Dopamine Formation From Phenylalanine: Independent Existence in Caudate Nucleus Synaptosomes*

S. P. Bagchi and T. M. Smith

Rockland Research Institute, Orangeburg, N.Y., U.S.A.

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Summary

Dopa and dopamine formations from phenylalanine and tyrosine in rat caudate nucleus synaptosomal preparation were studied. Phenylalanine and tyrosine, labelled with either $^{14}$C or $^3$H, were employed as cosubstrates leading to the formations of double labelled Dopa and dopamine. The ratio of the two isotopes in dopamine was found to be significantly different from that in Dopa. The results suggest that Dopa formed from the cosubstrates are compartmented.

Introduction

Data (Bagchi and Zarycki, 1972; Bagchi and Zarycki, 1973) indicate that the enzymatic steps for the formation of dopamine (DA) from phenylalanine in brain are the same as those for the formation of this amine from tyrosine; hydroxylation by tyrosine hydroxylase is followed by the decarboxylation of Dopa. Why the formation of brain dopamine may occur from an alternate precursor, phenylalanine, is not quite clear at the present time. Results (Bagchi and Zarycki, 1973; Bagchi and Zarycki, 1975) also indicate that the tyrosine intermediate formed from phenylalanine may be compart-

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mented and may not exchange freely with the endogenous tyrosine. We have now attempted to determine if the Dopa formed from phenylalanine in brain tissue through the tyrosine intermediate is also compartmented and not freely miscible with that Dopa derived from the cerebral endogenous tyrosine. The results may clarify whether the entire sequence, leading to DA from phenylalanine, may have a separate and distinct existence.

**Materials and Methods**

Female Wistar rats (150—200 gm, Carworth Division of Charles River Co., Wilmington, MA) were killed by decapitation and the brain region of caudate nuclei were dissected out. The mitochondrial-synaptosomal fraction (Ps) was prepared from the homogenate according to Whittaker (1972). The Ps fraction was then incubated at 37° with labelled phenylalanine in the presence of labelled tyrosine as a cosubstrate. The substrate mixtures were of either phenylalanine-U-14C (specific radioactivity 350 to 400 mCi/m mole from New England Nuclear, Boston, MA) with 3H-tyrosine (L-p-hydroxyphenyl[alanine-2, 3-3H]; specific radioactivity 22,000 mCi/m mole from Amersham Searle, Arlington Heights, Ill.) or of 3H-phenylalanine (L-phenyl[2, 3-3H]alanine; specific radioactivity 16,600 mCi/m mole from Amersham Searle) with 14C-tyrosine (L-tyrosine-U-14C; specific radioactivity 404 mCi/m mole from New England Nuclear). The incubation mixture contained 20 mg equivalent of caudate and the final concentrations of 0.05 M mercaptoethanol, 0.4 mM pargyline and 0.128 M sodium phosphate (pH 6.0) but no other addition. The final volume of the mixture was 0.675 ml. The incubations were done in open test tubes for 10 min at 37°. The reaction was stopped by the addition of 0.7 ml of 0.8 N perchloric acid followed by two extractions employing 2.0 ml of 0.4 N perchloric acid. The acid extract was analyzed for the separation and counting of the double labelled tyrosine, Dopa and DA essentially as described earlier (Bagchi and Smith, 1976). Paper chromatographic analysis of labelled DA indicated only a minor contamination (<5 %) by norepinephrine as found before (Bagchi and Smith, 1976; Bagchi and Zarycki, 1972). The ratio of the isotopes in the double labelled products, R (product), was the isotope in labelled phenylalanine substrate expressed as a percentage of the label in the tyrosine cosubstrate. By this definition, if the isotope ratio in any product is found to be higher than that in its precursor, a compartmentation may be indicated. The radiochemical purities of the labelled substrates used were checked as described before (Bagchi and Smith, 1976) and the results of such blank analyses usually indicated very low radioactivity (2—3 times background count rate) in the various compounds of our interest. The reported values were the averages of the indicated number of experimental determinations. The probability (p) values for the significance of difference between the isotope ratios were calculated as described by Snedecor and Cochran (1967) for two-tailed t-test.