Regulatory Properties of Soluble and Particulate Rat Brain Tyrosine Hydroxylase*

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SUMMARY

Rat brain tyrosine hydroxylase exists in two distinct physical forms, a soluble and a membrane-bound form. The two forms are brain-region specific, with the membrane-bound or particulate form predominating in the striatum, an area enriched in catecholamine nerve endings and associated with a specific membrane fraction. Whereas the soluble and the particulate enzymes exhibit an identical \( K_m \) for the substrate tyrosine (0.055 mM), the particulate form, which can be simulated by the addition to the soluble enzyme of microgram amounts of the sulfated mucopolysaccharide heparin, has a lower \( K_m \) (0.15 mM) for the synthetic co-factor 6,7-dimethyl-5,6,7,8-tetrahydropterin (DMPH) than does the soluble (0.75 mM) and a 2-fold increased apparent \( V_{max} \).

The particulate enzyme also has a greater affinity for the competitive feedback inhibitors norepinephrine and 3,4-dihydroxyphenylethylamine (dopamine). Dopamine exhibits a greater affinity for both forms of the enzyme than norepinephrine and, in addition, dopamine but not norepinephrine induces cooperative DMPH\(^4\) kinetics in soluble but not particulate tyrosine hydroxylase. It is suggested that dopamine may be the more significant regulatory metabolite of the striatal biogenic amine biosynthetic pathway, and that tyrosine hydroxylase activity may be regulated by alterations in the physical state of the enzyme.

In which catecholamines have been shown by histofluorescent techniques (8) to reside primarily in varicosities or nerve terminals but not in the midbrain, a region in which the catecholamines reside primarily in perikarya, or cell bodies. The regional specificity of this response as well as its short latency and duration suggested that tyrosine hydroxylase may exhibit regulatory properties more suitable to rapid and reversible alterations in activity in response to the demands of synaptic function than the previously described longer term changes.

The following experiments suggest that in rat brain tissue, tyrosine hydroxylase exists in two distinct physical forms, a soluble and a membrane-bound form. The two forms are brain-region specific, with the particulate form predominating in an area enriched in catecholamine nerve endings and associated with a specific membrane fraction. The particulate enzyme exhibits a decreased apparent \( K_m \) for the synthetic co-factor 6,7-dimethyl-5,6,7,8-tetrahydropterin relative to the soluble, and decreased \( K_i's \) for the competitive feedback inhibitors norepinephrine and 3,4-dihydroxyphenylethylamine (dopamine). In addition, the kinetic properties of the membrane-bound enzyme are simulated with the addition of the sulfated mucopolysaccharide heparin, which we have previously reported (9) to activate soluble dog hypothalamic tyrosine hydroxylase. Finally, we would like to report that soluble but not particulate tyrosine hydroxylase exhibits cooperative DMPH\(^1\) kinetics induced by the presence of dopamine but not norepinephrine. The data suggest that tyrosine hydroxylase activity may be regulated by alterations in its physical state.

EXPERIMENTAL PROCEDURE

Materials

L-[3,5-\(^3\)H]tyrosine (40 Ci per mmole) was obtained from the Radiochemical Centre (Amersham-Searle) and purified on a column of Dowex 50 W-X4 (H\(^+)\). \(^{14}C\)phenylalanine (5 mCi per mmole) and \(^{3}H\)tryptamine bisuccinate (10 mCi per mmole) were products of New England Nuclear. Purified catalase (powder form), heparin, pyridoxal-5'-phosphate, 3,4-dihydroxyphenylethylamine (dopamine) HCl, and L-norepinephrine bitartrate were Sigma products. L-3,4-dihydroxyphenylalanine, 6,7-dimethyl-5,6,7,8-tetrahydropterin, tryptamine-HCl and crystalline catalase were obtained

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\(^{1}\) The abbreviation used is: DMPH\(^4\), 6,7-dimethyl-5,6,7,8-tetrahydropterin.
from Calbiochem. All other reagents and chemicals were of maximum obtainable purity.

**Methods**

**Preparation of Tyrosine Hydroxylase**—Male Sprague-Dawley rats (150 to 200 g), obtained from Hilltop Labs (Chicaco), were killed by decapitation, and the brains were removed. The striate cortex, or the midbrain, or both, was immediately dissected free and chilled on ice. The tissue was homogenized in 10 volumes of ice-cold medium using a Thomas Teflon glass homogenizer with a 0.010 cm clearance. For the preparation of soluble tyrosine hydroxylase, a hypotonic homogenization medium consisting of 2 mM potassium phosphate buffer, pH 7.0, was routinely used. The homogenate was centrifuged at 45,000 x g for 20 min, and the clear supernatant was decanted. The pellet was rehomogenized in one-half of the original volume of buffer and centrifuged. To the combined supernatants were added 144 mg per ml of solid (NH₄)₂SO₄ with stirring. After 30 min, the suspension was centrifuged at 45,000 x g for 20 min, and the pellet was discarded. Solid (NH₄)₂SO₄ (160 mg per ml) was dissolved in the clear supernatant, the sample was immediately centrifuged, and the supernatant was discarded. The precipitate was dissolved in a minimal volume of 2 mM potassium phosphate buffer, pH 7.0, and dialyzed overnight against 100 volumes of the same buffer. After dialysis, the slightly cloudy solution was centrifuged to remove insoluble material, and the supernatant was diluted to yield 500 units of tyrosine hydroxylase activity per ml. Enzymatic activity of this preparation was stable when stored at 4°C as long as bacterial contamination could be avoided.

