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USE OF TYROSINE OR FOODS TO AMPLIFY CATECHOLAMINE

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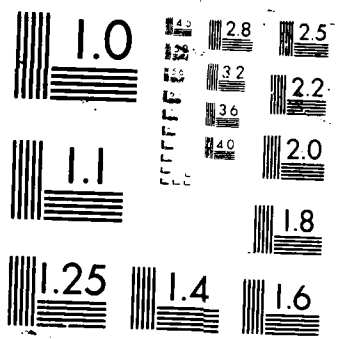
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This project examined the effects of supplemental tyrosine on catecholamine (CA) release and on various behaviors and brain functions thought to be mediated by CAs. It included studies on both human and experimental animals. The human studies focused on: a) development of a paradigm to produce short-term psychological stress in humans and evaluation of a treatment--administration of the A.A. tyrosine--that may mitigate some of the adverse behavioral and cardiovascular consequences of such stress; b) establishment of a collaborative research program with the USAF School of Aerospace Medicine to develop various nutritional and psychopharmacologic strategies to enhance performance in stressful environments. The animal studies focused on: a) developing a brain slice system in which tyrosine levels in the medium could affect release; b) examining the ability of supplemental tyrosine to suppress the neuro-chemical, behavioral and endocrine effects of experimental stress; c) determining whether particular stress situations altered plasma amino acid levels so as to affect tyrosine's availability to catecholaminergic neurons; d) determining whether tyrosine is (continued)

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toxic in doses that might be used to enhance CA release; e) setting up an isolated perfused retina experimental system in which tyrosine levels affect dopamine release from retinal amacrine cells; f) determining whether tyrosine-containing dipeptides constitute a useful source of circulating tyrosine.



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I. HUMAN STUDIES

A. Enhancement of Human Performance by Tyrosine During Psychological Stress

A number of animal studies (including several discussed below) have demonstrated that certain physiological and behavioral concomitants of acute stress can be ameliorated by administration of the amino acid tyrosine, given either acutely (in a single dose) or chronically in the diet. Tyrosine is the precursor of several central neurotransmitters involved in the regulation of the stress response -- especially norepinephrine. We are currently evaluating its beneficial effects in humans who are acutely stressed. Specifically, we are determining if tyrosine can mitigate the impaired performance that results when volunteers are exposed to acute psychological stress. Changes in vigilance, reaction time and mood state that occur as a consequence of stress are being measured. It will also be determined if the plasma concentration of various stress and other hormones such as cortisol, prolactin, and norepinephrine are altered by tyrosine administration. We will also determine whether tyrosine prevents or mitigates the increase in blood pressure that occurs as a consequence of psychological stress.

Research Plan

1. Experimental Approach: We are administering tyrosine and placebo to healthy volunteers, psychologically stressing them and testing selected aspects of behavior. We are also simultaneously monitoring blood pressure and heart rate. Blood samples are frequently withdrawn during the experimental session for assay of catecholamines, stress hormones and LNAA.

2. Methods: Thirty-two healthy males and females (in approximately equal numbers) will participate in this double-blind, crossover experiment. This sample size was selected based on significance levels of ALPHA=0.05 (two-sided) and BETA=0.20 (one-sided) to detect changes of approximately 0.75 standard deviations in performance. Each subject receives 100 mg/kg of tyrosine on one occasion and a matched placebo on another occasion. The substances are administered in capsule form in a randomized, counterbalanced order. There is a minimum washout period of five days between test sessions. After enrollment in the study, including a physical examination, subjects will participate in a 60 min. preliminary (non-treatment) session to allow them to practice the behavioral tests. Then, on two separate occasions, they take part in the actual testing sessions. On the morning of each testing session the subjects consume a standard breakfast meal (10 kcal/kg) at 8:30 a.m. The test session itself always begins at 11:00 a.m. and lasts until 3:30 p.m. Tyrosine or placebo are administered at 12:15 p.m., about one hour before the most stressful portion of the session.

