Pharmacology Biochemistry & Behavior, Vol. 12, pp. 525–532. Printed in the U.S.A.

Dietary Amino Acid Precursors: Effects on Central Monoamines, Aggression, and Locomotor Activity in the Mouse

JOHN B. THURMOND, NEAL R. KRAMARCY, STEPHEN M. LASLEY AND JOHN W. BROWN

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

Received 15 August 1979

THURMOND, J. B., N. R. KRAMARCY, S. M. LASLEY AND J. W. BROWN. *Dietary amino acid precursors: Effects on central monoamines, aggression, and locomotor activity in the mouse.* PHARMAC. BIOCHEM. BEHAV. 12(4) 525–532, 1980.—Behavioral and brain neurochemical changes were assessed in mice maintained on amino acid supplemented 12% protein diets for two and six weeks. Addition of 1, 2, or 4% L-phenylalanine increased aggression but only the 1% supplement increased locomotion. Addition of 0.25 or 0.5% L-tryptophan to the basal diet also increased aggression. All behavioral effects were noted after two weeks but not after six weeks on the diets, suggesting the development of behavioral tolerance. All groups of mice gained the same amount of weight on the various diets over a seven-week period and no ill effects were observed. Mice on 16% protein diets displayed only marginal differences in brain concentrations of phenylalanine, tyrosine, tryptophan, dopamine, norepinephrine, serotonin or 5-hydroxyindoleacetic acid. Brain concentrations of tyrosine, phenylalanine and tryptophan were significantly reduced in animals receiving supplements of leucine for two and six weeks. L-phenylalanine supplementation caused marked increases in brain phenylalanine and tyrosine concentrations after two and six weeks. Changes in brain dopamine and 5-hydroxyindoleacetic acid also were noted. Supplements of 0.25, 0.5, and 1.0% L-tryptophan increased brain indoles, and these changes also appeared to be sustained over the six-week period.

Amino acids Diet Monoamines Aggression Locomotion Tolerance

IT HAS recently been shown that the normal functioning of brain catecholaminergic and serotonergic systems is related to the availability of amino acid precursors in the diet [66]. Brain tryptophan and serotonin (5-HT) rise after an IP injection of tryptophan, or following dietary intake of tryptophan [18,67]. Brain tyrosine is elevated following an IP injection of tyrosine or after the consumption of a single, high-protein meal, and brain catecholaminergic synthesis is accelerated [68]. Brain dopamine (DA) concentration is increased in rats by dietary supplements of phenylalanine [24].

Tyrosine and tryptophan share the same blood-brain transport mechanism with at least four other neutral amino acids (phenylalanine, leucine, isoleucine, valine), and the relative concentration of these amino acids reaching the brain is probably more significant than their absolute quantities [6, 26, 46, 66]. Thus, a high relative concentration of phenylalanine or tyrosine in the blood not only favors brain catecholamine (CA) synthesis, but also diminishes 5-HT by competing with tryptophan for transport [26]. Also, phenylalanine inhibits tryptophan hydroxylase required for the synthesis of 5-HT; thus brains of animals on high phenylalanine diets contain more CA but less hydroxylated indole [11, 47, 61].

In recent years it has been shown that administration of neurotransmitter precursor amino acids can produce alterations in behavior. Decreases in dietary tryptophan, the amino acid manipulated in most studies, increases pain sensitivity [38] and facilitates mouse killing in rats [21]. Tryptophan injected (IP) in rats pretreated with a monoamine oxidase inhibitor (MAOI) increases locomotor activity [19] and produces a behavioral syndrome characterized by hyperactivity and stereotyped behaviors [34,41]. Except for findings from studies of experimental phenylketonuria in rats relating dietary administration of phenylalanine to deficits in learning [1,51], animal studies have not dealt with the behavioral effects of CA dietary precursors. Nor have animal studies examined the effects of additions of tryptophan supplements to the diet, with the exception of instances where it was added in order to reverse effects produced by a tryptophan-free diet [21].

We have obtained mixed behavioral effects due to dietary tryptophan supplements; however, we found that additions of phenylalanine to the diet increased open-field activity and tyrosine markedly increased aggressive behavior [55,56]. In the present experiment we have investigated the effects on aggression and open-field locomotor activity of varying levels of dietary phenylalanine and tryptophan. The effects of leucine were also determined, a neutral dietary amino acid which is unrelated metabolically to CA or indoleamine synthesis, but which competes for transport with the neurotransmitter precursor amino acids [26].

METHOD

Animals

Male CF-1 mice, 42 and 84 days old were obtained from

Carworth Farms (Wilmington, Massachusetts) and housed five per cage. 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 Manipulations

Mice were randomly designated as residents and immediately given free access to water and a semi-synthetic basal diet (all diet materials were obtained from ICN Pharmaceuticals, Cleveland, OH). The basal diet contained: 12% casein protein, 5% corn oil, 70% corn starch, 2% cellulose, 4% Salt Mixture XIV, 2.2% Vitamin Diet Fortification Mixture, and 4.8% dextrose. The animals were randomly assigned to one of nine groups (n=20 per group). Seven experimental groups received the basal 12% casein diet supplemented with (a) 4% L-leucine, (b) 1, 2, or 4% L-phenylalanine, (c) 0.25, 0.5 or 1.0% L-tryptophan. The eighth group of animals received a supplement of 4% casein to provide a total of 16% protein having the same balance of amino acids as the basal diet. A ninth group of animals was maintained on the semi-synthetic 12% basal casein diet with no supplement. 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 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, were given free access to Rat/Mouse Purina Chow. Mice on both the semi-synthetic diets and Purina Chow diets were weighed weekly to determine if any physical differences occurred as a result of the various dietary regimens.

