INHIBITORY EFFECT OF (+)-THREO-3,4-DIHYDROXY-PHENYLSERINE (DOPS) ON DECARBOXYLATION OF (-)-THREO-DOPS

Chiyoko INAGAKI, Hiroshi FUJIWARA and Chikako TANAKA

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

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The decarboxylation of racemic threo- and erythro-3,4-dihydroxyphenylserine (DOPS), norepinephrine (NE) precursors, has been reported in mammalian peripheral and central nervous system tissues in vivo (1-5). It has also been demonstrated both in vitro and in vivo that the rate of racemic erythro-DOPS decarboxylation is more rapid than that of racemic threo-DOPS (6). A recent report on the actions of the four stereoisomers of DOPS on the monoamine content of brain and heart stated that among four isomers, (+)-erythro-DOPS may be decarboxylated to (+)-NE and to a lesser extent, (-)-threo-DOPS may also be decarboxylated to (-)-NE in vivo (7). In the present work, we examined the enzymic decarboxylation of two optical isomers of threo-DOPS using rat kidney decarboxylase in vitro, and found the inhibition of (-)-threo-DOPS decarboxylation by (+)-threo-DOPS.

Kidneys of male Wistar rats weighing 200-250 g were used as the enzyme source. Homogenates of tissues with 15 volumes of distilled water were centrifuged at 8000 × g for 10 min and the supernatant fraction was used as L-aromatic amino acid decarboxylase. Medium (final volume 2 ml) containing 50 mM phosphate buffer (pH 8.2), 10⁻⁴ M pyridoxal phosphate, 2 × 10⁻⁴ M Pargyline, 1 to 3 mg of protein of the supernatant fraction and different amounts of substrate, was incubated at 37°C. Reaction was started by addition of the substrate and stopped by the addition of 4 ml of ice cold 0.4 N perchloric acid with 10 mg of sodium metabisulfite and 200 mg of ethylene-diaminetetraacetic acid disodium salt. The amount of reaction product, NE, was determined by the method of Bertler et al. (8), using Dowex 50 × 4 columns (0.7 mm in diameter, wet resin volume 1 ml). Protein concentration of the enzyme fraction was determined by the method of Lowry et al. (9). Other chemicals used were reagent grade.

Optical isomers of threo-DOPS, (-)-threo- and (+)-threo-DOPS correspond to L-threo- and D-threo-DOPS, respectively (6, 7). These compounds were synthesized in the Laboratory of Kyowa Hakko Kogyo Co. Ltd., Japan. Each purified isomer had a negative rotation of [α]_D^0 = -42.6 (purity: 99.5%) and a positive rotation of [α]_D^0 = +43.2 (C=1, N-HCL) (purity: 99.8%) respectively. Contamination by NE assayed in our laboratory was found to be 0.009% in (-)-threo-DOPS, 0.011% in (+)-threo-DOPS and 0.039% in racemic threo-DOPS.

As shown in Fig. 1, formation of NE by decarboxylation of (-)-threo-DOPS proceeded linearly for at least 30 min of incubation at 37°C in the presence of 10⁻³ M of (-)-threo-
FIG. 1. Time course of decarboxylation of (-)threo, (+)threo- and racemic threo-DOPS by supernatant fraction of rat kidney homogenate at 37°C. Concentration of each substrate in the reaction mixture was 10⁻³ M. The amount of NE formed was represented as nmoles/mg protein of enzyme preparation.

DOPS. This production of NE was not observed at 0°C. Further, nonenzymatic decarboxylation during the incubation at 37°C was undetectable. Incubation of the enzyme with (+)threo-DOPS (10⁻³ M) did not result in any detectable formation of NE. Assuming that racemic threo-DOPS contains 50% (-)threo-DOPS, the amount of NE from racemic threo-DOPS (10⁻³ M) would presumably be one half that from (-)threo-DOPS. However, when racemic threo-DOPS (10⁻³ M) was added as the substrate, the NE produced was one seventh of the NE amount from (-)threo-DOPS (10⁻³ M). This phenomenon suggested some inhibitory effect of (+)threo-DOPS on the decarboxylation of (-)threo-DOPS. To examine this effect of (+)threo-DOPS, (-)threo-DOPS at different concentrations was incubated with the enzyme in the absence or presence of (+)threo-DOPS (5×10⁻⁴ M, 10⁻³ M). Lineweaver-Burk plots of initial velocity of the (-)threo-DOPS decarboxylation yielded apparent Km of the enzyme for (-)threo-DOPS i.e. 1.37×10⁻³ M (Fig. 2,a). Lovenberg et al. reported Km values for other substrates with L-aromatic amino acid decarboxylase (10). Km for DOPS obtained in this work is larger than Km 2×10⁻³ M for 5-hydroxytryptophan (5HTP) and Km 4×10⁻⁴ M for 3,4-dihydroxyphenylalanine (dopa), and in the

![Figure 2](image)
same order as $K_m = 3 \times 10^{-3}$ M for tryptophan. This suggests that DOPS has a relatively low affinity for L-aromatic amino acid decarboxylase as compared with 5HTP or dopa.

Inhibition of (−)threo-DOPS decarboxylation was observed to be dependent on the concentration of (−)threo-DOPS (Fig. 2,a). The type of this inhibition was suggested to be non-competitive from Dixon plots of the data (Fig. 2,b). Apparent $K_i$ for (−)threo-DOPS was $1.87 \times 10^{-4}$ M. DOPS and other related compounds including dopa have been demonstrated to form complexes with pyridoxal phosphate (11). These complexes are inactive as pyridoxal phosphate. Substrate inhibition of decarboxylation of L-dopa can be explained by this inactivation of pyridoxal phosphate by L-dopa. Inhibition of this type is protected by addition of a sufficient amount of pyridoxal phosphate. Decarboxylation of (−)threo-DOPS in this study was carried out in the presence of $10^{-4}$ M of pyridoxal phosphate. Lineweaver-Burk plots formed a straight line and did not show any inhibitory effect of (−)threo-DOPS on the decarboxylation at the concentration examined. Under the same condition, however, addition of (−)threo-DOPS caused a remarkable inhibition of (−)threo-DOPS decarboxylation. The mechanism of this inhibition, which was demonstrated to be non-competitive concerning (−)threo-DOPS, is now under analysis in further experiments. The results obtained in this study suggest that (−)threo-DOPS is a more effective precursor of natural (−)NE than racemic threo-DOPS.

REFERENCES

EFFECTS OF CALCIUM IONS AND MAGNESIUM IONS ON 5-HYDROXYTRYPTAMINE-RECEPTOR INTERACTION

Isssei TAKAYANAGI, Chung Shin LIAO and Keijiro TAKAGI

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences,
University of Tokyo, Tokyo 113, Japan

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We have already reported (1–3) that Ca and Mg ions are involved in the interactions of a cholinergic drug (butyltrimethylammonium bromide), histamine and arternen with their receptors and are not involved in the interactions of competitive antagonists with their receptors. Woolly and Campbell (4) have, however, implicated Ca ions in the interaction