Alternatively, 0.32 M sucrose served as the homogenization medium, and the homogenate was treated by a modification of the procedure of Gray and Whittaker (10). The homogenate was centrifuged at 1,000 x g for 10 min, and the supernatant was carefully decanted. The pellet was resuspended in one-half of the original volume of 0.32 M sucrose, centrifuged, and the pellet, consisting of nuclei and cell debris, was discarded. The pooled suspension was then centrifuged at 100,000 x g for 60 min, and the supernatant (Fraction S1) was removed. The pellet (P1) consisting of synaptosomes, synaptic vesicles, and mitochondria was resuspended by homogenization in 5 ml per 0.5 g of starting tissue of ice-cold 2 mM potassium phosphate buffer, pH 7.0. The lysed suspension was again centrifuged at 100,000 x g for 60 min. The supernatant (Fraction S2) was used with or without (NH₄)₂SO₄ fractionation and after dialysis as an alternate source of soluble tyrosine hydroxylase. The pellet, Fraction P1, after resuspension by homogenization in phosphate buffer and dialysis against the same buffer, served as a source of particulate tyrosine hydroxylase. Particulate tyrosine hydroxylase activity, isolated in the presence of 1 mM CaCl₂, was stable for at least 5 days. Enzyme preparations isolated in the absence of the divalent cation decreased in activity to 50% in 16 hours and then remained constant for at least 5 days. The lost activity could not be recovered in any fractions.

To further purify the particle-bound tyrosine hydroxylase, Fraction P1 was resuspended in 0.32 M sucrose and layered on a discontinuous sucrose density gradient consisting of 7.5 ml each of 0.8, 1.0, 1.2, and 1.4 M sucrose (Gradient A). The gradient was centrifuged in a Beckman L2-65B centrifuge using a SW 27 rotor, at 80,000 x g for 120 min. The fractions were collected by aspiration, the sucrose was diluted to 0.32 M, and the fractions were pelleted at 200,000 x g for 30 min. Pellets were resuspended in 2 mM phosphate buffer, centrifuged at 50,000 x g for 20 min, and the particulate fractions, after resuspension and dialysis in the same buffer, served as sources of particulate tyrosine hydroxylase.

A second discontinuous sucrose gradient technique utilized Fraction P2 as the source of enzyme. The pellet was resuspended by extensive homogenization in ice-cold distilled water and layered on a gradient consisting of 7.5 ml each of 0.6, 0.8, 1.0, and 1.15 M sucrose (Gradient B). Treatment of this gradient followed the procedure outline for Gradient A.

Gradient subfractions were occasionally examined under an electron microscope. A sample of the washed and pelleted fraction was fixed for 5 hours in 2% OsO₄ in Veronal buffer, pH 7.5, and subsequently dehydrated through a graded series of ethanol solutions. The dehydrated pellet was embedded in an epoxy resin mixture and polymerized for 48 hours at 60°C. Thin sections were sliced with a diamond blade on a LKB Ultramicrotome and examined in a Zeiss 9S electron microscope.

A8aage—Tyrosine hydroxylase was assayed by an adaptation of the method of Nagatsu et al. (11). The incubation mixture contained 15 µM [3,5-3H]tyrosine (containing 106 cpm), 1.1 mM L-DOPA, 50 mM 2-mercaptoethanol, 0.435 mM FeSO₄, and 0.11 M Tris acetate buffer to give a final pH of 6.1 and enzyme in a final volume of 350 µl. Typical assays contained 10 to 150 units of tyrosine hydroxylase to yield 2,000 to 30,000 cpm over a background of 300 to 1,500 cpm. Some kinetic properties of tyrosine hydroxylase were also determined at 0.5 mM [3,5-3H]tyrosine (containing 5 x 106 cpm) to eliminate the possibility that the properties observed were characteristic only at suboptimal substrate concentrations. The assay mixture was incubated for 20 min at 37°C, and the reaction was stopped with 50 µl of glacial acetic acid. The mixture (300 µl) was placed on a 3.0 cm Dowex 50 W-X4 (H+), column, and the column was then washed twice with 600 µl of water. The column eluate and water wash were mixed with 50 µl of saturated ascorbic acid and counted in a Beckman LS-250 liquid scintillation spectrometer. The scintillation fluid contained (in 1 liter) 150 ml of Beckman Bio-Solv BB8-3 and 850 ml of toluene containing 3.4 g of 2,5-diphenyloxazol (PPO) and 0.41 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPPO). Enzyme activity was linear with time and protein over the range used in these experiments.

