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Effects of Dietary Tyrosine on L-Dopa- and Amphetamine-Induced Changes in Locomotor Activity and Neurochemistry in Mice

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THURMOND, J. B., G. B. FREEMAN, J. S. SOBLOSKY, J. R. IENI AND J. W. BROWN. *Effects of dietary tyrosine on L-dopa- and amphetamine-induced changes in locomotor activity and neurochemistry in mice.* PHARMACOL BIOCHEM BEHAV **37**(2) 259–266, 1990.—Recent findings suggest that intraperitoneal injections of L-tyrosine at high doses (100 mg/kg) alters amphetamine-induced changes in behavior by restoring amphetamine-induced decreases in whole brain norepinephrine (NE). The present study examined the motor effects of L-dihydroxyphenylalanine (L-dopa) and *d*-amphetamine sulfate in mice after treatment with a basal casein diet supplemented with L-tyrosine. The basal diet supplemented with 1–4% L-tyrosine, or 1–4% L-phenylalanine, produced no changes in motor activity in otherwise untreated mice. Whereas L-dopa (25–100 mg/kg) following inhibition of extracerebral decarboxylase by Ro 4-4602 (25 mg/kg) slightly decreased activity in diet control (casein) animals, this drug treatment enhanced motor activity in a dose-related fashion when L-tyrosine was added to the diet. Increases in motor activity following low doses of amphetamine (0.75–1.5 mg/kg) in casein control mice were antagonized by dietary L-tyrosine, but a higher dose of *d*-amphetamine (3 mg/kg) interacted with the addition of L-tyrosine producing an increase in motor activity. Neurochemical changes observed in brain concentrations of tyrosine, dopamine (DA), norepinephrine (NE), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), tryptophan, serotonin (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA) following drug and diet treatments suggest that 5-HT systems, in addition to catecholamine systems, may be involved in mediating these effects.

Catecholamines Tyrosine Locomotor activity Brain monoamines L-Dopa Amphetamine

THE role played by the precursor amino acids, phenylalanine, tyrosine and tryptophan in the regulation of biogenic amine synthesis and function has been investigated for more than 20 years. Manipulating blood concentrations of the amino acids produces concomitant changes in brain concentrations of tyrosine and/or tryptophan that result in changes in the synthesis of the catecholamines (CA) and/or serotonin (5-HT), respectively. Systemic administration of the precursor amino acids has been used to modify brain concentrations, based on the finding that the large neutral amino acids (including phenylalanine, tyrosine, tryptophan, leucine, isoleucine and valine) compete with each other for transport across the blood-brain barrier (4,13). Since phenylalanine, tyrosine and tryptophan share the same blood-brain transport system, a high relative concentration of phenylalanine or tyrosine in the blood not only favors brain CA synthesis, it also diminishes 5-HT by competing with tryptophan for transport (23). Brain tyrosine is elevated following administration of phenylalanine or tyrosine either by IP injection (16) or supplements to the diet (33,59). Treatments which raise or lower brain tyrosine concentrations (including administration of tyrosine or phenylalanine) produce variations in the rates at which brain dopa accumulates after inhibition of aromatic amino acid decarboxylase (9,17). 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 (45,63).

Recent findings suggest the hypothesis that administration of L-tyrosine, orally or systemically, can restore changes in behavior induced by manipulations that cause reductions of NE in the brain. Rats in chronic stress paradigms have reduced brain NE concentrations and are hypoactive in a novel environment (35); chronically stressed mice exposed to a novel acute stress prior to testing show a severe blunting of locomotor activity and decreased brain NE levels (57). In the former study just mentioned (35), it was observed that exposure to a tyrosine-enriched diet restored both

NE concentrations and locomotor behavior. It has been shown that injection of tyrosine to animals receiving IV amphetamine, in patterns resembling those of animals trained to abuse this substance, antagonize whole brain NE depletions induced by the stimulant (55); treatment with IP tyrosine was observed to antagonize amphetamine-induced NE depletions and reduce daily drug self-injection following chronic amphetamine exposure (15). These findings using brain monoamine precursors to affect neurotransmitter synthesis and behavior have clinical ramifications relevant to the human condition.

