 tolerance (13.92 min) and a smaller tolerance increase (7.05 min) during LBNP session 2. Subjects receiving tyrosine on the first day of testing experienced "first day" and LBNP stress along with tyrosine treatment and only LBNP stress on the second day (i.e., no "first day" stress or tyrosine). These subjects tolerated LBNP sessions 1 and 2 for 4.59 and 9.49 (significant) min longer when taking placebo. We suggest that the significant increase in LBNP session 1 tolerance observed in the placebo-tyrosine group was a result of the benefit of tyrosine and the lack of "first day" stress. The nonsignificant increase in LBNP session 2 tolerance was due to the increased stress inherent in LBNP session 2. We further suggest that the nonsignificant decrease with tyrosine treatment in LBNP session 1 tolerance among the tyrosine-placebo subjects occurred because the treatment reduced the "first day" stress. Tyrosine treatment did not, however, reduce the more severe stress of LBNP session 2 among these subjects.

The results of this study support the earlier findings of Conlay et al (1981, 1985) that tyrosine administration was effective in increasing the blood pressures of hypotensive animals. Tyrosine treatment significantly increased the pulse pressures of subjects, indicating increased cardiac output in response to LBNP stress. One possibility is that increased plasma tyrosine levels caused a corresponding increase in peripheral catecholamines which resulted in the observed increase in cardiac output. These results may be due to changes in central catecholamine levels.

We attribute the significant treatment by heart rate interaction to the same mechanism. While the significant decrease in heart rate between LBNP sessions 1 and 2 was not expected, it does support our proposal that LBNP session 2 was more stressful than LBNP session 1. This may be of particular interest to those using LBNP to study orthostasis as, to our knowledge, this is the first study to expose subjects to repeated LBNP sessions within a few hours.

The P300 component of the auditory evoked potential has been associated with timing or the "intensity" of an information-processing activity (Donchin, Kramer, & Wickens, 1986). In studies where subjects are required to attend to primary and secondary tasks simultaneously, P300 amplitude has been shown to decrease when the oddball task is secondary (Wickens, Isreal, & Donchin, 1977; Isreal, Chesney, Wickens, & Donchin, 1980). More recent work indicates that P300 amplitude decreases as task difficulty increases (Polich, 1987). Our results of greater P300-N300 amplitude in subjects ingesting tyrosine relative to placebo suggest that subjects found the oddball task less difficult when ingesting tyrosine. Arousal and sensory parameters did not change as a function of treatment as indicated by the lack of significant differences among other peak amplitudes (particularly N100). While this is, to our knowledge, the first time a direct effect on cognitive activity has been demonstrated with a neurotransmitter precursor, we interpret these results with caution and strongly suggest that further work be done in this area.

Data from this human study neither support nor contradict animal research indicating that tyrosine blocks a stress induced rise in plasma corticosterone (Reinstein et al, 1985). Even speculation of treatment effects are not reasonable due to strong treatment order effects evident in the raw plasma levels. The current work does not, however, support the work of (Allen, Davis, & Rowlands, 1982) indicating that LBNP stress has no effect on cortisol levels. This difference in results could be due to the shorter LBNP exposure (20 min) durations of the study conducted by Allen et al.

Responses to the POMS indicated that subjects overall were the most "stressed" during LBNP sessions 1 and 2, respectively, and the least "stressed" at the beginning of testing. While no discernible pattern of treatment related responses is evident from examining the mean response values at this time, the catecholamine data may provide covariates for later analysis.

CONCLUSIONS

The results of this study indicate that tyrosine reduces some physiological decrements caused by LBNP stress. During the first LBNP session, subjects could tolerate longer exposures to this stressor when they received tyrosine prior to exposure. Tyrosine pretreatment also allowed subjects to maintain significantly

higher pulse pressures throughout exposure to LBNP, an indication of increased cardiac output. The early EEG channel Pz P300-N300 amplitudes were larger in subjects pretreated with tyrosine. We cautiously interpret this as indicating that tyrosine pretreatment reduces the difficulty of the oddball task and may decrease the decrement in cognitive processing ability caused by LBNP stress. The pulse pressure, heart rate, behavioral task, and mood surveys indicate that subjects were more stressed by LBNP session 1 than session 2. Treatment order effects suggest that subjects re-exposed to LBNP a few days after their first exposure will experience less psychological stress, and possibly less of the physiological stress, experienced during their first exposure.

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