Dopa decarboxylase (3,4-dihydroxy-L-phenylalanine carboxy-lyase, EC 4.1.1.26) was assayed by measuring the evolution of 14CO₂ according to the following modifications of the procedure of Christenson et al. (13). The incubation mixture consisted of 3. Linearity of tyrosine hydroxylase activity as a function of protein concentration is maintained at less than 2 mg per ml in the case of the particulate enzyme and at less than 4 mg per ml for the (NH₄)₂SO₄ fractionated soluble enzyme. Nonlinearity above these concentrations appears to be a nonspecific protein effect, since addition of bovine serum albumin to the assay will also induce a nonlinear response in tyrosine hydroxylase activity. Whether this phenomenon is an artifact of the assay procedure or a property of tyrosine hydroxylase is a subject for further investigation. However, nonlinearity was also observed by Nagatsu et al. (12) for the bovine caudate enzyme using an alternate assay procedure.
0.1 M potassium phosphate buffer (pH 6.8), 1 mM L-dopa, 0.1 mM fluor described above and 95% ethanol in a ratio of 4:1.

through the rubber cap, and the mixture was incubated at 37°C for 20 min. The reaction was stopped by injecting 0.5 ml of perchloric acid, and the incubation was continued for an additional 2 hours to ensure complete removal of 14C02. The center well was cut from the caps directly into counting vials containing 10 ml of scintillation fluid consisting of the toluene described above and 95% ethanol in a ratio of 4:1.

Monoamine oxidase was assayed according to the procedure of Wurtman and Axelrod (15). Protein concentrations were determined by the method of Lowry et al. (16) using bovine serum albumin as the standard.

RESULTS

Distribution of Tyrosine Hydroxylase—Assay of tyrosine hydroxylase activity in specific brain-region homogenates reveals considerable heterogeneity in the distribution of the enzyme throughout the brain. In addition, depending on the specific region selected and independent of the absolute amount of tyrosine hydroxylase present, homogenization of tissue in isotonic (0.32 M) sucrose followed by lysis of the particulate material yields systematically varying percentages of particulate (Fraction P2) activity. Data for two representative regions, the midbrain and the striatum, are presented in Table I. As can be seen from the data, whereas 67% of midbrain tyrosine hydroxylase activity occurs in Fraction S1, only 14%

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Percentage of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Midbrain</td>
</tr>
<tr>
<td>-Ca++</td>
<td>+Ca++</td>
</tr>
<tr>
<td>Iosotonic (0.32 M) on sucrose</td>
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</tr>
<tr>
<td>10× g Pellet</td>
<td>8.5 (0.26)</td>
</tr>
<tr>
<td>10× g Soluble (S1)</td>
<td>67.0 (2.06)</td>
</tr>
<tr>
<td>Soluble of lysed 10× g pellet (S2)</td>
<td>15.0 (0.46)</td>
</tr>
<tr>
<td>Lysed 10× g particulate (P2)</td>
<td>9.5 (0.29)</td>
</tr>
<tr>
<td>Hypotonic (2 mM) potassium phosphate</td>
<td></td>
</tr>
<tr>
<td>10× g Soluble</td>
<td>88.1 (2.94)</td>
</tr>
<tr>
<td>10× g Particulate</td>
<td>11.9 (0.39)</td>
</tr>
</tbody>
</table>

* Details of the procedure are presented under "Results." 

Values in parentheses are 10⁻² × units of tyrosine hydroxylase activity per g of starting material.

0.1 M potassium phosphate buffer (pH 6.8), 1 mM L-dopa, 0.1 mM pyridoxal-5'-phosphate, enzyme, and 0.05 μCi of [1-14C]L-dopa in a final volume of 0.5 ml. The incubation was carried out according to a modification of the procedure of Ichihara et al. (14) for tryptophan hydroxylase, in a polypropylene tube 17 X 100 mm with a tight-fitting rubber cap from which was removed after removal of the 1-kg pellet.

Dopa decarboxylase, and monoamine oxidase Activities are presented as: for tyrosine hydroxylase, units per mg; for dopa decarboxylase, nanomoles of dopa decarboxylated in 30 min per mg of protein; and for monoamine oxidase, nanomoles of indole acetic acid formed in 20 min per mg of protein. See "Results" for details.

TABLE III
Subcellular distribution of striatal tyrosine hydroxylase, dopa decarboxylase, and monoamine oxidase Activities are presented as: for tyrosine hydroxylase, units per mg; for dopa decarboxylase, nanomoles of dopa decarboxylated in 30 min per mg of protein; and for monoamine oxidase, nanomoles of indole acetic acid formed in 20 min per mg of protein. See "Results" for details.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tyrosine hydroxylase</th>
<th>Dopa decarboxylase</th>
<th>Monoamine oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Ca++</td>
<td>+1 mM Ca++</td>
<td>-Ca++</td>
</tr>
<tr>
<td>10× g Pellet</td>
<td>150</td>
<td>150</td>
<td>32.0</td>
</tr>
<tr>
<td>10× g Soluble (S1)</td>
<td>427</td>
<td>319</td>
<td>57.9</td>
</tr>
<tr>
<td>Soluble of lysed (S2) 10× g pellet</td>
<td>1972</td>
<td>1292</td>
<td>376.3</td>
</tr>
<tr>
<td>10× g lysed particulate (P2)</td>
<td>364</td>
<td>430</td>
<td>10.1</td>
</tr>
<tr>
<td>Soluble -0.6 M sucrose interface</td>
<td>1510</td>
<td>650</td>
<td>40.7</td>
</tr>
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<td>0.0 to 0.8 M</td>
<td>80</td>
<td>26</td>
<td>4.5</td>
</tr>
<tr>
<td>0.8 to 1.0 M (P3)</td>
<td>193</td>
<td>433</td>
<td>3.8</td>
</tr>
<tr>
<td>1.0 to 1.15</td>
<td>1090</td>
<td>1850</td>
<td>12.0</td>
</tr>
<tr>
<td>Pellet</td>
<td>181</td>
<td>171</td>
<td>7.9</td>
</tr>
</tbody>
</table>

