

PHYSIOLOGICAL CONTROL OF BRAIN CATECHOL SYNTHESIS BY BRAIN TYROSINE CONCENTRATION

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Abstract—Treatments that raise or lower brain tyrosine concentrations in rats cause parallel changes in the rates at which their brains accumulate DOPA (after aromatic L-amino acid decarboxylase is inhibited by RO4-4602). When the neutral amino acid drug *p*-chlorophenylalanine (PCPA) was injected i.p. into rats (100 mg/kg), it decreased brain tyrosine concentrations over a wide range, partly by competing with tyrosine for uptake into the brain. Brain DOPA accumulation after RO4-4602 decreased in parallel. At this dosage, PCPA did not inhibit tyrosine hydroxylase activity *in vivo*, and, at very high concentrations (10^{-3} M) *in vitro*, it inhibited the enzyme only slightly. Hence, the decrease in DOPA accumulation probably derived from a decrease in the availability of tyrosine (the substrate for tyrosine hydroxylase) rather than from a change in enzyme activity. Similarly, the neutral amino acids, valine and isoleucine (which have been used previously to modify brain tyrosine concentrations and, hence, catechol synthesis), had no effect on tyrosine hydroxylase activity *in vitro* in concentrations up to 10^{-3} M. At high concentrations (10^{-3} M), leucine slightly inhibited the enzyme. When rats ingested a single meal containing 40 per cent casein, their brain evidenced increases in both tyrosine levels and catechol synthesis. Thus, the availability of tyrosine to the brain may be one of the factors normally controlling brain catecholamine synthesis.

Mammalian brains synthesize the catecholamines dopamine and norepinephrine and the indoleamine serotonin from the amino acid precursors L-tyrosine and L-tryptophan. The first (and probably the rate-limiting) step in the synthesis of each of these monoamine neurotransmitters involves the addition of a hydroxyl group to either the aromatic ring of tyrosine or the indole nucleus of tryptophan [1]; the enzymes tyrosine hydroxylase [2] and tryptophan hydroxylase [3] catalyze these reactions. DOPA and 5-hydroxytryptophan, the hydroxylated products, are then decarboxylated (by the enzyme aromatic L-amino acid decarboxylase [4]) to form dopamine and serotonin.

The rate of serotonin synthesis in rat brains can be accelerated by physiologic manipulations that raise brain tryptophan concentrations (e.g. feeding rats a single protein-free meal [5]). This finding is compatible with the view that the tryptophan hydroxylase enzyme is not saturated with its amino acid substrate *in vivo* [6]. Brain tryptophan concentration depends, in turn, upon the pattern of amino acids in the plasma: it varies directly with plasma tryptophan concentration and indirectly with the plasma concentrations of other neutral amino acids that compete with tryptophan for transport into the brain [7,8]. We recently described preliminary evidence that brain tyrosine concentrations might also influence the rates at which catecholamine neurons synthesize their neurotransmitters [9]. In that study the injection of amino acids that did or did not modify brain tyrosine levels caused parallel changes in the rates at which rat brains accumulated DOPA after decarboxylase inhibition.

We have now examined further the relationship between brain tyrosine level and catechol synthesis, which we found to be linear over a wide range. We

also found that the activity of the rate-limiting enzyme tyrosine hydroxylase was not affected by the experimental conditions used, and that a physiologic treatment that raised brain tyrosine also accelerated catechol synthesis.

MATERIALS AND METHODS

Groups of 6–8 male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), each weighing 150 g (except where noted), were housed in hanging cages, given access to water and a 26 per cent protein diet (Charles River Rat and Mouse Formula), and maintained under light (300 microwatts/cm²; Vita-Lite, Duro-Test Co., North Bergen, NJ) between 8 a.m. and 8 p.m. daily. Rats used for diet experiments were fasted overnight immediately prior to the study and then allowed to consume the experimental diet starting at 10 a.m. Diets were prepared in agar gel (35 g/1000 ml of water) with the compositions outlined in Table 1. All injections were intraperitoneal.

