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L-Tyrosine Ameliorates Some Effects of Lower Body Negative Pressure Stress

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DOLLINS, A. B., L. P. KROCK, W. F. STORM, R. J. WURTMAN AND H. R. LIEBERMAN. *L-Tyrosine ameliorates some effects of lower body negative pressure stress.* PHYSIOL BEHAV **57**(2) 223–230, 1995.—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 pharmacological quantities can reduce physiological and behavioral decrements induced by highly stressful conditions. The current study was designed to test the effects of tyrosine (100 mg/kg of body weight) on humans exposed to cardiovascular stress. Twenty participants were exposed to two Lower Body Negative Pressure (LBNP) sessions (-50 mm Hg for a maximum of 30 min) during each of two testing sessions of a repeated measure double-blind placebo-controlled study. The detected effects of tyrosine include an overall increase in pulse pressure (LBNP typically reduces pulse pressure) and an increase in auditory event related potential amplitude (P300-N300), an electrophysiological correlate of attention which may indicate enhanced cognitive activation.

Human Tyrosine Lower Body Negative Pressure (LBNP) Pulse pressure Electroencephalogram (EEG) Oddball paradigm Profile of Mood States (POMS) Reaction time

INTRODUCTION

TYROSINE, a large neutral amino acid normally present in protein foods, is the precursor of the catecholamine (CA) neurotransmitters dopamine (DA), norepinephrine (NE), and epinephrine. When systemically administered in pharmacological quantities, it can, under conditions such as stress, increase brain CA concentration and turnover (11,37,38). Because pure tyrosine is not normally ingested, any dose which significantly increases plasma levels above normal (approximately 70 ± 3.9 nmols/ml) would be considered a pharmacological dose. Plasma tyrosine levels peak approximately 2 h after oral ingestion of 100 mg of tyrosine and remain elevated for approximately 7 h (13). 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 appear to be system-specific and only present when needed (i.e., when local CA stores are diminished). Therefore, while more "specific" in its actions than most drugs, tyrosine's effects are likely to be less potent than a drug acting via the same neurotransmitter (20). 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 (DA or NE), they may require more of the precursor—tyrosine, the substrate for transmitter biosynthesis. Frequent neuronal firing enhances the kinetic properties of tyrosine hydroxylase causing this rate-limiting, catecholamine-synthesizing enzyme to be more susceptible to control by this amino acid than it is when neuronal firing is less frequent (22,36). Frequent neuronal firing may also deplete the tyrosine pools within nerve terminals (25,36).

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. Locomotor activity, rearing, and hole-poking behavior were significantly reduced, following 60 min of tail-shock stress in rats pretreated with saline but not with tyrosine (18,19,30). Tyrosine pretreatment has been found to restore normal levels of aggressive behavior in animals that have been subjected to cold-water stress (4). In a stressful behavioral procedure sometimes considered to be a learned helplessness paradigm and used to screen drugs for anti-depressant activity, the Porsolt swim test (28), significant dose-related potentiation of escape behavior following tyrosine administration has been observed (12). Specifically, animals pretreated with tyrosine (or phenylalanine

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which is metabolized to tyrosine) continued to swim significantly longer than placebo-treated controls. Tyrosine has also been reported to prevent decrements in performance among rats subjected to acute cold stress (29). Acute administration of tyrosine can lower blood pressure in spontaneously hypertensive rats that are subjected to stressful testing conditions (35) and, by acting on sympathetic instead of brain neurons, raise blood pressure in hypotensive animals (5,6). 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 (32). 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 (22).

Studies of tyrosine administration to unstressed humans have demonstrated possible beneficial effects in the treatment of essential hypertension (23) and depression (10). No adverse effects of such treatment were noted (13,21). However, participants in these studies were not subjected to 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 tyrosine study in which human volunteers were subjected to psychologically and physiologically stressful environmental conditions (2). The treatment employed was acute exposure (4 h) to a combination of hypobaric hypoxia (corresponding to 13,800 or 15,500 ft) and cold (60°F). Tyrosine appeared to have robust effects among those individuals who responded most adversely to the stressors on each behavioral task. 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. (See Owasoyo, Neri, and Lamberth (26) for a recent review of the effects of tyrosine on stress.)