Although brain NE concentrations are reduced by chronic stress (57) or by chronic amphetamine injections (15,55), the main action of amphetamine has been associated with release and/or inhibition of uptake of endogenous CA. Under certain conditions, acute injections of amphetamine have been observed to decrease brain NE concentrations (39). The locomotor stimulant action of amphetamine has been associated with CA release in the brain and particularly with the release of DA (10,22). On the other hand, amphetamine-induced increases in locomotor activity are potentiated by a tryptophan-free diet or by *p*CPA, whereas pretreatment with L-tryptophan antagonizes the enhanced response to amphetamine (26,33). In addition, it appears that the syndrome of hyperexcitability (19) produced by amphetamine may be due to the activation of 5-HT (as opposed to CA) receptors in the brain, probably by displacement of endogenous 5-HT (54). Amphetamine has been shown to release 5-HT, as well as CA, from neural tissue in vivo and in vitro (50,61).

There is ample evidence supporting the hypothesis that brain 5-HT systems are involved in the mediation of the behavioral effects of amphetamine (33,37). Administration of L-tryptophan has been found to produce dramatic reductions of amphetamine self-administration alluded to above (34,36). However, the role of the CA and 5-HT systems in mediating the behavioral effects of amphetamine, and the behavioral effects of the CA precursor, L-dopa, remains uncertain. The hyperactivity induced by L-dopa has been attributed to conversion to DA and NE (30,58). L-Dopa administration also reduces brain 5-HT and increases 5-HIAA in otherwise untreated rats (3) and in 6-hydroxydopamine (6-OHDA)-treated animals (8,27). Moreover, the hyperactivity syndrome produced by L-dopa plus pargyline is blocked by pretreatment with *p*-chlorophenylalanine (*p*CPA) or cinanserin, a 5-HT receptor blocker, but not with α-methyl-*p*-tyrosine (AMPT), a catecholamine (CA) synthesis inhibitor or pimozide, a DA receptor blocker (28). Thus, the syndrome produced by L-dopa plus pargyline may be mediated by a 5-HT mechanism.

Since CA function is apparently enhanced by administration of either tyrosine (9,17) or amphetamine (14), tyrosine would be expected to potentiate amphetamine-induced increases in brain NE. Moreover, if tyrosine administration can restore both brain NE concentrations and locomotor behavior following chronic stress (35), and if it can antagonize amphetamine-induced NE depletions and reduce self-administration of the drug (15), then perhaps tyrosine can alter the locomotor stimulant action of amphetamine commonly reported in rodents (24, 33, 43). The purpose of the present study was to examine this hypothetical link between tyrosine administration, enhanced drug-induced CA function, and stimulation of locomotor activity. Brain CA and 5-HT metabolism was manipulated by supplementing the diet with tyrosine, or phenylalanine, rather than injecting these amino acids systemically which is the method typically employed (15,16). It should be noted that although a tyrosine enriched diet was observed to restore both NE concentrations and locomotor behavior following chronic stress (35), restorations of amphetamine-induced decreases in brain NE following amphetamine self-administration were obtained with high doses (100 mg/kg IP injections) (15). The dose-dependent behavioral effects of L-dopa (plus decarboxylase inhibitor) and *d*-amphetamine were examined in the present study, alone or in combination with dietary tyrosine. In order to clarify further the role of the CA and 5-HT systems in mediating the drug and dietary amino acid effects, animals from each group were sacrificed and the whole brain concentrations of monoamines, their precursor amino acids and their metabolites were determined.

METHOD

Animals

Male CF-1 mice (Carworth Farms) 11 weeks old were housed 5 per cage under standard conditions. The room temperature was 21–23°C with light from 2100 to 0900 hr daily. Except where noted, the mice were given free access to Rat/Mouse Purina Chow and water.

Diets

Experimental mice were given free access for one week to water and a 12% casein basal diet (all diet materials were obtained from ICN Pharmaceuticals, Cleveland, OH) of the following composition (percentages of components based on weight): 12% casein protein, 5% corn oil, 70% corn starch, 2% cellulose, 4% Salt Mixture XIV, 2.2% Vitamin Diet Fortification Mixture, 4.8% dextrose. During the second week experimental groups received the 12% casein diet supplemented with 1%, 2%, or 4% L-tyrosine; other experimental groups received a supplement of 1%, 2%, or 4% L-phenylalanine. Control groups were maintained on the 12% basal casein 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 purpose of feeding.

Drugs

L-Dopa methyl ester HCl and *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) were dissolved in physiological saline and injected intraperitoneally (IP) in a volume of approximately 0.2 ml. The drug doses and their use in conjunction with a dietary supplement of 4% L-tyrosine are shown in the figures and tables. N-(D, L-seryl)-N-(2,3,4-trihydroxybenzyl) hydrazine (Ro 4-4602; Hoffmann-La Roche, Inc., Nutley, NJ) was dissolved in physiological saline. Mice were injected with 25 mg/kg of Ro 4-4602 and L-dopa at 90 and 60 min, respectively, prior to behavioral testing. Previous studies indicate that this dosage of Ro 4-4602 in mice selectively inhibits extracerebral aromatic L-amino acid decarboxylase activity and strongly affects L-dopa-induced alterations in motor activity (47). Amphetamine was injected IP 60 min prior to behavior testing. Mice not receiving L-dopa or amphetamine received 0.2 ml injections of the appropriate vehicle.

