Pharmacology Biochemistry & Behavior, Vol. 12, pp. 667–674. Printed in the U.S.A.

Behavioral and Neurochemical Effects of Dietary Tyrosine in Young and Aged Mice Following Cold-Swim Stress

KATHLEEN BRADY, JOHN W. BROWN AND JOHN B. THURMOND

Departments of Psychology, and Chemistry, Neuropsychopharmacology Program, University of Louisville, Louisville, KY 40208

Received 23 October 1979

BRADY, K., J. W. BROWN AND J. B. THURMOND. *Behavioral and neurochemical effects of dietary tyrosine in young and aged mice following cold-swim stress.* PHARMAC. BIOCHEM. BEHAV. 12(5) 667–674, 1980.—The effects of dietary supplements of L-tyrosine on aggressive behavior, locomotor activity, and brain neurochemical changes were assessed in young and aged mice following cold-swim stress. Male CF-1 mice, ages 3 months and 21 months, were maintained on a semi-synthetic basal diet for one week, pretested for aggressive behavior and locomotor activity, then switched to diets modified by the addition of tyrosine or casein (control). After one week on the diet supplements, half of the mice were stressed by cold water swim and all were again subjected to behavioral testing. Members of each group were sacrificed for analysis of amino acids and monoamines in brain tissue and corticosterones in blood. It was found that tyrosine supplementation induced a marked increase in aggressive behavior in young, nonstressed mice but not in aged mice. Stress decreased the aggressiveness of both young and aged mice, and tyrosine prevented this stress-induced decrease in both groups. Young mice exhibited no changes in locomotion as a function of stress or diet. However, tyrosine prevented decreases in locomotion observed on second testing of both stressed and nonstressed older mice. The effect of stress was to lower levels of brain norepinephrine (NE) and dopamine (DA) in both young and aged mice. Tyrosine supplementation increased brain tyrosine and DA in both groups. Brain serotonin levels were lower in the aged mice compared to the younger ones, and this was associated with relatively higher concentrations of 5-hydroxyindoleacetic acid. It appears that tyrosine supplementation was effective in reducing the effects of stress in the aged animals, possibly by virtue of its relationship to catecholamine metabolism.

Precursor amino acids Diet supplement Tyrosine Stress Aging Motor activity
Aggressive behavior Norepinephrine Dopamine Serotonin

SIGNIFICANT reductions in brain catecholamine levels and turnover occur with aging. Comparing senescent and mature C57BL/6J male mice, Finch found a 25% reduction in striatal DA concentrations and a 50% reduction in turnover of both DA and NE [17]. Synthesis in brain of ^{3}H-DA or ^{3}H-NE from ^{3}H-tyrosine or ^{3}H-DOPA injected IP was reduced 20–80% in the older animals. No age-dependent differences were found in the uptake of precursors. Comparing 4 months old and 25 months old male rats, Miller *et al.* reported a 50% reduction in hypothalamic DA and NE concentrations [35]. Similar findings have been reported for humans [5].

In general the plasma level of corticosterone does not change significantly with age, but adrenocortical responsiveness to stress appears to decline somewhat in both mice and rats. Finch *et al.* reported that plasma levels of corticosterone (and most other hormones) did not change with age in C57BL/6J and DBA/2J male mice, but after cold stress or challenge with ACTH, plasma steroid levels showed smaller increases in older mice [18,19]. These findings have been confirmed by others [12, 25, 40]. Glucocorticoids or stress reactions causing their production are known to stimulate the induction of certain enzymes such as tyrosine aminotransferase (TAT) in the liver [21]. This induction is delayed in aging rodents, although no significant age-related impairment in TAT induction was noted when corticosteroids were injected IP [19]. Roth has observed that *in vitro* binding of glucocorticoids declines significantly with aging in rat brain and muscle but not in liver [42].

Finch has speculated that age-related neurochemical changes originating at the brain-pituitary level may provide the basis of the observed decline in the stress response as measured by induction of liver enzymes [16]. Stress generally results in increased release of ACTH from the hypothalamus [34]. The roles of specific neurotransmitters in the control of this process have not yet been fully elucidated, but there is a considerable body of evidence suggesting that catecholamines are important [3, 26, 37, 44]. The feedback inhibitory effects of adrenal steroids on ACTH release may also be mediated by catecholaminergic mechanisms [27,31].

A number of investigators now have shown that brain monoamine levels can be raised by increasing dietary levels of tryptophan, tyrosine, or phenylalanine [11, 15, 22]. Research conducted in our laboratory has confirmed these neurochemical changes and demonstrated correlative alterations in behavior resulting from the different dietary regimens. Using male CF-1 mice, we found that dietary supplements of tyrosine increased brain levels of tyrosine and DA and at the same time increased aggressive behavior and

locomotor activity [52,53]. Others have reported that aggression in mice is increased by high doses of L-DOPA [57].

Little is known about the behavioral effects of stress in aging animals, although one would anticipate that recovery from stress as determined by measurements of active behavior would show a deficiency in comparing aged with young adult animals. Such a deficiency might be related to differences in adrenocortical response which may in turn be related to reductions in brain catecholamine levels and turnover occurring with age. Hence, the research described here was designed to determine the effect of dietary tyrosine supplements on both neurochemical and behavioral changes taking place in aged mice subjected to stress.

