Role of catechol structure in the inhibitory effects of D-isomers of amino acids on the decarboxylation of L-5-hydroxytryptophan

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Summary. D-3,4-dihydroxyphenylalanine, D-threo-3,4-dihydroxyphenylserine and pyrocatechol competitively inhibited aromatic L-amino acid decarboxylase, whereas no inhibition occurred with l-norepinephrine, l-epinephrine, dopamine or some other amino acids without catechol side chains.

Aromatic L-amino acid decarboxylase is shown to catalyze the decarboxylation of a wide variety of aromatic amino acids, including 5-hydroxytryptophan (5HTP) and 3,4-dihydroxyphenylalanine (DOPA), and is found in many tissues[2,3]. It decarboxylates only L-isomer of amino acids and no activity is detected with D-isomers[2]. In previous papers, we reported the inhibitory effect of D-isomer of DOPA or threo-3,4-dihydroxyphenylserine (threo-DOPS) on the decarboxylation of L-threo-DOPS[4,5] and L-5HTP[6]. Our findings herein clearly show that the catechol structure is required for D-amino acids to inhibit the enzymic decarboxylation of L-5HTP, and that the potency of the inhibitory effect is modified by the structure of the side chain.

Materials and methods. Male Wistar rats weighing 200–250 g were anesthetized with pentobarbital sodium and perfused through the aorta with 0.9% NaCl. Brain, liver and kidney were removed, weighed and homogenized with 9 vol. of 5 mM sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C and the supernatant fraction was used as aromatic L-amino acid decarboxylase. Medium (final volume 1 ml) containing 50 mM Tris-HCl buffer (pH 9.0), 0.4 mM pyridoxal phosphate (PALP), 0.2 mM pargyline, 0.5 to 2 mg of protein of the supernatant fraction and different amounts of substrate, were incubated at 37 °C for 20 min. Linearity of the reaction was maintained for 60 min. The reaction was terminated by placing the test-tube in a boiling water bath for 1 min. The amount of reaction product, 5-hydroxytryptamine (5HT), was determined by the method of Lovenberg using permutit columns[7]. Protein concentration was measured by the method of Lowry et al.[8]. The following stereoisomers and compounds were used: L- and D-5HTP, D-DOPA, D-tryptophan and 5HT creatinine sulfate complex (Sigma); D-threo-DOPS (Kyowa Hakko, Japan); D-α-alanine, D-phenylalanine, D-tyrosine, l-norepinephrine bitartrate and pyrocatechol (Wako, Japan); D-histidine, dopamine, pargyline hydrochloride and PALP (Nakarai, Japan); l-epinephrine (Merck).

Results and discussion. Christenson et al.[9] demonstrated that aromatic L-amino acid decarboxylase is a single, homogenous enzyme and is responsible for the decarboxylation of both DOPA and 5HTP. However, experimental evidence suggests the possibility of multiple forms of the decarboxylase, or that more than one decarboxylase for aromatic amino acids exists in some tissues[10–13]. In the present study using brain, liver and kidney, we found that, with all preparations, D-isomer of DOPA or threo-DOPS inhibited the decarboxylation of L-5HTP, while D-5HTP or D-tryptophan had no effect. The inhibition by D-DOPA or D-threo-DOPS was dependent on the concentration of the compound and from the Lineweaver-Burk plots of the data, the type of inhibition was suggested to be competitive

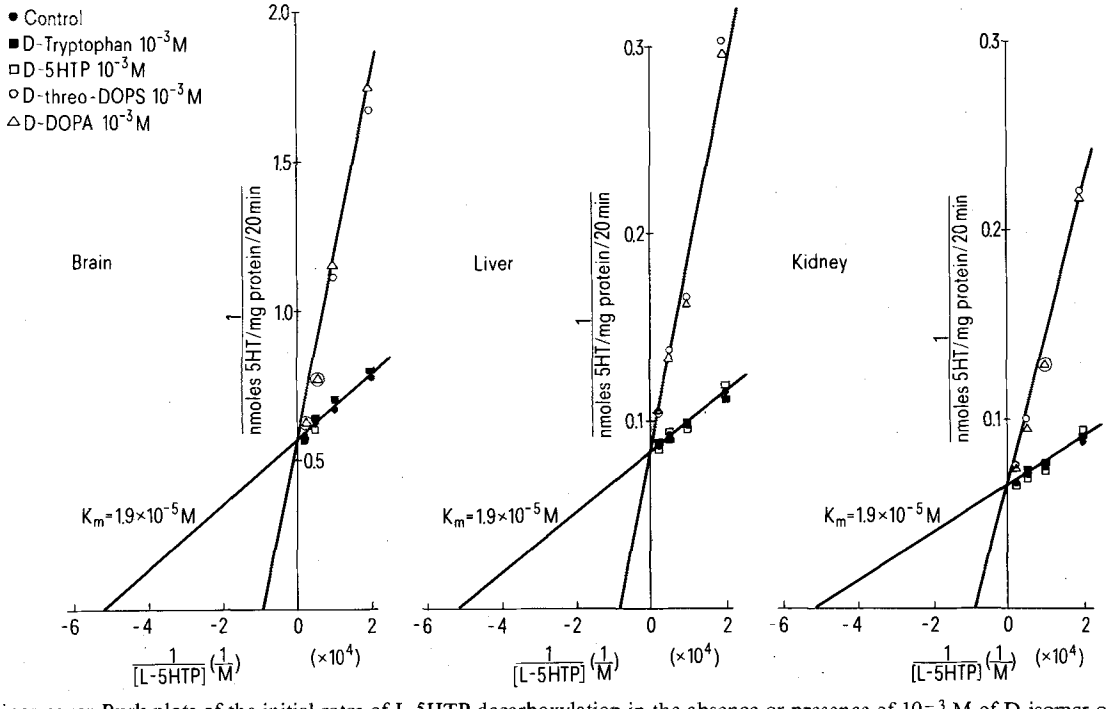

Fig. 1. Lineweaver-Burk plots of the initial rates of L-5HTP decarboxylation in the absence or presence of 10^{-3} M of D-isomer of DOPA, threo-DOPS, 5HTP or tryptophan. Supernatant fraction of the homogenate of rat brain, liver and kidney served as the enzyme preparation. Substrate (L-5HTP) and D-isomer were added simultaneously. The reaction medium was incubated at 37 °C for 20 min.