We estimated catechol synthesis in the rats' brains by measuring the accumulation of DOPA 30 or 60 min after the administration (800 mg/kg) of the DOPA decarboxylase inhibitor, RO4-4602 (generously provided by the Hoffman-LaRoche Co., Nutley, NJ) [6,9]. The accumulation of DOPA was linear for at least 60 min after decarboxylase inhibition; brain dopamine and norepinephrine levels were not significantly depressed during this interval. In some experiments, the methyl ester of *p*-chlorophenylalanine (PCPA) (Regis Chemical Co., Chicago, IL)—a synthetic amino acid that, among other actions, competes with tyrosine for uptake into the brain [9,10]—was administered (100 mg/kg) at various

Table 1. Composition of protein diets

| Dietary constituent | Casein diet* (g/1000 ml water) | | | | | |
|---------------------|--------------------------------|-----|-----|-----|-----|-----|
| | 0% | 8% | 18% | 24% | 40% | 75% |
| Casein† | 0 | 80 | 180 | 240 | 400 | 750 |
| Destrose | 270 | 241 | 207 | 184 | 133 | 3 |
| Sucrose | 221 | 198 | 167 | 167 | 151 | 4 |
| Dextrine | 270 | 241 | 207 | 184 | 133 | 3 |
| Salt mix‡ | 40 | 40 | 40 | 40 | 40 | 40 |
| Vitamin mix§ | 22 | 22 | 22 | 22 | 22 | 22 |

* Each diet also contained 150 ml of Mazola Oil (Best Foods, CPC International Inc., Englewood Cliffs, NJ).

† From General Biochemicals; Chagrin Falls, OH.

‡ Roger's-Harper's, Teklad Test Diets, Madison, WI.

§ From Nutritional Biochemicals, Cleveland, OH.

times before injection of RO4-4602. In the diet experiments, rats fasted overnight were allowed access to one of the test diets for 1 hr, injected with RO4-4602, permitted to eat for 30 min, and then killed. All amino acids were administered in doses of 100 mg/kg; in the subsequent discussion, doses are given as $\mu\text{M/kg}$.

Tyrosine hydroxylase activity was assayed by measuring the evolution of $^{14}\text{CO}_2$ produced by the enzymatic decarboxylation of carboxyl-labeled DOPA formed from carboxyl-labeled L-tyrosine [11]. Whole brains and corpora striata were homogenized in 5 and 10 vol. respectively, of 0.05 M Tris-acetate buffer, pH 6.0, containing 0.2 per cent Triton X-100 (Harleco, Philadelphia, PA). The homogenates were centrifuged at 10,000 g for 10 min, and the supernatant fluid was decanted for assay [2]. The assay medium contained (in a total vol. of 110 μl): 50 μl of brain homogenate; 65 nmoles of DMPH_4 (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride [synthetic cofactor obtained from Calbiochem, San Diego, CA]); 29 nmoles of pyridoxal phosphate; 4 nmoles of 2-mercaptoethanol; 240 units of catalase; 0.01 m-moles of phosphate buffer; and 10 μl of aromatic L-amino acid decarboxylase prepared from hog

kidneys [11]. Samples were preincubated at 37° for 2 min.

The reaction was started by adding 10 μl of L-[1- ^{14}C]tyrosine (sp. act., 54.6 mCi/m-mole [from New England Nuclear Corp., Boston, MA], diluted with 1 mM tyrosine to a final concentration in the assay medium of 0.1 mM) to the sample and incubating it at 37° for 30 min. The assay was stopped by injection of 0.5 ml of 10 per cent trichloroacetic acid through the rubber stopper. The acidified medium was shaken at 37° for an additional 2 hr to recover $^{14}\text{CO}_2$, which was trapped by folded filter paper strips in 0.2 ml of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The strips were then placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear), and their radioactivity was counted.

Within an enzyme range of 20–50 μl , the assay was linear with time for 45 min. Samples of boiled enzyme or complete assay mixtures containing 0.2 mM moniodotyrosine were used as blanks.

Brains used for amino acid analysis were homogenized in 5 vol. of 6 per cent trichloroacetic acid; the acid was then removed from the supernatant fraction by 3 or 4 washes with equal vol. of absolute ether. Samples were lyophilized overnight, made up to 1 ml with 0.2 N sodium citrate buffer, pH 2.2, and filtered through a Millipore filter (Millipore Corp., Bedford, MA) before being placed on the Beckman 119 or 121 C amino acid analyzer (Beckman Instruments, Inc., Fullerton, CA) [12].

Previously described methods were used to assay brains for tyrosine [13] and DOPA [14, 15].

