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STUDIES ON THE INTERACTION BETWEEN CATECHOLAMINES AND TYROSINE AMINOTRANSFERASE IN BRAIN

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Abstract—The tyrosine transaminating enzyme present in whole homogenates of brain tissue is not localized specifically in catecholamine-containing nerve terminals; its activity is not altered by a drug-induced increase or decrease in whole brain norepinephrine concentration; and it is not induced by the addition, *in vitro*, of norepinephrine. We conclude on the basis of these and other considerations that interactions between norepinephrine and the transamination of tyrosine are of no apparent significance in the regulation of brain catecholamine metabolism.

NOREPINEPHRINE, dopamine and serotonin appear to play important roles in the mammalian central nervous sytem, where they may act as synaptic transmitters. Each of these biogenic amines is formed within the brain from an essential aromatic amino acid: the catecholamines from tyrosine and phenylalanine,[1,2] and serotonin from tryptophan.[3] Most discussions of the regulation of biogenic amine biosynthesis have emphasized the roles of synthesizing enzymes. Yet, several variables, including endocrine state, diet, stress and time of day, can influence the concentration of tyrosine and tryptophan in both tissue and plasma, and it is possible that alterations in the availability of these amino acids might also lead to changes in synthesis rates (see review by Wurtman and Fernstrom[4]). This appears to be the case for serotonin; changes in brain and plasma tryptophan are accompanied by parallel changes in serotonin concentration in brain.[5,6] It has often been suggested that a similar interaction exists between tyrosine and catecholamine synthesis, and that by altering the availability of tyrosine, tyrosine aminotransferase (EC 2.6.1.5) might play a role in this interaction.[4,7–10] This hypothesis is made even more interesting by recent reports that liver and brain tyrosine aminotransferase can be induced by catecholamines.[9–13] Thus, there exists the possibility that end-product inhibition of catecholamine biosynthesis might be accomplished by end-product induction of the catabolism of the precursor. Such an action might serve to supplement the generally accepted end-product inhibition of tyrosine hydroxylase.[14] That is,

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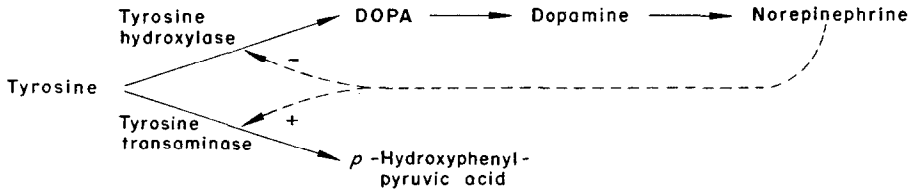

Although intriguing, this scheme must be questioned on several grounds. For example, tyrosine hydroxylase appears to be saturated by its precursor; studies in which brain tyrosine concentration has been manipulated have failed to find any change in catecholamines.[15,16,*] Moreover, the high K_m of tyrosine aminotransferase relative to the K_m of tyrosine hydroxylase and to the concentration of tyrosine in both blood and brain suggests that, were these two enzymes to compete for tyrosine, further induction of the transaminating pathway would be unnecessary. In addition, several investigators have failed to observe any induction of tyrosine aminotransferase by catecholamines.[7,17,18] Therefore, we have carefully examined the hypothesis that catecholamines can influence the rate of tyrosine transamination in the brain. We find no support for such an interaction.

MATERIALS AND METHODS

Animals. Male albino rats of the Sprague–Dawley strain (Zivic–Miller Laboratories, Inc., Pittsburgh, Pa.), weighing 150–250 g, were housed, one per cage, in metal cages with wire mesh floors for at least 1 week prior to sacrifice. Purina lab chow (Ralston Purina Company, St. Louis, Mo.) and tap water were freely available throughout the experiment. Room temperature was 23–25° and cool-white fluorescent lights were on from 600 to 1800 hr each day.

