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INHIBITION OF BRAIN GLUTAMATE DECARBOXYLASE BY GLUTARATE, GLUTACONATE, AND β-HYDROXYGLUTARATE: EXPLANATION OF THE SYMPTOMS IN GLUTARIC ACIDURIA?

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Summary

Glutaric aciduria is a disorder of lysine, tryptophan, and hydroxylysine metabolism characterized by intermittent metabolic acidemia, dystonia, athetosis and mental retardation. It is due to a recessively inherited deficiency of glutaryl-CoA dehydrogenase, the enzyme(s) which catalyze the dehydrogenation of glutaryl-CoA to glutaconyl-CoA and decarboxylation of the latter to crotonyl-CoA. Abnormal quantities of glutaric, β-hydroxyglutaric, and glutaconic acids are found in the urine of these patients.

The nature of the movement disorder prompted study of the effects of the abnormally excreted metabolites on brain glutamate decarboxylase, an enzyme implicated in the pathogenesis of Huntington's chorea. Glutamate decarboxylase activity was examined in rat and rabbit brain acetone powders, stabilized with pyridoxal phosphate and glutathione. Glutarate, β-hydroxyglutarate, and glutaconate were competitive inhibitors of this enzyme, K_i values being 1.3×10^{-3} mol/l, 2.5×10^{-3} mol/l, and 7.5×10^{-4} mol/l, respectively. This inhibition may explain the neurological accompaniments of this syndrome.

Introduction

Glutaric aciduria is an inborn error of amino acid metabolism in which glutaric, β-hydroxyglutaric, and (occasionally) glutaconic acids are excreted in abnormal amounts in the urine [1,2]. It is due to a recessively inherited

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deficiency of glutaryl-CoA dehydrogenase [3]. Clinical manifestations include intermittent compensated metabolic acidemia, mental retardation, and symptoms referable to dysfunction of the basal ganglia, i.e. spasticity, dystonia, athetosis, and grimacing.

It is thought that proper balance between acetylcholine, dopamine, and γ-aminobutyric acid (GABA)-containing fibers in the basal ganglia is necessary for normal movement, and that imbalance may be important in the pathogenesis of such disorders as Parkinson's disease and Huntington's chorea. For instance, the most prominent lesion in the former condition appears to be loss of dopaminergic nigro-striatal neurons[4,5], while findings in the latter have included deficiency of GABA [6] and glutamate decarboxylase (GAD, L-glutamate 1-carboxy-lyase, EC 4.1.1.15) in the basal ganglia [7—9].

The clinical similarity of glutaric aciduria to the juvenile form of Huntington's chorea prompted speculation that the movement disorder in the former might be caused by inhibition of brain glutamate decarboxylase by the accumulated five-carbon, dicarboxylic acids. The specific purpose of this paper is to show that, in fact, all three abnormal acids are powerful competitive inhibitors of this enzyme.

Materials and methods

DL-[1-^{14}C]Glutamic acid (spec. act. 38.7 mCi/mmole) DL-[5-^{14}C]glutamic acid (spec. act. 3.7 mCi/mmole) were purchased from New England Nuclear. L-Glutamic acid was obtained from Nutritional Biochemicals Corp., γ-amino-butyric acid from Mann Research Laboratories, pyridoxal 5′-phosphate and aminooxyacetic acid from Sigma Chemical Co., and glutathione (reduced) from Calbiochem. Glutaric acid was purchased from Aldrich Chemical Co. and glutaconic acid and β-hydroxyglutaric acid dimethyl ester were obtained from ICN-K&K Laboratories. β-Hydroxyglutaric acid was synthesized from its dimethyl ester by alkaline hydrolysis.

Acetone powders of rabbit and rat brain were prepared in standard fashion in the presence of pyridoxal 5′-phosphate and reduced glutathione [10,11]. Addition of further pyridoxal 5′-phosphate to the incubation mixture did not increase GAD activity. To reduce the concentration of glutamic acid and other possibly interfering water-soluble substances, the powder was washed once with ice-cold distilled water before the final acetone treatment and drying; this reduced the glutamic acid concentration to 1.6×10^{-9} mole/mg dry rabbit brain powder. The protein content of this powder [12] was 53.2 percent of the dry weight. Enzyme activity was stable for several months when the powder was stored in a vacuum desiccator at +1°C. Aliquots of the powder were homogenized in ice-cold distilled water (25 mg/ml) immediately prior to each experiment.

The standard incubation mixture consisted of 0.1 mmole potassium phosphate, 1 μCi (2.6×10^{-8} mole) of DL-[1-^{14}C]glutamic acid, from 10^{-7} to 10^{-5} mole of L-glutamic acid, the appropriate inhibitor, and 5 mg of brain acetone powder in a volume of 1 ml at pH 6.4. Inhibitor was added in water adjusted to pH 6.4. The reaction was started after a 5-min preincubation by adding the enzyme suspension and, after 10 min at 37°C, it was stopped

with 1 ml of 25 percent trichloroacetic acid. Evolved CO_2 was trapped in 10 percent KOH and counted in a Beckman LS-233 liquid scintillation counter.