RESULTS

Brain catechol synthesis at various intervals after PCPA. We have previously observed that PCPA administration produces parallel decreases in brain tyrosine concentration and catechol synthesis [9]. In our present study, we used PCPA given at various inter-

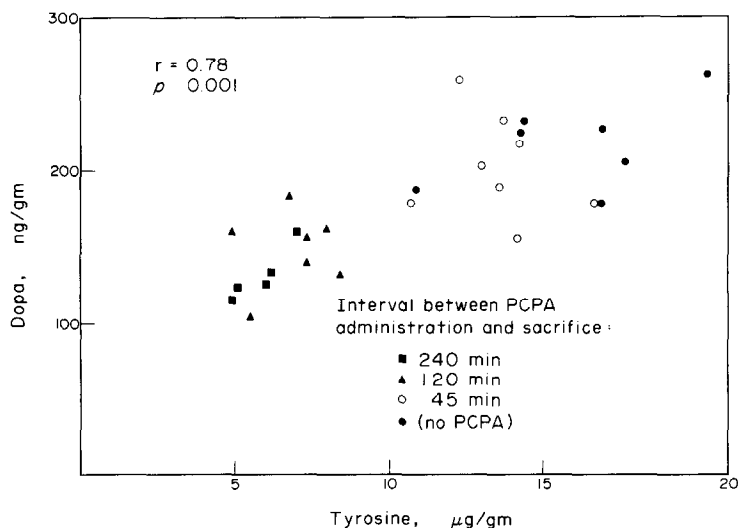


Fig. 1. Relationship between brain DOPA concentration and brain tyrosine level after PCPA administration. The animals were given injections of PCPA (100 mg/kg, i.p.) at various times and were killed 1 hr after a second injection of RO4-4602 (800 mg/kg). Each point represents DOPA and tyrosine values obtained from a single brain sample.

Table 2. Tyrosine hydroxylase activity of brain homogenates from animals given PCPA or valine

| Treatment | Number of animals | Per cent of control activity | Statistical significance* |
|-----------------------------|-------------------|------------------------------|---------------------------|
| 1 hr after valine injection | 4 | 110.5 \pm 3.2 | N.S. |
| 1 hr after PCPA injection | 4 | 92.6 \pm 23.3 | N.S. |
| 2 hr after PCPA injection | 5 | 112.2 \pm 6.3 | N.S. |

Male Sprague-Dawley rats weighing 200 g were injected (100 mg/kg, i.p.) with valine, PCPA, or their vehicle (water) and killed 1 or 2 hr later. A 50- μ l aliquot of whole brains homogenized in Tris-acetate buffer (pH 6.0) was used for assay of tyrosine hydroxylase activity. Tyrosine hydroxylase in control samples (9 animals) was 0.201 nM CO₂/mg protein/hr.

* N.S. = not significantly different from control animals.

vals before RO4-4602 to produce a range of decreases in brain tyrosine; we then determined the extent to which the rate of DOPA accumulation paralleled the tyrosine concentration in each rat's brain. Rats received PCPA either 15 min after RO4-4602 administration or 60 or 180 min before the decarboxylase inhibitor. All rats were killed 1 hr after the injection of RO4-4602.

The decreases in brain tyrosine concentrations after PCPA administration varied over a wide range, from 15.95 μ g/g (control) to 7.63 μ g/g (animals killed 4 hr after receiving the drug; $P < 0.001$) (Fig. 1). Brain DOPA accumulation showed a parallel decrease from 217 ng/g (control) to 149 ng/g ($P < 0.001$). Brain DOPA and tyrosine concentrations correlated significantly ($r = 0.78$; $P < 0.001$) over this range (Fig. 1).

Tyrosine hydroxylase activity after PCPA or neutral amino acid administration. PCPA inhibits the biosynthetic enzyme tryptophan hydroxylase [16] and has been widely used in the hope that it would specifically deplete brain serotonin. PCPA also inhibits phenyl-

alanine hydroxylase activity in the liver but has little or no effect on tyrosine hydroxylase activity. We have used PCPA to modify brain tyrosine levels (Fig. 1). In an earlier study, we showed similar effects with several of the branched-chain amino acids (leucine, valine, and isoleucine) which are in the same brain transport group as tyrosine [9], i.e. the large neutral amino acids [17]. In all these experiments, a parallel reduction in DOPA accumulation accompanied the decrease in brain tyrosine level (Fig. 1) [9].