To induce psychologic stress, a simulated public speaking procedure is being employed. This procedure has been used in many studies of stress and has been shown to produce both the subjective and

objective correlates of anxiety (Kemmer et al., 1986). Individuals who report that public speaking is not stressful for them, based on a standardized questionnaire, are excluded from the study. In addition to creating stress by this technique, an element of unavoidable aversive reinforcement is also included in the study as part of one of the performance tests to increase levels of stress. This is accomplished by using standard operant reinforcement procedures, specifically the loss of an anticipated, supplemental monetary reinforcement. Similar procedures have previously been employed in human studies of antianxiety drugs.

Concurrently, a series of behavioral tasks are administered to examine tyrosine's effects on mood and performance (Lieberman et al., 1983; 1986). Aspects of performance assessed include simple and choice reaction time, vigilance and complex information processing. Tests of these types of performance have been shown to detect the deterioration that occurs as a consequence of acute psychological stress. Mood-state is assessed using standardized self-report questionnaires. These questionnaires, as well as the endocrine dependent variables, should provide independent validation of the effectiveness of the stress procedures employed. In addition, we will also assess changes in spontaneous food consumption, particularly the ratio of protein/carbohydrate consumed following tyrosine or placebo administration.

B. Strategies to Enhance Human Performance

We have established, in collaboration with the Crew Performance Branch of the United States Air Force School of Aerospace Medicine (USAFSAM), a multi-disciplinary research program to develop new strategies to sustain and enhance human performance and health in stressful environments. The long range objective of this program is to attempt to apply recent scientific advances in neurophysiology, biochemistry and biological psychology to behavioral problems associated with sustained, stressful USAF operations. Initial human studies with the neurotransmitter precursor tyrosine are now being planned. In animals, tyrosine administration appears to protect against stress-induced deficits in noradrenergic neurotransmission. Such deficits appear to be associated with many of the undesirable effects acute stress has on behavior, notably a lack of responsiveness to the environment and a general inability to function (Lehnert et al., 1984a, b; Brady et al., 1980). When tyrosine is given to acutely stressed animals, either as a single dose or in the diet, it substantially reduces the adverse effects of several types of acute stress on various behaviors. Some preliminary observations from a study conducted with environmentally stressed soldiers suggest that such beneficial effects may generalize to humans.

In our initial human study at Brooks AFB, we will determine if administration of tyrosine can improve the ability of volunteers to maintain alertness and vigilance while they are undergoing simulated air combat maneuvers in a centrifuge.

II. ANIMAL STUDIES

A. Relationships Between the Frequency and Duration of Neuronal Firing; the Availability of Tyrosine; and Catecholamine Synthesis and Release:

1) Using the superfused rat caudate slice model described in our previous interim reports, we have shown that the tyrosine concentration needed to sustain dopamine release (i.e., to keep the S2/S1 ratio equal to unity) varies with the number of times the neuron is depolarized: When caudate slices were subjected to trains of electrical pulses (60 mA, 2 ms, 20Hz) for 30 secs (600 pulses) a 20 micromolar tyrosine concentration in the superfusion medium was needed to have the amount of dopamine released by the second pulse train equal to the amount released by the first train of pulses (i.e., S2=S1). In contrast, when the caudate slices were stimulated for 90 seconds (1800 pulses) a tyrosine concentration of 40-50 micromolar was required (see F1 (The total amount of dopamine released by 600 pulses equaled 3.92 pmol/mg; that released by 1800 pulses was 7.91 pmol/mg.) These observations affirm that, when the neuron is called upon to release dopamine for a sustained period (i.e., 30-90 secs), its ability to do so becomes very tyrosine-dependent. The concentrations of tyrosine needed to sustain dopamine release are on the order of those present in the blood, and higher than those usually present in cerebrospinal fluid.