Materials. Woelm Alumina (aluminum oxide, neutral, grade 1) was obtained from Waters Associates, Inc. (Framingham, Mass.); L-noradrenaline bitartrate and 6-hydroxydopamine hydrobromide from Regis Chemical Company (Chicago, Ill.) and sodium sulfite from Baker Chemical Company (Phillipburg, N.J.). Pyridoxal-5′-phosphate, α-ketoglutaric acid (monophosphate salt), *p*-hydroxyphenylpyruvic acid and Triton X-100 were obtained from Sigma Chemical Company (St. Louis, Mo.). Tryptamine-2-^{14}C-bisuccinate (47·3 Ci/m-mole) was purchased from New England Nuclear Corp. (Boston, Mass.). α-Methyltyrosine methyl ester hydrochloride was obtained from Regis Chemical Company (Chicago, Ill.). Pheniprazine hydrochloride (Catron) was furnished by Lakeside Laboratories (Milwaukee, Wis.), and reserpine (Serpasil) and its vehicle by CIBA Pharmaceutical Company (Summit, N.J.). All other chemical reagents were obtained from Fisher Scientific Company (Pittsburgh, Pa.) and were of the highest available purity. All drug doses were calculated as milligrams of the free base per kilogram of body weight.

Preparation of tissues. Animals were sacrificed by decapitation at 1600–1700 hr (unless otherwise noted). Brains (and sometimes livers) were removed and stored on dry ice until homogenization

* M. J. Zigmond and W. J. Shoemaker, unpublished observations.

Enzyme assays. The rate of conversion of tyrosine to *p*-hydroxyphenylpyruvic acid in whole brain homogenate was determined using modifications[10,19,20] of the method of Diamondstone.[21] With this procedure, *p*-hydroxyphenylpyruvic acid is converted to *p*-hydroxybenzaldehyde at a basic pH and the aldehyde is measured by spectrophotometry. Samples were stored below −20° for at least 48 hr prior to assay in an attempt to permit the degradation of enzymes known to metabolize *p*-hydroxyphenylpyruvic acid. Brain tissue was homogenized in 4 vol. of cold 0·1 M phosphate buffer (pH 7·6) and then sonicated (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) for 45 sec at 0° after the addition of Triton X-100 (1 %, v/v). Liver was homogenized in 9 vol. of 0·154 M KCl and then centrifuged at 0° for 30 min at 48,000 *g*. Whole sonicated brain homogenate, 100 μl, or high speed liver supernatant, 25 μl, was used for the enzyme assay. The enzyme preparation was added to 4·0 ml of an incubation mixture containing 400 μmoles sodium phosphate buffer (pH 7·6), 0·5 μmole pyridoxal-5′-phosphate, and 28 μmoles tyrosine, and the mixture was preincubated for 10 min while shaking at 37°. The reaction was initiated by the addition of 100 μl (45 μmoles) of α-ketoglutaric acid and was stopped after 30 min by the addition of 400 μl of 10 N KOH. Blanks were kept on ice. After incubation, brain samples and blanks were centrifuged at 0° for 5 min at 48,000 *g*. The liver samples and the supernatant of the brain samples were then allowed to stand at room temperature (24°) for 30–45 min and absorbance was read at 329 nm (uncorrected) using a Hitachi Perkin–Elmer spectrophotometer (Tokyo, Japan). Duplicate samples and a blank were assayed for each tissue. Values were calculated at micromoles of *p*-hydroxyphenylpyruvic acid produced per hr per g of tissue using an external standard curve. Monoamine oxidase was assayed by incubating ^{14}C-tryptamine with brain homogenate and extracting ^{14}C-indole-3-acetic acid with toluene at an acid pH, according to the method of Wurtman and Axelrod.[22] Experimental values were compared with control values using Student's *t*-test.

Norepinephrine assay. Norepinephrine concentration in brain tissue was determined by modifications of the methods of Crout[23] and Chang.[24] An aliquot of the homogenate used for enzyme assay was combined with an equal volume of 0·8 M perchloric acid and centrifuged at 0° for 10 min at 15,000 *g*. The supernatant was adjusted to pH 8·5 with NaOH and poured over a 400 mg Alumina column previously washed with 25 ml of 0·2 N NaOAc (pH 8·5). The column was then washed with 5 ml NaOAc and 5 ml H_2O, the norepinephrine was eluted with 3 ml of 0·2 N HOAc, and 500 μl of the eluate was added to 800 μl of 0·1 M sodium phosphate buffer (pH 7·0). Iodide reagent (0·1 M iodine and 0·3 M potassium iodide), 100 μl, was added to the reaction mixture, followed 2 min later by 300 μl of a freshly prepared solution of alkaline sodium sulfite (250 mg sodium sulfite/ml of H_2O diluted 10-fold with 5 N NaOH). After 2 additional min, 200 μl glacial acetic acid was also added. Blanks received alkaline sodium prior to iodine. Samples and blanks were then heated for 4 min in a 100° oven and allowed to stand at room temperature for 10–30 min. Fluorescence was read at 382/482 nm (uncorrected) using an Aminco–Bowman spectrophotofluorometer (American Instrument Company, Silver Springs, Md.). Values were calculated as micrograms of norepinephrine per g of fresh tissue weight using internal standards and blanks for each sample. No correction was made for recovery (86 per cent).