Motor Activity

Locomotor activity was determined using motimeters of a type previously described (31). Mice are tested individually, five at a time, in five identical motimeters. In this device the animal moves over aluminum contact plates mounted 4 mm apart in a clear 12 cm deep, 12 × 40 cm rectangular Plexiglas box (testing cage) and a count is recorded for every passage between two plates. The total contacts made for 20 minutes was recorded for each mouse.

Neurochemistry

Separate groups of animals (N = 5 per group) received diet and

drug treatments but were not subjected to behavioral tests. At the same time that these tests would have been initiated, these animals were killed by cervical dislocation, the brains rapidly removed, and split into two equal halves using a scalpel to slit down the center of the longitudinal fissure. Half of each brain was used to determine tyrosine (TYRO), tryptophan (TRYP), NE, and DA; the other half was used to determine 5-HT and the metabolites, HVA, DOPAC, and 5-HIAA. Monoamine levels and metabolites were determined utilizing high pressure liquid chromatography with electrochemical detection. NE and DA were determined using the method of Wagner *et al.* (62); 5-HT, HVA, DOPAC and 5-HIAA were determined by the technique of Perry and Fuller (44). Amino acid concentrations were determined fluorometrically, tyrosine according to the method of Wong *et al.* (65) as modified by Phillips (46) and tryptophan by the method of Denckla and Dewey (12).

Statistics

Based on tests of homogeneity of variance and of the distributions about the means, parametric statistics were deemed appropriate for the behavioral data, particularly in view of the characteristic robustness of the analysis of variance (64). Comparisons following the analysis of variance were calculated by Dunnett's test for comparing individual treatment means with the control group mean. Interactions were analyzed using analysis of variance for the simple effects of the drug doses for each category of diet (64). Similar analyses were applied to the neurochemical data.

RESULTS

Effect of Amino Acid Dietary Supplements on Body Weight

Mice in each of the diet supplement groups gained approximately the same amount of weight during the period of maintenance on the diets. No difference in weight gain were noted between animals on the "cake" diets used in this study and those fed Rat/Mouse Purina Laboratory Chow here or in earlier studies (60). The weights of the mice following 5 weeks of maintenance on the diets were (mean g ± SEM): casein basal diet, 35.7 ± 0.5; 4% L-phenylalanine supplement, 35.6 ± 0.5; 4% L-tyrosine, 36.2 ± 0.4.

Effects of L-Phenylalanine and L-Tyrosine on Motor Activity and Brain Monoamines

Figure 1 shows that neither L-phenylalanine nor L-tyrosine pretreatment alone affected motor activity, nor did either of these amino acid supplements to the diet appear to alter brain monoamine metabolism other than raising brain amino acid levels. Compared to controls (12% basal casein diet), L-phenylalanine produced an increase in brain phenylalanine, and brain tyrosine, with the greatest increase at the 4% supplement being (mean μg/g wet weight ± SEM): phenylalanine, 63.0 ± 12.4; tyrosine, 86.6 ± 12.6; control values were 16.5 ± 0.9 and 14.6 ± 0.9, respectively. Animals supplemented with L-tyrosine alone demonstrated a marked rise in brain tyrosine, $F(3,36) = 67.59$, $p<0.001$; no neurochemical changes were observed in brain NE, DA, DOPAC, HVA, TYRP, 5-HT, or 5-HIAA.

Effects of L-Dopa and d*-Amphetamine on Motor Activity and Brain Monoamines in Control Mice*

As shown in Fig. 1, L-dopa in the range used (25–100 mg/kg), in combination with Ro 4-4602 administered to animals maintained on a 12% casein diet, produced a slight decrease in motor activity. Others have reported a decrease in motor activity following Ro 4-4602 and L-dopa over this range of dosage levels (24). In contrast, amphetamine alone administered to casein control mice produced a dose-response increase in motor activity, $F(3,152) = 17.61$, $p<0.001$. Table 1 shows that L-dopa produced a dose-related increase in DA, $F(2,24) = 54.08$, $p<0.001$, DOPAC, $F(2,24) = 62.86$, $p<0.001$, and HVA, $F(2,24) = 207.96$, $p<0.001$. It can be seen in Table 2 that brain 5-HT also increased significantly when L-dopa was given with Ro 4-4602 at the 50 mg/kg dose but decreased following 100 mg/kg, $F(2,24) = 21.16$, $p<0.001$.

