Brain Neurotransmitters in Pyridoxine Deficiency

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Decarboxylation of the precursor acid is a necessary step in the formation of the putative neurotransmitters, such as dopamine (3,4-dihydroxyphenethylamine), noradrenaline, 5-hydroxytryptamine and \( \gamma \)-aminobutyric acid. As pyridoxal phosphate is the coenzyme of these decarboxylases it was decided to determine the brain concentrations of the various neurotransmitters in pyridoxine deficiency. The affinity of the coenzyme for the different apocarboxylases could vary. The activity of the decarboxylase with the most tightly bound coenzyme would be higher than those with lesser affinities between the apoenzyme and the coenzyme. In addition, deficiency of pyridoxine might alter the concentrations of the precursors of the various neurotransmitters to different extents. This would also result in non-parallel alterations in the brain concentrations of the various neurotransmitters. We have examined the effect of pyridoxine deficiency caused by dietary deprivation or the use of antagonists on the brain concentrations of the putative neurotransmitters.

Dietary pyridoxine deficiency was produced in rats during the postnatal period as described by Stephens et al. (1971). In other experiments pyridoxine antagonists, such as deoxypyridoxine and penicillamine, were administered for several weeks to weanling rats. Specific pyridoxine deficiency in these animals was characterized by determinations of erythrocyte and plasma alanine aminotransferase and aspartate aminotransferase activities as well as brain concentrations of pyridoxal phosphate. Deficient rats were compared with pair-weighed controls on a pyridoxine-supplemented diet. In some experiments, the effect of pyridoxine administration over a short time period to the deficient rats was assessed.

Rats were killed by guillotine with a minimum of stress between 13:00 and 14:00 h. Brains were dissected out, washed in 2M-NaCl, frozen in liquid N\(_2\) and stored at -40°C until used for the various assays. Pyridoxal phosphate was determined by the method described earlier (Dakshinamurti & Stephens, 1969). \( \gamma \)-Aminobutyric acid was determined as described by Fahn & Cote (1968). Brain monoamines were assayed by the method of Shellenberger & Gordon (1971). Internal and external standards were used for maximal accuracy, with recoveries of the order of 85–90\%. Plasma concentrations of free and total tryptophan as well as concentrations in the brain were determined by the method of Denckla & Dewey (1967). 5-Hydroxytryptamine and 5-hydroxyindolylacetic acid were assayed after administration of pargyline or probenecid by the method of Curzon & Green (1970). Brain tryptophan 5-hydroxylase activity was assayed by the method of Gal & Patterson (1973) with tetrahydrobiopterin as the cofactor. The aromatic amino acid decarboxylase inhibitor NSD-1036 was included in the incubation.

The nature of the results was the same regardless of the method used for pyridoxine depletion, either dietary or by administration of the antagonists. Deficient animals had significantly lower concentrations of brain \( \gamma \)-aminobutyric acid as compared with controls. This correlated well with brain concentrations of pyridoxal phosphate and the activity of glutamate decarboxylase. Brain concentrations of dopamine and noradrenaline were unaffected by pyridoxine deficiency. However, there was a significant \((P<0.005)\) decrease in the brain concentration of 5-hydroxytryptamine in deficient rats as compared with controls (Dakshinamurti et al., 1976). We ruled out the possibility that the decrease in brain 5-hydroxytryptamine was the result of the inanition and the resultant generalized malnutrition. We also ruled out the possibility that this decrease was due to an increased catabolism of 5-hydroxytryptamine or to a decrease in brain tryptophan. The possibility of a decrease in the activity of brain tryptophan hydroxylase was also excluded. Our observation of non-parallel changes in the brain concentration of catecholamines and 5-hydroxytryptamine respectively indicates that the syntheses of

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various amine transmitters are regulated differently. The existence of distinct substrate-specific aromatic amino acid decarboxylases could explain our results.


Inhibition of Kynurenine Hydrolase by Benserazide, Carbidopa and other Aromatic Hydrazine Derivatives: Evidence for Sub-Clinical Iatrogenic Niacin Deficiency

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It has been shown previously that kynurenine hydrolase (L-kynurenine hydrolase, EC 3.7.1.3) is inhibited by the hydrazide Benserazide (R, 4-4602, DL-serine 2-[(2,3,4-trihydroxyphenyl)methyl]hydrazide), a compound that is used clinically in the treatment of Parkinson’s disease (Bender et al., 1977). Benserazide is a potent inhibitor of aromatic amino acid decarboxylase (L-aromatic amino acid carboxy-lyase, EC 4.1.1.28) and is used in conjunction with dopa (3,4-dihydroxyphenylalanine) therapy. Since Benserazide does not cross the blood–brain barrier, it will inhibit only extra-cerebral aromatic amino acid decarboxylase, and hence allow a greater proportion of the administered dose of dopa to enter the brain for cerebral dopamine (3,4-dihydroxyphenethylamine) synthesis. This allows use of smaller doses of dopa, and hence decreases the incidence of dopa-induced side effects in Parkinsonian patients.

Since Benserazide is a hydrazide, it is not surprising that it inhibits kynurenine hydrolase, which is a pyridoxal phosphate-dependent enzyme. It is well established that many hydrazine derivatives inhibit pyridoxal phosphate-dependent enzymes, by formation of biologically inactive hydrazones of the cofactor (Vilter, 1964). In the present study the pattern of inhibition of kynurenine hydrolase by Benserazide has been studied. At the same time another hydrazine inhibitor of aromatic amino acid decarboxylase that is used in treatment of Parkinsonism together with dopa, Carbidopa (MK-486, S-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropionic acid monohydrate) has also been studied. For comparison, four other aromatic hydrazine derivatives have also been used: isonicotinic acid hydrazide, an anti-tuberculosis agent known to inhibit kynurenine hydrolase *in vivo* under clinical conditions (Roe, 1971), phenylhydrazine, Girard’s reagent T (2-hydrazino-NNN-trimethyl-2-oxoethanaminium chloride) and Girard’s reagent P (2-hydrazino-2-oxoethane-N-pyridinium chloride).

Liver from freshly killed rats was homogenized in 2ml of 0.15M-NaCl/g of tissue; 0.5ml of this homogenate and 0.5ml of 0.2M-sodium phosphate buffer, pH 7.0, were incubated together for 5 min at 30°C. For inhibition studies the inhibitor was dissolved in the buffer, so that it was present with the enzyme throughout this preincubation period. The reaction was initiated by addition of 0.2ml of a solution of dl-kynurenine; five concentrations of substrate were used, over the range 0.5–4.0mm. After incubation for a further 30 min the reaction was stopped by addition of 1ml of 1M-trichloroacetic acid. Denatured protein was removed by centrifugation and the supernatant was made approximately neutral by the addition of 0.5ml of 1M-NaOH. The volume of the supernatant was then adjusted to 5ml by the addition of 1M-sodium phosphate buffer, pH 5.5, and the fluorescence due to anthranilic acid measured (activation 310nm, 1978)