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BRIEF COMMUNICATION

Effects of Dietary Tyrosine, Phenylalanine, and Tryptophan on Aggression in Mice

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THURMOND, J. B., S. M. LASLEY, A. L. CONKIN, AND J. W. BROWN. *Effects of dietary tyrosine, phenylalanine, and tryptophan on aggression in mice.* PHARMAC. BIOCHEM. BEHAV. 6(4) 475–478, 1977. Dietary amino acid regimens designed to enhance catecholaminergic and serotonergic functioning were found to differentially affect territorial-induced attacks in mice. Male albino mice were maintained on a semi-synthetic 12% casein protein diet for 2 weeks, then switched to diets modified by the addition of a 4% L-amino acid supplement, or 4% casein (control). Measures of aggressive behavior and open-field locomotor activity were obtained before and after the dietary supplements were administered. Resident mice fed supplements of L-tyrosine displayed a marked increase in the number of attacks on intruders and shorter attack latencies, but their locomotor activity was unaffected. L-phenylalanine supplements alone or in combination with L-tyrosine reduced the latency to attack and increased motility but did not affect the number of attacks. As a whole, the group of animals fed L-tryptophan showed no changes in aggression or motility.

Amino acids Diet Mice Aggression Locomotion

IT IS well accepted that norepinephrine (NE), dopamine (DA), and serotonin (5-hydroxytryptamine, 5-HT) are putative CNS neurotransmitters associated with emotional behavior and locomotor activity [4]. NE and DA are synthesized in the brain from tyrosine, 5-HT from tryptophan [3]. Both amino acids are obtained from dietary protein. Generally, it has been assumed that variations in the quantity or balance of these dietary amino acids within the limits of adequate nutrition will have little influence on behavior. However, it has been found that rats fed dietary supplements of L-phenylalanine, the metabolic precursor of tyrosine, or L-tryptophan exhibit significant changes in motor activity and maze performance [5, 6, 8]. More recently, it has been shown that a positive correlation exists between the dose of tyrosine or tryptophan injected IP and the brain levels of these amino acids and the neurotransmitters synthesized from them [10].

This suggests that the balance of these amino acids in nutritionally adequate diets may provide a new and significant means of altering behavior. The study reported here was designed to test this hypothesis. Using a method devised in our laboratory [9], male CF-1 mice fed semi-synthetic diets containing 12% casein protein were tested for aggressive behavior and motor activity before and after amino acid supplementation of the diet. Based on current hypotheses concerning the influence of catecholaminergic and serotonergic systems on behavior, one might assume that tyrosine supplementation would stimulate greater activity and aggression, whereas tryptophan supplementation would have an opposing effect. A phenylalanine supplement also should favor aggression, not only because it is the dietary essential amino acid precursor of tyrosine, but because of its presumed ability – based on *in vitro* experiments – to inhibit tryptophan hydroxylase, the enzyme catalyzing the first step in serotonin synthesis from tryptophan [7]. Marked changes in behavior were indeed noted, and these concepts are partially supported by the results.

METHOD

Animals

CF-1 mice obtained from Carworth Farms at 84 days of age were housed 5 per cage at 21°C room temperature and maintained on a 12 hr light-dark cycle for the duration of the study.

Behavioral Measurements

A complete description of the apparatus used for producing and measuring territorial aggression has been published [9]. Briefly, the test animal (resident mouse) takes up lone residence for 24 hr in a 60 cm square box containing a small 30 cm high tower in the center and access through a 12 cm long tube to a standard mouse cage with food, water, and bedding. After this interval, a naive intruder mouse is introduced. Typically, the resident mouse

attacks the intruder within the first several min of the test, either after the intruder climbs down the tower or by climbing the tower to reach the intruder. Most of the resident's aggression displayed toward the intruder takes place during the first 15 min of the test. The latency (in min) to first attack and the number of attacks over a 20 min observation period are used to quantify the level of aggression. In addition to measuring aggressive behavior, locomotor behavior was assessed with use of a 60 cm square open-field marked off into 16 equal squares [2].