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 (CV) stress. It seemed likely that tyrosine would prove effective in reducing the effects of CV stress in humans. Lower Body Negative Pressure (LBNP), was chosen to induce CV stress. LBNP is a technique used to simulate a gravitational load (orthostasis) by exposing the lower body to subatmospheric pressures. This causes blood and interstitial fluids to pool in the lower extremities resulting in decreased venous return and increased sympathetic drive (3). Studies have shown that LBNP induces a variety of hemodynamic changes including increases in heart rate and narrowing of pulse pressure, as well as decreases in cardiac output, stroke volume, left ventricular ejection time, and venous pressure (15,33). Brief exposures to LBNP (−50 mm Hg) have been shown to increase CA levels (14), suggesting that some of the above CV effects might be attributable to an increase in sympathetic activity. Subjects exposed to LBNP typically respond initially with decreased blood pressure and increased heart rate. Such changes persist until the CV system is no longer able to maintain homeostasis. At this point, blood pressure and heart rate rapidly fall and consciousness is lost if exposure to LBNP is not halted.

METHODS

Subjects

Twenty-two healthy adult males who had no previous experience with LBNP (mean age = 28.13 ± 4.71 yr) were paid participants in this study. Two of the subjects were unable to tolerate LBNP to −50 mm Hg in at least one of the four LBNP sessions for reasons unrelated to the experimental treatment and were dropped from the analyses. All subjects met the USAFSAM medical requirements for human subjects (as specified by the USAFSAM Advisory Committee on Human Experimentation) and signed an informed consent form prior to testing (in accordance with AFR 169-3 and the MIT Committee on the Use of Humans as Experimental Subjects).

Procedure

A double-blind, placebo-controlled, crossover design was used to control for subject and experimenter bias. The subjects participated in a one h training session to familiarize them with the laboratory, procedures, and performance tasks prior to actual testing. Each subject was then tested on two occasions separated by at least seven days. Two 39 min periods of LBNP were included in each 4.5 h test session. Subjects were not tested on Mondays or following holidays to reduce the effects of irregular activity schedules.

Subjects were asked to refrain from alcohol, nicotine, and caffeine consumption for 24 h prior to testing and to consume only water from 2400 h the night before until their arrival at the laboratory at 0730 h. They were served a breakfast of granola bars and noncaffeinated beverages. Capsules containing tyrosine (50 mg/kg) or placebo (cellulose) were ingested at 0748 and 0900 h. A divided dose was administered to maintain circulating tyrosine levels throughout testing. Table 1 provides a schedule of times for events occurring during each test session.

LBNP Subjects were tested while they were supine, face up, and with the lower half of their body in the LBNP chamber. A surgical rubber seal (3 mm thick) was placed around the subject at the level of illiac crest to ensure air tight integrity. A padded seat was adjusted to prevent the subject from sliding into the chamber when a vacuum was applied. The vacuum source, a Hoover PowerMAX II WET/DRY Vac (Model C2079, Hoover Company, North Canton, OH) was located in a nearby-electrically shielded and sound-attenuated chamber to prevent extraneous noise and electrical signals from interfering with subject testing. Vacuum was applied via a 3.8 cm flexible hose connecting the vacuum source to the LBNP chamber. Pressure was regulated by opening and closing a valve (5.08 cm aperture) mounted on the side of the LBNP chamber. The negative pressure was calibrated with a Hi-Performance Gauge (Model 61A-1D-0800, Wallace & Tierman, Belleville, NJ) and monitored via a digital display driven by a pressure transducer. As a safety precaution, vacuum pressure was active only while the subject pressed on a positive pressure switch held in his left hand.