2) When the slices are electrically-stimulated (and releasing dopamine) in a medium that lacks tyrosine, their actual tyrosine concentrations diminish markedly (i.e., by about 50%, after two 90-second trains of stimuli). In contrast, tissue levels of other large neutral amino acids that are not catecholamine precursors (e.g., valine; phenylalanine; tryptophan) are unaffected (see Figure 2). These observations suggest a second mechanism for the precursor-dependence of dopamine release, i.e., the fact that, if supplemental precursor is not supplied, the catecholaminergic neurons run out of tyrosine. (The other mechanism involves the activation by phosphorylation of tyrosine hydroxylase that occurs when the neurons fire frequently. This activation changes the enzyme's kinetics so that it becomes limited not by the concentration of cofactor, as is usually the case, but by the extent to which it is saturated with tyrosine.) Of course, catecholaminergic neurons in the intact animal's or person's brain and periphery are continuously perfused with blood, which contain tyrosine; hence, it is unlikely that nerve terminals are actually as depleted of tyrosine as occurs in our superfused slices. However, tyrosine is an unusually lipid-soluble amino acid, only poorly soluble in water, which diffuses into and out of cells relatively slowly; hence, it seems not unlikely that some depletion of the amino acid does occur within catecholaminergic terminals, when nerves fire frequently and continuously.

B. Neurochemical and Behavioral Consequences of Acute, Uncontrollable Stress: Effects of Dietary Tyrosine

Acute, uncontrollable stress increases norepinephrine (NE) turnover in the rat's brain (thereby depleting NE) and diminishes the animal's subsequent tendency to explore a novel environment. We determined whether supplemental dietary tyrosine could prevent some of these changes. Rats given a control diet or diets enriched with tyrosine or tyrosine plus valine were exposed to tail-shock stress or to no stress over a 60-min period. Exposure to the stress caused an increase in NE turnover, decreasing NE and increasing 3-methoxy-4-hydroxy-phenylethylene glycol sulfate (MHPG-SO₄) concentrations within the locus coeruleus, hypothalamus and hippocampus. No changes were detected in serotonin (5-HT) levels or turnover. Behavioral deficits following the stress were observed using measures of locomotion and of exploration in a novel open-field environment: stressed animals displayed much less spontaneous motor activity, hole-poking or frequency of standing on their hind legs than control animals. Animals receiving the tyrosine-enriched diet displayed neither the stress-induced depletion of NE nor the behavioral depression. These preventive effects of tyrosine were abolished by co-administration of valine, a large neutral amino acid that competes with tyrosine for transport across the blood-brain barrier. Since tyrosine alone in animals not subjected to stress, did not change NE turnover nor the behaviors studied, our observations affirm that catecholaminergic neurons respond to the precursor amino acid only when they are physiologically active. Supplementary tyrosine may be useful therapeutically in people exposed chronically to stress.

We have affirmed, using a larger series of animals, that pretreatment with supplemental tyrosine (provided, in this case, by putting free tyrosine into the diet), not only "protects" against stress-induced depletion of brain norepinephrine, and against the behavioral deficits that follow stress, but also suppresses the stress-induced increase in adrenocortical secretion (Figure 3) (displayed, in rats, as an increase in plasma corticosterone levels). This effect may allow tyrosine to be used to minimize the secondary consequences of severe stress, when it is appropriate and necessary to do so.

C. Alteration of Plasma Amino Acid Levels

Tyrosine and tryptophan are the circulating precursors for the catecholamine neurotransmitters and serotonin. The administration of these amino acids, or dietary manipulations which change their plasma concentration or plasma "ratio" (their concentration divided by the summed concentrations of the other large neutral amino acids) have been shown to accelerate the synthesis and release of their neurotransmitter products within physiologically active neurons. Since exercise could then influence neurotransmission by altering plasma amino acid concentrations, we measured these concentrations in thirty-seven subjects completing the

Boston Marathon. Marathon running increased the plasma concentrations of tyrosine and phenylalanine, as well as their plasma "ratios"; no changes were noted in the plasma tryptophan nor tyrosine ratios. The metabolic alterations induced by marathon running may therefore increase tyrosine's availability for neuro-transmitter synthesis.

