Subcellular Distribution of Tyrosine Hydroxylase and Monoamine Oxidase in the Bovine Caudate Nucleus

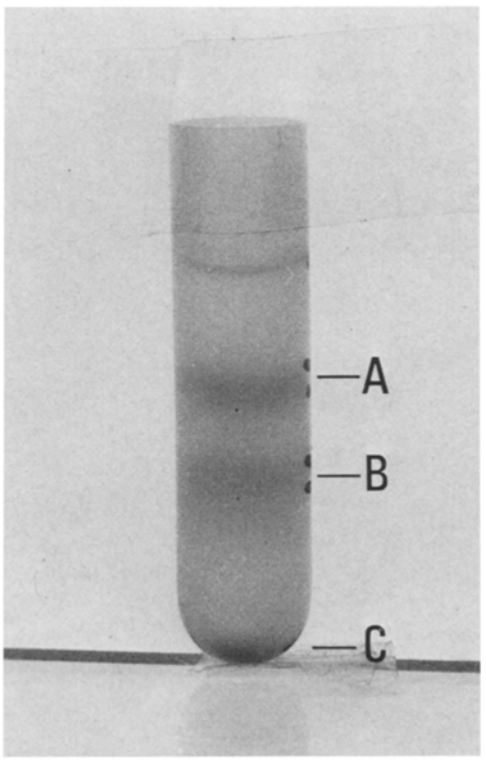
Tyrosine hydroxylase[1], which catalyzes the hydroxylation of tyrosine to DOPA, was shown to be the rate-limiting step in the biosynthesis of catecholamines[2]. The biosynthesis of catecholamines in sympathetically innervated tissues such as the brain, heart, spleen, vas deference and adrenal medulla, was found to be regulated by the feed-back inhibition of tyrosine hydroxylase by catecholamines[3–5]. Catecholamines are mostly localized in granular structures in the adrenal medulla[6] and in the sympathetic nerve endings[7]. Since there are indications that newly synthesized norepinephrine inhibits tyrosine hydroxylase, the intracellular localizations of both tyrosine hydroxylase and the end product catecholamine are expected to be in close contacts. Tyrosine hydroxylase was reported to be particle-bound[1,8]. On the other hand, there are some reports indicating that tyrosine hydroxylase is exclusively localized in the soluble fraction in the adrenal medulla[9,10] and bovine splenic nerve[11].

Present communication describes the subcellular distribution of tyrosine hydroxylase in the bovine caudate nucleus. Another important catabolic enzyme for catecholamines, monoamine oxidase, was also examined.

The bovine brain was obtained fresh, packed in ice, from the slaughterhouse. The caudate nucleus was dissected and homogenized in 10 vol. of 0.32 *M* sucrose with an Ultra-Turrax homogenizer. All fractionation procedures were carried out at 0–4 °C by the method of WHITTAKER[12]. The homogenate was centrifuged at low speed (1000 *g*) for 10 min. The precipitate, which consists of nuclei, myelin fragments and cell debris, was discarded. The supernatant was centrifuged at 18,500 *g* for 60 min. The precipitate, crude mitochondrial fraction, containing myelin, nerve ending particles and mitochondria, was further separated at 55,500 *g* for 120 min on a density gradient consisting of equal volumes of 0.8 and 1.2 *M* sucrose. Three fractions, i.e. myelin and membrane fragments (A), nerve endings (synaptosomes) (B) and mitochondria (C), were separated as shown in the Figure.

Separation of granulated vesicles in the nerve ending fraction were tried by differential centrifugation method of MAYNERT et al.[13] after hypotonic treatment of the crude mitochondrial fraction. Microsomes and the soluble fraction were further separated from the 18,500 *g* supernatant by centrifugation at 107,000 *g* for 60 min. A part of each fraction was centrifuged at 107,000 *g* for 60 min, and the pellets of tissue fractions were examined in an electron microscope. The electron-microscopic photographs showed that the purity of each fraction was fairly high. Details of the morphological data will be published elsewhere.

Enzyme activities were determined simultaneously on the various fractions. Tyrosine hydroxylase activity was measured by using L-tyrosine-^{14}C as substrate. DOPA-^{14}C formed by the enzyme reaction was isolated by an alumina column and measured[1]. The incubation mixture contained 200 µmoles of acetate buffer (pH 6.0), 0.1 µmole of L-tyrosine containing 1.1×10^5 cpm of L-tyrosine-^{14}C, 100 µmoles of mercaptoethanol, 1 µmole of 2-amino-4-hydroxy-6,7-dimethyl-tetrahydropteridine, and 0.5 ml of the various fractions as enzyme in a total volume of 1.0 ml. Monoamine oxidase activity was measured by the disappearance of kynuramine[14]. The incubation mixture contained 75 µmoles of phosphate buffer (pH 7.4), 0.25 µmole of kynuramine and 0.5 ml of the various fractions as enzyme in a total volume of 1.5 ml. Protein was measured by the method of LOWRY et al.[15].