During LBNP exposure, pressure was initially reduced to −20 mm Hg for three min. Pressure was then reduced an additional 10 mm Hg every 3 min until the internal chamber pressure was −50 mm Hg. Pressure was maintained at −50 mm Hg for the next 30 min or until presyncopal symptoms were observed. The subject's electrocardiogram (ECG) and general condition were monitored continuously throughout each LBNP session. Heart rate and blood pressure (auscultatory cuff) were recorded a minimum of once every three min (more frequently if irregular shifts were observed) throughout the LBNP session and until physiological indices returned to baseline levels. Pulse pressures were calculated by subtracting the diastolic from the systolic blood pressure. Blood samples (20 cc) were drawn: (a) prior to the onset of LBNP; (b) prior to LBNP pressure release or as quickly as possible after pressure release; and (c) at the end of each testing session. Behavioral testing continued regardless of when LBNP was returned to the ambient level.

TABLE 1

APPROXIMATE TYROSINE/LBNP STUDY TIME LINE*

Start Time	Event	Start Time	Event
0725	Arrive Laboratory	1019	Simple RT 2
0728	Profile of Mood States 1	1025	Profile of Mood States 3
0735	Eat Breakfast	1030	Dual Vigilance Task 2
0748	TREATMENT Dose 1	1042	Blood Draw 3
0820	Enter LBNP Chamber	1045	Begin LBNP Session 2
0822	Insert IV Catheter	1107	Four-choice RT Task 3
0850	Four-choice RT Task 1	1118	Evoked Potential task 2
0857	Blood Draw 1	1124	Profile of Mood States 4
0900	TREATMENT Dose 2	1125	Blood Draw 4
0902	Begin LBNP Session 1	1125	LBNP Off
0907	Dual Vigilance Task 1	1129	Simple RT 3
0937	Simple RT 1	1138	Dual Vigilance Task 3
0940	Profile of Mood States 2	1150	Four-choice RT Task 4
0943	Blood Draw 2	1158	Blood Draw 5
0944	LBNP Off	1158	Profile of Mood States 5
0950	Evoked Potential Task 1	1200	Exit LBNP Chamber
1009	Four-choice RT Task 2	1215	Leave Laboratory

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

Electrophysiological Measures

Heart rate and skeletal muscle activity were monitored throughout the two LBNP periods of each testing session. Data for the initial (baseline through three min of LBNP at −50 mm Hg) and final portions of LBNP session 1, all of LBNP session 2, and electroencephalographic auditory event related potential (ERP) sampling were recorded on FM magnetic tape (Honeywell Model 101, Denver, CO) for off-line analysis. Electrophysiologic signals were amplified using Data Inc. differential amplifiers (Model 2124, Ft. Collins, CO). Electrode impedances were held below 5K ohms at all sites.

Electrocardiography

Silver/silver chloride electrodes (Cleartrace Model 1700-030, Medtronic Andover Medical, Haverhill, MA) were placed at standard lead II and V configurations throughout testing. Amplifier gains were set to 2K with high- and low-pass filters set to 0.5 and 50 Hz, respectively. 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 every 3 min throughout data acquisition. Eleven data points from a strip-chart electrocardiogram were substituted for missing digitized data.

Electromyography

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

Event Related Potentials

Cortical activity was recorded from 10–20 system Fz and Pz positions referenced to linked earlobes (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 recording period. Amplifier gains were set to 10 K and the high- and low-pass filters were set to 0.5 and 50 Hz respectively. The electrooculogram (EOG) was recorded from above and below the right eye using Grass E-5 electrodes (Grass Instruments Inc., Quincy, MA). The electrooculography amplifier high- and low-pass filters were set to 0.5 and 1000 Hz respectively (60 Hz notch filter active) and the gain was set to 10 K.