RESULTS

Tyrosine aminotransferase activity in brain tissue. Tyrosine aminotransferase activity was observed in whole brain homogenates (Table 1). The reaction requires α-ketoglutaric acid. In the absence of tyrosine, the reaction rate is 90 per cent lower than that observed with $7{\cdot}0 \times 10^{-3}$ M tyrosine, while essentially no enzyme activity is observed at 0°. (In subsequent experiments, the 0° "blank" is subtracted from activity observed at 37°.) The reaction product (prior to the addition of KOH) has an R_f identical to that of *p*-hydroxyphenylpyruvic acid (0·74) using ascending paper chromatography and a butanol–acetic acid–water (4 : 1 : 1) solvent system. After the addition of KOH, both compounds have an absorbance spectrum with a peak at 329 nm (uncorrected).

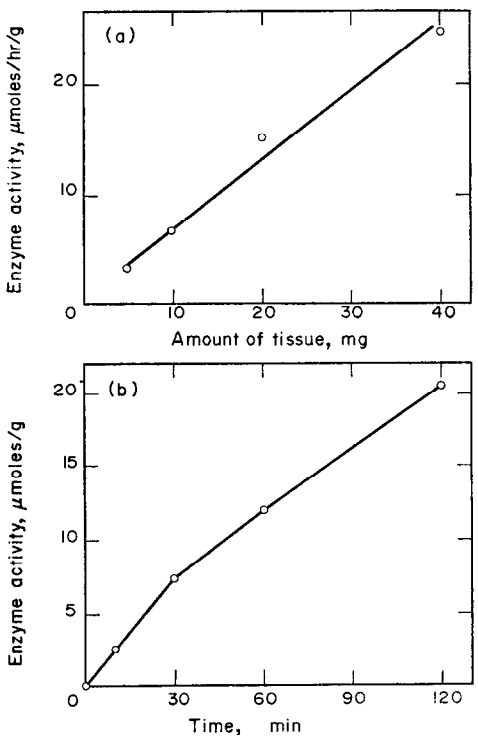

FIG. 1. Tyrosine aminotransferase activity in brain as a function of the amount of tissue and of incubation time. (a) Five, 10, 20 or 40 mg tissue was incubated for 30 min; (b) 20 mg tissue was incubated for 10, 30, 60 or 120 min. Each point is the mean of two determinations.

In the range routinely used in our assay procedure (20 mg tissue incubated for 30 min), enzyme activity increases linearly with time and with the amount of tissue present (Fig. 1a, b). The enzyme is not saturated at the concentration of tyrosine used (7×10^{-3} M tyrosine; Fig. 2). Higher tyrosine concentrations are not possible due to the relative insolubility of this amino acid. However, since the medium concentration of tyrosine is approximately 100 times greater than that normally found in brain tissue,[25] it can be assumed that small changes in tissue tyrosine concentration do not influence the assay. These observations are in general agreement with those of previous investigators.

TABLE 1. TYROSINE AMINOTRANSFERASE ACTIVITY IN BRAIN HOMOGENATE*

Reaction mixture	Temp.	Gross enzyme activity (μmoles/hr/g)	Net enzyme activity (μmoles/hr/g)
Complete	37°	16·12	15·05
Without tyrosine	37°	2·18	1·31
Without α-ketoglutarate	37°	0·00	
Complete	0°	1·07	0·00

* All results are the mean of two determinations. Net enzyme activity refers to total activity at 37° minus activity observed at 0°. (In all subsequent tables, this subtraction has already been performed.)