A CaCl₂ was added after removal of the 1-kg pellet.

TABLE II
Subcellular distribution of striatal tyrosine hydroxylase Activities are presented as: for tyrosine hydroxylase, units per mg; for dopa decarboxylase, nanomoles of dopa decarboxylated in 30 min per mg of protein; and for monoamine oxidase, nanomoles of indole acetic acid formed in 20 min per mg of protein. See "Results" for details.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific activity</th>
<th>Percent- age of recovery</th>
<th>Specific activity</th>
<th>Percent- age of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradient A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>300</td>
<td>15.9</td>
<td>9.0</td>
<td>4.5</td>
</tr>
<tr>
<td>0.32 to 0.8 M Sucrose interface</td>
<td>393</td>
<td>22.4</td>
<td>379</td>
<td>24.1</td>
</tr>
<tr>
<td>0.6 to 1.0 M Sucrose</td>
<td>636</td>
<td>46.0</td>
<td>670</td>
<td>42.1</td>
</tr>
<tr>
<td>1.0 to 1.2</td>
<td>309</td>
<td>24.1</td>
<td>308</td>
<td>21.6</td>
</tr>
<tr>
<td>1.2 to 1.4</td>
<td>166</td>
<td>7.3</td>
<td>126</td>
<td>7.7</td>
</tr>
<tr>
<td>Gradient B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>2483</td>
<td>54.1</td>
<td>1086</td>
<td>24.1</td>
</tr>
<tr>
<td>Soluble -0.6 M sucrose interface</td>
<td>106</td>
<td>1.3</td>
<td>50.2</td>
<td>1.2</td>
</tr>
<tr>
<td>0.6 to 0.8 M</td>
<td>287</td>
<td>11.9</td>
<td>480</td>
<td>13.1</td>
</tr>
<tr>
<td>0.8 to 1.0</td>
<td>1145</td>
<td>21.3</td>
<td>2329</td>
<td>43.2</td>
</tr>
<tr>
<td>1.0 to 1.15</td>
<td>468</td>
<td>8.7</td>
<td>982</td>
<td>12.7</td>
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<tr>
<td>Pellet</td>
<td>227</td>
<td>2.7</td>
<td>317</td>
<td>5.7</td>
</tr>
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</table>

* See "Results" for details of procedures. 

+ Units per mg.

Expressed as percentage of total activity recovered from gradient.

The 1.0 to 1.2 M sucrose interface from Gradient A was layered on Gradient B after hypotonic shock.

Searle). The reaction was initiated by injecting substrate concentrations were determined by the method of Lowry et al. (16) using bovine serum albumin as the standard.


A considerable percentage of the remaining tyrosine hydroxylase of midbrain and striatal specific region selected and independent of the absolute amount of tyrosine hydroxylase present, homogenization of tissue in isotonic (0.32 M) sucrose followed by lysis of the particulate material yields systematically varying percentages of particulate (Fraction P2) activity. Data for two representative regions, the midbrain and the striatum, are presented in Table I. As can be seen from the data, whereas 67% of midbrain tyrosine hydroxylase activity occurs in Fraction S1, only 14% of the caudate enzyme is associated with this fraction. Lysis of the pellet (Fraction P1) in 2 mM KPO₄, pH 7.0, solubilizes a considerable percentage of the remaining tyrosine hydroxylase activity. In the case of the striatum, a major fraction (30 to 55%) of the total tyrosine hydroxylase remains "particle-
bound" in Fraction P2, even after repeated washings and homogenizations.

As can be seen from Table I, Part b, homogenization of tissue in hypotonic potassium phosphate buffer, although very effective in solubilizing midbrain tyrosine hydroxylase, fails to release more than 60% of the total striatal enzyme. Systematic variation of buffer composition, ionic strength, pH, and temperature have all failed to lower, below a value of 30% of the total, the amount of particle-bound tyrosine hydroxylase activity in the lysed particulate fraction of the striatum (Table I), and had little or no effect on the distribution of the midbrain enzyme.