Auditory ERP activity was recorded using the "oddball" paradigm (34). During this paradigm, subjects are exposed to a series of frequent and infrequent stimuli, and are usually asked to count the number of rare stimuli. The P300 wave is a large positive slow wave that occurs approximately 300 msec after the onset of the rare stimuli in the series. The latency and/or amplitude of the P300 wave vary, in a systematic manner, with a wide variety of testing circumstances (8). In this study, 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, 50 msec, stimuli were played at the rate of 1.01 tone/sec. The infrequent tones (20%) were randomly distributed throughout the frequent tones. Subjects were not told the correct number of infrequent tones until all testing was complete. The tones were generated by modifying an Z-200 microcomputer so that 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 shielded with sound suppressors to prevent the impingement of extraneous sounds. A 50 msec stimulus marker was recorded on magnetic tape prior to the onset of each stimulus, simultaneous with the electroencephalographic and electrooculographic signals. There were two evoked potential sessions during each testing session.

Electrophysiologic data for each trial were sampled at a rate of 512 samples per second for 600 msec. Averaging was time-locked relative to the stimulus marker. Trials with EOG peak-to-peak values greater than 45 μV were omitted from averaging. Many trials contained an artifact corresponding to the QRS complex of the ECG. The data acquisition software was revised to

detect the onset of the ECG-QRS complex and to omit averaging of cortical activity for 117 msec following the onset of the QRS complex. The data for subjects with an average of fewer than 30 trials were omitted from further processing. The P100, N100, P200, N200, P300, and N300 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 subject and treatment condition throughout this process to prevent experimenter bias during peak and trough identification. Peak minus trough values for the P100-N100, P200-N200, and P300-N300 components of each subject's electroencephalogram (EEG) were calculated for analysis.

Cortisol Levels

Blood was drawn from an antecubital vein of the right arm through an indwelling IV 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. The samples were immediately transferred to chilled centrifuge tubes (containing one ml of EDTA solution) and placed on ice. The plasma and cellular components of the blood were then separated by centrifugation. Two 0.5 ml aliquots of the plasma fraction were stored (−80°C) for cortisol analysis, which was performed using a commercially available radioimmunoassay kit.

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 (see Table 1). 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 game I/O card (model B107, 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 (min to complete) were: Dual Task Information Processing (30), Four-choice Visual Reaction Time (5), and Simple Reaction Time (5). These tasks were selected because previous studies have shown them to be sensitive to the effects of sleep deprivation, stress and other pharmacological agents (2,17,21). Details concerning these tasks and their administration are published elsewhere (7).

A computerized version of the Profile of Mood States (POMS, 24) was administered five times during each testing session. The POMS is a 65 question self-report mood questionnaire which yields six factors when analyzed: Tension/Anxiety, Depression/Dejection, Anger/Hostility, Vigor/Activity, Fatigue/Inertia, and Confusion/Bewilderment. The terms "Flushed," "Light-headed," "Heaviness in legs," and "Sweaty" were added to the usual 65 POMS questions to monitor specific reactions to LBNP induced stress. The additional questions are referred to as the LBNP Stress Factor.

Analyses

Except where noted, all analyses of variance were calculated using a between-groups (order), within-subjects (treatment and time), repeated measures factorial designs. The Greek symbol delta "Δ" is used to indicate "a change of."

RESULTS

Pulse Pressures

The mean pulse pressures for which full data were available (i.e., from resting until 6 min of LBNP at −50 mm Hg; $N = 20$) are plotted in Fig. 1. Mean pulse pressures (averaged over LBNP sessions 1 and 2) measured during LBNP following tyrosine treatment were significantly greater (Δ + 3.3 mm Hg) than those following placebo treatment [$F(1,18) = 8.22$, $p < 0.01$]. Mean pulse pressures decreased significantly from 47.8 mm Hg during LBNP session 1 to 42.3 mm Hg during LBNP session 2 [$F(1,18) = 23.23$, $p < 0.001$]. The change in mean pulse pressure between baseline and six min of LBNP at −50 mm Hg (52.72, 47.59, 45.95, 43.65, 41.21, and 39.76 mm Hg, respectively) was significant [$F(5,90) = 28.59$, $p < 0.001$]. Contrasts comparing each pulse pressure with the average of subsequent pulse pressures were all significant ($p < 0.01$) except the comparison between the final two pulse pressures. No significant order (test day) effects or interactions were found. Separate analyses of the two LBNP sessions indicate that the mean pulse pressure was higher when tyrosine was ingested, relative to placebo, during both LBNP session 1 (Δ +3.74, $F(1,18) = 5.69$, $p < 0.001$) and 2 (Δ +2.83, $F(1,18) = 4.46$, $p < 0.05$).

