INHIBITION OF BRAIN GLUTAMATE DECARBOXYLASE BY 4,5-DIHYDROXYISOPHTHALIC ACID AND RELATED COMPOUNDS

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Hydroxybenzoic and phthalic acids and their related compounds were tested for inhibitory activity to brain glutamate decarboxylase. Of mono-, di-, and trihydroxybenzoic acids, gallic acid was the most inhibitory, giving 50% inhibition at a concentration of 0.17 mm. Dihydroxybenzoic acids were less inhibitory than the trihydroxyacids but more than monohydroxybenzoic acids. Of the phthalic acid-related compounds tested, 4,5-dihydroxyisophthalic acid was the most potent inhibitor, producing 50% inhibition at 0.61 uM. The inhibition of these compounds was competitive with respect to L-glutamate. The Ki values were 0.02, 1.2 and 4.9 uM for 4,5-dihydroxyisophthalic acid, 5-hydroxyisophthalic acid and gallic acid, respectively.

When administered intraventricularly to mice, 4,5-dihydroxyisophthalic acid produced a significant decrease in the r-aminobutyric acid content of the brain, resulting in induction of convulsions.

Brain glutamate decarboxylase (L-glutamate 1-carboxy-lyase, EC 4. 1. 1. 15) is the enzyme that catalyzes the synthesis of r-aminobutyric acid, an inhibitory transmitter in invertebrate nervous system and probably also a major inhibitory transmitter in the vertebrate central nervous system1-3). This decarboxylase is believed to be the rate-limiting enzyme which determines the levels of r-aminobutyric acid in the brain.

Numerous substances have been tested for their ability to inhibit brain glutamate decarboxylase1-3). Although several potent inhibitors were found in the course of these studies, most of them are not sufficiently specific to inhibit glutamate decarboxylase without simultaneously inhibiting other enzymes4-11).

In a previous paper from this group10), 4,5-dihydroxyisophthalic acid was isolated from cultures of Streptomyces toyocaensis No. 1039 as a potent inhibitor of brain glutamate decarboxylase. In the present experiments, hydroxybenzoic and phthalic acids and their related compounds were tested for the ability to inhibit this enzyme. The results indicate that 4,5-dihydroxyisophthalic acid is among the most effective, competitive inhibitors, having a Ki value of 0.02 uM, and that its intraventricular administration causes a decrease in the brain r-aminobutyric acid content in mice.

Materials and Methods

Materials

Pyridoxal phosphate was obtained from Calbiochem (U.S.A.) and DL-[1-14C]glutamate (13.1 Ci/mole) from New England Nuclear (U.S.A.). Glutathione (reduced form) and 3-mercaptopropionic acid were purchased from Sigma (U.S.A.). 2-Hydroxybenzoic acid, 3-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, gallic

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acid, gallic acid trimethyl ether, 2-hydroxy-5-sulfobenzoic acid, 2-hydroxy-5-nitrobenzoic acid, 2-
hydroxy-4-aminobenzoic acid, 2-hydroxy-4-aminobenzoic acid (sodium salt), 2-hydroxy-5-aminobenzoic acid, 3-hydroxyanthranilic acid and 3-hydroxy-4-aminobenzoic acid were obtained from Tokyo Kasei (Tokyo). 5-Hydroxysolphthalic acid was purchased from Eastman Organic Chemicals (U.S.A.). Phthalic, isophthalic and tere-
phthalic acids were from Tokyo Kasei. 4,5-Dihydroxyisophthalic acid and its methyl and ethyl ethers were prepared as described previously^{10}.

Enzyme Preparation
Solubilized glutamate decarboxylase was obtained from bovine brain acetone powder by the method described previously^{9} and stored at −80°C until use.