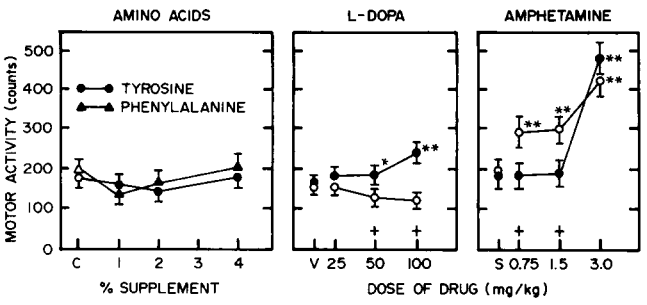

FIG. 1. Effects of L-dopa or *d*-amphetamine on locomotor activity in mice following two weeks on a 12% casein basal diet (control) or one week on the 12% casein diet plus one week of amino acid dietary supplements. The dietary supplements consisted of a balanced 12% casein basal diet (control) plus 1–4% L-phenylalanine or 1–4% L-tyrosine. Also shown are dose-effect curves of L-dopa and *d*-amphetamine on activity in mice maintained on a 12% casein diet (○) or a 12% casein diet plus 4% L-tyrosine (●). All injections were IP. L-Dopa was administered along with 25 mg/kg Ro 4-4602. Each point represents the mean ± SEM of 20 independent determinations. The symbol (+) indicates a significant difference ($p<0.01$) between corresponding points in the curves for mice maintained on casein and casein plus tyrosine. Asterisks denote significant differences between drug-treated mice and saline-injected controls or vehicle-injected (25 mg/kg Ro 4-4602 + 0.9% saline for L-dopa); *$p<0.05$; **$p<0.01$.

Amphetamine alone also apparently stimulated catecholamine function as shown in Table 1, producing dose-related increases in both NE, $F(2,24) = 6.94$, $p<0.01$, and DA, $F(2,24) = 14.59$, $p<0.001$. However, in contrast to L-dopa, a dose-related decrease in DOPAC was observed following amphetamine, $F(2,24) = 47.27$, $p<0.001$, as well as a decrease in HVA, $F(2,24) = 6.24$, $p<0.01$. Although these results may seem unexpected, other studies have reported similar effects of amphetamine (5,49). Significant rises in brain tryptophan, $F(2,24) = 17.03$, $p<0.001$, and 5-HT, $F(2,24) = 10.31$, $p<0.001$, also occurred as shown in Table 2, and this was accompanied by a decrease in 5-HIAA when L-tyrosine was added to the diet, $F(2,24) = 7.84$, $p<0.001$.

Effects of L-Tyrosine Supplement on the Motor Actions of L-Dopa and d*-Amphetamine*

Whereas L-dopa alone (with Ro 4-4602) slightly lowered motor activity, when combined with L-tyrosine it resulted in a dose-related rise in activity (Fig. 1). Hence, the main effect of L-tyrosine was significant, $F(1,52) = 22.99$, $p<0.001$, as was also the effect of L-dopa with L-tyrosine across the dose range 0–100 mg/kg, $F(3,152) = 9.20$, $p<0.01$, and the interaction of effects produced by this combination, $F(3,152) = 7.62$, $p<0.01$.

In casein control animals, amphetamine administration increased motor activity in a dose-related manner over the range 0.75–3.0 mg/kg, $F(3,152) = 17.61$, $p<0.001$ (Fig. 1). A dose-related increase in activity was also observed in animals receiving the L-tyrosine dietary supplement, $F(3,152) = 36.97$, $p<0.001$.

TABLE 1

EFFECTS OF ADMINISTRATION OF TYROSINE DIET SUPPLEMENT ALONE OR COMBINED WITH L-DOPA OR AMPHETAMINE ON MEASURES OF CATECHOLAMINE METABOLISM IN WHOLE MOUSE BRAIN