Behavioral Tests

A complete description of the apparatus used for producing and measuring territorial aggression has been published [54]. 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. 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.

All 20 animals in a given condition were tested after two weeks on the diets. All four cages of diet-fed mice in a particular condition were transferred to the testing room containing the five identical test boxes for assessing aggressive behavior and a 60 cm square open-field marked off into 16 equal squares for assessing locomotor behavior [7]. Locomotor behavior was measured by gently placing each diet-fed mouse in the center of the open-field apparatus and counting the number of squares crossed for five 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 intruder mice were introduced, and the ensuing aggression was measured.

At the conclusion of this test period, this entire procedure was replicated with separate groups of 20 animals in each diet condition, except that the mice were maintained on the diet for six weeks instead of two weeks. These animals were behaviorally tested for aggression and locomotor activity in the same manner. The observers of behavior were blind to the animal's treatment condition.

Biochemical Determination

Separate groups of animals (n=5), maintained for two weeks on the semi-synthetic diets, were sacrificed by cervical dislocation. Their brains were rapidly removed and immediately homogenized in ten volumes of cold acidified n-butanol. Serotonin, 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), and norepinephrine (NE) in whole brain were determined spectrophotofluorometrically according to the method of Chang [9] and Cox and Perhach [10]. Amino acid fractions were also analyzed, tyrosine according to the method of Wong *et al.* [65] as modified by Phillips [48], phenylalanine according to McCaman and Robins [39], and tryptophan according to Denckla and Dewey [13].

Five additional groups of five animals were designated for six weeks diet administration: (a) 12% casein (basal) control, (b) 4% casein, (c) 4% pehnylalanine, (d) 1% tryptophan, and (e) 4% leucine supplements. At the conclusion of the six-week period the animals were sacrificed by cervical dislocation and their brains homogenized and analyzed for the neurotransmitters and amino acids as before.

Statistical Analysis

Control (12% casein) groups and groups on the supplemented diets were compared by separate one-way analyses of variance followed where significant by tests for comparing individual treatment means with the control group mean.

RESULTS

Weight Gain

Average weights varied from 32 g to 36 g over the period of the experiment. Weight gain over the period of maintenance on the diets was significant for each condition ($p<0.01$, in each case).

Behavioral

In all analyses reported here, animals on 12% casein (basal) diets were considered as controls. Figure 1 and Fig. 2 show results of behavioral testing after two weeks and six weeks, respectively, on the semi-synthetic diets. All results are shown as percentage of control activity. Addition of 4% casein, which maintained the amino acid balance of the basal diet but increased its protein content from 12% to 16%, had little effect on aggressive behavior or locomotion after two or six weeks of feeding. A slight increase in aggression was suggested, but differences were not statistically significant.

Addition of 1, 2, or 4% phenylalanine to basal diets resulted in a significant increase in aggression as measured by either decreased attack latency, $F(3,76)=2.78$, $p<0.05$, or increased number of attacks, $F(3,76)=2.75$, $p<0.05$, after two weeks. However, only the group on the 1% phenylalanine supplement showed significantly increased locomotion, $F(3,76)=9.86$, $p<0.001$, within this period. After six weeks, no significant behavioral differences were