METHOD

Animals

Male albino CF-1 mice were obtained from Carworth Farms (Wilmington, MA). Young mice (2 months old) and older retired breeders (12 months old) were housed in specially constructed cages designed to reduce damage from aggressive encounters. These cages consisted of ten compartments with small holed (6 mm square) hardware cloth partitions which allowed some contact (sniffing, touching) but prevented fighting. At the time of experimentation, the young mice were 3 months old whereas the aged mice were 21 months old. Both groups of mice were housed under identical conditions (5 per polypropylene cage) during the period of the experiments. The laboratory was maintained at a temperature of 21°C, with a light cycle of 12 hr on, 12 hr off governed by three 100 W red bulbs superimposed for 12 hr with bright fluorescent lights. All procedures were performed during the first half of the dim portion of the cycle, between 12 noon and 5 p.m.

Diet

All mice were given free access to water and Rat/Mouse Purina Chow until initiation of the experiment. At this time, all mice designated as "residents" in behavioral tests were given free access for one week to water and a semi-synthetic basal diet (all diet materials were obtained from ICN Pharmaceuticals, 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. The animals were then assigned to one of eight groups (N=20 per group). During the second week, four of these groups received the 12% casein diet supplemented with 4% L-tyrosine, and the other four groups received a supplement of 4% casein (to provide a total of 16% protein having the same balance of amino acids as the basal diet). The supplements replaced equal weights of dextrose; thus, all diets were isocaloric. All dietary materials were thoroughly mixed with enough water to make a batter, then oven-dried at 105°C for 40 min. The result was a cream-colored cake which could be easily cut into pieces for purposes of feeding. Other mice, designated as "intruders" in the behavioral test, were continued on Rat/Mouse Purina Chow. Animals were weighed weekly to determine if any major physical differences occurred as a result of the dietary regimens.

Behavior

A complete description of the apparatus used for producing and measuring territorial aggression has been published [51]. Briefly, the test animal (resident mouse) takes up lone residence for 24 hr in a 60 cm square box containing a 30 cm high tower in the center and has 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 placed on the tower and aggressive behavior is observed. Typically, the resident mouse intercepts and attacks the intruder within the first several min of the test. 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. An attack is defined as a bout of activity lasting 1–5 sec during which at least one bite is inflicted.

In order to obtain a measure of recovery from the cold water stress, locomotor activity was measured by using the motimeter described by Knoll [28]. In this device the animal moves over aluminum contact plates mounted 4 mm apart in a clear Plexiglas box (testing cage) and a count is recorded for every passage between two plates. Each mouse was tested singly in one of five identical testing cages thus permitting the automatic recording of locomotor activity.

Stress

The stress apparatus consisted of a 20×48 cm Plexiglas tank, 23 cm deep, partially filled with water to a depth of 11 cm. Water temperature ranged from 2° to 6°C. Forty-eight minutes prior to observing aggressive behavior, half of the animals on each diet supplement (4% tyrosine and 4% casein) were cold water stressed for 3 min. Each animal was removed from its behavioral test chamber and placed gently in the tank of water and permitted to swim for the required period. Immediately after removal from the tank, rectal temperature was recorded by inserting a telethermometer probe 3.5 cm into the rectum. Nonstressed animals were treated identically, except that they were placed for 3 min in a clean polypropylene mouse cage during this interval. This control procedure was used rather than placing the animals into ambient temperature water since Stone [48] has shown that the behavioral and neurochemical effects of acute swim stress are due to hypothermia rather than forced swimming per se.

Procedure

Two sets of behavioral measures were recorded, the first to provide a baseline for all animals prior to treatment, and the second set to determine the diet and stress treatment effects. One week after mice were placed on the 12% basal casein diet, the first set of behavioral tests was administered. On the basis of these tests, the young and aged animals were randomly divided into eight groups, with the constraint that each group showed comparable levels of aggression and locomotor activity. Two groups of young and two groups of aged mice were then fed the 4% tyrosine supplement for one week; the remaining groups were fed the 4% casein supplement. Following this, one group on each diet supplement was cold-water stressed whereas the other was not, then all groups were subjected to a second series of behavioral tests.

Biochemical Determinations

Separate groups of young and aged animals (N=5 per group) were maintained on dietary supplements and treated identically except that they were not subjected to the second set of behavioral tests. At the same time that these tests would have been initiated, the animals were sacrificed by

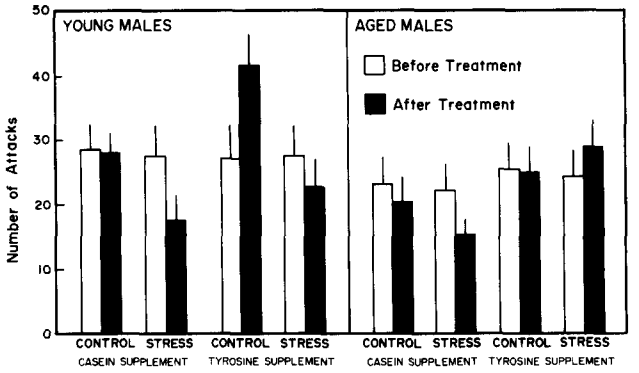

FIG. 1. Comparison of the effects of cold-swim stress on number of attacks in young and aged male mice. Animals were maintained on a 12% casein basal diet for one week (before treatment) then switched to supplements consisting of the basal diet plus 4% casein or 4% L-tyrosine. Results are given as mean with SEM of 20 determinations.