To determine whether the fall in DOPA accumulation was related to a drug-induced decrease in tyrosine hydroxylase activity rather than to altered substrate (tyrosine) availability, we measured tyrosine hydroxylase activities in the brains of animals receiving either PCPA or one of the other neutral amino acids. Animals were injected with PCPA (37 μ M/kg) or with the neutral amino acid valine (85 μ M/kg) (which lowered brain tyrosine to 67 per cent of control and brain DOPA accumulation to 69 per cent of control); they were killed 1 or 2 hr later. Whole brains were removed, homogenized in 0.05 M Tris-acetate buffer, and assayed for tyrosine hydroxylase activity. Neither valine nor PCPA, administered *in vivo*, significantly changed tyrosine hydroxylase activity, as assayed *in vitro* (Table 2).

In other studies, PCPA or one of the other neutral amino acids was added to the assay mixture in various concentrations, *in vitro*, and tyrosine hydroxylase activity was determined with homogenates of whole brains or striata as the enzyme source. The striata showed considerably greater activity. The branched-chain amino acids valine and isoleucine had no effect on the tyrosine hydroxylase activity of whole brain or caudate homogenates, even when they were present in concentrations as high as 1 mM. Leucine at this concentration did somewhat inhibit tyrosine hydroxylase activity in homogenates of whole brains. PCPA had no effect on tyrosine hydroxylase activity in concentrations of 1 μ M to 0.1 mM, but, at a concentration of 1 mM, it did suppress enzyme activity by 40 per cent (Table 3). In contrast, phenylalanine concentrations as low as 0.1 mM significantly inhib-

Table 3. Effects of neutral amino acids, added *in vitro*, on tyrosine hydroxylase activities of homogenates of whole brains or caudate nuclei

| Amino acid added to the medium | Control value | Tyrosine hydroxylase activity at various amino acid concentrations (nmoles ¹⁴ CO ₂ /hr/mg protein) | | | |
|-----------------------------------|------------------|---|--------------------|--------------------|--------------------|
| | | 10 ⁻⁶ M | 10 ⁻⁵ M | 10 ⁻⁴ M | 10 ⁻³ M |
| A. Caudate nuclei | | | | | |
| PCPA | 2.70 | 2.31 | 2.60 | 2.40 | 1.56 |
| Valine | 2.70 | 2.28 | 2.68 | 2.69 | 2.50 |
| B. Whole brains | | | | | |
| PCPA | 0.145 | 0.154 | 0.140 | 0.140 | 0.089 |
| Isoleucine | 0.145 | 0.136 | 0.156 | 0.154 | 0.155 |
| Leucine | 0.169 | --- | 0.155 | 0.149 | 0.113 |
| Phenylalanine | 0.150 | 0.154 | 0.172 | 0.095 | 0.023 |

PCPA or one of the neutral amino acids (in a vol. of 10 μ l) was added to the assay mixture, containing 50 μ l of homogenate prepared from whole brains or caudate nuclei from untreated animals, and the evolution of ¹⁴CO₂ was measured.

Data are given as averages of duplicate determinations.

ited the conversion of labeled tyrosine to DOPA, and hence to $^{14}\text{CO}_2$ (Table 3). This concentration of phenylalanine is close to that normally found in the brain.

To determine whether the concentration of branched-chain amino acids examined *in vitro* for possible inhibition of tyrosine hydroxylase were as high as the brain levels attained *in vivo* after administering the described doses, we gave rats valine, isoleucine or leucine and then measured their brain concentrations of these substances after 1 hr. The valine concentration was 217 μM in valine-treated rats (as compared to 71 μM in control animals), the isoleucine concentration rose to 66 μM (as compared to 41 μM in control animals), and the leucine concentration was 68 μM in leucine-treated rats (as compared to 42 μM in control rats). Hence, the *in vitro* concentrations of these compounds needed to inhibit tyrosine hydroxylase (i.e. 1 mM for leucine and greater than 1 mM for valine and isoleucine) (Table 3) were considerably greater than those occurring *in vivo*. (Although we did not determine PCPA levels in brain, they were probably less than 0.1 mM: Gal *et al.* found a maximum PCPA concentration of 0.72 $\mu\text{M/g}$ 6 hr after injecting rats with 405-mg/kg doses of the ethyl ester of PCPA [19].)