	TYRO	NE	DA	DOPAC	HVA
Controls (casein)	15.1 ± 0.8	0.41 ± 0.01	1.13 ± 0.03	0.16 ± 0.01	0.21 ± 0.008
Casein +					
dopa (50 mg/kg)	17.5 ± 1.6	0.43 ± 0.01	2.64 ± 0.19‡	3.63 ± 0.42‡	2.31 ± 0.17‡
dopa (100 mg/kg)	18.0 ± 0.9	0.38 ± 0.01	3.93 ± 0.31‡	7.61 ± 0.85‡	3.17 ± 0.06‡
Controls (casein + 4% tyrosine)	55.2 ± 6.3‡	0.45 ± 0.02	1.12 ± 0.03	0.20 ± 0.03	0.29 ± 0.005‡
Casein + 4% tyrosine +					
dopa (50 mg/kg)	59.5 ± 5.4	0.44 ± 0.01	2.51 ± 0.11¶	5.55 ± 0.53#††	2.76 ± 0.14#**
dopa (100 mg/kg)	64.6 ± 4.0	0.42 ± 0.03	3.75 ± 0.24#	11.48 ± 0.43#††	3.85 ± 0.09#**
Controls (casein)	22.0 ± 1.1	0.52 ± 0.01	1.11 ± 0.05	0.19 ± 0.01	0.26 ± 0.02
Casein +					
amphetamine (1.5 mg/kg)	20.7 ± 1.4	0.64 ± 0.03*	1.66 ± 0.16‡	0.12 ± 0.01‡	0.22 ± 0.003†
amphetamine (3.0 mg/kg)	22.8 ± 2.8	0.68 ± 0.06†	1.68 ± 0.06‡	0.09 ± 0.01‡	0.22 ± 0.004†
Controls (casein + 4% tyrosine)	62.4 ± 1.8‡	0.53 ± 0.02	1.05 ± 0.05	0.17 ± 0.01†	0.31 ± 0.02‡
Casein + 4% tyrosine +					
amphetamine (1.5 mg/kg)	55.2 ± 3.5	0.52 ± 0.01††	1.33 ± 0.06§**	0.09 ± 0.01#**	0.21 ± 0.01#
amphetamine (3.0 mg/kg)	63.6 ± 2.2	0.51 ± 0.02**	1.34 ± 0.05§**	0.09 ± 0.01#	0.22 ± 0.004#

Following one week of maintenance on a 12% basal casein diet or a 12% basal casein diet supplemented with 4% L-tyrosine, mice were injected IP with L-dopa dissolved in saline (30 min after pretreatment with Ro 4-4602, 25 mg/kg IP) and sacrificed after 60 min. Controls were sacrificed after IP injection of Ro 4-4602, 25 mg/kg followed by saline. Amphetamine was dissolved in saline and injected IP (controls received saline) following one week on casein (controls) or one week on tyrosine supplement. Animals were sacrificed 60 min after injection with amphetamine. Results are expressed as mean μg/g wet weight ± SEM (N = 5 for each determination).

*$p<0.05$; †$p<0.01$; ‡$p<0.001$, significantly different from controls (casein). §$p<0.05$; ¶$p<0.01$; #$p<0.001$, significantly different from controls (casein + 4% tyrosine). **$p<0.05$; ††$p<0.01$; ‡‡$p<0.001$, significantly different from corresponding casein + drug animals.

However, the stimulating effect of amphetamine on activity was apparently antagonized by L-tyrosine at the lower dose level but potentiated at the high dose (3.0 mg/kg). Thus, as was the case with L-dopa and L-tyrosine, not only was the main effect of L-tyrosine significant, $F(1,152) = 6.19$, $p<0.05$, so also was the interaction effects due to amphetamine and L-tyrosine given in combination, $F(3,152) = 5.38$, $p<0.05$.

Effects of 4% L-Tyrosine Supplement Combined With L-Dopa or d*-Amphetamine on Brain Monoamines*

Table 1 shows that following supplementation with dietary L-tyrosine, L-dopa produced ($df = 2,24$, $p<0.001$ in each case) dose-related increases in brain DA ($F = 37.50$), DOPAC ($F = 139.51$), and HVA ($F = 302.65$); in Table 2 at the higher dose (100 mg/kg) L-dopa increased 5-HIAA ($F = 106.08$). Significant main effects were obtained due to L-tyrosine in that increases were observed in ($df = 2,24$, $p<0.001$ in each case) brain tyrosine ($F = 186.40$), and in DOPAC ($F = 28.68$) and HVA ($F = 23.93$) as shown in Table 1; L-tyrosine also decreased 5-HT ($F = 110.00$) but increased 5-HIAA ($F = 216.80$) (Table 2). Interaction effects which were significant and examination of the data presented in Tables 1 and 2 reveal that L-tyrosine supplementation potentiated L-dopa-induced increases in DOPAC, $F(2,24) = 7.58$, $p<0.01$, and HVA, $F(2,24) = 4.43$, $p<0.05$, but antagonized the L-dopa induced rise in 5-HT which occurred at the 50 mg/kg dose, $F(2,24) = 9.06$, $p<0.01$. Table 2 also shows that the L-dopa (100 mg/kg) and L-tyrosine interacted to produce an increase in 5-HIAA when compared to animals treated with L-dopa alone (with Ro 4-4602), $F(2,24) = 58.34$, $p<0.001$.