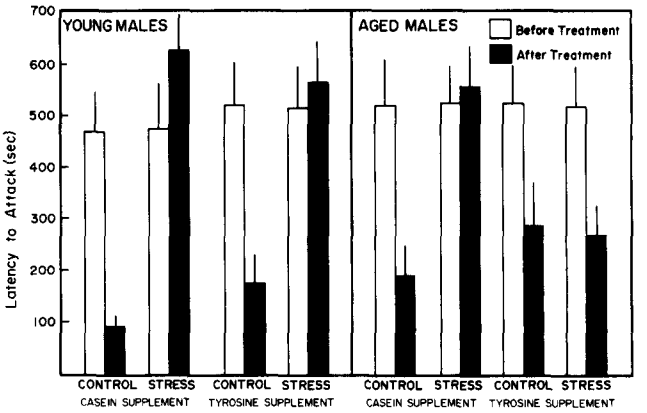

FIG. 2. Comparison of the effects of stress on latency to attack in young and aged mice maintained on the casein and tyrosine dietary supplements. Results are given as mean with SEM of 20 determinations.

cervical dislocation, their brains rapidly removed and homogenized in ten volumes of cold acidified n-butanol. Serotonin (5-HT),5-hydroxyindoleacetic acid (5-HIAA), DA and NE in whole brain were determined spectrophotofluorometrically according to the method of Chang [6] and Cox and Perhach [7]. Amino acids were also analyzed, tyrosine (TYR) according to the method of Wong *et al.* [54] as modified by Phillips [38], and tryptophan (TRP) according to Denckla and Dewey [10].

Blood was collected at the time of sacrifice, and plasma corticosterone levels were determined by the method of Silber *et al.* [46].

Statistical Analyses

The data were analyzed with use of multivariate analyses of variance; for the behavioral data, analyses of covariance were conducted using the pre-test baseline data obtained for aggression scores and activity measures as covariates [36]. Where the overall analysis of treatment effects indicated statistical significance, planned orthogonal comparisons were made to determine more specifically the interactive effects of age, diet, and stress on the dependent measures recorded [30].

RESULTS

Weight Gain

The young and aged mice in each of the diet supplemented groups gained approximately the same amount of weight during the period of maintenance on the diets. No differences in weight gain were noted between animals on the "cake" diets used in these studies and those fed either Rat/Mouse Purina laboratory chow or powdered semi-synthetic diets of the same composition used in earlier studies. The young mice ranged between 25–41 g in weight before administration of the diet and 31–42 g following diet treatment; for aged mice, the range was 33–48 g before diet treatment and 34–50 g afterwards.

Behavioral

The multivariate analysis of covariance indicated significant effects due to the type of diet: $F(3,147)=9.39$, $p<0.001$, stress, $F(3,147)=12.36$, $p<0.001$, age, $F(3,147)=2.65$, $p<0.05$, and age×stress interaction, $F(3,147)=3.96$, $p<0.01$. Univariate tests provided by the analysis indicated sigcant effects on number of attacks due to diet, $F(1,149)=14.10$, $p<0.001$, stress, $F(1,149)=9.05$, $p<0.01$, age ×stress interaction, $F(1,149)=8.51$, $p<0.01$; effects on latency to attack due to stress, $F(1,149)=35.97$, $p<0.001$, age×stress interaction, $F(1,149)=9.58$, $p<0.01$, diet×stress interaction, $F(1,149)=6.19$, $p<0.05$; effects on locomotor activity due to diet, $F(1,149)=9.93$, $p<0.01$.

Figure 1 shows the effects of diet, stress and age on the number of attacks inflicted by resident (treated) mice on intruder mice before and after treatment. The results of planned comparisons made following significant effects obtained based on the overall analyses of variance indicated the significance of specific treatment effects. In Fig. 1 it can be seen for the young nonstressed animals that the number of attacks increased markedly after treatment with the tyrosine supplemented diet, $F(1,4)=8.04$, $p<0.05$. However, the number of attacks in the young tyrosine supplemented mice decreased after stress relative to the nonstressed group, $F(1,4)=14.86$, $p<0.05$. By comparison, the effect of stress was more severe when no tyrosine supplement was provided (i.e., in mice fed the 4% casein supplement), but the difference here did not reach statistical significance. Whereas aggression decreased following stress in the aged males fed the casein supplement, a slight increase in number of attacks was evidenced by the aged animals on the tyrosine supplement, $F(1,4)=14.06$, $p<0.05$. Compared to the young nonstressed mice, which showed a marked increase in aggression after the tyrosine supplement, the number of attacks following tyrosine in the aged control animals did not change.

The data in Fig. 2 showing the effects of diet, stress, and age on latency to attack generally are consistent with those obtained for number of attacks, as would be expected since these two measures of aggression tend to co-vary inversely with changes in levels of aggression. Comparison of control and stress groups shows that latency to attack in the young animals increased following stress in both the tyrosine supplemented group, $F(1,4)=14.68$, $p<0.05$, and the casein supplemented group, $F(1,4)=27.7$, $p<0.01$. In the aged mice, latency to attack increased following stress when the casein supplement was provided, $F(1,4)=10.41$, $p<0.05$, but la-

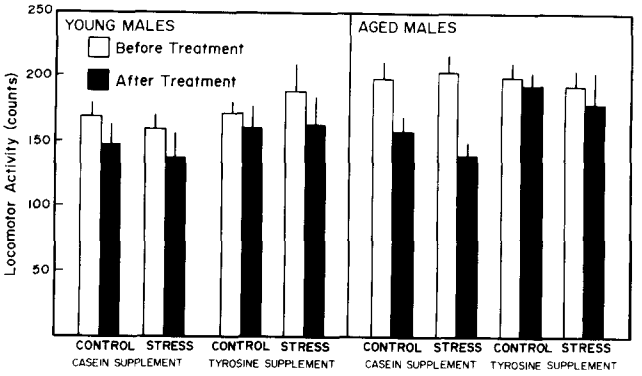

FIG. 3. Comparison of the effects of stress on locomotor activity in young and aged mice maintained on the casein and tyrosine dietary supplements. Results are given as mean with SEM of 20 determinations.

tency did not change in those fed the tyrosine supplement.