Dietary Regimens

One hundred of the 84 day old mice were randomly designated as residents and placed immediately ad lib on water and a semi-synthetic diet (all diet materials were obtained from ICN, Cleveland, OH) of the following composition: 12% casein protein, 5% corn oil, 70% corn starch, 2% cellulose, 4% Salt Mixture XIV, 2.2% Vitamin Diet Fortification Mixture, 4.8% dextrose. After maintenance on this diet for 2 weeks, the animals were randomly assigned to one of 5 groups (N = 20 per group). Four experimental groups received the semi-synthetic 12% casein diet supplemented with: (a) 4% L-tyrosine, (b) 2% L-tyrosine plus 2% L-phenylalanine, (c) 4% L-phenylalanine, or (d) 4% L-tryptophan. The fifth group of animals received a supplement of 4% casein to provide a total of 16% balanced protein. This served as a control group. The supplements replaced equal weights of dextrose; thus, all diets were isocaloric. The dietary materials for the resident mice were thoroughly mixed with enough water to make a batter, then cooked at relatively low temperature of 105°C for 40 min. The result was a cream-colored cake which could be easily cut into pieces for purposes of feeding. Another 200 mice, designated as intruders, were maintained ad lib on Rat/Mouse Purina Chow and water.

Procedure

The different dietary conditions were arranged in staggered fashion so that all 20 animals in a given condition could be tested after exactly 2 weeks on the 12% casein control diet and again after 1 week on the supplemented diet. All 4 cages of diet-fed mice in a particular condition were transferred to the testing room containing the 5 identical test boxes for assessing aggressive behavior and the open-field box for assessing locomotor behavior. The light cycle in the testing room was identical to that where the mice were housed, and was governed by two 100 W red bulbs superimposed for 12 hr with the brighter fluorescent lights of the room. Locomotor behavior was measured 3 hr following onset of the dim phase of the light cycle by gently placing each diet-fed mouse in the center of the open-field apparatus and counting the number of squares crossed for 2 min. Immediately after the open-field test, a spot of blue liquid food dye was rubbed on the mouse's head for identification during the aggression test, and the mouse was placed on the tower in the middle of the test box. On the next day, exactly 24 hr later, the 5 intruder mice were removed from their cage. Each was placed on the tower in a test box, and the ensuing aggression was recorded during a 20 min observation period.

RESULTS

The effects of the dietary supplements on locomotion in the open-field and on weight gain are shown in Fig. 1. The