Tyrosine aminotransferase activity at different times of day. Liver tyrosine aminotransferase activity fluctuates widely during the day, normally remaining low during the light period and rising to a maximum shortly after the onset of darkness.[19] Under certain conditions, this diurnal rhythm is accompanied by parallel rhythmicity in the response of the enzyme to drug treatment.[26,27] Thus, prior to our examination of the effects of pharmacological agents on brain tyrosine aminotransferase, we first determined whether this enzyme shows any diurnal fluctuation. Rats were sacrificed at 1200, 1600, 2000, 2300, 200 and 800 hr. Liver and brain were removed and assayed for enzyme activity (Fig. 3). Liver tyrosine aminotransferase activity shows a characteristic 3-fold diurnal rhythm with a maximum 2 hr after the beginning of the dark period. However, no significant fluctuation in the activity of the enzyme is seen in brain. A similar distinction has been observed between liver tyrosine aminotransferase and that portion (less than 15 per cent[28,29]) of the brain enzyme which is recoverable in the high speed supernatant fraction of an isotonic homogenate.[30]

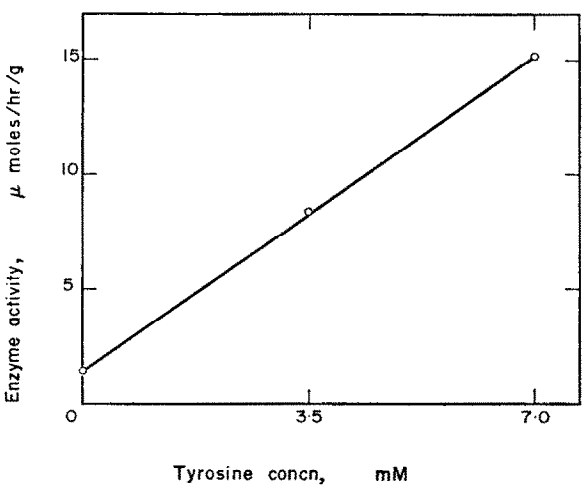

FIG. 2. Tyrosine aminotransferase activity in brain as a function of tyrosine concentration. Tissue was incubated with 0, 3·5 or 7 mM tyrosine for 30 min. Each point is the mean of two determinations.

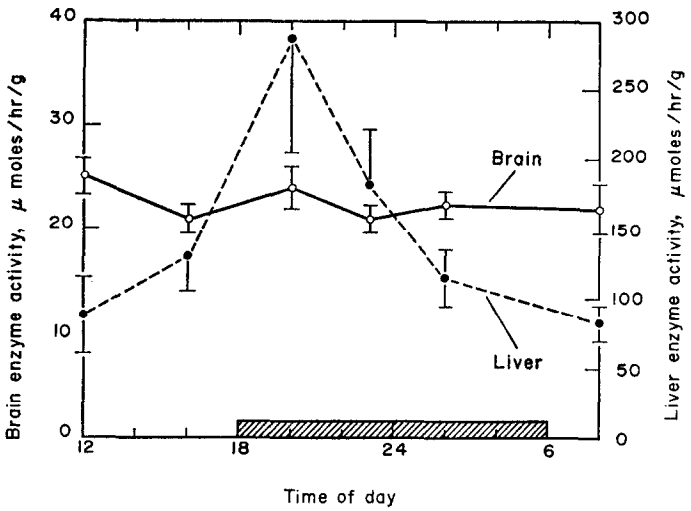

FIG. 3. Tyrosine aminotransferase activity in brain and liver as a function of time of day. Animals housed with lights off from 1800 to 600 (dark bar) were killed at 1200, 1600, 2000, 2300, 200 and 800. Enzyme activity was measured in brain (solid line) and liver (dotted line). Each point is the mean of four determinations. Estimated standard errors of the mean are indicated by vertical lines.