Heart Rate

Heart rate increased significantly from 65.9 beats per min (BPM) during baseline to 80.6 BPM after the first 15 min of

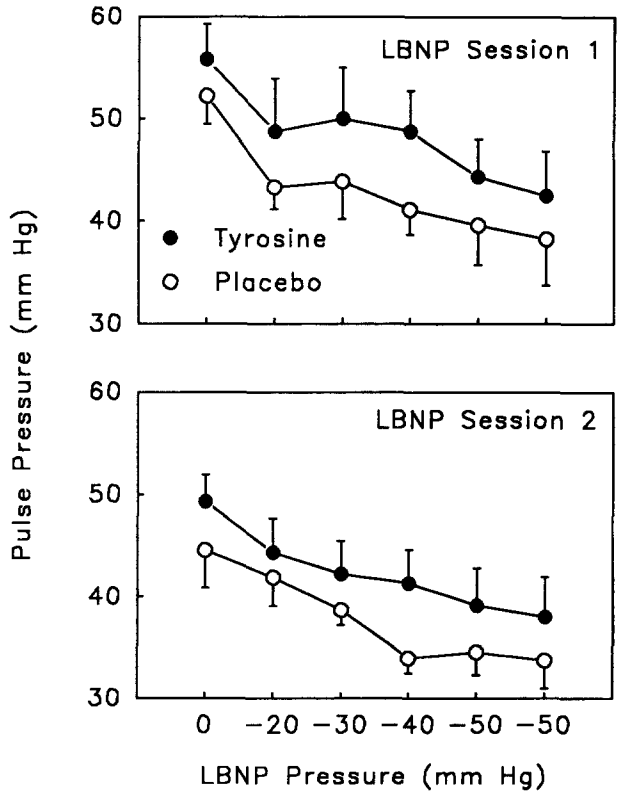

FIG. 1. Average pulse pressures from baseline through 6 min of LBNP at −50 mmHg (vertical bars are SEM, $N = 20$; data sampled at 3 min intervals).

LBNP exposure [$F(4,72) = 61.30, p < 0.0001$]. Contrasts comparing each heart rate with the average of subsequently measured heart rates were all significant ($p < 0.001$). The mean heart rate during LBNP session 1 was significantly faster [$F(1,18) = 26.87$, $p < 0.0001$] than that during LBNP session 2 (Δ +4.4 BPM). A significant [$F(1,18) = 8.28, p < 0.01$] treatment by LBNP session interaction was also found, indicating that the decrease in heart rate between LBNP sessions 1 and 2 was greater when the subjects ingested tyrosine (Δ −5.68 BPM) than when they ingested placebo (Δ −3.06 BPM). No other effects were significant. Separate analyses for the two LBNP sessions indicate that heart rate, averaged over placebo and tyrosine conditions, increased significantly over baseline during both sessions.

Event Related Potentials

Sixteen subjects produced artifact-free data for both treatment conditions during ERP session 1 while only eight subjects produced complete data during ERP session 2. Paired *t*-tests were used to compare the treatment effects of the P100-N100, P200-N200, and P300-N300 amplitudes for Channels Fz and Pz. A significant [$t(15) = 2.13, p < 0.05$] increase in channel Pz P300-N300 ERP session 1 amplitude of 1.41 μV occurred when subjects ingested tyrosine, as illustrated in Fig. 2. A similar though nonsignificant increase of 0.10 μV was observed in Fz P300-N300 amplitude during ERP session 1. No significant treatment differences were found among the P100-N200 or P200-N200 amplitudes of either ERP session or among the P300-N300 amplitudes for ERP session 2.