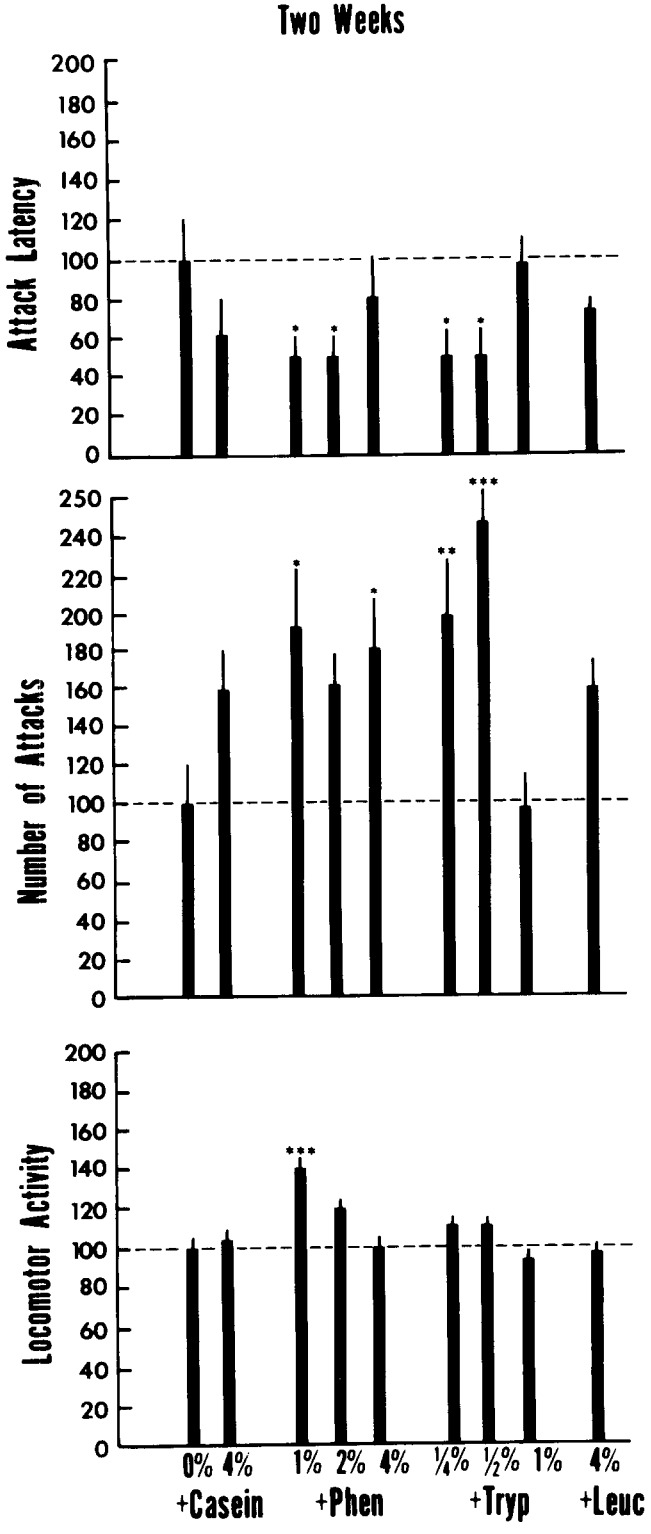

FIG. 1. Comparison of the attack latency, number of attacks, and locomotor activity following two weeks maintenance on the various dietary supplements consisting of 12% casein basal diet plus 4% casein (balanced diet), 4% L-leucine, 1%–4% L-phenylalanine, and 0.25%–1.0% L-tryptophan. Results are given as mean with SEM of 20 determinations and expressed as a percentage of the basal 12% casein control values (100). Significance levels are based on analyses of variance followed where significant by comparisons of the control mean with individual treatment means (two-tailed tests). $*p<0.05$; $**p<0.01$; $***p<0.001$.

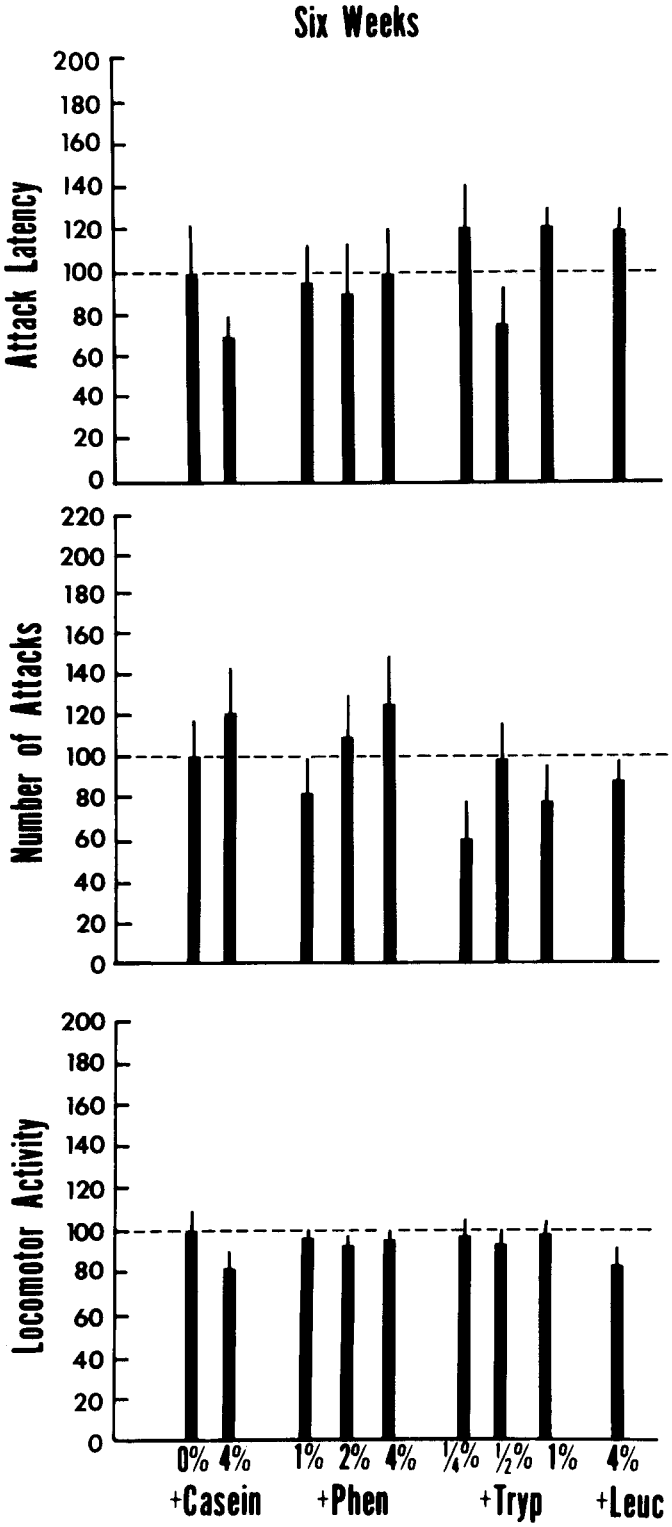

FIG. 2. Comparison of the attack latency, number of attacks, and locomotor activity following six weeks maintenance on the various dietary supplements. See Fig. 1 for explanatory details. Results are given as mean with SEM of 20 determinations and expressed as a percentage of the basal 12% casein control values (100). See Fig. 1 for explanations of statistical procedures.