Effect of destruction of catecholamine-containing nerve terminals on brain tyrosine aminotransferase activity. When administered by way of the cerebrospinal fluid, 6-hydroxydopamine causes a large and permanent decrease in the concentration of norepinephrine and dopamine in brain.[31] This depletion is associated with a loss of fluorescence[32] and cellular damage,[33] and is generally believed to be due to a selective degeneration of catecholamine-containing terminals. In addition to loss of catecholamines, 6-hydroxydopamine produces a parallel decrease in tyrosine hydroxylase,[31] an enzyme which participates in the formation of catecholamines from tyrosine, and may also be the rate-limiting enzyme in this biosynthesis. If tyrosine aminotransferase plays a role in the regulation of norepinephrine metabolism, a portion of the enzyme might also be localized in norepinephrine-containing nerve terminals, and 6-hydroxydopamine might be expected to lead to the partial disappearance of this enzyme. We administered two 200-μg doses of 6-hydroxydopamine or the vehicle (20 μl of 0·9% NaCl, 0·1% ascorbic acid), 24 hr apart, into the cerebrospinal fluid by way of the lateral ventricle.[34] Two days after the first injection, the animals were killed and brains were assayed (Table 2). 6-Hydroxydopamine reduces the concentration of norepinephrine in whole brain by 86 per cent ($P < 0{\cdot}001$), suggesting large-scale degeneration of catecholamine-containing terminals. However, no change is observed in tyrosine aminotransferase activity. Similar results are apparent 45 days after 6-hydroxydopamine treatment. Thus, unlike tyrosine hydroxylase, tyrosine aminotransferase does not appear to be specifically localized in norepinephrine-containing terminals. This observation is consistent with the homogeneous regional distribution of tyrosine aminotransferase in brain tissues,[20,28,29] which contrasts with the heterogeneous distribution of both catecholamines[35] and tyrosine hydroxylase.[36] It is also consistent with the observation that only 12–31 per cent of the transaminating enzyme is located in the nerve ending fraction of brain homogenate.[20,28]

TABLE 2. EFFECT OF 6-HYDROXYDOPAMINE ON BRAIN TYROSINE AMINOTRANSFERASE ACTIVITY*

Group (N)	Norepinephrine concn (μg/g)	Enzyme activity (μmoles/hr/g)
Control treatment (5)	0·35 ± 0·04	19·72 ± 0·75
6-Hydroxydopamine treatment (4)	0·05 ± 0·01†	19·72 ± 0·40

* Animals received 200 μg 6-hydroxydopamine or its vehicle by intraventricular administration 24 and 48 hr prior to sacrifice at 1600 hr. All values are means ± estimated standard error of the mean. Control values were pooled with values from animals treated with reserpine vehicle.
† $P < 0·001$.

Effect of decreased norepinephrine concentration on brain tyrosine aminotransferase activity. Like 6-hydroxydopamine, both reserpine and α-methyltyrosine deplete the brain of norepinephrine. However, unlike 6-hydroxydopamine, neither of these drugs is associated with gross or permanent damage to the nerve terminal. Reserpine appears to act by blocking uptake and storage in neuronal vesicles,[37] while α-methyltyrosine inhibits tyrosine hydroxylation.[38] If norepinephrine normally exerts an excitatory influence on tyrosine aminotransferase, drug-induced depletion of norepinephrine might be expected to decrease the activity of this enzyme.

Reserpine (1 mg/kg), i.p.) was administered to animals 24 and 4 hr before sacrifice. α-Methyltyrosine was given 9 hr (200 mg/kg, i.p.) and 4 hr (100 mg/kg, i.p.) before sacrifice. Brains were removed and assayed for norepinephrine and tyrosine aminotransferase. The livers of animals receiving α-methyltyrosine were also removed for tyrosine aminotransferase assay. Values were compared with those obtained from animals receiving identical injections of vehicle solutions (Tables 3 and 4). Reserpine and α-methyltyrosine reduce the concentration of brain norepinephrine by 74 and 77 per cent respectively ($P < 0·001$). In the liver, α-methyltyrosine causes a 32 per cent rise ($P < 0·05$) in tyrosine aminotransferase. However, neither reserpine nor α-methyltyrosine produces any observable change in brain tyrosine aminotransferase.

TABLE 3. EFFECT OF RESERPINE ON BRAIN TYROSINE AMINOTRANSFERASE ACTIVITY*

Group (N)	Norepinephrine concn (μg/g)	Enzyme activity (μmoles/hr/g)
Control treatment (5)	0·35 ± 0·04	19·72 ± 0·75
Reserpine treatment (5)	0·09 ± 0·01†	19·25 ± 0·24

* Animals received reserpine (1 mg/kg, i.p.) or its vehicle 24 and 4 hr prior to sacrifice at 1600 hr. Values are expressed as mean ± estimated standard error of the mean. Control values were pooled with values from animals treated with 6-hydroxydopamine vehicle.
† $P < 0·001$.