To show that GABA was a product of the reaction, the following incubate was prepared: 0.1 mmole potassium phosphate, 2 μCi (5.4×10^{-7} mole) of DL-[5-^{14}C]glutamic acid, 1 μCi (2.6×10^{-8} mole) of DL-[1-^{14}C]glutamic acid, and 25 mg of rabbit brain acetone powder in a volume of 1 ml at pH 6.4. After a 20 min incubation at 37°C the CO_2 was trapped and counted (see above). An aliquot of the reaction mixture was chromatographed on Whatman paper No. 1 using *n*-butanol/glacial acetic acid/water (12 : 3 : 5, by vol.) as the developing system. The distribution of radioactivity was determined by liquid scintillation counting of 5 mm broad bands of the chromatogram. The same technique was used to check the radiopurity of the DL-[1-^{14}C]- and DL-[5-^{14}C]glutamic acids. Both were found to be more than 99 percent pure, and did not contain any radioactivity above the background level corresponding to GABA.

Results

Incubation with a substrate containing both [1-^{14}C]- and [5-^{14}C]glutamate resulted in production of $^{14}CO_2$ corresponding to 3.8 percent of the substrate. The amount of [^{14}C]GABA, as measured by paper chromatography, corresponded to 4.0 percent of the substrate.

Time curves obtained with 10^{-2} and 10^{-3} mol/l glutamate showed that $^{14}CO_2$ production was linear with time for at least 30 min. The production of $^{14}CO_2$ from [1-^{14}C]glutamate was strongly inhibited by aminooxyacetic

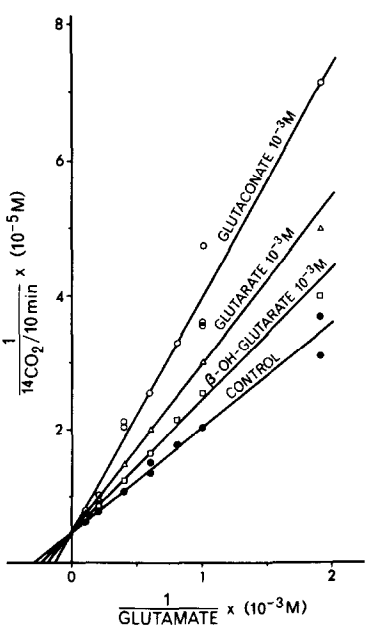

Fig. 1. Effects of 10^{-3} mol/l glutaconate, glutarate, and β-hydroxyglutarate on rabbit brain glutamate decarboxylase. The relationship between reaction velocity and glutamate concentration is shown as a Lineweaver-Burk plot.

acid; at 10^{-2} mol/l glutamate the addition of 10^{-4} mol/l aminooxyacetic acid inhibited more than 90 percent, and at 10^{-6} mol/l inhibition was 37 percent.

Glutaric, glutaconic, and β-hydroxyglutaric acids were found to competitively inhibit glutamate decarboxylation, glutaconic being the most potent inhibitor. Fig. 1 shows Lineweaver-Burk plots of results obtained using acetone powder of rabbit brain, with the inhibitors present at 10^{-3} mol/l. The K_M glutamate for the reaction was 2.9×10^{-3} mol/l, and the V_{max} was 2.2×10^{-9} mol/min. The K_i for glutaconic, glutaric and β-hydroxyglutaric acids were calculated to be 7.5×10^{-4} mol/l, 1.3×10^{-3} mol/l and 2.5×10^{-3} mol/l, respectively. Experiments using rat brain powders gave similar results.

Discussion

The production of equimolar amounts of $^{14}CO_2$ and [^{14}C]GABA from [1,5-^{14}C]glutamate demonstrates that the enzyme examined in these studies was glutamate decarboxylase. Two forms of this enzyme have been demonstrated in brain; one, GAD-I, occurs mainly in the synaptosomes of neuronal tissue, while GAD-II is found in glial cells and cerebral blood vessels [13,14]. The properties of the two differ in that the former is inhibited by carbonyl trapping agents like aminooxyacetic acid while the latter is stimulated by anions and carbonyl trapping agents [11,14].

The specific enzyme assayed in these experiments is GAD-I. The preparation of the enzyme and assay conditions were those used in previous investigations of this enzyme, enzyme activity was inhibited almost completely by 10^{-4} mol/l aminoxyacetic acid, and its K_M glutamate (2.9×10^{-3} mol/l) was in the correct range [15].

All three acids excreted in excess in glutaric aciduria (glutarate, β-hydroxyglutarate, and glutaconate) were powerful competitive inhibitors of GAD-I in rat and rabbit brain, glutaconate being the most powerful of the three. The K_i glutarate of 1.3×10^{-3} mol/l is in good agreement with the K_i of 3.5×10^{-3} mol/l recently published using highly purified mouse brain GAD-I [16]; glutaconate and β-hydroxyglutarate were not studied by these authors.

Glutarate dehydrogenase activity (2.5×10^{-5} moles CO_2/h/g wet weight) is present in rat brain mitochondria (Goodman, S.I., unpublished data). If this observation can be extended to man, generalized glutaryl-CoA dehydrogenase deficiency might lead to intracellular accumulation of the abnormal acids in brain, and inhibition of GAD-I might then cause the associated neurological dysfunction.

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