TABLE 1

EFFECTS OF PRECURSOR AMINO ACIDS ON CONCENTRATIONS OF NOREPINEPHRINE (NE), DOPAMINE (DA), SEROTONIN (5-HT), 5-HYDROXYINDOLEACETIC ACID (5-HIAA), TRYPTOPHAN (TRYP), TYROSINE (TYRO) AND PHENYLALANINE (PHEN) IN MOUSE BRAIN TWO WEEKS FOLLOWING DIETARY SUPPLEMENTS

Treatment	NE	DA	5-HT	5-HIAA	TRYP	TYRO	PHEN
Control (basal diet)	100 ± 8	100 ± 16	100 ± 6	100 ± 2	100 ± 9	100 ± 8	100 ± 7
+4% casein	112 ± 2	94 ± 18	107 ± 3	92 ± 9	85 ± 5	128 ± 10	123 ± 4*
+1% phenylalanine	116 ± 2	88 ± 4	131 ± 5†	98 ± 14	75 ± 9	124 ± 16	145 ± 11†
+2% phenylalanine	108 ± 6	81 ± 4	111 ± 11	68 ± 2‡	100 ± 9	263 ± 16‡	185 ± 13‡
+4% phenylalanine	104 ± 2	104 ± 7	104 ± 13	68 ± 4‡	77 ± 13	689 ± 124†	367 ± 91*
+0.25% tryptophan	90 ± 4	77 ± 4	128 ± 9*	131 ± 9*	92 ± 5	88 ± 9	91 ± 5
+0.50% tryptophan	116 ± 2	102 ± 7	135 ± 2‡	152 ± 6‡	129 ± 13	90 ± 6	114 ± 3
+1.00% tryptophan	112 ± 4	94 ± 4	165 ± 6‡	162 ± 11‡	186 ± 10‡	96 ± 6	88 ± 3
+4% leucine	78 ± 2*	88 ± 7	78 ± 9	72 ± 2‡	37 ± 3‡	29 ± 2‡	64 ± 2‡

Results are given as mean ± SEM (n=5 for each determination) and expressed as a percentage of basal diet control values (100). Treatment means were compared with basal diet control group values following analyses of variance where significant (two-tailed tests). *$p<0.05$; †$p<0.01$; ‡$p<0.001$. Mean μg/g ± SEM for 12% casein basal control diet: NE=0.50 ± 0.04; DA=1.13 ± 0.18; 5-HT=0.54 ± 0.03; 5-HIAA=0.85 ± 0.02; TRYP=4.33 ± 0.37; TYRO=12.62 ± 1.03; PHEN=17.00 ± 1.17.

TABLE 2

EFFECTS OF PRECURSOR AMINO ACIDS ON CONCENTRATIONS OF NOREPINEPHRINE (NE), DOPAMINE (DA), SEROTONIN (5-HT), 5-HYDROXYINDOLEACETIC ACID (5-HIAA), TRYPTOPHAN (TRYP), TYROSINE (TYRO) AND PHENYLALANINE (PHEN) IN MOUSE BRAIN SIX WEEKS FOLLOWING DIETARY SUPPLEMENTS

Treatment	NE	DA	5-HT	5-HIAA	TRYP	TYRO	PHEN
Control (basal diet)	100 ± 6	100 ± 10	100 ± 2	100 ± 3	100 ± 6	100 ± 7	100 ± 8
+4% casein	112 ± 6	111 ± 4	104 ± 4	92 ± 6*	130 ± 8*	84 ± 4	98 ± 4
+4% phenylalanine	100 ± 4	152 ± 2‡	91 ± 11	56 ± 2‡	111 ± 12	251 ± 15‡	350 ± 21‡
+1% tryptophan	100 ± 4	78 ± 4	173 ± 9‡	160 ± 6‡	190 ± 13	79 ± 6*	96 ± 2
+4% leucine	110 ± 6	100 ± 7	102 ± 7	99 ± 5	48 ± 6‡	31 ± 2‡	79 ± 6
Purina Lab Chow	108 ± 15	96 ± 4	115 ± 6	89 ± 3	119 ± 6	112 ± 5	132 ± 12

Results are given as mean ± SEM (n=5 for each determination) and expressed as a percentage of basal diet control values (100). Treatment means were compared with basal diet control group values following analyses of variance where significant (two-tailed tests). *$p<0.05$; †$p<0.01$; ‡$p<0.001$. Mean μg/g ± SEM for 12% casein basal control diet: NE=0.49 ± 0.03; DA=1.14 ± 0.11; 5-HT=0.54 ± 0.01; 5-HIAA=0.88 ± 0.03; TRYP=3.53 ± 0.22; TYRO=14.34 ± 0.95; PHEN=15.05 ± 1.21.

noted, although a slight, dose-dependent increase in attack frequency is suggested by the data.

Supplementation of diets with 0.25% or 0.50% tryptophan significantly increased aggression after two weeks as measured by either the attack latency, $F(3,76)=3.53$, $p<0.02$, or number of attacks, $F(3,76)=5.46$, $p<0.01$. However, animals on the 1.0% tryptophan supplement were not significantly different from controls, and no changes in aggression were noted at any level of tryptophan supplementation after six weeks. No differences in locomotion were noted after either two or six weeks on these diets.

Animals receiving a 4% leucine supplement displayed no significant behavioral effects after two or six weeks and appeared similar in almost all behavioral measures to the group on the casein supplement.