D. Tyrosine Toxicity in Rats

We have initiated studies to determine whether the kinds of tyrosine doses that we administer to rats (e.g., to suppress stress responses) might conceivably be associated with toxicity. For these studies, supplemental tyrosine is added to the rats' diets, in doses considerably greater than those required to treat stress responses; we then monitor food consumption, weight gain, the general appearance of the animals, and brain levels of tyrosine, the catecholamines and their metabolites, and related compounds. Since it is known that tyrosine toxicity requires concurrent protein deprivation, our test diets include some (5-10% casein) known to provide inadequate protein for rats.

In general, we fail to find any evidence of gross toxicity. Minor changes, such as reductions in food consumption, do not occur unless the animal's diet contains both far too much tyrosine (5%) and far too little protein (5-10%). (To put the tyrosine dose into perspective, a 5% protein diet to which no supplemental tyrosine had been added would contain only 0.2% tyrosine.) The ability of a food containing supplemental (5%) tyrosine to raise brain tyrosine levels varies in inverse proportion to its protein content: hence, the rise in brain tyrosine is less than half as great, when the food contains 20% protein (the amount in standard chow diets) as when it contains only 5% protein. (This probably reflects the action of the other large neutral amino acids in the dietary protein, i.e., their ability to compete with tyrosine for transport across the blood-brain barrier.) The 5% tyrosine, 5% protein diet causes a small but significant rise in brain levels of the norepinephrine metabolite MHPG sulfate; other diets have no effect, and none of the diets affects brain levels of norepinephrine, dopamine, or the dopamine metabolite DOPAC (see tables). These observations provide reassurance that tyrosine toxicity will not be a problem in any of the circumstances in which supplemental amounts of the amino acid are likely to be given, for their neurological effects, to humans.

E. The Effects of Light on Retinal Dopamine in the Rat

The rat retina contains several classes of neural cells for the reception and processing of photic information. One of these cell types, the amacrine cells, is located in the inner nuclear layer, and the cells have intricate synaptic arborizations in the inner plexiform layer of the retina. Approximately 10% of all amacrine cells synthesize and release the neurotransmitter, dopamine (DA). Light activates tyrosine hydroxylase (Iuvone et al. 1978), the rate-limiting enzyme in dopamine synthesis in brain neurons and retinal amacrine cells, accelerating its synthesis and release. Our laboratory has shown that retinal levels of tyrosine, the amino acid precursor of dopamine, may also influence retinal dopamine turnover (Gibson et al., 1983). The function of the dopaminergic amacrine

neurons is unclear; Ames and Pollen (Ames et al., 1969) found that dopamine applied to the rabbit retina altered the spontaneous and evoked activity of ganglion cells. In rats, retinal dopamine depletion alters the timing of visual evoked potentials (Dyer et al., 1981).

F. Use of Parenteral Dipeptides to Increase Serum Tyrosine Levels and to Enhance Catecholamine-Mediated Neurotransmission

The use of intravascular tyrosine (TYR) to enhance catecholamine release in hemorrhagic shock and other cardiovascular diseases, or as a constituent of nutrient mixtures used for total parenteral nutrition, is limited by the amino acid's unusually poor solubility in water. We have thus examined the ability of various TYR-containing dipeptides, which are more water soluble than the amino acid, to raise plasma TYR; to restore blood pressure in hemorrhaged hypotensive rats; and to lower blood pressure in spontaneously hypertensive rats (SHR). TYR-PRO, TYR-ALA, and TYR-TYR given intra-arterially (i.a., 12-25 mg/kg) all caused prolonged increases in plasma TYR. The increase after TYR-PRO was dose-related in the range of 12.5 - 50 mg/kg. All of the dipeptides also caused significant elevations in blood pressure among hypotensive rats. Moreover, given intraperitoneally (100 mg/kg) all of them also lowered blood pressure in SHR's. These observations suggest that TYR-containing dipeptides may be useful in some clinical situations where maintaining or elevating plasma TYR levels would be desirable.