Following 4% L-tyrosine supplement Table 1 shows that amphetamine increased DA, $F(2,24) = 3.91$, $p<0.05$, but caused marked reductions in ($df = 2,24$, $p<0.001$ in each case) DOPAC ($F = 33.75$) and HVA ($F = 24.28$). The effects on indoleamines (Table 2) were also pronounced and perhaps unexpected in that brain tryptophan levels rose, $F(2,24) = 8.40$, $p<0.01$, as did also 5-HT, $F(2,24) = 12.94$, $p<0.001$; 5-HIAA, however, following the higher dose (3.0 mg/kg) was reduced, $F(2,24) = 10.57$, $p<0.01$.

In mice receiving the 4% tyrosine supplement, Table 1 shows that amphetamine produced a number of significant main effects on catechol parameters ($df = 1,24$, $p<0.001$ in each case); thus, in comparison to casein controls (administered amphetamine only), L-tyrosine increased brain tyrosine ($F = 422.71$), but decreased NE ($F = 21.58$), DA ($F = 13.12$), and DOPAC ($F = 15.24$). The lower values obtained also for HVA (Table 1) did not reach statistical significance ($F = 2.55$). Thus, in contrast to the interactive effects produced on the various neurochemical parameters obtained by combining L-dopa with L-tyrosine pretreatment, L-tyrosine was observed to antagonize changes induced by amphetamine. The only significant interaction was that due to an amphetamine-induced rise in brain tryptophan which occurred at the 1.5 mg/kg dose in L-tyrosine supplemented animals (Table 2), but not at this dose in the casein controls, $F(2,24) = 7.82$, $p<0.01$.

DISCUSSION

In agreement with the findings of previous investigations (16, 60, 68), administration of phenylalanine resulted in large increases in brain phenylalanine and brain tyrosine. Treatment with phenylalanine or tyrosine lead to only slight fluctuations in brain NE and DA that were not significant. These results are as expected since

TABLE 2

EFFECTS OF ADMINISTRATION OF TYROSINE DIET SUPPLEMENT ALONE OR COMBINED WITH L-DOPA OR AMPHETAMINE ON MEASURES OF 5-HYDROXYTRYPTAMINE METABOLISM IN WHOLE MOUSE BRAIN

	TRYP	5-HT	5-HIAA
Controls (casein)	5.3 ± 0.2	0.55 ± 0.02	0.35 ± 0.006
Casein +			
dopa (50 mg/kg)	5.7 ± 0.3	0.63 ± 0.02*	0.36 ± 0.02
dopa (100 mg/kg)	5.4 ± 0.3	0.51 ± 0.02*	0.34 ± 0.08
Controls (casein + 4% tyrosine)	5.6 ± 0.4	0.41 ± 0.01‡	0.42 ± 0.01‡
Casein + 4% tyrosine +			
dopa (50 mg/kg)	5.3 ± 0.3	0.56 ± 0.005#††	0.37 ± 0.01
dopa (100 mg/kg)	5.1 ± 0.2	0.42 ± 0.003††	0.50 ± 0.01#††
Controls (casein)	5.3 ± 0.3	0.52 ± 0.01	0.40 ± 0.02
Casein +			
amphetamine (1.5 mg/kg)	5.9 ± 0.1	0.55 ± 0.01	0.37 ± 0.01
amphetamine (3.0 mg/kg)	7.8 ± 0.6†	0.63 ± 0.02‡	0.40 ± 0.03
Controls (casein + 4% tyrosine)	5.9 ± 0.1	0.48 ± 0.02	0.43 ± 0.01
Casein + 4% tyrosine +			
amphetamine (1.5 mg/kg)	7.7 ± 0.3¶††	0.58 ± 0.03#	0.39 ± 0.02
amphetamine (3.0 mg/kg)	7.1 ± 0.2*	0.61 ± 0.01#	0.36 ± 0.01#

Results are expressed as mean μg/g wet weight ± SEM (N = 5) for each determination. See Table 1 for experimental details.