Biochemical

Tables 1 and 2 summarize biochemical data from analyses of mouse brains after two weeks and six weeks, respectively, on the semisynthetic diets. Results are expressed as percent of control (basal diet) brain concentrations. Animals receiving a 4% casein supplement displayed only marginal differences in brain concentration of tryptophan, tyrosine or phenylalanine after either two or six weeks. No differences were noted at either time in the brain content of NE, DA, or 5-HT.

Phenylalanine supplementation (1, 2, and 4%) resulted in large increases after two weeks in both brain phenylalanine and tyrosine. There were no increases in either NE or DA. A decrease in the brain concentration of 5-HIAA was seen with both 2 and 4%. The amino acid increases and 5-HIAA decreases were sustained for six weeks. There was a significant increase in DA after six weeks on the 4% supplement.

Tryptophan supplementation at 0.25, 0.50, and 1.0% of diet for two weeks resulted in consistent increases in both brain 5-HT and 5-HIAA concentrations. Significant in-

creases in brain tryptophan concentrations were noted only at the 1% level of supplementation. Analyses of brains from animals on the 1% supplement for six weeks indicate that these biochemical differences were sustained. The data also suggest that some decreases in brain tyrosine and DA resulted after this period of tryptophan supplementation.

Dietary supplements of 4% leucine resulted in striking decreases in brain tryptophan, tyrosine and phenylalanine concentrations. In general these effects were sustained after six weeks. After two weeks both the catechols and the indoles appeared to be somewhat diminished, although only NE and 5-HIAA were significantly below controls. After six weeks, however, these values had returned to control levels. Thus, the leucine-supplemented animals stood in sharp contrast biochemically to the casein-supplemented groups, although the two sets appeared to be quite similar behaviorally. The data given in Table 2 for animals maintained on Rat/Mouse Purina Chow also show that the semi-synthetic casein control diet does not result in artificial neurochemical control values.

DISCUSSION

It is apparent that the source or level of dietary protein in the present study had no significant effect on the measured behaviors. Only marginal differences were noted in brain amino acids or their metabolites from animals on Purina Chow (22% protein) or the semi-synthetic diets containing 12 or 16% casein protein. Weight gains of the animals was the same on all diets at all times, and no physical differences between animals in the various groups were apparent. Animals on the 16% casein diets appeared to be slightly more aggressive than those on 12% casein, but the difference was not significant. Similarly, no significant behavioral changes in leucine-supplemented animals were observed at either two or six weeks.

Tryptophan supplementation at low levels significantly increased aggression after two weeks. No differences in aggression were obtained, however, at the highest level of supplementation at either two or six weeks. Others have reported that administration of 5-HTP decreased aggression in mice [30] and muricide in rats [35] whereas a tryptophan-free diet increased muricide in rats [21]. Also it has been reported that lesioning of the dorsal raphe nucleus or removal of the olfactory bulbs, reducing the 5-HT level, increased aggression in rats [33]. Inhibition of 5-HT synthesis by PCPA or PCMA reportedly increases aggression in rats [49] and mice [29].

In the present study, no differences in motor activity after either two or six weeks on the tryptophan supplemented diets were observed. The findings of others vary. some have observed that administration of tryptophan, 5-HTP (IP) or 5-HT (intraventricularly) resulted in decreased motor activity [17, 23, 43]. Others, administering tryptophan or 5-HTP together with MAO or peripheral decarboxylase inhibitors have reported an increase in mouse activity [40], and stereotyped hyperactivity in rats, which can be blocked by PCPA [32,41]. Some evidence has indicated that peripherally formed tryptamine, which can pass the blood-brain barrier [25], is responsible for this hyperactivity when it occurs [19]. It has been shown recently that brain tryptamine changes contribute to enhanced motor activity produced by tranylcypromine plus tryptophan in rats [41].

Phenylalanine supplementation at the lowest (1%) level was correlated with a significant increase in locomotor activity after two weeks in the present study. No differences were noted at higher levels of supplementation after either two or six weeks. These findings are in general agreement with the reports of others. DOPA administered with or without PCPA to retard 5-HT formation caused increased locomotor activity [37]. Intraventricular administration of NE or DA also increased locomotor activity [27]. Furthermore, DA agonists such as apomorphine or releasers such as amphetamine or methylphenidate increase motor activity [14,16]. On the other hand, 6-OHDA and pimozide, a DA receptor blocker, cause a decrease in locomotor activity [2, 8, 28, 36].

All levels of phenylalanine supplements tested significantly increased aggression after two weeks. Studies in which DOPA was utilized to increase brain CA have yielded conflicting results. Some have reported that DOPA at high doses, or at lower doses in the presence of MAOI, results in increased aggression and excitation [70] whereas others have reported the opposite effect [30,62]. Apomorphine, a DA agonist, has been reported to increase aggression in rats [20,31] but decrease aggression in mice [30]. Amphetamine at low doses reportedly increases mouse aggression, but at high doses decreases this behavior [30]. Inhibitors of dopamine-β-hydroxylase have been reported to both increase and decrease aggression [30, 42, 50, 52].

Differences in brain CA levels in aggressive and non-aggressive animals also are not consistent. Although some studies have reported no differences in DA levels between isolated aggressive mice and aggregated non-aggressive mice in whole brain, olfactory bulbs, mesencephalon, or diencephalon [12,59], recent studies indicate that steady-state DA levels are higher in the hypothalamus of aggressive rats [4] and aggressive mice [57] when compared to non-aggressive animals. These results are consistent with earlier reports of higher steady-state levels and higher turnover of CA in the whole brain of fighting mice [63].