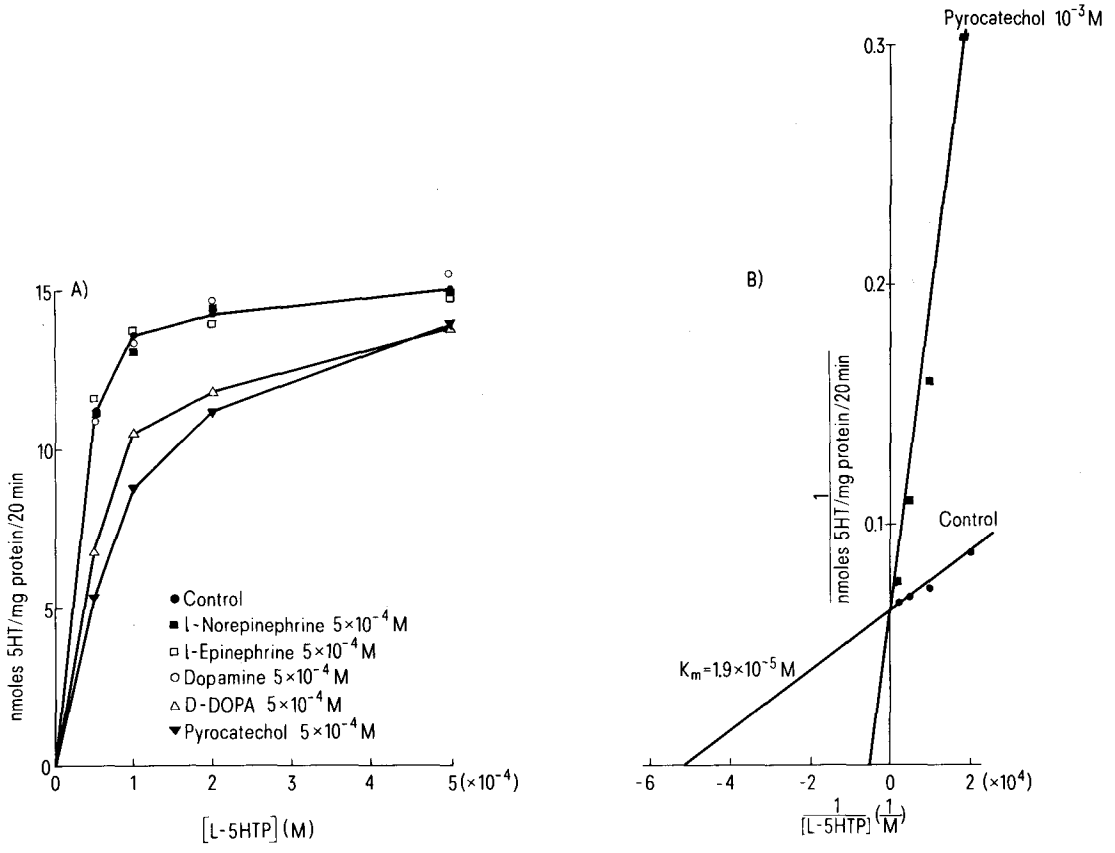

Fig. 2. *A* Plots of reaction velocity as a function of substrate concentration in the absence or presence of 5×10^{-4} M of catecholamines and pyrocatechol. Supernatant fraction of rat kidney homogenate was used as the enzyme preparation. Substrate (L-5HTP) and D-isomer were added simultaneously. The reaction was carried out for 20 min at 37 °C. *B* Lineweaver-Burk plots of the initial rates of L-5HTP decarboxylation in the absence or presence of pyrocatechol. Assay conditions were as in A.

(figure 1). The apparent K_i for these D-isomers was calculated as 1.7×10^{-4} M, regardless of the source of enzyme. The competitive type of inhibition and K_i value did not vary even when D-DOPA or D-threo-DOPS was preincubated with the enzyme from kidney in the presence of PALP for 15 min before adding the substrate. No effect was also observed with D-isomers of alanine, phenylalanine, tyrosine and histidine. These results suggest that the 3,4-dihydroxyphenyl group plays an important role in the mechanism of inhibition of D-isomers. If the interaction of 3,4-dihydroxyphenyl group with apoenzyme actually did produce an inhibitory effect on the decarboxylation of L-5HTP, compounds possessing the catechol structure should have a similar effect. However, no effect was found with l-norepinephrine, l-epinephrine and dopamine, while pyrocatechol inhibited the decarboxylation of L-5HTP (figure 2, A). The difference in K_i values between D-DOPA (or D-threo-DOPS) ($K_i = 1.7 \times 10^{-4}$ M) and pyrocatechol ($K_i = 0.9 \times 10^{-4}$ M) for the decarboxylation of L-5HTP also was observed (figure 2, B). These findings indicate that the inhibitory effect of the compounds possessing the catechol structure is limited by the structure of the side chain.

1 The authors express their gratitude to M. Ohara for assistance in preparation of the manuscript.

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