To further characterize the subcellular distribution of particulate tyrosine hydroxylase in the striatum after isotonic sucrose homogenization, Fraction P1 from 20 pairs of caudates (1.75 g of tissue) was resuspended in 15 ml of 0.32 M sucrose, layered on 20 ml of 0.6 M sucrose, and centrifuged in an SW 27 rotor at 50,000 × g for 60 min. This procedure yielded an essentially myelin-free pellet which contained 95% of the initial tyrosine hydroxylase activity. The pellet was resuspended in 10 ml of 0.32 M sucrose, divided into two 5-ml fractions, and one fraction was made 1 mM in CaCl2. This CaCl2 concentration yields near maximal binding of enzymatic activity to membrane without producing significant clumping of subcellular fractions. The fractions were layered on sucrose Gradient A, and the layers were collected, the sucrose was diluted to 0.32 M, and the fractions were pelleted at 200,000 × g for 30 min and resuspended in 5 ml each of 2 mM potassium phosphate buffer in the presence or absence of 1 mM CaCl2. The fractions which equilibrated between 1.0 and 1.2 M sucrose were layered on Gradient B and further fractionated. The specific activities of these samples are presented in Table II. The fractions with highest specific activity include the soluble of the lysed synaptosomal material and the membrane fraction which sediments as the synaptosomal ghosts (17). Fractionation of the midbrain P1 fraction on Gradient A also yields a fraction with highest specific activity for tyrosine hydroxylase corresponding to the synaptosomal fraction. The identity of these fractions was confirmed with electronmicroscopic examination.

An alternative approach to isolation of particle-bound tyrosine hydroxylase involved application of lysed Fraction P2 particulate material directly to Gradient B followed by centrifugation as described under "Methods." This procedure had the advantages of eliminating one gradient as well as increasing over-all yield of particulate enzyme with near comparable specific activity as that obtained from the two-gradient procedure. Results of a typical fractionation are presented in Table III, along with comparable data for the distribution of dopa decarboxylase and monoamine oxidase. In the presence of 1 mM CaCl2, the particulate fraction with highest tyrosine hydroxylase specific activity was obtained at the 0.8 to 1.0 M sucrose interface, similar to the results described in Table II. The fraction so obtained (P3) is distinct from the mitochondrial fraction, as characterized by high monoamine oxidase activity, and contains a relatively small amount of
dopa decarboxylase, 85% of which was solubilized by hypotonic lysis. Fraction P3 contained about 40% of the tyrosine hydroxylase activity of the crude homogenate.

**Kinetic Properties of Two Forms of Brain Tyrosine Hydroxylase**—We have previously reported (9) that dog hypothalamic soluble tyrosine hydroxylase is markedly activated by low concentrations of the sulfated mucopolysaccharide heparin but not by the chondroitin sulfates nor by hyaluronic acid. Both rat midbrain and striatal soluble tyrosine hydroxylase are also activated by heparin. The data in Fig. 1 represent the effects of increasing concentrations of heparin on the activity of soluble striatal tyrosine hydroxylase. The activation curve is sigmoidal and a Hill plot of the data (18) yields a value for \( n_H \) near two, and a \( K_m \) of 0.5 \( \mu \)M, assuming a molecular weight for heparin of 16,000. In an effort to elucidate the mechanism of this activation and its relationship to the particulate characteristics of tyrosine hydroxylase, several kinetic parameters of the soluble and particulate enzyme from rat striatum were determined. Unless otherwise specified, soluble tyrosine hydroxylase was obtained from Fraction S2. The kinetic parameters of Fraction S1 soluble and of the soluble fraction obtained by hypotonic homogenization were identical with those of Fraction S2. Particulate enzyme was obtained from the 0.8 to 1.0 M sucrose interface (Fraction P3) after sucrose gradient centrifugation on Gradient B of the lysed 100,000 × g particulate material. This fraction, as well as both the whole unfractionated 100,000 × g particulate fraction (P2) and the particulate material obtained from hypotonic homogenization, yielded identical kinetic parameters. In addition, the particulate enzyme obtained from the 0.8 to 1.0 M

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**Fig. 3.** The effect of tyrosine concentration on the activity of soluble striatal tyrosine hydroxylase. The concentration of heparin was 28.6 \( \mu \)g per ml. Assay conditions were as described under “Experimental Procedure.” Velocity is in nmoles of dopa formed in 20 min.

**Fig. 4.** Reciprocal plot of the activity of soluble (a) and particulate (b) striatal tyrosine hydroxylase as a function of DMPH4 concentration. Assay conditions were as described under “Experimental Procedure.” The final concentration of heparin, when added, was 28.6 \( \mu \)g per ml. Velocity is in \( 10^{-3} \times \) pmoles of dopa formed in 20 min.

**Fig. 5.** The effect of norepinephrine (NE) on the activity of soluble striatal tyrosine hydroxylase as a function of DMPH4 concentration, in the absence (a) and presence (b) of 28.6 \( \mu \)g of heparin per ml. Other assay conditions are described under “Experimental Procedure.” Norepinephrine was added as the bitartrate. Velocity is in \( 10^{-3} \times \) pmoles of dopa formed in 20 min.
sucrose interface in the presence of 1 mM CaCl₂, dialyzed, and assayed in the absence of CaCl₂, also appeared identical with respect to the kinetic parameters measured. No effects of the addition of Ca²⁺ to the assay mixture, on the soluble or particulate enzymes alone, were observed.