All levels of phenylalanine supplementation resulted in large increases in brain phenylalanine and brain tyrosine, the amino acid derived from it which serves as the immediate precursor for CA synthesis. These amino acid increases were sustained over the six-week interval. No changes in brain catechols were observed after two weeks, although DA was significantly increased after six weeks on the 4% supplement. Similar increases following administration of phenylalanine or tyrosine in these amino acids in the brain have been reported [67]. Also, administration of tyrosine systemically in rats pretreated with a peripheral decarboxylase inhibitor has been shown to increase brain DOPA [68]. Oral administration of phenylalanine, as part of a procedure to produce experimental phenylketonuria in rats, reportedly increased brain NE or DA [1,24]. It is often assumed that the concentration in brain of both of these catechols is under feed-back control on tyrosine hydroxylase, the first enzyme in the pathway to their formation [44,45]. Evidently, however, some changes in this system occur after prolonged periods of amino acid administration. This was confirmed in another study in our laboratory in which animals maintained on phenylalanine supplements showed an approximately 50% increase in brain DA compared to controls after five weeks but not after one week [56]. The finding that dietary supplements of tryptophan increased brain tryptophan, 5-HT and 5-HIAA agrees with reports of similar increases obtained in other studies [18, 22, 67, 69]. It is noteworthy that these levels were also sustained after six weeks on the dietary regimen.

Thus, the present findings indicate that changes in brain amino acid, catechol or indole content resulting from the amino acid supplements, were relatively stable over the six-week period. Behavioral differences seemed to be much less permanent, however, appearing within two weeks and disappearing after six weeks of the dietary regimens. A number of studies in animals and humans have indicated that tolerance develops to drug-induced alterations of monoamine neurotransmitter metabolism. Tolerance in mice has been reported to develop to increased locomotor activity induced by repeated injections of 5-HTP following peripheral decarboxylase inhibition [40]. Also, rapid tolerance to increased motor activity in rats was observed following repeated injections of PCMA [15]. Marked increases in psychic and motor activity have been produced in chronic schizophrenic patients by oral administration of 5-HTP with a peripheral decarboxylase inhibitor, but these effects were greatly attenuated with continued maintenance at very high doses [69]. These instances of behavioral tolerance, including the present results, may be explained at a biochemical level by studies of the adenylate cyclase system responsible for mediating the effects of catecholamines. Chronic receptor stimulation with catecholamines produces a subsensitivity of the adenylate cyclase to adrenergic agonist [5, 60, 64]. The application of adrenergic agonists to tissues frequently results in a subsensitive response (i.e., tolerance) to the subsequent addition of agonists [53,58].

Presumably because of competition for uptake by a common carrier, a given amino acid may exert an indirect influence on the brain level of another amino acid and neurotransmitters or other metabolites derived from it [6,46]. We observed that after six weeks on the highest level of tryptophan supplementation, brain tyrosine and DA levels were somewhat below control levels. Similar effects have been observed caused by the administration of 5-HTP [3]. We also noted that phenylalanine supplements significantly reduced 5-HIAA levels at both two and six weeks. Surprisingly, no major changes in tryptophan or 5-HT were observed. This might be expected not only by reason of "competition for carrier", but also because phenylalanine has been noted to have a direct inhibitory effect in vitro on tryptophan hydroxylase [47]. Indeed, others have reported that phenylalanine administration reduces brain tryptophan und 5-HT [24]. Also as anticipated, leucine supplements caused a striking reduction in brain tyrosine, phenylalanine and tryptophan, a finding generally in accord with the observations of others [67].

In addition to 5-HIAA, other indices of monoamine release such as HVA and DOPAC might indicate the variables responsible for the behavioral changes observed. Also, it should be noted that analysis of whole brain constituents provides only a baseline for further, more specific analyses of biochemical events [45].