Enzyme Assay
Glutamate decarboxylase was assayed as described previously^{9}. The reaction mixture contained in a total volume of 0.5 ml: 50 mm potassium phosphate buffer, pH 6.2; 50 mM L-[1-14C]glutamate (0.1 Ci/mole, adjusted to pH 6.2 with sodium hydroxide); 5 mM glutathione; 0.05 mM pyridoxal phosphate; 0.8 − 1.0 mg protein of enzyme. The reaction was started by the addition of enzyme and conducted at 37°C for 60 minutes with gentle shaking on a Dubnoff metabolic shaker. At the end of incubation, 0.5 ml of 2 M sulfuric acid was added to the assay tubes and the mixtures were further incubated at 37°C for at least 60 minutes with gentle shaking. The [14C]-CO₂ evolved was counted in a toluene-based fluid.

Protein was determined by the method of LOWRY et al.^{11}.

Animal Experiments
4,5-Dihydroxyisophthalic acid dissolved in saline was intraventricularly injected to ddY mice (24−26 g) using a Hamilton microsyringe by the method of HALEY and MCCORMIC^{12}. The animals were sacrificed by decapitation at appropriate intervals, and whole brains were immediately removed, rinsed with cold water and then frozen in dry ice-acetone. The frozen brains were homogenized in 0.4 M perchloric acid and centrifuged at 10,000 × g for 20 minutes. The resultant supernatant was neutralized with 5 M potassium bicarbonate. After the precipitate formed was removed by centrifugation, aliquots of the supernatant were assayed for γ-aminobutyric acid content according to an enzymatic method^{12}.

Results and Discussion

As shown in Table 1, all benzoic acid derivatives with one, two or three hydroxyl groups showed inhibitory activity to glutamate decarboxylase. Among them, gallic acid, having three hydroxyl group, was the most effective, inhibiting the enzyme activity 50% at a concentration of 0.17 mM. Its inhibitory activity was abolished by the methylation of the hydroxyl groups. The introduction of sulfo, nitro or amino group in the molecule caused no increase in the inhibitory activity.

Of the three forms of phthalic acid, isophthalic acid was the most potent inhibitor (Table 2). Inhibitory activity of this acid was potentiated approximately 50 times by the introduction of one hydroxyl group at the 5 position, and 50 more times by the introduction of two hydroxyl groups at the 4 and 5 positions. Thus, 4,5-dihydroxyisophthalic acid, which inhibited decarboxylase activity 50% at a concentration of 0.61 μM, was approximately 2,500 times more potent than isophthalic acid (Table 2). Inhibitory activity of the former acid was strikingly reduced by either methylation of hydroxyl group at the 5 position or esterification of both carboxy groups. These results indicate that free hydroxyl groups are essential for the activity to inhibit decarboxylase activity.

The inhibitory activity of 4,5-dihydroxyisophthalic acid and the related compounds was competitive with respect to L-glutamate. The Kᵢ values were 0.02, 1.2 and 4.9 μM for 4,5-dihydroxyisophthalic acid, 5-hydroxyisophthalic acid and gallic acid, respectively.

When intraventricularly administered to mice at a dose of 5 − 10 μg/body, 4,5-dihydroxyisophthalic acid caused convulsions 15 minutes after injection. As shown in Fig. 1, γ-aminobutyric acid content of
the brain was lowered 27% (P < 0.05) and 21% (P < 0.01) at 30 and 60 minutes, respectively, after administration of the agent at a dose of 10 μg/body. After 24 hours, γ-aminobutyric acid content of the brain returned to the normal levels. On the other hand, intraperitoneal administration of 4,5-dihydroxyisophthalic acid to mice produced neither convulsions nor decreases in γ-aminobutyric acid levels of the brain, indicating that this acid can not pass through the blood brain barrier.

The present experiments demonstrate that 4,5-dihydroxyisophthalic acid is the most potent inhibitor of brain glutamate decarboxylase among the structurally related compounds so far studied. Since this compound is low in toxicity (data not shown), it seems to be a useful research tool for studying the function of γ-aminobutyric acid in the brain nervous system.

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