Figure 3, showing the effects of the treatments on locomotor activity, reveals that motor activity decreased slightly between the before and after treatment measures for all conditions, possibly reflecting habituation to the motimeter tests. Levels of motor activity in the young stressed animals after treatment can be seen in Fig. 3 to be approximately the same as those for control (nonstressed) mice, indicating that 2 hr was sufficient for these animals to evidence recovery from the cold water swim. Aged mice fed the tyrosine supplement, in both the control and stressed groups, evidenced smaller drops in motor activity after treatment than did the aged control animals under the same conditions. This finding is significant as indicated by the results of the multivariate analysis of variance which yielded an overall effect of diet on locomotor activity, $F(1,149)=9.93$, $p<0.01$.

Body Temperature

Rectal temperatures recorded for animals that were not cold-swim stressed averaged 38.4°C. Mean temperature for the young and aged mice immediately following stress were 24.9°C and 28.6°C, respectively ($p<0.001$). Thus, immediately following the cold-swim, the drop in body temperature was less for the aged mice than for the young ones, a finding no doubt related to the insulation provided by the fat deposits typical of older, heavier mice. At the time of aggression testing, rectal temperatures for both the young and aged mice had returned to approximately 37.0°C and did not differ significantly.

Because of the difference in temperature between the two age groups observed immediately after stress, a multivariate analysis of covariance was conducted on all of the behavioral data using the rectal temperature of each animal measured immediately after stress as a fourth covariate (along with the three pretreatment behavioral measures of number of attacks, latency to attack, and locomotor activity). The results of this analysis indicated that the main effects of the diet factor on the posttreatment behavioral measures remained significant, $F(3,146)=9.40$, $p<0.001$, as did also the main effect of age, $F(3,146)=3.5$, $p<0.05$. There was no change in the significant effects obtained as a result of the planned comparisons when rectal temperature was included as a covariate.

Plasma Corticosterone

Although the cold swim provides a rather severe stress as borne out by behavioral and neurochemical data (see below), no statistically significant changes were noted in plasma corticosterone levels in the young mice; plasma concentrations for young animals averaged 10.8 μg/100 ml $\pm$ 3.3. Plasma corticosterone levels rose significantly following cold swim in the aged mice, from 13.6 μg/100 ml $\pm$ 5.5 to 18.7 μg/100 ml $\pm$ 5.9, $t(27)=2.36$, $p<0.05$.

Neurochemical

Table 1 summarizes data on brain catecholes, indoles and amino acids in the eight groups of mice tested. The data were analyzed with use of multivariate analysis of variance procedures in which the three independent variables (diet, stress, and age) were manipulated and six dependent measures (NE, DA, 5-HT, 5-HIAA, TYR, and TRP) were analyzed. A set of planned comparisons, identical to those used for determining effects of behavior, were also used in this analysis.

The multivariate analysis of variance indicated significant effects of diet, $F(6,27)=14.94$, $p<0.001$, stress, $F(6,27)=7.23$, $p<0.001$, and age, $F(6,27)=8.42$, $p<0.001$. Univariate tests provided by the analysis indicated significant effects on NE due to stress, $F(1,32)=41.53$, $p<0.001$; DA due to diet, $F(1,32)=36.19$, $p<0.001$, and stress, $F(1,32)=5.75$, $p<0.05$; 5-HT due to age, $F(1,32)=21.32$, $p<0.001$; 5-HIAA due to age, $F(1,32)=16.43$, $p<0.001$; TYR due to diet, $F(1,32)=77.78$, $p<0.001$, and age, $F(1,32)=5.01$, $p<0.05$; TRP due to stress, $F(1,32)=6.93$, $p<0.05$.

There were no significant effects revealed by the univariate tests for either the diet or age factor in the analysis of NE. The stress induced decrease in levels of NE shown in Table 1 for the aged mice is significant for both the tyrosine-fed group, $F(1,4)=27.24$, $p<0.001$, and the group fed the casein supplement, $F(1,4)=12.84$, $p<0.05$. The higher concentrations of DA shown in Table 1 for animals fed the tyrosine supplement was significant for young nonstressed mice compared to those on the casein supplement, $F(1,4)=9.35$, $p<0.05$, and between the aged groups of mice under the same conditions, $F(1,4)=12.56$, $p<0.05$. Also, the aged stressed animals fed tyrosine had higher DA levels than their stressed counterparts on the casein supplement, $F(1,4)=12.83$, $p<0.05$.

It is apparent from the data of Table 1 that the young animals evidenced higher brain concentrations of 5-HT across all treatment conditions compared to the aged mice. The mean 5-HT level for the four groups of young animals was 0.904 μg/g whereas that for the aged animals was 0.648 μg/g, hence the significant effect of age on 5-HT indicated by the univariate test noted above. The main contribution to this effect can be seen to come from a comparison between the 5-HT levels of the young and aged animals who were fed the tyrosine supplement and subjected to stress, $F(1,4)=13.71$, $p<0.05$. The age factor also significantly affected 5-HIAA, as noted above, the aged animals having higher levels of this 5-HT metabolite than the younger animals. In particular, examination of Table 1 shows that the mean level of 5-HIAA was considerably higher for the aged animals on the tyrosine supplement who were not stressed than the young animals under the same treatment conditions, $F(1,4)=12.67$, $p<0.05$.

