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Inhibition of Rat Brain Dopamine Beta Hydroxylase by Manganese Ions

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T S Padmashree, M N Subhash .

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- *Department of Neurochemistry, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Abstract

The effect of manganese on partially purified dopamine beta hydroxylase from rat brain was studied. The activity was measured at optimal copper concentrations and at 0.5 to 5.0 micro molar concentration of manganese in the assay system. A two phase inhibition of the enzyme is observed. At lower concentration a 25% decrease in enzyme activity is observed which returns to baseline at 2.5 micromolar concentration. At higher concentration, again the activity decreases and at 5.0 micro molar concentration nearly 60% inhibition is observed. This variable effect of manganese might be responsible for the differential effect seen in biogenic amine metabolism in manganese neurotoxicity.

Key words -

Dopamine beta hydroxylase,
Rat brain,
Manganese,
Invitro effect

Clinical, neuropathological and biochemical investigations in humans exposed to manganese (Mn) have suggested central nervous system dysfunction due to disturbances in the neuro transmitter system. Experimental studies on animals have substantiated this suggestion by reporting alterations in biogenic amines after Mn intoxication. The signs of chronic Mn poisoning closely resemble those of Parkinson's disease [1] and the underlying biochemical lesion has been found to be a deficiency of dopamine (DA) in basal ganglia region [2]. The fact that L-Dopa, to a certain extent, is useful in the treatment of Mn poisoning has led to the speculation of involvement of daopaminergic system. Major differences do exist in the metabolism of brain biogenic amine during short and long term exposure to Mn.

In general it is suggested, that chronic exposure to Mn results in a decrease in biogenic amines while short term exposure has either no effect or shows increased levels. This has been attributed to the effect of Mn on enzymes involved in the biogenic amine metabolism [3]. The observation of various studies, relating to dose and mode of exposure and the effect on biogenic amines, have led us to investigate the in vitro effect of Mn on partially purified dopamine betahydroxylase (DBH) (EC. 1. 14. 17.1) from rat brain.

Material and Methods

Ten male adult rats were taken up for this study. The animals were killed by decapitation and brain removed immediately. An aliquot of (100-200µ l) crude tissue extracts (containing approximately 10 mg brain tissue) and equal volume of cancavlin A sepharose suspension (15 gm % in acetate buffer pH 7.4) were taken in borosilicate centrifuge tubes. After mixing for 15 min the mixture was centrifuged at 9000 X g for 3 min and the supernatant was discarded. The pellet was washed three times with ice cold deionized water and resuspended in 200µ l of incubation buffer. Aliquots of this suspension, containing DBH adsorbed to concavlin A sepharose, was used to assay. The method of Sperk et al [4], using tyramine as substrate at an optimal copper concentration of 30µ M was employed. The octopamine formed was spectrophotometrically measured [5]. The enzyme activity was assayed at Mn concentrations ranging from 0.5 to 5.0µ M. The assays were done in duplicate for each Mn concentration and enzyme activity was expressed as nanomoles of octopamine liberated per milligram tissue per 30 min.

Results

DBH activity was measured while the enzyme was bound to concavlin A sepharose. This procedure removed the endogenous inhibitory substances known to be present in crude brain tissue extracts. When compared to the baseline value of 0.48 units the DBH activity is found to be decreased (27%) at 1.5µ M of Mn which returns to baseline value at 2.5µ M. Further addition of Mn shows a steady decline of enzyme activity and at 5.0µ M concentration of Mn about 60% of enzyme activity is inhibited.

Table I - Effect of manganese on partially purified rat brain dopamine beta hydroxylase activity .

Mean ± SD of ten experiments in duplicate (@) nanomoles of octopamine liberated/mg tissue/30 min. The incubation mixture contained in a final volume of 400 µ l: 3 mM tyramine, 0.6 mM pargyline, 40 mM fumarate, 4 mM ascorbic acid, 30 µ M copper sulphate, 700 units of catalase, 50 mM sodium acetate buffer pH 5.5 and aliquot of partially purified enzyme preparation. The assay was performed at varying concentrations of manganese

±µµ

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±µµ

Discussion

It is evident from clinical reports that animal experiments that Mn essentially affects the CNS [6]. In spite of several investigations the mechanism of brain damage due to Mn remains unexplained. Though the exact interactions between trace metals and enzymes have not yet been clarified, Mn has been shown to affect enzymes like tyrosine hydroxylase [7], Monoamine oxidase [8], [9], [10] and DBH in brain. In the present study we have estimated brain DBH in presence of external Mn to understand the effect of the metal on the enzyme. In animal experiments this metal is reported to decrease dopamine (DA) and nonepinephrine (NE) during long term exposure [8]. However in the early phases of short term exposure elevated levels of NE have been reported. This variable effect in DA and NE has been attributed to the inhibition of tyrosine hydroxylase and DBH respectively. In the present in vitro experiment DBH is inhibited at very low concentrations (1.5μ M) as well as at very high concentrations (5.0μ M) of Mn.

In short term exposure when Mn concentration of brain is low, either no changes in DA and NE levels or a slight increase in NE might be expected. After exposing the animals for long term, the Mn concentration in brain will be very high and at such concentrations the DBH is found to be inhibited. At this stage a substantial decrease in NE is expected. The initial increase in NE and decrease after long term exposure may be due to the variable inhibition of DBH which is reflected in this study. Though it has not been possible to establish the critical level of Mn in brain which is responsible for biochemical effect on biogenic amine metabolism, this study has shown that above 2.5 (M concentration of external Mn, a substantial inhibition of DBH is observed. This variable inhibition in enzyme activity may thus account for an early increase observed in NE [11] and DA [12], while the substantial inhibition in DBH in later stages may account for the decreased NE observed after long term exposure [13]. Similar observations on the influence of Mn on tyrosine hydroxylase activity have been reported by Bonilla [7]. The present study, carried out with external Mn, approximately equivalent to the levels reported in rat brain after exposure to Mn [10], demonstrates marked alternations in DBH activity. The inhibition of DBH, an important enzyme in biogenic amine metabolism, by Mn may lead to alterations in neurotransmitter system in brain which might be responsible for the symptoms seen in Mn neurotoxicity.

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