The effect of the pH of the assay medium on tyrosine hydroxylase activity is presented in Fig. 2. Soluble tyrosine hydroxylase exhibits a sharp pH optimum near 5.9 in Tris acetate buffer. The addition of 28.6 µg per ml of heparin to the assay causes a slight shift of the optimum to pH 6.2, similar to the optimum pH obtained for particulate enzyme.

Dialyzed soluble tyrosine hydroxylase, both in the presence and absence of 28.6 µg per ml of heparin, as well as particulate tyrosine hydroxylase, required the addition of FeSO₄ or catalase to the assay for any activity to be observed. In all cases, activation by Fe³⁺ exhibited typical Michaelis-Menten saturation kinetics with half-maximal activation achieved at a Fe³⁺ concentration of 9.5 × 10⁻⁵ M. Concentrations of FeSO₄ above 5 × 10⁻⁴ M yielded a marked inhibition of enzymatic activity.

As previously reported for bovine adrenal tyrosine hydroxylase (19), the rat brain enzyme is also stimulated by catalase, but only to about 80% of the activity obtained in the presence of 0.436 mM Fe³⁺ and at a catalase concentration (15,000 units per ml, specific activity, 21,000 units per mg), nearly 10-fold greater than was used by those authors.

The effects of tyrosine concentration on the activity of tyrosine hydroxylase are presented in Fig. 3. (In this and in all subsequent kinetic studies, each assay contained 50 µg of S₂ protein, specific activity 1420 units per mg, or 65 µg of P₃ protein, specific activity 1150 units per mg.) As was reported for the hypothalamic enzyme, whereas the addition of heparin produces a 2-fold increase in the apparent Vₘₐₓ, addition of the mucopolysaccharide has no effect on the apparent Kₘ for tyrosine, with a value near 50 µM observed in both cases. The particulate enzyme also exhibits a similar Kₘ for tyrosine.
particulate form of the enzyme has a \( K_m \) for \( \text{DMPH}_4 \) near 0.15 mM, similar to the value obtained for the soluble form of the enzyme in the presence of heparin. The addition of heparin to the particulate enzyme affects neither the \( K_m \) nor the \( V_{\text{max}} \) of \( \text{DMPH}_4 \) kinetics for the soluble and particulate enzymes in the presence or absence of heparin at a tyrosine concentration of 0.5 mM yielded identical \( K_m \) values with those obtained at the lower tyrosine concentration.

The effects of the feedback inhibitor norepinephrine on the \( \text{DMPH}_4 \) kinetics of tyrosine hydroxylase are depicted in Figs. 5 and 6. In all three cases, soluble, soluble plus heparin (Fig. 5), and particulate (Fig. 6), norepinephrine functions strictly as a competitive inhibitor, yielding linear reciprocal plots in all cases. The \( K_i \) for the soluble enzyme is near 0.7 mM, whereas the particulate enzyme and the soluble enzyme in the presence of heparin both exhibit \( K_i \) values near 0.15 mM.

Fig. 7 shows a similar plot for the inhibition of soluble tyrosine hydroxylase by dopamine as a function of \( \text{DMPH}_4 \) concentration. In contrast to the effects of norepinephrine on the kinetics for \( \text{DMPH}_4 \), the addition of low concentrations of dopamine to the soluble enzyme induces a sigmoidal response of the enzyme to the co-factor in addition to apparently competitive effects on the affinity of the enzyme for \( \text{DMPH}_4 \), suggesting a second-site inhibitory binding of dopamine to soluble tyrosine hydroxylase. Hill plots of the data (Fig. 8) yield values of \( n_H \) for \( \text{DMPH}_4 \) in the presence of the inhibitor near 1.0.

On the other hand, as can be seen from Fig. 9, the addition of heparin to the soluble enzyme (Fig. 9a) or the binding of tyrosine hydroxylase to the membrane fraction (Figure 9b) desensitizes the enzyme to the sigmoidal response induced by dopamine. The inhibition curves reflect strictly competitive inhibition with respect to co-factor, with a \( K_i \) for dopamine for both the soluble enzyme in the presence of heparin and for the particulate enzyme near 35 \( \mu \)M, a value indicating the affinity of brain tyrosine hydroxylase for dopamine to be five times greater than that for norepinephrine.

**DISCUSSION**

Brain tyrosine hydroxylase was first reported to be associated with the synaptosomal fraction of striate cortex homogenates by McGeer et al. (20). This observation is supported by evidence from histofluorescent studies of the brain by Fuxe (8) that the striatum is enriched in catecholaminergic nerve terminals. The data presented above are consistent with the predominantly synaptosomal nature of striatal tyrosine hydroxylase and further suggest that the particulate tyrosine hydroxylase of midbrain is also synaptosomal, although the majority of the enzyme from the midbrain, an area rich in catecholamine cell bodies, is soluble after isotonic homogenization. In addition, the data suggest that concomitant with the synaptosomal distribution of this enzyme in at least two regions of rat brain, tyrosine hydroxylase exists in two distinct physical forms, a soluble form and a particulate or membrane-bound form, with the amount of the latter paralleling the relative concentration of catecholamine nerve terminals in the striatum and midbrain (8).