As might be expected, treatment with the tyrosine supplement raised the levels of TYR in both age groups and under stressed and nonstressed conditions (Table 1). The

TABLE 1

EFFECTS OF DIET SUPPLEMENT, STRESS, AND AGING ON CONCENTRATIONS OF NOREPINEPHRINE (NE), DOPAMINE (DA), SEROTONIN (5-HT), 5-HYDROXYINDOLEACETIC ACID (5-HIAA), TYROSINE (TYR), AND TRYPTOPHAN (TRP) IN MOUSE BRAIN. RESULTS ARE GIVEN AS MEAN μg/g WET WEIGHT ± SD (N=5 FOR EACH DETERMINATION)

Group	NE	DA	5-HT	5-HIAA	TYR	TRP
Young tyrosine nonstressed	0.501 ±0.025	2.264 ±0.974	0.746 ±0.113	0.787 ±0.321	56.861 ±24.116	3.467 ±0.429
Young tyrosine stressed	0.416 ±0.043	1.577 ±0.464	1.003 ±0.337	0.820 ±0.119	43.734 ±12.031	3.168 ±0.740
Young casein nonstressed	0.530 ±0.087	1.270 ±0.260	0.931 ±0.166	0.743 ±0.270	14.182 ±4.253	2.824 ±0.495
Young casein stressed	0.463 ±0.055	0.978 ±0.616	0.939 ±0.146	0.814 ±0.089	17.141 ±6.942	3.763 ±0.238
Aged tyrosine nonstressed	0.614 ±0.070	2.416 ±0.232	0.633 ±0.160	1.272 ±0.369	31.253 ±5.295	2.936 ±0.530
Aged tyrosine stressed	0.396 ±0.070	2.132 ±0.628	0.584 ±0.070	0.979 ±0.098	47.082 ±14.001	3.486 ±0.400
Aged casein nonstressed	0.537 ±0.091	1.264 ±0.199	0.710 ±0.141	1.075 ±0.167	12.407 ±1.689	2.803 ±0.489
Aged casein stressed	0.387 ±0.035	0.968 ±0.104	0.665 ±0.149	0.947 ±0.047	9.194 ±1.076	3.370 ±0.713

results of planned comparisons among the different treatment groups indicated significantly higher levels of brain tyrosine for young animals fed the tyrosine supplement whether stressed, $F(1,4)=13.87$, $p<0.05$, or nonstressed, $F(1,4)=35.74$, $p<0.01$, and for aged animals when stressed, $F(1,4)=28.17$, $p<0.01$; the lesser increase observed for aged nonstressed animals on the tyrosine supplement nearly reached significance as well, $F(1,4)=6.97$, $p<0.06$. Comparisons between the young and aged mice concerning these diet effects indicated that the young mice fed tyrosine who were not stressed had significantly higher levels of brain tyrosine than the group of aged mice receiving identical treatment conditions, $F(1,4)=12.87$, $p<0.05$.

As indicated above, a significant overall effect on TRP was obtained due to the effects of stress, and Table 1 shows that levels of brain tryptophan in the stressed animals exceeded those of the nonstressed animals for all groups except the young mice fed the tyrosine supplement. However, only the increase in brain tryptophan seen in the young stressed animals fed the casein supplement is significantly higher than those who were not stressed, $F(1,4)=7.93$, $p<0.05$.

DISCUSSION

Our data showing increased levels of brain tyrosine and DA in both young and old animals following tyrosine supplementation confirm and extend the findings of Wurtman *et al.* [56] and Gibson and Wurtman [20]. These investigators have demonstrated that tyrosine supplementation in conjunction with a central L-aromatic decarboxylase inhibitor resulted in an increase in DOPA, the immediate precursor of DA. Some age-related differences in brain tyrosine also were noted, but these may be of marginal significance in that Finch [16] reported no differences in tyrosine uptake ability. Brain NE levels showed no significant changes as a result of the diet treatment. This was not unexpected, since others have shown that NE levels are closely regulated by feedback on tyrosine hydroxylase [55].

Prior work in our laboratory has demonstrated that maintaining male albino mice on a semi-synthetic 12% casein protein diet supplemented with 4% tyrosine results in a marked increase in aggressive behavior [52]. Those findings were replicated here, although only in the younger nonstressed mice did the tyrosine supplement produce a large increase in levels in aggression. Also, the tyrosine supplement was somewhat effective in reducing the detrimental effects of the cold swim stress on behavior in the young mice. Administration of the stressor to the young mice produced comparable drops in brain NE concentrations in the tyrosine and casein supplemented groups, but brain TYR and DA levels were higher for mice given the tyrosine supplement.

The tyrosine supplement produced rather different behavioral results in the aged mice. It had only a mild stimulating effect on aggressive behavior but had surprisingly strong effects in reducing the debilitating effects of the cold swim stress on aggression and motor activity. Indeed, the aged animals fed the tyrosine supplement evidenced slightly higher levels of aggression (in terms of number of attacks) after the stress than the control group of tyrosine-fed animals that were not stressed.