REFERENCES

1. Airakeinen, M. M., M. L. Leppänen, H. Turakka, M. Marvola and E. J. MacDonald. Protective effect of tryptophan and 5-hydroxytryptophan on experimental phenylketonuria induced with phenylalanine + p-chlorophenylalanine in rats. *Med. Biol.* **53:** 481–488, 1975.
2. Anden, N.-E., S. G. Butcher, H. Corrodi, K. Fuxe and U. Ungerstedt. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.* **11:** 303–314, 1970.
3. Andrews, D., R. Patrick and J. Barchas. The effects of 5-hydroxytryptophan and 5-hydroxytryptamine on dopamine synthesis and release in rat brain striatal synaptosomes. *J. Neurochem.* **30:** 465–470, 1978.
4. Barr, G. A., N. S. Sharpless and J. L. Gibbons. Differences in the level of dopamine in the hypothalamus of aggressive and non-aggressive rats. *Brain Res.* **166:** 211–216, 1979.
5. Baudry, M., M.-P. Martres and J. C. Schwartz. Modulation in the sensitivity of noradrenergic receptors in the CNS studied by the responsiveness of the cyclic AMP system. *Brain Res.* **116:** 111–124, 1976.
6. Blasberg, R. and A. Lajtha. Substrate specificity of steady-state amino acid transport in mouse brain slices. *Archs Biochem. Biophys.* **112:** 316–377, 1965.
7. Blizzard, D. A. Situational determinants of open-field behavior in *Mus musculus*. *Br. J. Psychol.* **62:** 245–252, 1971.
8. Burkhard, W. P., M. Jalfre and J. Blum. Effects of 6-hydroxydopamine on behavior and cerebral amine content in rats. *Experientia* **25:** 1295–1296, 1969.
9. Chang, C. C. A sensitive method for spectrophotofluorometric assay of the catecholamines. *Int. J. Neuropharmac.* **3:** 643–649, 1964.
10. 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.
11. Culley, W., R. Saunders, E. Mertz and D. Jolly. Effect of tryptophan deficient diet on brain serotonin and plasma tryptophan level. *Proc. Soc. exp. Biol. Med.* **113:** 645–648, 1963.
12. Da Vanzo, J. P., M. Daugherty, R. Ruckart and L. Kang. Pharmacological and biochemical studies in isolation-induced fighting mice. *Psychopharmacologia* **9:** 210–219, 1966.
13. Denckla, W. D. and H. K. Dewey. The determination of tryptophan in plasma, liver, and urine. *J. Lab. clin. Med.* **69:** 160–169, 1967.
14. Eichelman, B. S. and N. B. Thoa. The aggressive monoamines. *Biol. Psychiat.* **6:** 143–164, 1973.
15. El-Yousef, M. K., L. Steranka and E. Sanders-Bush. Rapid tolerance to the motor effects of p-chloroamphetamine in rats. *Psychopharmacology* **55:** 109–114, 1977.
16. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
17. Everett, G. M. Effect of 5-HTP on brain levels of dopamine, norepinephrine, and serotonin in mice. In: *Advances in Biochemical Pharmacology, Vol. X.*, edited by E. Costa, G. L. Gessa and M. Sandler. New York: Raven Press, 1974, pp. 261–262.
18. 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.
19. Foldes, A. and E. Costa. Relationship of brain monoamine and locomotor activity in rats. *Biochem. Pharmac.* **24:** 1617–1621, 1975.
20. Gianutsos, G. and H. Lal. Modification of apomorphine induced aggression by changing central cholinergic activity in rats. *Neuropharmacology* **16:** 7–10, 1977.
21. Gibbons, J. L., G. A. Barr, W. H. Bridger and S. F. Leibowitz. Manipulations of dietary tryptophan: effects on mouse killing and brain serotonin in the rat. *Brain Res.* **169:** 139–153, 1979.