Centrifugation of crude mitochondrial fraction of the bovine caudate nucleus through discontinuous density-gradient solution of sucrose (WHITTAKER[12]). 1.0 ml of the crude mitochondrial fraction was overlaid with two 1.5 ml sucrose layers (1.2 and 0.8 *M*). The tube was photographed after it had been centrifuged at 55,500 *g* for 120 min. The subcellular components present in the crude mitochondrial fraction separated into 3 fractions: A, myelin fraction; B, nerve endings; and C, mitochondria in the bottom of the tube.

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The results are shown in the Table. High tyrosine hydrolylase activities were seen in the supernatant and in nerve endings (synaptosomes) and microsomes. The enzyme activity in mitochondria was significantly lower than that in nerve endings or microsomes. In contrast, monoamine oxidase activity was seen predominantly in mitochondria. The low activity of monoamine oxidase in nerve endings may be attributed to the mitochondria which are contained in the fraction. Granulated vesicles, which were isolated after hypotonic treatment of the nerve-ending fraction, contained relatively high tyrosine hydroxylase activity and low monoamine oxidase activity.

These results showed that there exist 2 forms of tyrosine hydroxylase in the homogenate of the bovine caudate nucleus; soluble and particle-bound enzymes. The particle-bound enzyme appears to be localized in nerve endings, and probably in granulated vesicles which stores catecholamines. These results agree with UDENFRIEND's scheme[16] that the enzymes necessary for catecholamine synthesis may actually be organized into a single particle which stores catecholamines[17].

Subcellular distribution of tyrosine hydroxylase and monoamine oxidase in the bovine caudate nucleus

Subcellular fractions	Tyrosine hydroxylase pmoles/mg protein/min (37 °C)	Monoamine oxidase
Homogenate	12	755
Myelin (A)[a]	3	320
Nerve endings (B)[a]	17	1300
Mitochondria (C)[a]	9	3030
Microsomes	20	800
Soluble fraction	17	0
Granulated vesicles[b]	18	390

[a] These subcellular fractions were isolated from crude mitochondrial fraction by the method of WHITTAKER[12]. [b] Granulated vesicles were isolated from crude mitochondrial fraction by the method of MAYNERT[13].

Zusammenfassung. Die subzelluläre Lokalisation von Tyrosin-Hydroxylase und Monoaminoxydase im Nucleus caudatus des Rindergehirns wurde untersucht. Tyrosin-Hydroxylase fand sich grösstenteils in den Synaptosomen, den Mikrosomen und dem Zytoplasma. Eine bedeutende Aktivität von Monoaminoxydase war in den Mitochondrien vorhanden.

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3,5-Dihydroxyphenylpropionic Acid, a Further Metabolite of Sinapic Acid

Sinapic acid, a widely distributed phenolic compound in plants[1,2] and a constituent of human dietary materials[3,4] has recently been reported[5] to give rise on oral administration to the rat to a number of phenolic metabolites including dihydrosinapic acid, 3-hydroxy-5-methoxyphenylpropionic acid and 3-hydroxy-5-methoxycinnamic acid. Further investigation has resulted in the identification of an additional metabolite, excreted by sinapic fed rats, which has been shown to possess identical chromatographic and spectral characteristics (Table) with synthetic 3,5-dihydroxyphenylpropionic acid. mp 125°, obtained from 3,5-dihydroxycinnamic acid[5] by sodium amalgam reduction.

The metabolite was isolated from the urines of 6 rats which had each received a single initial dose of 200 mg of sinapic acid in admixture with the standard diet[6]. After a collection period of 7 days, the phenolic metabolites were obtained from the seven 24 h urine samples of each animal by ethereal extraction[5] and the 3,5-dihydroxyphenylpropionic acid separated from the other metabolites by band chromatography in solvents B and D. The metabolite, 3,5-dihydroxyphenylpropionic acid was shown to be absent from the urines of a control group of 6 rats receiving the standard diet only.

Since earlier investigations had shown that other phenolic acids are dehydroxylated by the intestinal microflora[7,8], the effect of an oral antibiotic known to inhibit bacterial dehydroxylation[7] was studied. Administration of 30 mg chloramphenicol daily to each rat of an experimental and control group of 6 animals resulted in complete suppression of the metabolites 3,5-dihydroxyphenylpropionic acid, 3-hydroxy-5-methoxyphenylpropionic acid and 3-hydroxy-5-methoxycinnamic acid; the formation of dihydrosinapic acid under these conditions was reduced but not abolished indicating that the hydrogenation of sinapic acid is effected in part by tissue enzymes or a chloramphenicol resistant strain of intestinal microorganism.

Administration of 200 mg sinapic acid to a group of 3 rabbits under similar experimental conditions has been found to give rise to 3,5-dihydroxyphenylpropionic acid

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