Much of the literature suggests an age-related vulnerability of the DA system [17], but no differences in DA or NE brain concentrations were observed in the present study between the nonstressed young and aged mice. This may have resulted from the use of whole brain analyses which would not reflect a decline in a specific brain region. It was anticipated, however, that the cold-swim stress would exacerbate age-related changes in the catecholamines (CA).

While the stress resulted in a decrease in CA in both age groups, very marked drops in NE levels occurred in the aged animals following stress. Others have shown that a cold stress-induced depletion of NE is much more severe in 7 months old than in 3 months old rats [41]. Moreover, the time required for restoration of NE levels in the older animals was twice as long as that for the younger group.

Brain TRP levels generally were higher in stressed than nonstressed animals. Stress has been shown to result in an increase in 5-HT synthesis in the rat brainstem-mesencephalon [49] and various other brain regions [4]. Bliss [4] has suggested that while foot shock accelerates the metabolism of 5-HT in rats, it is rapidly resynthesized so that decrements are rarely found; however, the accelerated metabolism of serotonin with foot shock was indicated by increases in 5-HIAA. Curzon [9] suggested a possible mechanism for a stress-induced decrease in 5-HT levels. Serotonergic mechanisms have been implicated in the regulation of adrenocorticotropin hormone (ACTH). Injection of cortisol results in a decrease in brain levels of 5-HT and 5-HIAA within a matter of hours. Thus, there may be a feedback mechanism whereby 5-HT stimulation facilitates corticosterone production which in turn decreases 5-HT. Azimitia and McEwen [1] reported that tryptophan hydroxylase, the enzyme catalyzing the rate determining step in 5-HT synthesis, is regulated by corticosterone. Thus, stress-induced increases in corticosterone may result in a decrease in the synthesis of 5-HT.

Although the cold swim provided a rather severe stress, no significant changes in the concentrations of plasma corticosterone were noted in the young mice. Data from prior studies indicate that the plasma corticosterone response to stress in mice is quite variable, depending upon both the strain of mouse and type of stress. For example, following cold exposure, C57BL/10J mice have shown an increase in corticosterone response, CBA/J animals exhibit no response, and BALB/cJ mice show a significant reduction [43]. Levine and Treiman [29] have shown that not only does the magnitude of the plasma corticosterone response to stress vary but the time of this response differs greatly among genetic strains of mice.

One might speculate on a possible peripheral mechanism for the increase in tryptophan. Curry and Curry [8] reported the finding in an *in vitro* study (perfused rat pancreas) that hypothermia directly inhibits insulin secretion and that there exists a direct relationship between tissue temperature and the total quantity of insulin released. They suggest that rewarming could possibly result in a hypersecretion of insulin. Fernstrom *et al.* [14] have shown that insulin increases total serum tryptophan and decreases serum concentrations of other amino acids which compete with it for uptake by brain. The result is that insulin secretion increases brain tryptophan and 5-HT concentrations.

Serotonin has been implicated in temperature control. Studies in mice after prolonged exposure to cold have shown a brief initial increase in 5-HT followed by a marked decrease [24]. Others have reported that intra-hypothalamic injection of 5-HT in the rat resulted in hyperthermia when low doses were employed [39]. Barofsky and Feldstein [2] postulated that 5-HT was hyperthermic in the mouse but that its metabolite, 5-hydroxytryptophol, was hypothermic. Others have obtained results which support this idea [45].

Neurochemically, the young and aged mice differed regarding brain levels of 5-HT and 5-HIAA as a function of the kind of diet supplement and the effects of stress. Aged animals taken as a whole had relatively lower levels of 5-HT and higher levels of 5-HIAA. When these results are considered in relation to changes in brain CA concentration with stress, they suggest that all three monoamines acting in concert may have been responsible for the behavioral changes observed. Other investigators have postulated a reciprocal relationship between CA and 5-HT functions to account for affective aggressive behavior in mice [32]. There also is evidence that a number of behaviors in rodents may be related to the ratio of brain DA and NE concentrations. Increased shock-induced fighting in rats has been found due to treatment with 6-hydroxydopa, which reduced brain NE but left brain DA unaffected [50]. In another study, rats administered PCPA in combination with 6-hydroxydopa showed higher levels of aggression than animals receiving 6-hydroxydopa alone [13]. Although brain NE and 5-HT were lowered substantially, DA was changed little. It has been suggested that a ratio which takes into account the DA÷(NE+5-HT) relationship might best account for the facilitation of aggression. The significant increases in 5-HIAA which we observed in the aging mice may be taken to indicate, as others [47] have reported, an enhanced metabolism of 5-HT. When the ratios of DA÷(NE+5-HIAA) were calculated, these turned out to be highly correlated with the mean aggression scores for both young and aged groups of animals, $r=.889$, $t(14)=7.05$, $p<0.001$. The aged mice fed the tyrosine supplement had the highest levels of NE and 5-HIAA of any groups in the experiment. This may account for the failure of tyrosine to facilitate aggression in these older animals, although DA concentrations were high. The DA÷(NE+5-HIAA) ratio also was lower in the aged casein supplemented animals, and so was the mean level of aggression. On the other hand, the aged tyrosine fed animals showed a high level of aggression following the cold-swim stress compared to the young animals on tyrosine; associated with this enhanced behavioral effect was an increased DA÷(NE+5-HIAA) ratio which resulted mainly from the relatively low brain levels of NE and 5-HIAA in this group.