*$p<0.05$; †$p<0.01$; ‡$p<0.001$, significantly different from controls (casein). §$p<0.05$; ¶$p<0.01$; #$p<0.001$, significantly different from controls (casein + 4% tyrosine). **$p<0.05$; ††$p<0.01$, significantly different from corresponding casein + drug animals.

inferences concerning the enhancement of CA metabolism by administration of precursor amino acids is typically based on other indices such as the accumulation of brain dopa (18,69). Indeed, Gibson *et al.* (16) found that phenylalanine given IP slightly decreased brain DA and NE while tyrosine had no effect. Oral administration of phenylalanine (the route used in the present study), as part of a procedure to produce experimental phenylketonuria in rats, reportedly increased brain DA and NE (1,20).

Since tyrosine serves as the immediate precursor for CA synthesis, the possibility that either phenylalanine or tyrosine might contribute to drug effects mediated by brain monoamines seems likely. In support of this view, our data indicate that tyrosine dietary supplement enhanced L-dopa-induced or amphetamine-induced stimulated activity. However, tyrosine or phenylalanine given alone failed to alter locomotor activity. Prior studies have usually examined open-field activity and the results obtained following administration of phenylalanine or tyrosine have varied widely. Thus, open-field activity reportedly increased in mice given phenylalanine in the diet (60), but acute administration produced decreased activity in rats (56) and no change in mice (16). Dietary administration of tyrosine had no effect on open-field activity in mice, but acute IP administration stimulated activity (16). While it is apparent that the route of administration may play a role in the results obtained, it should also be noted that the biochemical effects of these amino acids are complex and any of several causal factors may have been responsible for the differing observations. Compared to tyrosine, phenylalanine more strongly inhibits both tyrosine hydroxylase (40) and tryptophan hydroxylase (32). Brain NE levels, and DA levels to a lesser extent, are closely regulated by feedback on tyrosine hydroxylase (67). Brain concentrations of phenylethylamine increase following administration of phenylalanine, but not tyrosine, a condition believed to affect motor activity (51,56).

Previous findings regarding the effects of L-dopa on motor activity have been mixed. In agreement with previous findings (24), L-dopa in the presence of a decarboxylase inhibitor produced a dose-dependent decrease in locomotor activity. When combined with L-tyrosine pretreatment, L-dopa given with Ro 4-4602 led to a dose-dependent increase in activity. Also, previous findings from our laboratory (33) indicated that L-dopa failed to increase activity at any dose (12.5–100 mg/kg), and decreased activity at 12.5 mg/kg. However, in this prior study, 4% L-tyrosine had no effect on these effects of L-dopa, but a different decarboxylase inhibitor was used. These results are consistent with those of other investigators (21,33) showing that L-dopa produces a biphasic effect on locomotor activity with low dosages decreasing and higher dosages increasing activity. Thus, pretreatment with dietary tyrosine in the present study apparently interacted with low doses of L-dopa to produce the same behavioral effect as would be achieved with a high dose. Interestingly, this occurred even though we found that neither L-dopa alone, nor tyrosine alone, stimulated activity over the range of doses employed. It is important to note that values of brain dopa following L-dopa, with or without pretreatment with dietary L-tyrosine, were approximately the same (3.7–6.7 μg/g wet tissue) and that brain dopa after tyrosine pretreatment alone was nondetectable. These findings are at variance with the suggestion that the biphasic effect of L-dopa on locomotor activity may be due to a combination of pre- and postsynaptic actions of L-dopa (7), with the low dosages leading to depressed firing of DA neurons involved in spatial movements and higher dosages leading to a nonimpulse related release of DA thus producing stimulation of motor activity. That is, in the present study, differences in motor activity were obtained not by changing the dosage of L-dopa but by pretreating the

animals with tyrosine.

Our finding that L-dopa (100 mg/kg) produced a rise in 5-HIAA agrees with other investigators showing that L-dopa administered to rats at high dosages increases 5-HIAA and reduces brain 5-HT (2). This occurred because L-dopa must be decarboxylated in brain to exert its stimulant effects on motor activity (52) and such decarboxylation occurs apparently in both CA and 5-HT neurons, with the DA formed displacing the stored 5-HT (42,66). The action of L-dopa to reduce brain content of 5-HT led to the postulate that this monoamine may play a role in L-dopa-induced locomotion (8). Although 5-HIAA was increased by L-dopa plus tyrosine in the present study, indicating a possible release of 5-HT from serotonin neurons, our biochemical results do not provide compelling evidence that the concomitant locomotor activity induced was due to increased potentiation of serotonergic systems. Not only were 5-HT levels affected slightly by L-dopa (100 mg/kg) plus tyrosine, catecholamine function was markedly enhanced as evidenced by large increases in DA, DOPAC and HVA. Moreover, it should be noted that intraventricular administration of serotonin has an inhibitory effect on spontaneous motor activity (20) and attenuates DA-stimulated activity (29). The hyperactivity induced by L-dopa has been attributed to conversion to NE as well as the formulation of DA (30,58). However, our data indicate that brain NE concentrations following treatment with L-dopa alone, or with tyrosine, remained stable. Others (27) also obtained evidence that formation of DA rather than NE is important for the L-dopa-induced stimulated activity.