TABLE 4. EFFECT OF PHENIPRAZINE AND α-METHYLTYROSINE ON TYROSINE AMINOTRANSFERASE ACTIVITY*

Group (N)	Brain		Liver
	Norepinephrine concn (μg/g)	Enzyme activity (μmoles/hr/g)	Enzyme activity (μmoles/hr/g)
Control treatments (5)	0·35 ± 0·03	20·63 ± 0·36	308·0 ± 30·8
α-Methyltyrosine (5)	0·08 ± 0·01†	19·92 ± 1·19	406·6 ± 18·0‡
Pheniprazine (5)	0·55 ± 0·02†	19·72 ± 1·03	209·4 ± 41·8§

* Animals received α-methyltyrosine (200 mg/kg, i.p., 9 hr and 100 mg/kg, i.p., 4 hr prior to sacrifice) or pheniprazine (5 mg/kg, i.p., 6 hr prior to sacrifice). Control animals received vehicle injections at the same times. All animals were killed at approximately 1600 hr. Since no vehicle effects were observed, the values from control animals were pooled. All values are the means ± estimated standard error of the mean.

† $P < 0.001$.

‡ $P < 0.05$.

§ $P < 0.10$.

Thus, a decrease in norepinephrine concentration, like destruction of the entire terminal, is not associated with any measureable change in tyrosine aminotransferase activity in homogenates of brain tissue. One might argue that α-methyltyrosine was administered over too brief a time (9 hr) to effect a change in enzyme activity *in vitro*. Reserpine, however, was given 24 hr prior to sacrifice, and drug-induced changes in tyrosine hydroxylase activity *in vitro* had been observed after periods at least as short as 12 hr.[39] Moreover, it is noteworthy that an α-methyltyrosine-induced change in liver tyrosine aminotransferase was observed after 9 hr. Our failure to observe a change in brain transaminase activity after reserpine or α-methyltyrosine is consistent with the observations of Fuller,[30] but contradicts those of Gibb and Webb,[10] who report a fall in brain tyrosine aminotransferase activity under conditions similar to ours.

Effect of increased norepinephrine concentration on brain tyrosine aminotransferase activity. Pheniprazine (Catron) causes an increase in brain norepinephrine, apparently by inhibiting the enzyme, monoamine oxidase, which catabolizes intracellular norepinephrine.[40] By reasoning similar to that outlined in the previous experiment, one might expect that pheniprazine would indirectly alter the activity of enzymes participating in the feedback loops by which norepinephrine regulates its biosynthesis. Monoamine oxidase inhibitors have recently been shown to inhibit tyrosine hydroxylation.[41] Tyrosine aminotransferase has not been measured under these conditions.

We first administered pheniprazine (5 mg/kg, i.p.) or saline vehicle at various times of day. At 1900 hr, brains were removed, and norepinephrine concentration and monoamine oxidase activity were measured (Table 5).

Monoamine oxidase activity is reduced by 95 per cent within 7 min of the pheniprazine injection and remains at that level for at least 8 hr. Norepinephrine rises gradually to a maximum of 130 per cent of control within 2 hr and remains elevated at this level for at least the next 6 hr. Similar observations have been made previously.[42] This sustained elevation in norepinephrine level is consistent with the hypothesis that norepinephrine regulates its own rate of formation.

TABLE 5. EFFECT OF PHENIPRAZINE ON NOREPINEPHRINE AND MONOAMINE OXIDASE IN BRAIN*

Time	Monoamine oxidase activity (% Control)	Norepinephrine concn (% Control)
0	100 ± 7	100 ± 2
2 min	41 ± 1	123 ± 6
7 min	7 ± 1	107 ± 2
25 min	5 ± 1	115 ± 6
2 hr	6 ± 1	130 ± 19
8 hr	12 ± 1	128 ± 16

* Animals were killed at 1900 hr after the administration of 5 mg/kg of pheniprazine (i.p.). Monoamine oxidase activity and norepinephrine concentration were determined in brain tissue. Time indicated is the interval between the pheniprazine injection and decapitation. Monoamine oxidase and norepinephrine values are the means of three determinations (calculated as a per cent of control values) ± estimated standard error of the mean.