The major limitation to the use of parenteral TYR for treating severe hypotension stems from its limited water-solubility (0.453 g/liter at 25 °C, Table I). For this reason and because of the widely-held view that TYR is always a nonessential amino acid, TYR is also usually omitted entirely, or included in only low concentrations, in solutions prepared for total parenteral nutrition. We therefore tested the ability of more water-soluble TYR-containing dipeptides to serve as substitutes for TYR. These dipeptides would be useful clinically if: a) they were rapidly cleaved to their constituent amino acids; when administered intravascularly; b) they then caused a sustained increase in plasma TYR concentrations; and c) they thus enhanced catecholamine synthesis in, and release from, activated neurons. Experiments were performed to determine the ability of the TYR-containing dipeptides, L-tyrosyl-L-proline (TYR-PRO), L-tyrosyl-L-alanine (TYR-ALA), L-alanyl-L-tyrosine (ALA-TYR), and L-tyrosyl-L-tyrosine (TYR-TYR) to: 1) increase plasma TYR; 2) increase BP in hypotensive rats; and 3) decrease BP in hypertensive rats.

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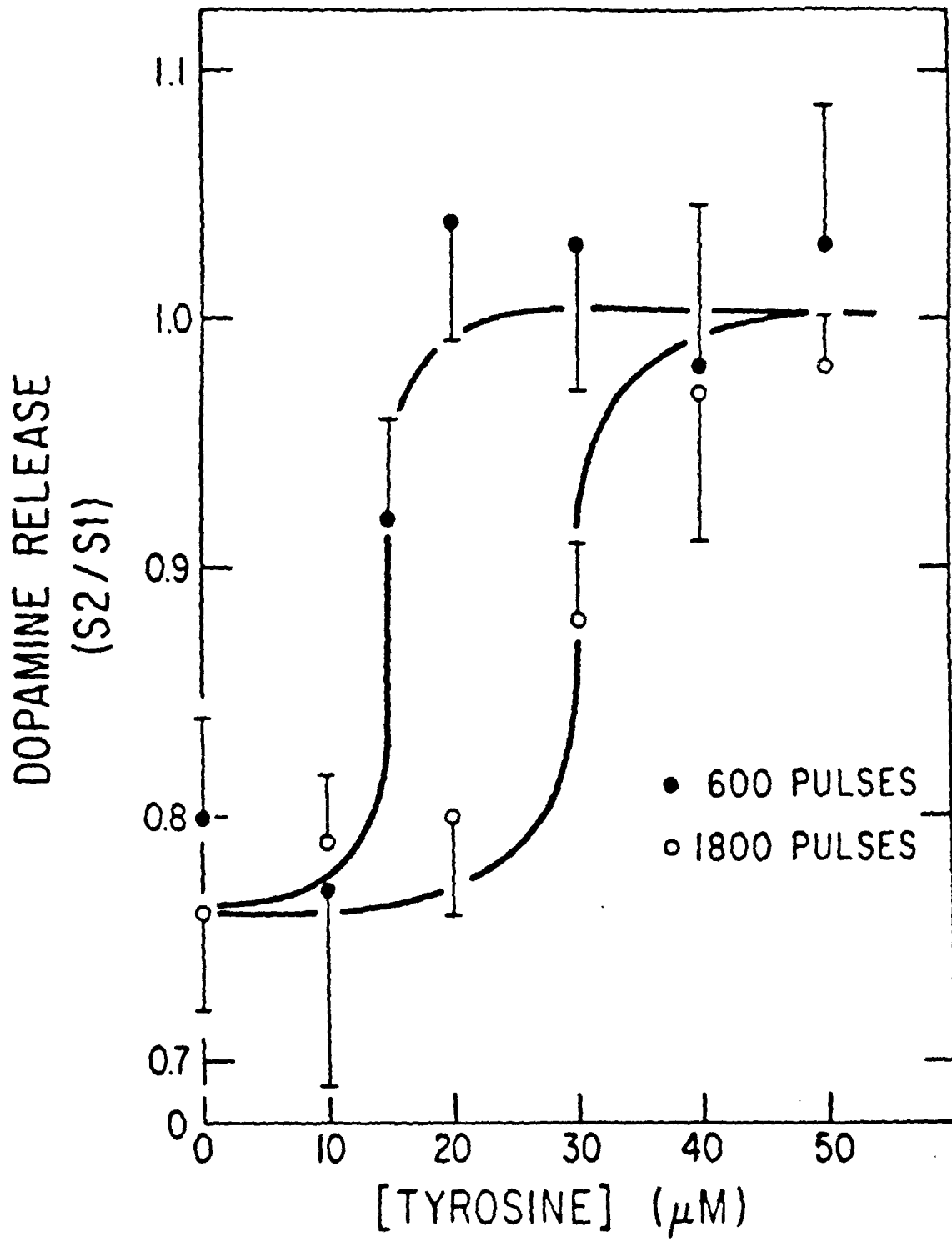