Using the coupled decarboxylation system and the synthetic cofactor DMPH₄, we determined an *in vitro* K_m for tyrosine of approximately 0.10 mM in whole brain homogenates. From the DOPA accumulation data obtained with PCPA, we obtained a double reciprocal plot of substrate concentration versus velocity, as described by Carlsson *et al.* [6]. Using weighted least-squares analysis [20], we estimated the *in vivo* K_m of tyrosine hydroxylase for tyrosine to be 0.043 mM. The concentration of tyrosine in brains of normal rats (fed *ad lib.* with rat chow) was 0.088 mM, a value which is very close to the measured K_m ; hence, tyrosine hydroxylase is probably not saturated with its amino acid substrate *in vivo*. Treatment with PCPA (or other neutral amino acids) lowered the brain tyrosine concentration to 0.036 mM, which is well below its K_m (for tyrosine hydroxylase) and thus could account for the reduced rate of tyrosine hydroxylation.

Brain catechol synthesis after ingestion of a protein meal. To determine whether the brain tyrosine level normally varies and, if so, whether such variations are sufficient to modify catechol synthesis, we measured catechol synthesis (by decarboxylase inhibition) in animals consuming a single high-protein meal which would be expected to elevate brain tyrosine. Groups of rats fasted overnight were given access to either a carbohydrate (protein-free) or 40 per cent casein meal for 1 hr; they were then injected with RO4-4602 and killed 30 min later. In all of twelve similar experiments, the ingestion of the casein meal caused 60–70 per cent increases in both brain tyrosine concentration ($P < 0.001$) and brain DOPA accumulation ($P < 0.001$) (Table 4A). The ingestion of a carbohydrate meal generally increased brain tyrosine level and brain DOPA accumulation (i.e. compared to these levels in animals that continued to be fasted). Occasionally, the consumption of the carbohydrate meal did not elevate brain tyrosine (Table 4B)—a finding that was especially common in studies on

Table 4. Effect of single meals containing 0 or 40 per cent protein on the synthesis of DOPA in rat brains

| Treatment | Brain tyrosine level ($\mu\text{g/g}$) | Brain DOPA level (ng/g) |
|-------------------|--|-------------------------|
| A. 200-g rats | | |
| Fasted | 12.9 ± 0.6 | 66.5 ± 4.2 |
| Protein-free diet | $18.8 \pm 0.6^*$ | $116.5 \pm 6.0^\dagger$ |
| 40% protein diet | $26.9 \pm 0.7^*$ | $108.0 \pm 5.3^\dagger$ |
| B. 100-g rats | | |
| Fasted | 14.6 ± 0.5 | 53.0 ± 4.2 |
| Protein-free diet | 15.0 ± 0.5 | 53.1 ± 3.6 |
| 40% protein diet | $25.3 \pm 0.9^*$ | $82.3 \pm 7.0^\dagger$ |

Male Sprague-Dawley rats weighing 200 g (A) or 100 g (B) were fasted overnight and given access to food at 10 a.m. At 11 a.m., they received RO4-4602 (800 mg/kg, i.p.). They were killed at 11:30 a.m. Animals on the protein-free and 40 per cent protein diets consumed 6.6 and 9.2 g, respectively, in group A, and 8.2 and 9.7 g, respectively, in group B.

* $P < 0.001$ differs from fasted animals.

† $P < 0.01$ differs from fasted animals.

younger animals (weight, 100 g). In all these cases, brain DOPA accumulation also failed to increase. The rate of DOPA accumulation tended to be somewhat lower in the younger rats (Table 4).

Effect of varying the protein content of the diet on catechol synthesis. In order to vary the rats' brain tyrosine concentrations physiologically, we fed fasted rats single meals in which the protein content ranged from 8 to 75 per cent; brain tyrosine level and DOPA accumulation were measured after decarboxylase inhibition, as above.