Cortisol Levels

The cortisol levels of ten subjects were analyzed (Fig. 3). Samples were randomly selected with the constraint that five received placebo first and five received tyrosine first. Mean plasma cortisol levels increased significantly over time from a baseline of 7.11 to 11.29 μg/dl at the end of testing [$F(4,32) = 3.48, p < 0.01$]. Contrasts comparing each cortisol level with the average of subsequent levels indicate that the baseline level was significantly less than the remaining four levels [$F(1,8) = 14.326, p < 0.005$]. There were no significant treatment, order, or interaction effects.

Performance Tasks

The number of correctly identified repeated digits on the Dual Task changed significantly during testing [$F(2,36) = 5.63, p < 0.007, N = 20$]. Contrasts indicate that the number of correctly identified repeated digits decreased significantly from 15.22 to 13.52 between the first and second test administrations [$F(1,18) = 23.56, p < 0.001$]. Treatment and testing order effects were not sigificant. No differences were found among subject estimates of the proportion of letters presented or the number of digits erroneously identified as repeated.

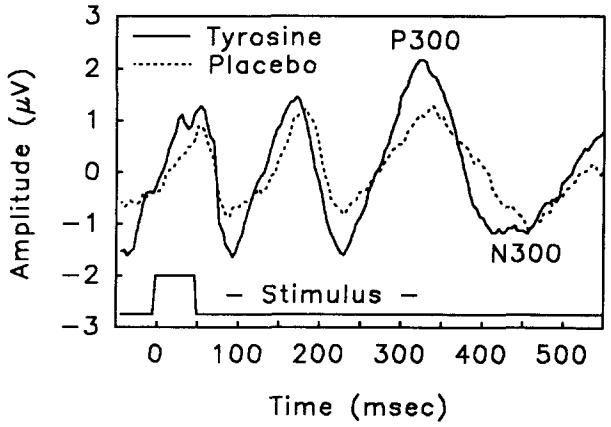

FIG. 2. Grand average event related potentials for Channel Pz ($N = 16$).

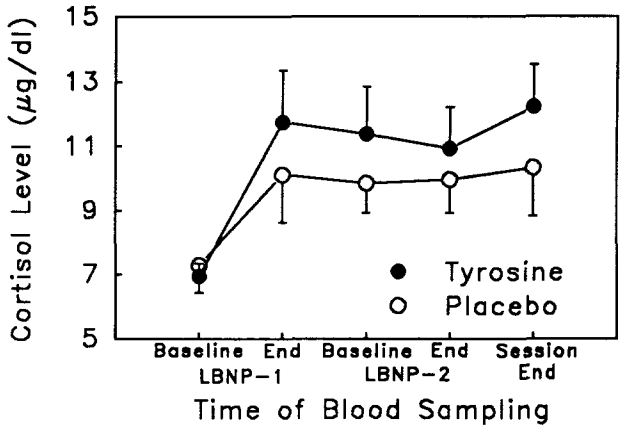

FIG. 3. Mean (SEM) plasma cortisol levels throughout testing ($N = 10$).

Four-choice RT data for two subjects and simple RT data for three subjects were lost through experimenter error, thus only 18 and 17 subjects, respectively, were included in these analyses. A significant increase (+12.2 msec) in Four-choice RT latencies occurred when subjects ingested tyrosine [$F(1,16) = 7.81$ $p < 0.01$]. The treatment by order interaction was also significant [$F(1,16) = 10.69, p < 0.005$] on this measure. Analysis of the data, divided on the basis of treatment order, indicates that the increase in RT latencies is attributable to the responses of subjects receiving tyrosine followed by placebo [$F(1,7) = 28.09, p < 0.001$] rather than to a treatment effect influencing both groups of subjects. Significant differences were found in the number of correct [$F(3,48) = 2.805, p < 0.05$] and incorrect [$F(3,48) = 3.55, p < 0.02$] responses over time. Contrasts indicate that the number of correct responses during the first Four Choice RT task was significantly greater than the average number of correct responses recorded during subsequent administrations of the task [$F(1,16) = 7.46, p < 0.02$]. The number of incorrect responses during the first Four Choice RT task was significantly less than the average number of incorrect responses recorded during subsequent administrations [$F(1,16) = 8.88, p < 0.01$]. No significant differences were found, in either measure, among the final three administrations of the task. No significant changes occurred in the number of premature or time-out errors. Significant treatment by order [$F(1,15) = 6.09, p < 0.02$] and treatment by time [$F(2,30) = 4.65, p < 0.02$] interactions were observed in simple RT latencies. These effects, however, were attributable to a baseline offset which occurred during the 0937 h test. No other differences were observed in simple RT latency or in the number of premature or time-out errors.