Particulate tyrosine hydroxylase has been observed and reported by several investigators in both brain tissue (12) and in peripheral systems (21). The enzyme from bovine adrenal has been the subject of greatest scrutiny, particularly because of its abundance in this organ. Petrack et al. (22) confirmed the observation of Nagatsu et al. (21) that the majority of the enzyme from this tissue source was particle-bound. In apparent contradiction to these results, Musacchio (23) and Laduron and Belpair (24) found that rat and bovine adrenal tyrosine hydroxylase were localized in the high speed supernatant. Further, in an extensive examination of the bovine adrenal enzyme, Wurzburger and Musacchio (25) concluded that the particulate nature of tyrosine hydroxylase from this tissue source was an artifact of preparation in sucrose homogenization media and could be explained by random aggregation of the protein and adsorption to subcellular organelles. Over the time course of our experiments, in which brain removal, dissection, homogenization and subcellular fractionation, dialysis, and assays are completed within 36 hours, this kind of aggregation of the soluble enzyme has not been observed. However, when the soluble fraction is stored in isotonic sucrose at 4° for 3 days, 5 to 10% aggregation can be obtained. Under the hypotonic conditions which yield a high percentage of
particulate tyrosine hydroxylase in the striate cortex, no ag-
regation has been observed. That nonspecific adsorption to
subcellular organelles is not responsible for the particulate
state of the majority of striate tyrosine hydroxylase activity
is indicated by the failure in our experiments of soluble enzyme
to bind to particulate fractions from brain regions relatively
low in concentration of catecholamine nerve terminals, even
when the fractions are combined in isotonic sucrose.

Further, sucrose density gradient centrifugation of the lysed
synaptosomal fraction (Table II) yields a particulate fraction
highly enriched in tyrosine hydroxylase activity with sedimenta-
tion and electronmicroscopic characteristics of "synaptosomal
ghosts" described by Whittaker et al. (17). Our attempts to
replicate the findings of Fahn et al. (26), who reported that
bovine caudate nucleus tyrosine hydroxylase was associated
with synaptic vesicles (the M2 fraction of DeRobertis et al.
(27)), demonstrate that most of the particulate tyrosine hy-
droxylase from rat striate cortex in the M2 fraction sediments
with the synaptosomal membranes at the 0.8 to 1.0
m sucrose interface and little with the vesicular fraction (0.6 to 0.8
m sucrose interface) when the M2 fraction is further fractionated
on Gradient B. In addition, the nearly complete solubiliza-
tion of dopa decarboxylase, reportedly a synaptosomal enzyme
(28), following hypoosmotic lysis of Fraction P1 (Table III),
the presence of considerable particulate tyrosine hydroxylase
activity following hypotonic homogenization conditions which
should not produce synaptosomes (Table III), and the absence
of a significant amount of intact synaptosomes in electronmi-
crographs of the lysed synaptosomal fraction support the sug-
gestion that striate particulate tyrosine hydroxylase is bound to
synaptic membranes rather than entrapped within synaptosomes.

A considerable literature exists which describes the response
of the level of tyrosine hydroxylase activity in both peripheral
and central nervous systems to pharmacological and nonphar-
macological manipulations, which suggests that induction or
repression of new enzyme synthesis may be a mechanism in-
volved in the regulation of catecholamine biosynthesis (re-
viewed in Reference 29). The differential kinetic properties
of soluble and particulate tyrosine hydroxylase reported above
provide a mechanism, via an alteration in the physical state
of previously existing enzyme, by which the activity of striatal
nerve ending tyrosine hydroxylase might be subject to rapid
modulation.

Membrane binding of tyrosine hydroxylase does not affect
the $K_m$ of the enzyme for the substrate tyrosine or the activa-
tion by ferrous ion. Rather, the primary effect of binding to
the synaptic membrane on the kinetics of tyrosine hydroxylase
is mediated through an alteration in the $K_m$ of the enzyme for
the cofactor. The particulate enzyme exhibits a $K_m$ for DMHP,
nearly an order of magnitude lower than the soluble enzyme
(Fig. 4). This effect of membrane binding is of particular
significance since the reported (30) competitive feedback reg-
ulation of tyrosine hydroxylase is also mediated through cofac-
tor binding. Thus, the alteration in kinetic properties in vitro of
tyrosine hydroxylase accompanying binding to the nerve ending membrane could reflect a marked faclilitation of
the response of the enzyme to both the pteridine co-factor as
well as the end product inhibitors in vivo.

In addition, soluble tyrosine hydroxylase exhibits coopera-
tivity of co-factor binding induced by dopamine (Fig. 7), which
is abolished by binding of the enzyme to the synaptic membrane
(Fig. 9). We have previously reported cooperativity of ligand
binding by soluble tyrosine hydroxylase for the heparin activa-
tion of the hypothalamic enzyme (9) but hyperbolic kinetics
for both substrate and co-factors. Similarly, the activation
of the striate soluble enzyme by the sulfated mucopolysac-
charide is characterized by a Hill slope near two but simple
Michaelis-Menten saturation curves for DMHP, and tyrosine
both in the presence and absence of heparin. In the presence
of low concentrations of dopamine, however, striatal tyrosine
hydroxylase exhibits cooperativity of DMHP binding in addi-
tion to apparent competitive inhibition by the catecholamine.
The dopamine-induced cooperativity of co-factor binding,
which is abolished by binding of the enzyme to the synaptic
membrane, adds another facet to the potential regulatory
properties of tyrosine hydroxylase by providing a pathway
by which the level of activity of the catecholamine biosyn-
thetic apparatus may be synergistically altered from near
inactivity at low concentrations of co-factor, with tyrosine
hydroxylase in the soluble state, to relatively high activity
at the synaptic membrane.