A second group of rats was given either pheniprazine (5 mg/kg) or saline vehicle and killed after 6 hr. Brain norepinephrine and brain and liver tyrosine aminotransferase activity were measured (Table 4). Despite a 57 per cent increase in brain norepinephrine, pheniprazine causes no change in brain tyrosine aminotransferase. Liver tyrosine aminotransferase is reduced by 32 per cent ($P < 0{\cdot}10$). Thus, elevated levels of norepinephrine, reported to decrease tyrosine hydroxylase activity, have no effect on the rate of tyrosine transamination in brain homogenates.

Effect of norepinephrine concentration in vitro *on tyrosine aminotransferase activity.* These observations suggest that the concentration of norepinephrine does not influence tyrosine aminotransferase activity in brain. It is possible, however, that such an interaction is obscured by a large pool of tyrosine aminotransferase localized in non-norepinephrine-containing neurons. Such a pool would not normally be accessible to the monoamine. In order to study the influence of norepinephrine on whole brain tyrosine aminotransferase, sonicated brain homogenates were incubated with approximately $3–30 \times 10^{-5}$ M norepinephrine for 30 min. The average concentration of norepinephrine in brain tissue is approximately 3×10^{-6} M. It is estimated, however, that the concentration of norepinephrine in the terminal is much higher.[43]

Norepinephrine does not increase tyrosine aminotransferase activity at any concentration studied (Table 6). Similar studies using a lower range of norepinephrine concentration (10^{-8} to 10^{-5}) also failed to demonstrate enzyme induction by norepinephrine. At the highest concentration (250 μM), a slight inhibition is observed. Other investigators have observed no induction of the enzyme by norepinephrine at concentrations of norepinephrine as high as 10^{-3},[7,17] and similar observations have been made using liver tyrosine aminotransferase.[18]

DISCUSSION

We have observed that the brain, like the liver, can transaminate tyrosine to *p*-hydroxyphenylpyruvic acid, but have failed to obtain any evidence in brain tissue to

suggest that this reaction can be influenced by norepinephrine. Tyrosine aminotransferase was first observed in brain by Canellakis and Cohen,[44] and it may provide the primary route of catabolism for tyrosine in the central nervous system. The enzyme has been studied by several investigators.[17,20,28,45] Our observations are consistent with those of other laboratories, differing primarily in the amount of enzyme activity reported. Most such differences can be explained in terms of the method used. Our method, which yielded values somewhat higher than most, was designed to measure total enzyme activity in whole brain.

TABLE 6. EFFECT OF INCUBATION WITH L-NOREPINEPHRINE ON BRAIN TYROSINE AMINOTRANSFERASE ACTIVITY *in vitro**

Concn of norepinephrine in medium (μM)	Enzyme activity (μmoles/hr/g)
0	14·65
30	14·49
50	13·54
100	14·06
250	9·35

* Brain homogenate was incubated with various concentrations of norepinephrine for 30 min and tyrosine aminotransferase activity was measured. All values are the mean of two determinations.

Several factors serve to distinguish the brain enzyme from liver tyrosine aminotransferase: First, liver tyrosine aminotransferase shows a 4-fold diurnal rhythm. We observe no such rhythm in brain tissue (Fig. 3). Second, under certain experimental conditions, drug-induced fluctuations in brain norepinephrine are accompanied by fluctuations in liver tyrosine aminotransferase.[26,27,46] We have observed drug-induced changes in both brain norepinephrine and liver tyrosine aminotransferase under conditions which do not lead to a change in the brain enzyme (Tables 3 and 4). Additional distinctions between the two enzymes have also been reported by other investigators. These include substrate specificity,[44] subcellular distribution[28] and response to drug and hormone treatment.[30] Thus, it seems possible that the brain and liver each contain a different enzyme capable of catalyzing the same reaction but having several different properties.

The control of liver tyrosine aminotransferase activity has been studied extensively. The diurnal rhythm in this enzyme in the rat normally parallels the rhythm in food ingestion.[27,47] Wurtman *et al.*[27] have presented evidence suggesting that the rise in liver tyrosine aminotransferase activity following the onset of darkness is due to an induction of the enzyme by the ingestion of protein-rich food during this time. They hypothesize that the transaminating pathway serves as a shunt for the diversion of excess tyrosine during periods of high food (and thus tyrosine) intake.[4] The observation that catecholamines, as well as amino acids, can induce liver tyrosine aminotransferase[9,11–13] led to the suggestion that liver tyrosine aminotransferase might play a special role in regulating the tyrosine available for the biosynthesis of catechol-