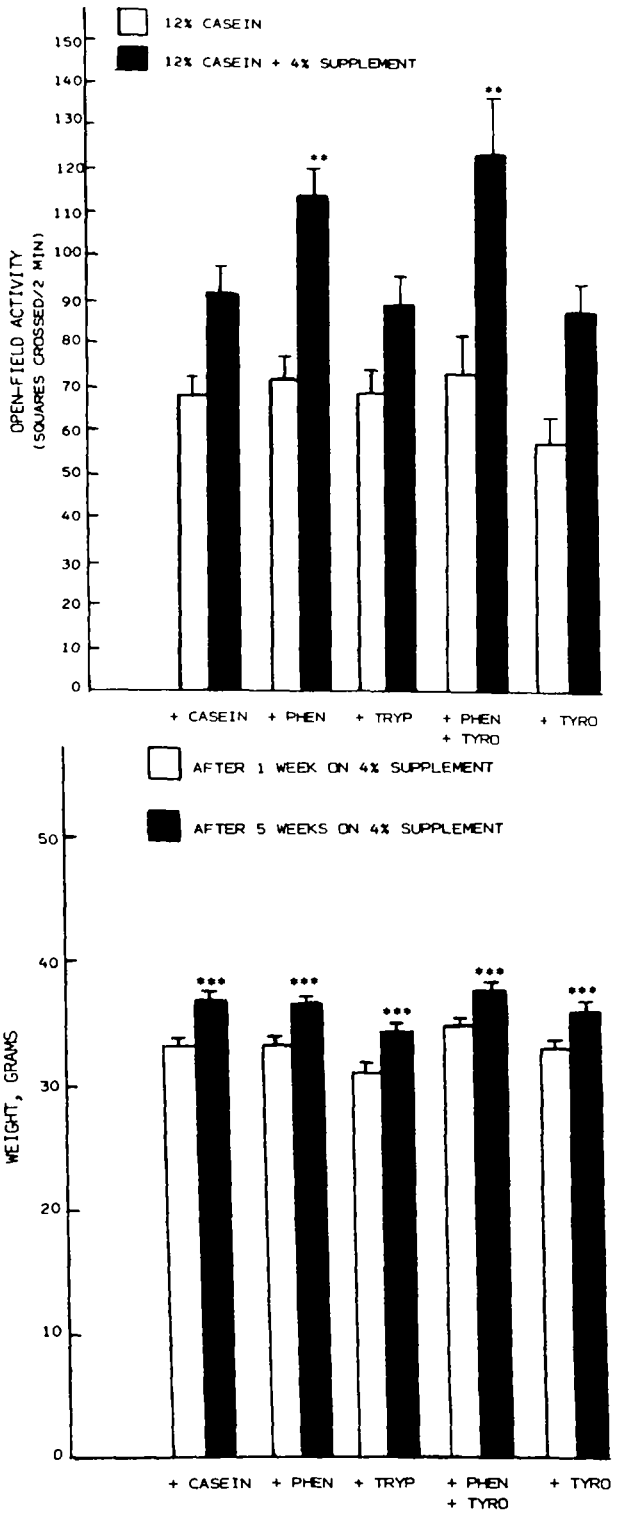

FIG. 1. Effect of dietary supplements on open-field activity (top graph) after 2 weeks on casein and 1 week on casein plus the amino acid supplement, and on weight gain (bottom graph). Each bar represents the mean and S. E. M. for 20 resident mice. The *t*-test for independent groups (two-tailed) was used to compare the open-field activity after 1 week on the casein supplement and 1 week on the amino acid supplements. The *t*-test for correlated means was used to compare weights of the same mice after 1 week and 5 weeks on the dietary supplements. $*p<0.05$, $**p<0.02$, $***p<0.001$.

mice in each group gained approximately the same amount of weight over a 5 week period of maintenance on the diets ($t>6.75$, $df = 18$, $p<0.001$ in each case) indicating that the supplements produced no ill effects. Compared to the casein supplemented control group, an increase of about 30% in open-field locomotor behavior resulted from supplements of L-phenylalanine alone ($t = 2.84$, $df = 38$, $p<0.02$) or in combination with L-tyrosine ($t = 2.41$, $df = 38$, $p<0.02$). No significant changes in open-field locomotion was observed due to the supplements of L-tryptophan or L-tyrosine alone, compared to the casein supplemented control group.

In Fig. 2 are presented the effects of the dietary supplements on aggression. Mice fed supplements of L-phenylalanine alone or in combination with L-tyrosine did not increase their number of attacks significantly but did attack with a shorter latency ($t = 4.13$, $df = 19$, $p<0.001$ for L-phenylalanine; $t = 2.56$, $df = 19$, $p<0.05$ for L-phenylalanine and L-tyrosine). These were mild effects, however, compared to increases in the number of attacks resulting from the L-tyrosine supplement alone, which produced a marked increase in number of attacks ($t = 5.18$, $df = 19$, $p<0.001$) as well as a decrease in attack latency ($t = 4.00$, $df = 19$, $p<0.001$). The L-tryptophan supplement resulted in no significant changes in aggressive behavior on the average. However, closer examination of the data revealed that individually some of the animals displayed marked increases in aggression whereas others showed substantial decreases.

DISCUSSION

In the present study, animals fed casein diet supplement with L-tyrosine became much more aggressive than those fed either L-phenylalanine alone, or in combination with L-tyrosine. L-phenylalanine produced only moderate increases in aggression, perhaps because of its competition with tyrosine for active transport into the brain [1]. We have no explanation for the greater increase in open-field locomotor behavior produced by L-phenylalanine supplements compared to that produced by L-tyrosine.