Profile of Mood States

There were no significant main effects attributable to Tyrosine treatment among the POMS scales. The subjects' responses on every POMS scale changed significantly as testing progressed. A significant treatment by time interaction was found among the Vigor/Activity scale responses [$F(4,72) = 3.11, p < 0.02$], in-

dicating that subjects felt less "Vigor" during the first half of a testing session and more "Vigor" during the second half when ingesting tyrosine, as compared to placebo ingestion. The treatment, order, and interaction effects of all other POMS factors were nonsignificant. Response averages indicate that the greatest Tension/Anxiety, Depression/Dejection, Anger/Hostility, Fatigue/Inertia, Confusion/ Bewilderment, and LBNP-stress occurred during LBNP session 2, and the least when testing began. Responses also indicate that subjects felt the greatest "Vigor" prior to LBNP session 1 and the least during LBNP session 2. These differences were significant at the $p < .05$ level, using Duncan's multiple range test.

LBNP Tolerance

Of the 20 subjects tested, nine subjects tolerated LBNP at −50 mm Hg for the full 30 min of testing. The remaining 11 subjects tolerated LBNP an average of 2.5 min longer when taking tyrosine than when taking placebo. Subjects also tolerated an average of 8.76 min more LBNP on the second day of testing than on the first, regardless of treatment. The LBNP tolerance treatment and order effects were nonsignificant, but a significant treatment by order interaction was found [$F(1,9) = 12.91$, $p < 0.005$]. The data were divided on the basis of treatment order and analyzed separately to interpret this significant interaction. Average (SEM) LBNP tolerance times by treatment and treatment order may be found in Table 2. Tyrosine treatment significantly increased the LBNP tolerance of subjects receiving placebo followed by tyrosine [$F(1,5) = 6.46$, $p < 0.05$] and decreased the LBNP tolerance of subjects receiving tyrosine followed by placebo [$F(1,4) = 13.50$, $p < 0.02$]. Paired t-tests were used to examine within order treatment effects. Tyrosine treatment increased the average LBNP tolerance of subjects receiving placebo followed by tyrosine from 18.61 to 32.53 min during LBNP session 1 [$t(5) = 4.20$, $p < 0.0085$], but had no effect during LBNP session 2. Tyrosine treatment decreased the LBNP session 2 tolerance of subjects receiving tyrosine on the first day of testing by an average of 9.4 min [$t(4) = 3.77$, $p < 0.02$], but had no effect on LBNP session 1 tolerances for this group.

TABLE 2

AVERAGE (SEM) LBNP TOLERANCE TIMES (MIN) BY TREATMENT AND TREATMENT ORDER

Group/Treatment	LBNP Session 1	LBNP Session 2	Test Day
Pla-Tyr ($N = 6$)			
Placebo	18.61 (0.72)	24.13 (2.57)	1
Tyrosine	32.53 (2.96)	31.18 (3.36)	2
Tyr-Pla ($N = 5$)			
Placebo	32.96 (3.94)	36.82 (1.95)	2
Tyrosine	28.37 (4.01)	27.33 (3.67)	1

DISCUSSION

The results of this study indicate that tyrosine treatment reduces physiological decrements attributable to LBNP. Measures which improved with tyrosine ingestion include LBNP tolerance, maintenance of pulse pressure, self-reported feelings of "Vigor," and channel Pz ERP P300-N300 component amplitudes during the "odd-ball" task.