amines. A similar hypothesis has been set forth for brain tyrosine aminotransferase, following the report that reserpine and α-methyltyrosine (drugs which normally deplete the brain of catecholamines) decrease tyrosine aminotransferase activity and that these effects are blocked by the administration of DOPA (3,4-dihydroxyphenylalanine, a precursor of catecholamines which can restore brain catecholamine concentration).[10] Investigators of the enzyme in both brain[10] and liver[9] have suggested that a high concentration of catecholamines (in plasma or in brain) induces tyrosine aminotransferase (in liver or in brain), resulting in a decrease in the amount of tyrosine available for catecholamine synthesis and a subsequent restoration of dopamine and norepinephrine levels. A fall in the concentration of catecholamines, on the other hand, would remove enzyme induction, increase available tyrosine, and again restore catecholamine levels.

Our observations in the central nervous system fail to support this hypothesis: First, tyrosine aminotransferase activity in brain is not altered by the intraventricular administration of 6-hydroxydopamine (Table 2), suggesting that the enzyme is not specifically localized in catecholamine-containing nerve terminals. Second, the enzyme activity is not altered by either increases or decreases in the concentration of norepinephrine in the brain (Tables 3 and 4). Third, we are unable to demonstrate any induction of tyrosine aminotransferase activity *in vtiro* by norepinephrine (Table 6).

Additional studies are needed to determine whether the localization of brain tyrosine aminotransferase would permit any interaction between the enzyme and catecholamine biosynthesis. It is certainly possible that some of the enzyme is present in catecholamine-containing neurons where it metabolizes tyrosine otherwise available for other pathways. However, in this study, currently available techniques have failed to uncover any evidence that norepinephrine has an influence in such a reaction. This is not altogether surprising. As other investigators have pointed out,[17,20,28] it is doubtful that any significant interaction occurs between tyrosine aminotransferase and tyrosine hydroxylase. Moreover, if competition between the two tyrosine-metabolizing enzymes were to exist, there would be little or no value in an interaction between norepinephrine and the tyrosine transaminating enzyme. The arguments can be summarized as follows.

First, brain tyrosine hydroxylase has a K_m of from 10^{-6} to 10^{-4},[48,49] while the concentration of tyrosine in whole brain is 1×10^{-4} M.[25] This suggests that tyrosine hydroxylase is normally saturated or nearly saturated by tyrosine, a hypothesis supported by the failure of fluctuations in brain tyrosine level to alter substantially the level or rate of synthesis of brain catecholamines.[15,16,*] Thus, under normal conditions, tyrosine concentration does not appear to be of significance in the control of catecholamine metabolism. Moreover, since brain tyrosine transaminase has a K_m of 10^{-3} to 10^{-2},[17,50] and therefore is probably unsaturated, any increase in the availability of tyrosine would be followed by an increase in the rate of tyrosine transamination without the necessity of enzyme induction. Second, tyrosine aminotransferase appears to be localized on the inner surface of mitochondria,[20] while the outer surface of the mitochondrial membrane contains monoamine oxidase.[51] Presumably, catecholamines would be required to bypass monoamine oxidase in order to exert any regulatory influence on tyrosine aminotransferase.[17] Third, no direct evidence is

* M. J. Zigmond and W. J. Shoemaker, unpublished observations.

available in these (Table 6) or other experiments[7,17] to suggest that brain tyrosine aminotransferase can be induced by norepinephrine. However, it should be emphasized that our observations cannot exclude the possibility that tyrosine transamination in brain is in some way related to the biosynthesis of catecholamines, but only that this pathway does not appear to be modulated by feedback from brain catecholamines. Even this conclusion must be qualified, since our measurements were made only in homogenates of brain tissue. Experiments are now in progress in our laboratory to determine whether subtle changes in brain tyrosine aminotransferase activity *in vivo* might occur which are not detectable by assay *in vitro*.

In conclusion, while there is considerable evidence demonstrating that the level of norepinephrine in brain is controlled by feedback from norepinephrine to the synthesizing enzymes, tyrosine hydroxylase, and possibly dopamine-β-hydroxylase (see review by Weiner[14]), we can find no evidence to suggest that feedback to tyrosine aminotransferase plays a role in such regulation.

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