REFERENCES

1. Azimitia, E. C. and B. S. McEwen. Corticosterone regulation of tryptophan hydroxylase in the midbrain of the rat. *Science* **166:** 1274–1276, 1979.
2. Barofsky, I. and A. Feldstein. Serotonin and its metabolites and their respective role in the production of hypothermia in the mouse. *Experientia* **26:** 990–991, 1970.
3. Bhattacharya, A. and B. Marks. Effects of pargyline and amphetamine upon acute stress responses in rats. *Proc. Soc. exp. Biol. Med.* **130:** 1194–1198, 1969.
4. Bliss, E. L., J. Ailion and J. Zwanziger. Metabolism of norepinephrine, serotonin and dopamine in rat brain with stress. *J. Pharmac. exp. Ther.* **164:** 122–134, 1968.
5. Carlsson, A. and B. Winblad. Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. *J. Neural Trans.* **38:** 217–276, 1976.
6. Chang, C. C. A sensitive method for spectrophotofluorometric assay of the catecholamines. *Int. J. Neuropharmac.* **3:** 643–649, 1964.

7. Cox, R. H. and J. L. Perhach. A sensitive, rapid and simple method for the simultaneous spectrophotofluorometric determinations of norepinephrine, dopamine, 5-hydroxytryptamine, and 5-hydroxyindoleacetic acid in discrete areas of brain. *J. Neurochem.* **20:** 1777–1780, 1973.
8. Curry, D. L. and K. P. Curry. Hypothermia and insulin secretion. *Endocrinology* **87:** 750–755, 1970.
9. Curzon, G. Relationships between stress and brain 5-hydroxytryptamine and their possible significance in affective disorders. *J. Psychiat. Res.* **9:** 243–252, 1972.
10. Denckla, W. D. and H. K. Dewey. The determination of tryptophan in plasma, liver, and urine. *J. Lab. clin. Med.* **69:** 160–169, 1967.
11. Eccleston, D., G. Ashcroft and T. Crawford. 5-hydroxyindole metabolism in rat brain. A study of intermediate metabolism using the technique of tryptophan loading. II. *J. Neurochem.* **12:** 493–503, 1965.
12. Eleftheriou, B. Changes with age in pituitary-adrenal responsiveness and reactivity to mild stress in mice. *Gerontologia* **20:** 224–230, 1974.
13. Ellison, G. C. and D. E. Bresler. Tests of emotional behavior in rats following depletion of norepinephrine, serotonin, or of both. *Psychopharmacologia* **34:** 275–288, 1974.
14. Fernstrom, J. D., M. J. Hirsch and D. V. Faller. Failure of brain tryptophan levels to correlate with serum free tryptophan, or its ratio to the sum of the other serum neutral amino acids. *Biochem. J.* **160:** 589–595, 1976.
15. Fernstrom, J. and R. Wurtman. Brain serotonin content: physiological regulation by plasma amino acids. *Science* **178:** 414–416, 1972.
16. Finch, C. E. Neuroendocrine mechanisms and aging. *Fedn Proc.* **38:** 178–183, 1979.
17. Finch, C. Catecholamine metabolism in the brains of aging male mice. *Brain Res.* **52:** 261–276, 1973.
18. Finch, C. E., V. Jonec, J. R. Wisner, Jr., Y. N. Sinha, J. S. de Vellis and R. S. Swerdloff. Hormone production by the pituitary and testes of male C57BL/6J mice during aging. *Endocrinology* **101:** 1310–1317, 1977.
19. Finch, C., J. Foster and A. Mirsky. Aging and the regulation of cell activities during exposure to cold. *J. gen. Physiol.* **54:** 690–712, 1969.
20. Gibson, C. J. and R. J. Wurtman. Physiological control of brain norepinephrine synthesis by brain tyrosine concentration. *Life Sci.* **22:** 1399–1406, 1978.
21. Goldstein, L., E. Stella and E. Knox. The effect of hydrocortisone on tyrosine α-ketoglutonate transaminase and tryptophan pyrrolase activities in the isolated, perfused rat liver. *J. biol. Chem.* **237:** 1723–1726, 1962.
22. Grahame-Smith, D. G. Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.* **18:** 1053–1066, 1971.
23. Green, H., S. M. Greenberg, R. W. Erickson, J. L. Sawyer and T. Ellison. Effect of dietary phenylalanine and tryptophan upon rat brain amine levels. *J. Pharmac. exp. Ther.* **136:** 174–178, 1962.
24. Harri, M. and R. Tirri. Serotonin and temperature regulation. *Acta physiol. scand.* **75:** 631–635, 1969.
25. Hess, G. and G. Riegle. Adrenocorticol responsiveness to stress and ACTH in aging rats. *J. Gerontol.* **25:** 354–358, 1970.
26. Hiroshige, T. and K. Abe. Role of brain biogenic amines in the regulation of ACTH secretion. In: *Neuroendocrine Control,* edited by K. Yagi and S. Yoshida. New York: Wiley, 1973, pp. 205–228.
27. Kizer, J., M. Palkovits, J. Zivin, M. Brownstern, J. Saaverda and I. Kopin. The effects of endocrinological manipulations on tyrosine hydroxylase and dopamine-β-hydroxylase activities in individual hypothalamic nuclei of the adult male rat. *Endocrinology* **95:** 799–812, 1974.
28. Knoll, J. Motimeter, a new sensitive apparatus for the quantitative measurement of hypermotility caused by psychostimulants. *Archs int. Pharmacodyn.* **130:** 141–154, 1961.
29. Levine, S. and D. M. Treiman. Differential plasma corticosterone response to stress in four inbred strain of mice. *Endocrinology* **75:** 142–144, 1964.
30. Lindman, H. R. *Analysis of Variance in Complex Experimental Designs.* San Francisco: Freeman, 1974, pp. 156–164.
31. Maas, J. and M. Mednieks. Hydrocortisone-mediated increase of norepinephrine uptake by brain slices. *Science* **171:** 178–179, 1971.
32. Mabry, P. D. and B. A. Campbell. Serotonergic inhibition of catecholamine-induced behavioral arousal. *Brain Res.* **49:** 381–391, 1973.
33. McCaman, M. W. and E. Robins. Fluorometric method for the determination of phenylalanine in serum. *J. Lab. clin. Med.* **59:** 885–890, 1962.
34. Martin, C. *Textbook of Endocrine Physiology.* New York: Oxford U. Press, 1976, pp. 12–15.
35. Miller, A., C. Shaar and G. Riegle. Aging effects on hypothalamic dopamine and norepinephrine content in the male rat. *Exp. Aging Res.* **2:** 475–480, 1976.
36. Morrison, D. F. *Multivariate Statistical Methods.* New York: McGraw-Hill, 1967, pp. 159–203.
37. Naumenko, E. Hypothalamic chemoreactive structures and the regulation of pituitary-adrenal function. Effects of local injections of norepinephrine carbachol and serotonin into the brain of guinea pigs with intact brain and after mesencephalic transection. *Brain Res.* **11:** 1–10, 1968.
38. Phillips, R. E. Tyrosine in serum. In: *Manual of Fluorometric Clinical Procedures.* Palo Alto: G. K. Turner Associates, 1972.
39. Rewerski, W and P. Kubikowski. Influence of biogenic amines on the central regulation body temperature in rats. *Acta physiol. pol.* **6:** 777–779, 1969.
40. Riegle, G. Chronic stress effects on adrenocorticol responsiveness in young and aged rats. *Neuroendocrinology* **11:** 1–10, 1973.
41. Ritter, S. and N. L. Pelzer. Magnitude of stress-induced brain norepinephrine depletion varies with age. *Brain Res.* **152:** 170–175, 1978.
42. Roth, G. Age-related changes in specific glucocorticoid binding by steroid responsive tissues of rats. *Endocrinology* **94:** 82–90, 1974.
43. Sakellaris, P. C., A. Peterson, A. Goodwin, C. M. Winget and J. Vernikos-Danellis. Response of mice to repeated photo-period shifts: susceptibility to stress and barbiturates. *Proc. Soc. exp. Biol. Med.* **149:** 667–680, 1975.
44. Scapugnini, U., G. VanLoon, G. Moberg, P. Preziosi and W. Ganony. Evidence for central norepinephrine-mediated inhibition of ACTH secretion in the rat. *Neuroendocrinology* **10:** 155–160, 1972.
45. Sheard, M. H. and G. K. Aghajanian. Neural release of brain serotonin and body temperature. *Nature* **216:** 495–496, 1967.
46. Silber, R. H., R. D. Busch and R. Oslapas. Practical procedure for estimation of corticosterone or hydrocortisone. *Clin. Chem.* **4:** 278–285, 1958.
47. Simpkins, J. W., G. P. Mueller, H. H. Huang and J. Meites. Evidence for depressed catecholamines and enhanced serotonin metabolism in aging male rats: possible relation to gonadotropin secretion. *Endocrinology* **100:** 1672–1678, 1977.
48. Stone, E. A. Behavioral and neurochemical effects of acute swim stress are due to hypothermia. *Life Sci.* **9:** 877–888, 1970.
49. Thierry, A., F. Javoy, J. Glowinski and S. Kety. Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the CNS of the rat. 1. Modifications of norepinephrine turnover. *J. Pharmac. exp. Ther.* **103:** 163–171, 1968.
50. Thoa, N. B., B. Eichelman, J. S. Richardson and D. Jacobowitz. 6-hydroxydopa depletion of brain norepinephrine and the facilitation of aggressive behavior. *Science* **178:** 75–77, 1972.
51. Thurmond, J. B. Technique for producing and measuring territorial aggression using laboratory mice. *Physiol. Behav.* **146:** 879–881, 1975.