Amphetamine increased locomotor activity, thus replicating a commonly reported finding in rodents (24, 33, 43). Pretreatment by supplementing the diet with tyrosine potentiated the rise in activity produced by amphetamine (3 mg/kg), thereby implicating CA function. The biochemical data provide further evidence of CA involvement since amphetamine alone (without tyrosine) led to a rise in both brain NE and DA levels, which has been the usual finding when short-term effects of amphetamine have been measured (11, 14, 33); brain concentrations of NE are depleted during chronic amphetamine administration (35) or chronic stress (57). Surprisingly, pretreatment with tyrosine antagonized these effects of amphetamine, thus leading to reduced brain concentrations of NE and DA, yet amphetamine administration nevertheless produced an increase in locomotor activity. Moreover, this occurred despite the fact that no significant increases in brain DA or NE were observed following tyrosine administration alone, nor were any expected. Inferences concerning the enhancement of catecholamine metabolism by administration of precursor amino acids are typically based on indirect indices; modifications of brain DA or NE per se following these manipulations are seldom observed. For example, it has been demonstrated that tyrosine supplementation in conjunction with a central L-aromatic decarboxylase inhibitor resulted in an increase in brain dopa, the immediate precursor of DA (18,69). The effect of dietary tyrosine on reducing amphetamine-induced rises in brain NE and DA in the present study may be related to the close regulation of NE levels by feedback on tyrosine hydroxylase (67). Amphetamine is often used as a prototype activator of CA mechanisms in the central nervous system (14). However, compared to the marked L-dopa-induced changes observed in DA metabolites, treatment with amphetamine decreased DOPAC and antagonized the rise in HVA obtained following administration of tyrosine. Previous researchers have reported similar complex changes in DA metabolism following amphetamine treatment, i.e., DOPAC decrease accompanied by a dose-dependent decrease or increase of HVA levels (5,49). It should be noted that making correlations between whole brain neurochemistry and behavior is difficult, particularly in the dopaminergic area, where DA neurons may behave differently when exposed to amphetamine and other agents.

L-Dopa (100 mg/kg) combined with the tyrosine dietary supplement did not affect 5-HT but increased 5-HIAA. In contrast, amphetamine (3 mg/kg) following tyrosine supplementation produced a dose-dependent rise in 5-HT and left 5-HIAA unperturbed. Brain tryptophan also rose significantly in animals given amphetamine, lending further support to a growing body of evidence implicating both catecholaminergic and serotonergic processes in the action of this drug (38). For example, selective depletion of DA in brain blocks the ability of amphetamine to produce increases in locomotor activity or stereotyped behaviors (25), but the enhancement of locomotor activity by amphetamine is increased by injection of PCPA (53), lesions of the midline raphe nuclei (41), or injections of 5,6-dihydroxytryptamine (6). While these findings suggest that serotonergic function facilitates the locomotor stimulant action of amphetamine, other findings as noted in the Introduction support an inhibitory role (26,33). Also, it has been shown that direct infusion of amphetamine into the dorsal raphe nucleus of the rat inhibited the activity of 5-HT neurons at this site (48). If 5-HT does play a role then it may be that of inhibiting catecholaminergic function since injection of 5-HT into the nucleus accumbens reportedly attenuates DA-stimulated activity (29).

The data we obtained relating increased brain tryptophan and 5-HT to the locomotor stimulant action of *d*-amphetamine should not be compared directly with the *d*-amphetamine-induced hyperactivity syndrome in rats which is apparently mediated by 5-HT (54). This syndrome produced by *d*-amphetamine, which was reportedly accompanied by a rise in brain 5-HT, results from doses of 15 mg/kg or greater. We observed a rise in brain 5-HT concentrations in the present study with use of a relatively low dose of amphetamine (3 mg/kg) which caused a marked increase in locomotor activity in mice but no signs of the syndrome caused by high dosages in rats. It is cautioned that the actions of *d*-amphetamine on 5-HT systems in mediating behavioral effects may be different in these two situations.

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