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, R. A., J. C. Gillin and R. J. Wyatt. The inhibitory effect of intraventricular administration of serotonin on spontaneous motor activity of rats. *Psychopharmacology* **51:** 81–84, 1976.
24. 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.
25. Green, H. and J. L. Sawyer. Correlation of tryptamine-induced convulsions in rats with brain tryptamine concentration. *Proc. Soc. exp. Biol. Med.* **104:** 153–155, 1960.
26. Guroff, G. and S. Udenfriend. Studies on aromatic amino acid uptake in rat brain *in vivo:* uptake of phenylalanine and of tryptophan; inhibition and stereoselectivity in the uptake of tyrosine by brain and muscle. *J. biol. Chem.* **237:** 803–806, 1962.
27. Herman, Z. S. Behavioural changes induced in conscious mice by intraceretroventricular injection of catecholamines, acetylcholine and 5-hydroxytryptamine. *Br. J. Pharmac.* **55:** 351–358, 1975.
28. Herman, Z. S., K. Kmieciak-Kolada and R. Brus. Behaviour of rats and biogenic amine level in brain after 6-hydroxydopamine. *Psychopharmacologia* **24:** 407–416, 1972.
29. Hodge, G. K. and L. L. Butcher. 5-hydroxytryptamine correlates of isolation-induced aggression in mice. *Eur. J. Pharmac.* **28:** 326–337, 1974.
30. Hodge, G. K. and L. L. Butcher. Catecholamine correlates of isolation-induced aggression mice. *Eur. J. Pharmac.* **31:** 81–93, 1975.
31. Isaacson, R. L., B. Yongue and D. McClearn. Dopamine agonists: their effect on locomotion and exploration. *Behav. Biol.* **23:** 163–179, 1978.
32. Jacobs, B. L. Evidence for the functional interaction of two central neurotransmitters. *Psychopharmacologia* **39:** 81–86, 1974.
33. Jacobs, B. L. and A. Cohen. Differential behavioral effects of lesions of the median or dorsal raphe nuclei in rats: open field and pain-elicited aggression. *J. comp. physiol. Psychol.* **90:** 102–108, 1976.
34. Jacobs, B. L., E. E. Eubanks and W. D. Wise. Effect of indole-alkylamine manipulations on locomotor activity in rats. *Neuropharmacology* **13:** 575–583, 1974.
35. Kulkarni, A. S. Muricidal block produced by 5-hydroxytryptophan and various drugs. *Life Sci.* **7:** 125–128, 1968.
36. Laverty, R. and K. M. Taylor. Effects of intraventricular 2, 4, 5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behaviour and brain catecholamine metabolism. *Br. J. Pharmac.* **40:** 836–846, 1970.
37. Lycke, E., K. Modigh and B. E. Roos. Aggression in mice associated with changes in the monoamine metabolism of the brain. *Experientia* **25:** 951–953, 1969.
38. Lytle, L. D., R. B. Messing, L. Fisher and L. Phebus. Effects of long-term corn consumption on brain serotonin and the response to electric shock. *Science* **190:** 692–694, 1975.
39. MaCaman, M. W. and E. Robins. Fluorometric method for the determination of phenylalanine in serum. *J. Lab. clin. Med.* **59:** 885–890, 1962.
40. Magyar, R. L., J. C. Gillin and R. J. Wyatt. Tolerance to the increased locomotor activity produced by L-5-hydroxytryptophan following peripheral decarboxylase inhibition in mice. *Psychopharmacology* **56:** 343–350, 1978.
41. Marsden, C. A. and G. Curzon. The contribution of tryptamine to the behavioural effects of L-tryptophan in tranylcypromine-treated rats. *Psychopharmacology* **57:** 71–76, 1978.
42. Masur, J., S. Czeresnia, H. Skitnevsky and E. A. Carlini. Brain amine levels and competitive behavior between rats in a straight runway. *Pharmac. Biochem. Behav.* **2:** 55–62, 1974.
43. Modigh, K. Effects of L-tryptophan on motor activity in mice. *Psychopharmacologia* **30:** 123–134, 1973.
44. Nagatsu, T., M. Levitt and S. Udenfriend. The initial step in norepinephrine biosynthesis. *J. biol. Chem.* **239:** 2910–2917, 1964.
45. Neff, N. H. and E. Costa. Application of steady-state kinetics to the study of catecholamine turnover after monoamine oxidase inhibition of reserpine administration. *J. Pharmac. exp. Ther.* **160:** 40–47, 1968.
46. Oldendorf, W. Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. *Am. J. Physiol.* **221:** 1629–1633, 1971.
47. Peters, S. A. V. Inhibition of brain tryptophan-5-hydroxylase by amino acids—the role of L-tryptophan uptake inhibition. *Biochem. Pharmac.* **21:** 1051–1053, 1972.
48. Phillips, R. E. Tyrosine in serum. In: *Manual of Fluorometric Clinical Procedures.* Palo Alto: G. K. Turner Associates, 1972.
49. Pradhan, S. N. Aggression and central neurotransmitters. *Int. Rev. Neurobiol.* **18:** 213–262, 1975.
50. Ross, S. B. and S.-O Ogren. Anti-aggressive action of dopamine-β-hydroxylase inhibitors in mice. *J. Pharm. Pharmac.* **28:** 590–593, 1976.
51. Schalock, R. L., W. J. Brown, J. H. Copenhaver and R. Gunter. Model phenylketonuria (PKU) in the albino rat: behavioral, biochemical, and neuroanatomical effects. *J. comp. physiol. Psychol.* **89:** 655–666, 1975.
52. Scheel-Kruger, J. and A. Randrup. Aggressive behavior provoked by pargyline in rats pretreated with diethyldithiocarbonate. *J. Pharm. Pharmac.* **20:** 948–949, 1968.
53. Sharpless, S. K. Supersensitivity-like phenomena in the central nervous system. *Fedn Proc.* **34:** 1990–1997, 1975.
54. Thurmond, J. B. Technique for producing and measuring territorial aggression using laboratory mice. *Physiol. Behav.* **146:** 879–881, 1975.
55. 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.
56. 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.
57. Tizabi, Y., N. B. Thoa, G. D. Maengwyn-Davies, I. J. Kopin and D. M. Jacobowitz. Behavioral correlation of catecholamine concentration and turnover in discrete brain areas of three strains of mice. *Brain Res.* **166:** 199–205, 1979.
58. Trendelenburg, U. Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.* **18:** 629–640, 1966.
59. Valzelli, L. 5-hydroxytryptamine in aggressiveness. *Adv. Biochem. Psychopharmac.* **11:** 255–263, 1974.
60. Vetulani, J., R. I. Stawarz and F. Sulser. Adaptive mechanisms of the noradrenergic cyclic AMP generating system in the limbic forebrain of the rat: Adaptation to persistent changes in the availability of norepinephrine. *J. Neurochem.* **27:** 661–666, 1976.
61. Wang, H., V. Harwalker and H. Waisman. Effect of dietary phenylalanine and tryptophan on brain serotonin. *Arch. Biochem. Biophys.* **96:** 181–184, 1962.
62. Welch, B. L. and A. S. Welch. Aggression and the biogenic amine neurohumors. In: *Aggressive Behavior,* edited by S. Garattini and E. B. Sigg. Amsterdam: Excerpta Medica, 1969, pp. 188–202.
63. Welch, B. L. and A. S. Welch. Isolation reactivity and aggression: evidence for an involvement of brain catecholamine and serotonin. In: *Physiology of Fighting and Defeat,* edited by B. E. Eleftheriou and J. P. Scott. Chicago: University of Chicago Press, 1971, pp. 91–142.
64. Williams, B. J. and J. H. Pirch. Correlation between brain adenylate cyclase activity and spontaneous motor activity in rats after chronic reserpine treatment. *Brain Res.* **68:** 227–234, 1974.

65. 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.
66. Wurtman, R. J. and J. D. Fernstrom. L-tryptophan, L-tyrosine, and the control of brain monoamine biosynthesis. In: *Perspectives in Neuropharmacology*, edited by S. H. Snyder. New York: Oxford University Press, 1972, pp. 143–193.
67. Wurtman, R. J. and J. D. Fernstrom. Control of brain monoamine synthesis by diet and plasma amino acids. *Am. J. clin. Nutr.* **28:** 638–647, 1975.
68. Wurtman, R. J., F. Larin, S. Mostafapour and J. D. Fernstrom. Brain catechol synthesis: control by brain tyrosine concentration. *Science* **185:** 183–184, 1974.
69. Wyatt, R. J., J. Kaplan and T. Vaughan. Tolerance and dependence to serotonin: a speculation. *Arch. Gen. Psychiat. Chicago* **29:** 597–599, 1973.
70. Yen, N. 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.