52. 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:** 475–478, 1977.
53. Thurmond, J. B., S. M. Lasley, N. R. Kramarcy and J. W. Brown. Differential tolerance to dietary amino acid induced changes in aggressive behavior and locomotor activity in mice. *Psychopharmacology* **66:** 301–308, 1979.
54. Wong, P. W. K., M. E. O'Flynn and I. Inouye. Micromethods for measuring phenylalanine and tyrosine in serum. *Clin. Chem.* **10:** 1098–1104, 1964.
55. Wurtman, R. J., E. L. Cohen and J. D. Fernstrom. Control of brain neurotransmitter synthesis by precursor availability and food consumption. In: *Neuroregulators and Psychiatric Disorders*, edited by E. Usdin, D. A. Hamburg and J. Barchas. New York: Oxford U. Press, 1977, pp. 103–121.
56. Wurtman, R. J., F. Larin, S. Mostafapour and J. D. Fernstrom. Brain catechol synthesis: control by brain tyrosine concentration. *Science* **185:** 183–184, 1974.
57. Yen, H. C. Y., M. H. Katz and S. Krop. Effects of various drugs on 3,4-dihydroxyphenylalanine (DL-DOPA)-induced excitation (aggressive behavior) in mice. *Toxic. appl. Pharmac.* **17:** 597–604, 1970.