With increasing protein content in the meal, brain tyrosine level also increased; however, at very high concentrations of protein (75 per cent casein), the food-induced increase in brain tyrosine was somewhat suppressed (Table 5), possibly because the very large increases in plasma neutral amino acid concentrations suppressed tyrosine uptake into the brain. Among animals consuming meals of different composition, the rates at which the brain accumulated DOPA (after RO4-4602 administration) roughly paralleled the food-induced increases in brain tyrosine concentration (Table 5). DOPA accumulation was less rapid in animals consuming the 75 per cent protein meal than in those eating 8 per cent casein, even though the brain tyrosine levels were not significantly different (Table 5). This disparity could reflect a greater inhibition by phenylalanine of tyrosine hydroxylase activity after the high-protein meal (Tables 3 and 6).

DISCUSSION

Our observations affirm that the concentration of tyrosine in rat brain influences the rate at which the rat brain synthesizes catecholamines. This influence is possible because the tyrosine hydroxylase enzyme is not saturated with its amino acid substrate (tyrosine) *in vivo* and becomes less so after short-term fasting or the administration of another neutral amino acid. Our data further demonstrate that the relationship between brain tyrosine concentration and cat-

Table 5. Effect of varying the protein content of the diet on catechol synthesis

| Treatment | Brain tyrosine level ($\mu\text{g/g}$) | Brain DOPA level (ng/g) | Serum tyrosine level ($\mu\text{g/ml}$) |
|-----------------|--|------------------------------------|---|
| Fasted | 12.25 ± 0.45 | 66 ± 7 | 20.69 ± 1.08 |
| 8% casein diet | $19.84 \pm 0.81^*$ | $123 \pm 11^\dagger$ | 23.31 ± 1.21 |
| 18% casein diet | $20.75 \pm 1.32^*$ | $91 \pm 4^\ddagger$ | $30.20 \pm 3.23^\ddagger$ |
| 40% casein diet | $24.64 \pm 1.68^*$ | $106 \pm 8^\ddagger$ | $54.66 \pm 6.08^*$ |
| 75% casein diet | $21.05 \pm 1.43^*$ | 88 ± 4 | $48.65 \pm 3.90^*$ |

Male Sprague-Dawley rats were fasted overnight and allowed access to one of the test diets at 10 a.m. One hr later, they received an injection of RO4-4602 (800 mg/kg, i.p.). They were decapitated 30 min after injection. Blood was collected from the necks, and whole brains were analyzed for tyrosine [13] and DOPA [14, 15].

* $P < 0.01$ differs from fasted animals.

† $P < 0.001$ differs from fasted animals.

‡ $P < 0.05$ differs from fasted animals.

Table 6. Effect of varying the protein content of the diet on brain phenylalanine concentration

| Treatment | Brain phenylalanine level ($\mu\text{g/g}$) |
|-------------------|---|
| Fasted | 13.12 ± 0.97 |
| Protein-free diet | $16.65 \pm 0.51^*$ |
| 8% casein diet | $18.41 \pm 0.71^\dagger$ |
| 18% casein diet | $18.06 \pm 0.71^\dagger$ |
| 40% casein diet | $21.95 \pm 1.31^\dagger$ |
| 75% casein diet | $32.08 \pm 0.67^\dagger$ |

Male Sprague-Dawley rats were fasted overnight and allowed access to one of the test diets at 10 a.m. One hour later they received an injection of RO4-4602 (800 mg/kg, i.p.). They were killed 30 min later, and whole brains were analyzed for phenylalanine [18].

* $P < 0.01$ differs from fasted animals.

† $P < 0.001$ differs from fasted animals.

echol synthesis (obtained over a wide range of tyrosine concentrations [Fig. 1]) is not due to an effect of other neutral amino acids (besides phenylalanine and, possibly, leucine) on the activity of tyrosine hydroxylase (Tables 2 and 3); moreover, brain tyrosine concentrations probably affect catechol synthesis under normal conditions (i.e. after rats consume nutrients in proportions similar to those they are likely to eat naturally) (Tables 4 and 5).

The broad correlation between brain tyrosine level and catechol synthesis was clearly evident (Fig. 1) in animals acutely treated with PCPA, a drug widely used to inhibit tryptophan hydroxylase activity and serotonin synthesis [16]. Tagliamonte *et al.* [10] have observed decreased conversion of [^3H]tyrosine to [^3H]dopamine and [^3H]norepinephrine in PCPA-treated rats; the level of endogenous tyrosine and the specific activity of [^3H]tyrosine in the brain were also reduced. These authors attributed their findings to an impaired transport of tyrosine from plasma to brain.