Figure 1

Effect of Stimulation on LNAA Levels in Striatal Slices

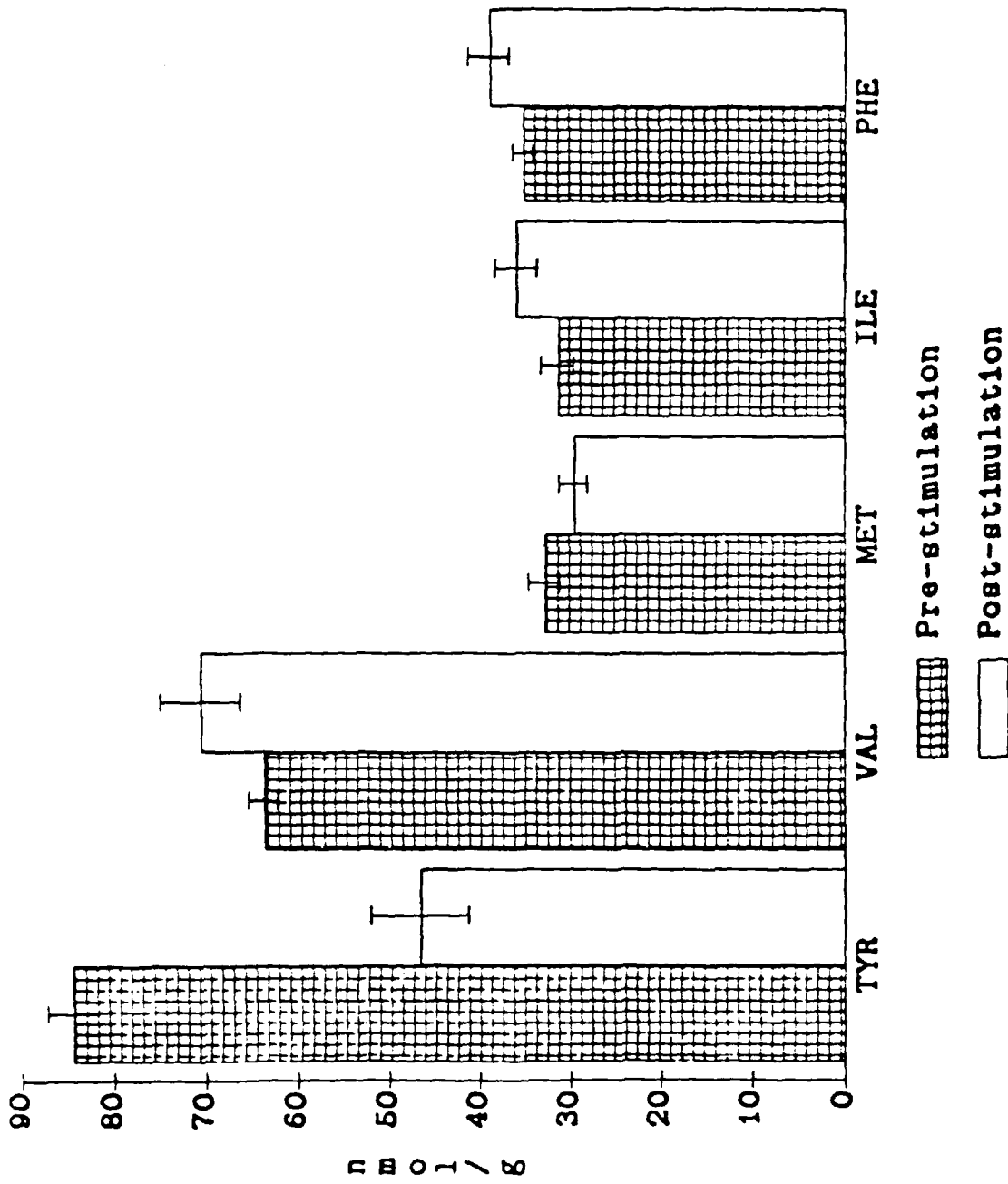


Figure 2

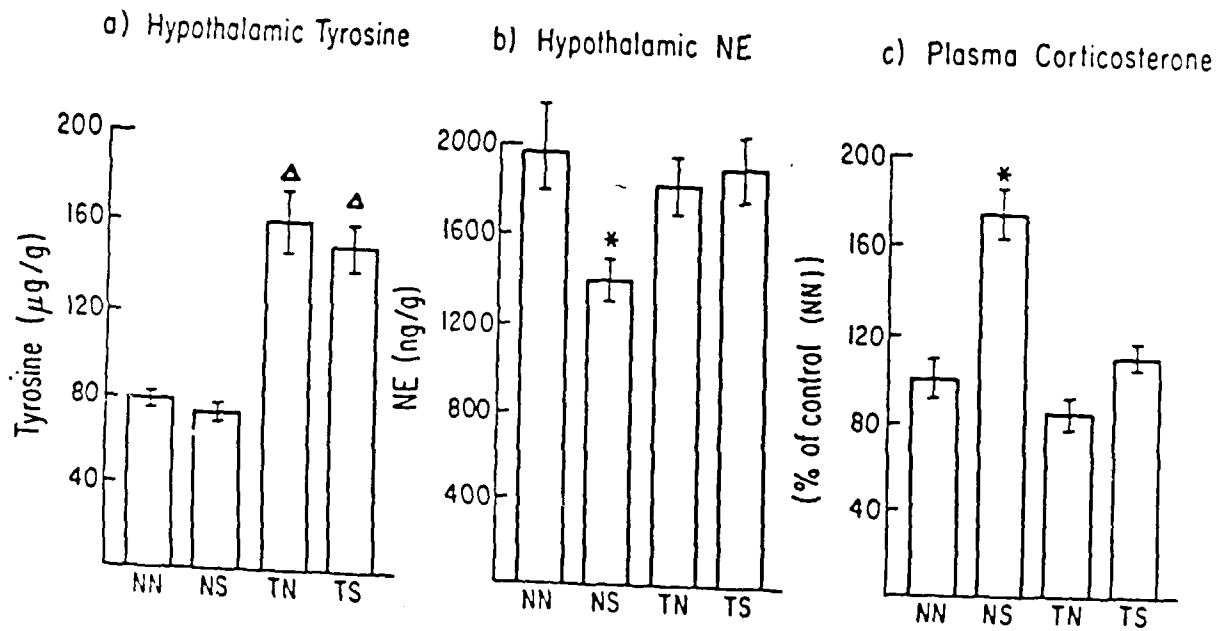


FIG. 3

Effects of tyrosine and stress on hypothalamic tyrosine and norepinephrine (NE) and on plasma corticosterone. Units are: tyrosine ($\mu\text{g/g}$), NE ($\mu\text{g/g}$); corticosterone (percentage of mean control (NN) value of 37.4 $\mu\text{g}/100\text{ ml}$).

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