Effect of D-penicillamine on Vitamin B_6 Activation of Aspartate Aminotransferase in Synaptosomes of the Developing Brain

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Abstract

The activity of aspartate aminotransferase a vitamin B_6 containing enzyme was measured in the laysed synaptosomes prepared from rat brain, during development. Administration of vitamin B_6 increased the enzyme activity throughout development. The stimulating effect of this vitamin was prevented when the animals were previously treated with D-penicillamine. However, penicillamine alone had no effect on the pattern of the enzyme activity during development. Similar results were observed when the synaptosomal preparations were incubated in the presence of the vitamin, the drug or both. It is concluded that vitamin B_6 content of the brain might be unaffected by D-penicillamine treatment.

Introduction

Penicillamine (ß,ß dimethylcyteine) is an effective therapeutic agent for Wilson's disease. The isomers of penicillamine are known to be toxic by reason of its liability to antagonize vitamin B_6 (Rumsby and Shepherd, 1979; Otomo et al., 1980). Several authors have shown that vitamin B_6 deprivation in animals during early development produces abnormalities in the central nervous system. In the brain of vitamin B_6-deficient animals a reduction in amino acid metabolism (Wasynczuk et al., 1983), serotonin (Dakshinamurti and Paulose, 1983), dopamine (Guilarte, et al., 1987) and gamma aminobutric acid (Tunnicliff and Ngo, 1986) concentrations have been reported. Furthermore, evidence is also avilable for brain morphological changes of the vitamin-deficient rats (Chang, et al., 1981).

Aspartate aminotransferase (AST), a vitamin B_6 containing enzyme has most of its activity in the nerve ending (Synaptosomal) fraction (Subolokshmi and Murthy, 1985). Because penicillamine may be prescribed for patients with Wilson's disease during the growth period, we have studied the effects of D-penicillamine on AST activity in rat brain synaptosomes during development.

Materials and Methods

Four groups of male and female (n= 4 and 4 in each group) Wistar rats were caged at room temperature (about 20°C) separately. They were fed a mixture of wheat, barley, dried whey and 10 vitamin mixture granulated by a feed producer as formulated by our Nutrition Department.

Two groups (A and B) were given D-penicillamine (500 mg/l) in their drinking water, the others (C and D) had drinking water only. Group A and C were injected with vitamin B_6, (pridoxine hydrochloride, 500 mg free base / kg kg body weight /day). Group D was regarded as control. The day on which sperm was identified in the smear was designated gestational day 1.

All experiments were carried out on the animals bred in the four separate groups. The birth date of all animals were carefuly recorded after daily inspection. Animals of both sexes were used for 10 days, then only male animals were used.

The animals were decapitated at 1, 3, 7, 10 and 35 days after birth and synaptosomes were prepared. The entire forebrain of 2 - 12 rats (about 1g wet wt. tissue) were homogenised in 10 ml of 0.32M sucrose solution. The homogenate was centrifuged at 1000g for 10 min at 4°C, the pellet discarded and the supernatant was centrifuged at 18,000g for 20 min (eg. Messripour and Clark, 1985). The resulting pellet was suspended in an incubation medium containing: 125mM NaCl, 5 mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$, and 15 mM sodium phosphate buffer (pH = 7.4). The assay of AST activity was measured in the laysed synaptosomes. The suspension was exposed to Triton x-100 (0, 1% V/V) and homogenised. This was centrifuged at 30,000g for 20 min. at 4°C and the enzyme activity was measured by the coupled reaction (Martinez-Carrion, et al, 1967) in which the synaptosolic fraction was added to the reaction mixture containing NADH and malate dehydrogenase. Reaction was initiated by the addition of alpha-ketoglutarate and the oxidation of NADH at 340 nm was monitored against a blank containing all components except alpha-ketoglutarate. Synaptosolic protein was measured by the Lowry method, et al. (1951).

Results

Rat brain developmental changes synaptosomal AST activity and the effects of vitamin B_6 and D-penicillamine on the enzyme activity is shown in Fig. 1. The activity of enzyme in newborn control rats was 162. 5±6 nmol/min /mg protein, which increased to 1132.5± 121 nmol/min /mg protein 35 days after birth. Administration of vitamin B_6 to normal rats resulted in a significant increase in the enzyme activity at about every stage of brain development. As can be seen in Fig. 1, when the vitamin was injected into rats treated with D-penicillamine, the pattern of the enzyme activity was similar to that of control animals.

Table 1 indicates the *in vitro* influence of vitamin B_6 and D-penicillamine on the activity of AST in adult rat brain synaptosomes. When the synaptosomal preparation was incubated with 10 mM vitamin B_6 in 100 mM potassium phosphate buffer pH 7. 4 at 37°C for 30 min. the enzyme activity increased by about 20%, whereas when the synaptosomes were incubated in the presence of added vitamin at 0°C the enzyme activity was not significantly different from that of the control group. The stimulating effect of the vitamin on the synaptosomal AST activity was prevented by 0.1 mM D-penicillamine. However, the presence of 0.1 mM D-penicillamine alone caused no significant effects.