As can be seen from Table II, the presence of Ca$^{2+}$
during the isolation procedure, which produces no change in the total
synaptosomal tyrosine hydroxylase, markedly increases the
amount of bound enzyme in the synaptosomal membrane
fraction. Although the membrane binding induced by Ca$^{2+}$
appears to be irreversible, further characterization of the effects
on cations on this phenomenon suggests that an equally effec-
tive but reversible binding of tyrosine hydroxylase to the same
membrane fraction is promoted by Mg$^{2+}$. The subcellular
regional specificity of the desensitization to dopamine and
concomitant activation of enzymatic activity associated with
membrane binding of the enzyme, and the dependence of these
alterations in tyrosine hydroxylase activity on cations, suggests
that the subcellular regional changes in cation concentrations
associated with neural activity may play an important role in
triggering modulations of catecholamine neurotransmitter
biosynthesis.

The kinetic properties of membrane-bound tyrosine hy-
droxylase are remarkably similar to those of the soluble enzyme
in the presence of heparin. Both membrane binding and the
presence of heparin produce identical shifts in the pH activity
profile of the soluble enzyme (Fig. 2), as well as similar altera-
tions in the $K_m$ of tyrosine hydroxylase for DMHP (Fig. 4)
and the $K_i$ of the enzyme for dopamine and norepinephrine
(Fig. 5 to 9). In addition, both membrane binding and the
addition of heparin desensitize the soluble enzyme to the dopa-
mine-induced cooperativity of co-factor binding (Fig. 9). The
similarities of these effects suggest that both forms of activation
proceed by a common mechanism and suggest two specula-
tions. First, the site of binding for tyrosine hydroxylase on
the membrane surface may contain a functionally important
sulfated mucopolysaccharide. This is consistent with the ob-
servations of heparin-like compounds in brain tissue (31) and
the relatively high concentration of sulfated mucopolysac-
charides in synaptosomal and synaptic vesicle fractions from
brain (32). Second, the 2-fold increase in the apparent $V_{max}$
of soluble tyrosine hydroxylase associated with the addition
of heparin to the soluble enzyme preparation may also be an
integral feature of the membrane-bound enzyme. In pre-
liminary studies of the characteristics of the binding of tyrosine
hydroxylase to synaptic membrane fractions, we have observed
an increase in total enzymatic activity concomitant with a
cation-induced shift of soluble enzyme to the particulate frac-
tion with trypsin, a procedure which was recently utilized by
Petrack et al. (22) in their studies of the adrenal enzyme. This
approach to the kinetics of tyrosine hydroxylase has recently
been challenged by Musacchio et al. (33), who have shown that
the proteolytic digestion alters the molecular weight of tyro-
sine hydroxylase from 140,000 for the "native" soluble enzyme
to 94,000 for the trypsin-treated enzyme. This may explain
the order of magnitude difference in $K_m$ for their
solubilized enzyme as compared to the $K_m$ reported above.

In summary, we would suggest that rat brain particulate
 tyrosine hydroxylase, characterized by a 2-fold increased $V_{\text{max}}$
and a $K_m$ for DMPH$_4$ nearly an order of magnitude less than
the soluble enzyme, may reflect a more functionally active
form of the enzyme at the synapse. In addition, the decreased
$K_i$ of the particulate enzyme for norepinephrine and dopamine
facilitates a more sensitive response by the enzyme to altera-
tions in levels of co-factor or catecholamine product. Con-
comitantly, soluble tyrosine hydroxylase exists in a relatively
inactive conformation which is favored by the presence of low
concentrations of dopamine. The conformational transition
to a more active state, which accompanies binding of the enzyme
to the synaptic membrane, may be promoted in vitro by the
addition of heparin. Further, the specific action of dopamine
 (but not norepinephrine) as an allosteric inhibitor of tyrosine
 hydroxylase, and the greater affinity of the enzyme for dopamine
compared with norepinephrine as a competitive inhibitor,
suggest that dopamine may be the more significant regulatory
product of the striatal nerve ending catecholamine biosynthetic
pathway.

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REFERENCES
6. Segal, D. S., Sullivan, J. L., Kuczynski, R. T., and Mand-
ell, A. J. (1971) Science 175, 847
7. Sullivan, J. L., Segal, D. S., Kuczynski, R. T., and Man-
15. Wurtman, R. J., and Axelrod, J. (1963) Biochem. Pharmacol. 12, 1439
27. De Robertis, E., Rodriguez De Lorenzo Arnoll, G., Sal-
Oregon State University Press, in press
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