Treatments with neutral amino acids, which also inhibit the transport of tyrosine into the brain, caused parallel reductions in brain catechol synthesis [9]. These reductions in synthesis cannot be attributed to changes in the kinetic properties of tyrosine hydroxylase (e.g. direct inhibition), inasmuch as the concentrations of neutral amino acids used to inhibit tyro-

sine transport had no effect on brain tyrosine hydroxylase activity *in vitro* (Table 3).

In choosing a 40 per cent protein meal for our original diet studies as a means for physiologically increasing brain tyrosine level, we made the following two assumptions:

(1) Added protein would cause a greater increase in plasma tyrosine than in other neutral amino acids because the protein contributes tyrosine both directly and indirectly—i.e. as phenylalanine, much of which is transformed to tyrosine in passing through the liver from the portal to the systemic circulation.

(2) Brain tyrosine levels change postprandially as a function of the ratio between plasma tyrosine level and the concentrations of competing neutral amino acids (i.e. just as brain tryptophan had been shown to vary with the plasma tryptophan/competitor ratio [7, 8]).

The latter assumption has been confirmed in studies by Fernstrom and Faller, who showed that for each of the six neutral amino acids tested (tyrosine, tryptophan, phenylalanine, leucine, isoleucine and valine), its brain concentration after food consumption could be predicted by the ratio of its plasma concentration to the sum of the five competitors [21].

In considering factors regulating catecholamine synthesis, investigators have often ignored the effect of substrate (i.e. tyrosine) concentration on the basis that its brain concentration would be well above the K_m , as determined *in vitro*. The problem of knowing what K_m value to compare *in vivo* brain tyrosine levels to is complicated by the fact that at least three different methods are used to assay tyrosine hydroxylase [2, 11], and that different investigators utilize different cofactors for these assays (e.g. the presumed natural cofactor, tetrahydrobiopterin, or the artificial cofactors, 6-methyltetrahydropteridine [6-DMPH₄] and 6,7-dimethyltetrahydropteridine [DMPH₄]). The apparent K_m values obtained for the substrates (tyrosine and oxygen) also vary depending upon the pterin used; for example, the tyrosine K_m for adrenomedullary tyrosine hydroxylase can vary by a factor of 10 depending on which cofactor is chosen [22]. Differences in the media used for homogenization and in the buffer systems employed in the assay mixture can also cause considerable differences in tyrosine hydroxylase activity, and in its affinity for its cofactors

and substrate [23]. Thus, from the presently available, often conflicting literature, it is very difficult to draw simple conclusions regarding the degree to which tyrosine hydroxylase is saturated *in vivo*.

Measurement of the *in vivo* tyrosine K_m by a method similar to that used by Carlsson for tryptophan and tryptophan hydroxylase [6] might provide a better index of the affinity of tyrosine hydroxylase for its substrate *in vivo* (even though interpretation of such data is complicated by the possibility that the tyrosine concentration in homogenates of whole brain is not equal to its concentration at the locus of tyrosine hydroxylase). Using this method [20], we calculated a K_m for whole brain. This value was approximately equal to the whole brain tyrosine concentration. Therefore, even in the normally fed rat, the enzyme is only about half saturated. Fasting or the administration of a neutral amino acid further reduces the saturation of the enzyme and, correspondingly, the catechol synthesis rate.

It is well established that the amount and the activity of tyrosine hydroxylase in a given tissue can control the rate at which that tissue converts tyrosine to DOPA. Thus, treatments that accelerate catecholamine release from adrenal medulla or nerve terminals (by increasing presynaptic inputs) rapidly activate existing tyrosine hydroxylase—either by decreasing the end-product inhibition of the enzyme [24] or by allosterically changing its affinity for its substrate and cofactor [25]. When prolonged, such treatments can also increase the amount of tyrosine hydroxylase enzyme protein in the tissue, probably by accelerating its synthesis [26, 27]. As demonstrated in this study, the substrate control of tyrosine hydroxylation probably serves a physiological function very different from the enzyme responses to enhanced neuronal activity; it allows neurotransmitter synthesis to be modulated by the plasma amino acid pattern, which in turn varies with food consumption, the actions of various hormones, and general metabolic state. Of course, it remains to be determined whether the changes in neuronal catecholamine synthesis (induced by modifying precursor levels or by other means) actually influence the quantities of neurotransmitter which are released into synapses and which act on postsynaptic receptors.

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