Tyrosine treatment caused a significant increase in the duration of LBNP session 1 tolerance of subjects receiving placebo followed by tyrosine, but no difference in the duration of LBNP session 2 tolerance. Subjects receiving tyrosine followed by placebo had lower LBNP session 2 tolerances when ingesting tyrosine, relative to placebo, but no tolerance differences during LBNP session 1. The average LBNP session 2 tolerances for all subjects decreased by 0.47 min (N = 11) and the average LBNP session 1 tolerance increased by 5.5 min when taking tyrosine, relative to placebo. As indicated above, all subjects had higher LBNP tolerances on the second day of testing, regardless of treatment. The treatment by order interaction suggests that the subjects experienced more "novelty" stress on the first day of testing than on the second day. This may have resulted in complex interactions among LBNP tolerance, the level of stress, and the effects of tyrosine (see Table 2).

The results of this study are consistent with the earlier findings of Conlay et al. (5,6) that tyrosine administration is effective in increasing the blood pressures of hypotensive animals. These studies have shown that tyrosine treatment significantly increases the pulse pressures of subjects, indicating increased cardiac output in response to LBNP. A possible mechanism for this effect is that elevated plasma tyrosine levels lead to an increase in CA, from peripheral sympathetic neurons and adrenomedullar cells, and these, in turn, enhanced cardiac output. These results may also be due to changes in central CA levels. We attribute the significant treatment by time interaction to the same mechanism. While the observed decrease in heart rate between LBNP sessions 1 and 2 was not expected, it is consistent with our proposal that LBNP session 2 was more difficult than LBNP session 1.

The P300 component of the auditory ERP has been associated with timing or the "intensity" of an information-processing activity (9). 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 (16). More recent work indicates that P300 amplitude decreases as task difficulty increases (27). 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). It is, however, also possible that subjects may have attended and/or responded to more of the infrequently presented stimuli after ingesting tyrosine, causing an increase in average response amplitude that was unrelated to processing ability. While this is, to our knowledge, the first time a direct effect on cognitive activity has been associated with the ingestion of a neurotransmitter precursor, we interpret these results with caution and suggest that further work be done in this area.

Data from this human study, which indicate that there were no significant differences in plasma cortisol levels attributable to tyrosine treatment, neither support nor contradict animal research indicating that tyrosine blocks a stress induced rise in plasma corticosterone (31). The current observations are not in agreement with another study (1) which indicated that LBNP stress has no effect on cortisol levels. This difference in results may be due to the shorter LBNP exposure periods (20 min) of that study.

While responses on the Dual and Four-choice RT tasks, as well as the POMS scales, changed significantly with repeated LBNP exposure and sometimes changed with treatment order, there were no main effects for tyrosine treatment. This result could be attributed to factors other than an insensitivity of these measures to tyrosine administration. The catecholamine depletion experienced during LBNP exposure may not have been severe enough, in either duration or extent, to induce differential responding, attributable to the treatment, on the behavioral tasks used. It is more likely, however, that the activity and distractions

which surrounded each subject during testing, and were necessary to ensure his safety, may have obscured any behavioral task differences which might otherwise have been observed.

In summary, the results of this study indicate that tyrosine reduces some physiological decrements caused by LBNP exposure. During the first LBNP session, subjects could tolerate longer exposures to this stressor when they received tyrosine, relative to placebo, prior to exposure. Tyrosine pretreatment also allowed subjects to maintain significantly higher pulse pressures throughout exposure to LBNP, an indication of increased cardiac output. Channel Pz P300-N300 auditory ERP amplitudes were larger in subjects pretreated with tyrosine. We cautiously interpret this as indicating that tyrosine pretreatment enhances central, presumably cortical, information processing and may ameliorate the decrement in cognitive processing ability caused by LBNP. The pulse pressure, heart rate, behavioral task, and mood surveys indicate that subjects were more stressed by LBNP session 2 than session 1. Treatment order effects suggest that subjects reexposed to LBNP a few days after their initial exposure will manifest less psychological "novelty" stress and physiological change, than during their first exposure.

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