Effect of homocysteine on brain glutamate decarboxylase and interaction with pyridoxal phosphate

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There is ample experimental evidence which indicates that 4-aminobutyric acid is an inhibitory neurotransmitter in mammalian brain. Changes in 4-aminobutyric acid concentrations in the central nervous system have therefore prompted speculation regarding its involvement in neurological aberrations.

Individuals homozygous for cystathionine β-synthase deficiency (an autosomal recessive inherited disorder of methionine metabolism) are affected by a wide variety of clinical abnormalities. Glutamate decarboxylase activity is decreased in the presence of homocysteine. The normal condensation of serine and homocysteine to produce cystathionine is markedly affected, resulting in an accumulation of methionine, homocysteine and the dialpohide homocystine in glutamate or pyridoxal phosphate.

The biochemical aetiology of the convulsive episodes is not well defined, although it has been shown that administration of homocysteine to experimental animals results in the production of severe convulsive seizures. It has also been reported that homocysteine inhibits several pyridoxal phosphate-dependent bacterial enzymes.

In view of these facts, together with the suggestion that the convulsant properties of many compounds may act through a disturbance of brain 4-aminobutyric acid metabolism, we decided to ascertain whether the convulsant action of homocysteine might function in this manner, possibly by interference with the 4-aminobutyric acid-synthesizing enzyme glutamate decarboxylase (EC 4.1.1.15) which has an essential requirement for pyridoxal phosphate.

Glutamate decarboxylase was partially purified from mouse brain, after homogenization and ultrasonication, by (NH₄)₂SO₄ precipitation (60% satd.) and gel-exclusion chromatography. The active fractions eluted from a Sephadex G-200 column were concentrated by ultrafiltration, by using a macrosolute concentrator (Amicon, type B 15), and used for enzyme assays in vitro. Glutamate decarboxylase activity was quantified by measuring the evolution of 14CO₂ from L-[1-14C]glutamic acid. Assay mixtures, in a total volume of 0.3 ml, contained (final concn.) 0.2 M-potassium phosphate, pH 6.8, 0.025 µCi of L-14C[glutamic acid and partially pure glutamate decarboxylase. Glutamic acid and pyridoxal phosphate concentrations were varied according to the experiment, and homocysteine, when added, was present at final concentrations of 0.1–10 mM. Incubations were allowed to proceed for 90 min at 37°C in reaction vessels fitted with air-tight stoppers from which were suspended small glass wells. Evolution of 14CO₂ was determined essentially by the method of Wu et al. (1973), except that ethanolamine was used as a CO₂-trapping agent.

The rate of 14CO₂ evolution was markedly decreased in the presence of homocysteine when the concentration of either glutamate or pyridoxal phosphate was varied in the assay, the rate decreasing with increasing concentrations of homocysteine. The observed decrease in 14CO₂ evolution can be interpreted as follows. Either, there may be direct inhibition of glutamate decarboxylase by homocysteine binding at the glutamate-binding site, or homocysteine may interact with free pyridoxal phosphate to form a complex which could still bind to the coenzyme-binding site but block the binding of glutamate. Brain glutamate decarboxylase is particularly sensitive to depletion of pyridoxal phosphate both in vitro (Roberts et al., 1964) and in vivo (Minard, 1967); moreover, the activity of some decarboxylases may be regulated by a decarboxylation-dependent transamination reaction (O’Leary & Baughn, 1977) whereby the resulting inactive apo-decarboxylase is normally re-activated by endogenous pyridoxal phosphate. The formation of a homocysteine–pyridoxal phosphate complex could interfere with the regulatory control of the physiological action of 4-aminobutyrate, by preventing re-formation of the holoenzyme.

In this study, we have shown that there is a direct interaction between homocysteine and pyridoxal phosphate. The absorption spectrum of pyridoxal phosphate has a characteristic peak at 388 nm, representative of the free and homocysteine–pyridoxal phosphate complex. The formation of this complex was a time-dependent process, attaining an apparent equilibrium within 40 min. It was thus possible to calculate a dissociation constant of 0.48 mM for this reaction, by using the A₃₇₈ values obtained with various concentrations of homocysteine.

It is proposed that the interaction of homocysteine and pyridoxal phosphate produces a thiazine derivative of the homocysteine–pyridoxal phosphate complex. The formation of this complex could interfere with the physiological action of 4-aminobutyrate.

Our results demonstrate that mouse brain glutamate decarboxylase activity is decreased in the presence of homocysteine.

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