The L-tryptophan supplement produced inconsistent effects on aggression, a finding which has been obtained using smaller supplements of L-tryptophan in other studies of dietary effects on aggression in our laboratory (to be published). It should be noted that, although the dietary supplement of L-tryptophan administered in the present study constituted an unusually large amount of this particular amino acid, none of the animals showed ill effects after 5 weeks on the diet, and locomotor behavior patterns in the open-field did not differ from those of the casein controls.

ACKNOWLEDGEMENTS

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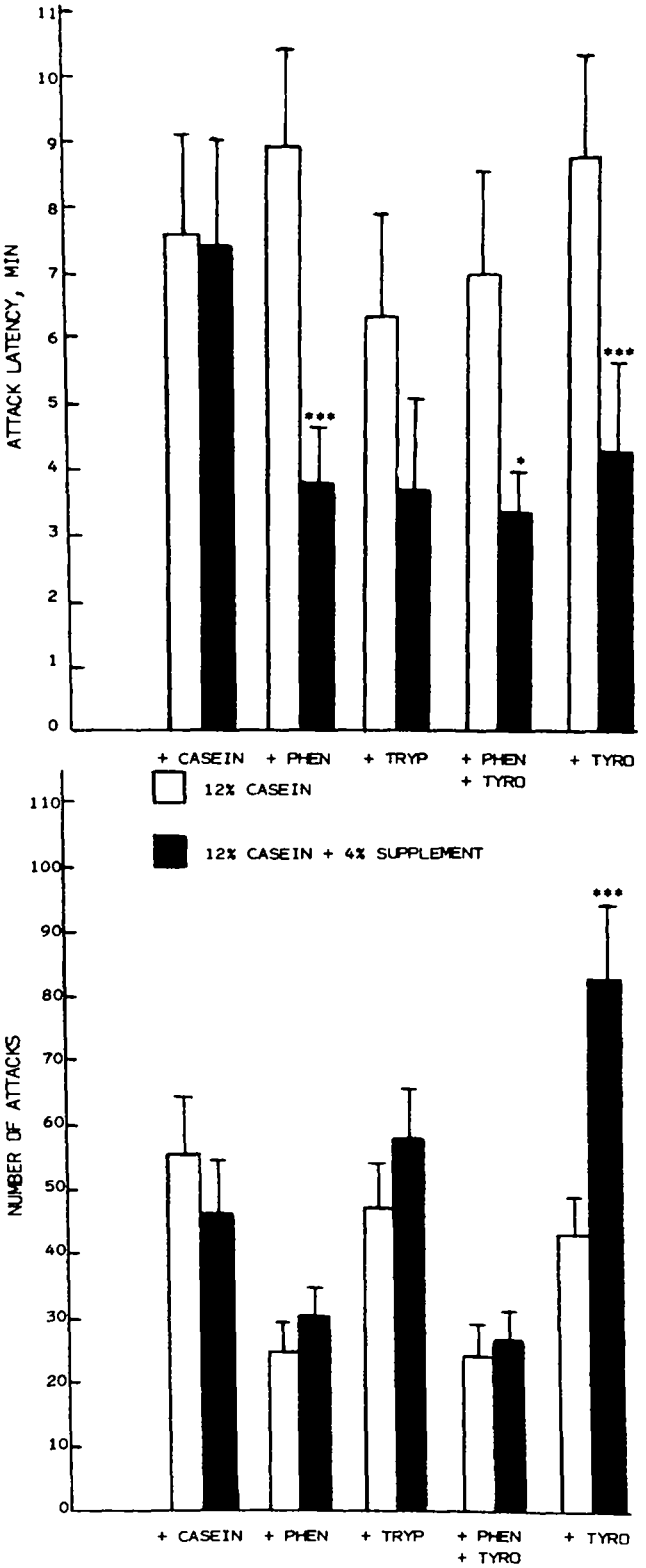

FIG. 2. Effect of dietary supplements on aggression after 2 weeks on casein and 1 week on casein plus the amino acid supplement. Each bar represents the mean and SEM for 20 resident mice. The t-test for correlated means (two-tailed) was used in comparing effects of supplements to the diet on behavior of the same mice observed during successive sessions. $*p<0.05$, $**p<0.02$, $***p<0.001$.

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