Discussion

Penicillamine is known to increase urinary excretion of vitamin B_6 (Rumsby and Shepherd, 1979, Otomo, et al. 1980), which could result in a vitamin B_6 deficiency. In principle because AST requires pyridoxal 5'-phosphate (PLP) as a co-factor, alternation in the availability of PLP should have an effect on the activity of this enzyme. However, the results of this study showed that long-term treatment of D-penicillamine had no effect on the developmental characteristics of AST activity in rat brain synaptosomes, while in both *in vivo* and *in vitro* experiments the drug prevented enzyme activation by the added vitamin B_6. Studies on isolated AST from pig heart have indicated that various forms of the apoenzymes react with PLP at different rates, in some forms, PLP is bound in an active mode, in others the co-enzyme is bound in a nonactive mode (Martinez-Carrion, et al., 1967), This leads us to speculate that activation of the synaptosomal enzyme by vitamin B_6 is related to the nonactive binding of PLP to the opoenzyme, which may be removed from the protein site by penicillamine, whereas actively bound PLP is not affected. This possibility is suported by the suggestion made by Russell et al. (1985) that the PLP content of tissues containing proteins with a strong affinity for PLP should be more resistant to the effects of a vitamin B_6 deficiency.

In conclusion, since AST activity may be taken as a measure to evaluate endagenous vitamin B_6 status (Russell et al. 1985), it is unlikely that D-penicillamine affects vitamin B_6 content of the brain during development.

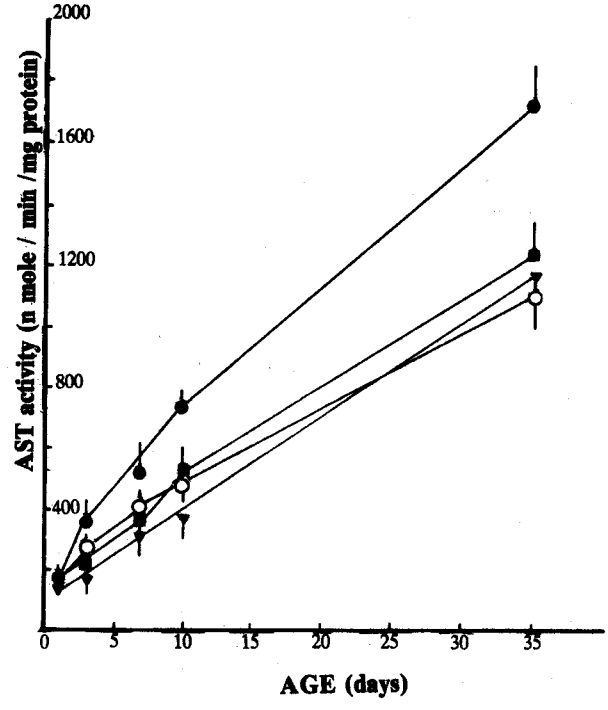
Addition (s)	Synaptosomal AST activity (nmol/min/mg protein) Incubated at 0° C	Incubated at 37° C
None	1943 ± 32	1917 ± 28
Vitamin B_6 (10 mM)	1928 ± 37	2371 ± 29
Penicillamine (0, 1 mM)	1945 ± 33	1930 ± 11
Vitamin B_6 + Penicillamine	1983 ± 42	1941 ± 21

TABLE 1. Effect of vitamin B_6 and penicillamine on synaptosomal aspartate aminotransferase activity.

Rat brain synaptosomes were suspended in the incubation medium, pH = 7.4 (see materials and methods). Aliquots of the synaptosomal suspensions (1 ml, about 4mg of protein) were incubated for 30 min at either 0°C or 37° C in the presence of added vitamin B_6 (10 mM) and or penicillamine (0.1 mM), and in their absence. Aspartate aminotransferase activity was measured in the laysed synaptosmes. Results are expressed as n mol/min/mg synaptosmal protein + SEM of four separate experiments.

Legend to Fig 1

Effects of vitamin B_6 and penicillamine on development of the synaptosomal aspartate aminotransferase.
Four groups of rats were treated in separate cages as follows: (group C (●) was injected with vitamin B_6 (500 mg/kg/day), group B (■) was given D-penicillamine in their drinking water (500 mg/L), group A (▼) was treated with both D-penicillamine and vitamin B_6, and group D (○) received neither (control). The brain synaptosomal



preparations were exposed to Triton- X 100 for lasing and centrifuged at 30000 g, for 20 min at 4° C. The enzyme activity was measured in the synaptosolic fractions in triplicate. Each point is the mean of four separate experiments + SEM.

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