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Effect of Manganese on Striatal Biogenic Amines in Rats

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Abstract

The effect of long term exposure in manganese on the striatal content of biogenic content of biogenic amines and their metabolising enzymes in rats has been investigated. The levels of dopamine and serotonin were measured by HPLC in rat striatum after 90 days of exposure to manganese. The activity of dopamine beta hydroxylase and monoamine oxidase was also measured in striatum. The levels of manganese in striatum, measured by atomic absorption spectrophotometry, were found to be increased above control values ($p<0.05$) and striatal dopamine beta hydroxylase was decreased significantly ($p<0.01$) after exposure to manganese. Monoamine oxidase did not show any significant change. No statistically significant change was observed in the levels of striatal dopamine and serotonin after exposure to manganese though dopamine was found to be slightly decreased. Experimental evidence has been presented to show that long term exposure to manganese causes inhibition of dopamine beta hydroxylase and depletion of dopamine in rat striatum.

Key words -
Striatum,
Dopamine beta hydroxylase,
Monoamine oxidase,
Dopamine,
Serotonin,
Manganese,
Rats

The neurological syndrome of manganese (Mn) toxicity has been attributed to the alterations in neurotransmitter metabolism in the central nervous system (CNS). The signs of Mn toxicity closely resemble those of paralysis agitans [1]. It is also observed that L-dopa, to some extent, is helpful in treating the Mn toxicity cases. The major underlying biochemical lesion in Mn toxicity has been found to be a deficiency of dopamine (DA) in brain. Many experimental studies have shown alterations in brain biogenic amines in animals after exposure to Mn [2], [3], [4]. It is also believed that enhanced turnover of brain catecholamines is responsible for locomotor signs in animals and manical symptoms in humans [5]. Most of the studies are done on whole brain. Very few reports are available on the effect of Mn on different regions of the brain. In view of the reports of greater accumulation of Mn in corpus striatum [6] and the involvement of

dopaminergic system in Mn neurotoxicity, we attempted to study the changes in biogenic amine metabolism in rat striatum. This paper reports the observations made during experimental studies on striatal catecholamines in rats during prolonged exposure to low level Mn.

Materials and Methods

Ten male albino rats (approximate weight 220 g) were given manganese chloride (0.54 mg MnCl25H20 /ml-equal to 0.15 mg of manganese/ml) in drinking solution for 90 days. A group of ten rats which received sodium chloride in place of MnCl2, formed the control group. At the end of the experimental period, the rats from both the groups were sacrificed by decapitation, and their brain removed immediately. The striatium was dissected on ice, adopting the method described by Glowinski and Iversen [7]. The wet weight was determined to the nearest milligram.

Biogenic amine assay

The striatal tissues were homogenized in 0.1 M perchloric acid and the homogenate was centrifuged at 10,000 × g for 5 min. An aliquot of 100µ l of supernatant was used for the simultaneous assay of DA and serotonin (5-HT) by HPLC-ECD method as described by Sasa and Blank [8]. For the separation of DA and 5-HT, Tracor HPLC equipped with BAS electrochemical detector and SCX strong cation exchange column (Partisil;-10, Whatman USA) was used. DA and 5-Ht were expressed asµ g/.gm wet weight of the tissue.

Enzyme assay

DBH: The striatal tissues were homogenized in 50 mM acetate buffer (pH 6.0) containing 1% triton-X. The homogenates were then subjected to quick freezing and thawing in liquid nitrogen and centrifuged at 20,000 × g for 10 min. The supernatant was used for DBH assay after subjecting to partial purification using Concavlin A Sepharose. The method was essentially that of Sperk et al [9] except that tyramine was used as substrate, at an optimal copper concentration of 30µ M. Liberated octopamine was estimated by the method of Nagatsu and Udenfriend [10].

MAO: Striatal MAO was assayed flurometrically by the method of Snyder and Hendley [11] in whole homogenates of the tissue prepared in 0.1M Na-K phosphate buffer (pH 7.8). The activity was expressed in units. Protein was estimated in tissue homogenates by the method of Bradford [12].

Manganese

Striatal Mn was estimated in supernatants of homogenates prepared in 0.1M ice cold perchloric acid, by using atomic absorption spectrophotometer. In brief, the procedure for the analysis of Mn was: an aliquot of 10µ l supernatant (diluted ten times) was injected into the graphite tube. The sample was

dried at 130° C for 20s with a ramp of 8, ashed at 700° for 15s at a ramp of 6 and atomized at 2500° C for 3.5s spontaneously. The absorbance was recorded in height mode. The instrument was calibarated with 2.5 and 5.0µ g% Mn standards. All solutions were prepared with deionized water, double distilled in quartz. Pye-Unicam flameless atomic absorption spectrophotometer with graphite furnace and an auto sampler were used. Values were expressed asµ g per gram tissue weight.

Results

The average daily intake of water in both control and experimental group was 33 ml. There was no significant difference in weight gain between the two groups. The total intake of manganese in the experimental rats was about 5 mg Mn/day.

Effect on Mn on BDH and MAO in striatum

The results expressed in Table I show that after exposure to Mn for 90 days the striatal DBH is significantly decreased ($p<0.01$) whereas no change is observed in the content of MAO. There is a significant increase ($p<0.05$) in the accumulation of Mn in striatum after 90 days of exposure to Mn. The striatum of Mn treated rats showed 3.15µ g of Mn/gm tissue when compared to 1.23µ g in control rats.

Table I - Dopamine, serotonin, DBH, MAO and Mn content in striatum of manganese exposed rats .
Values are for ten rats in each group, assayed in duplicate

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Effect of Mn on striatal content of DA and 5-HT

After exposure to Mn for 90 days, no statistically significant change was noted in striatal content of either DA or 5-HT though DA was found to be slightly decreased when compared to control rats.

Discussion

The behavioural symptoms noted in Mn neurotoxicity in humans have been attributed to the alterations in neurotransmitter levels. Previous studies have indicated a derangement in the metabolism of DA in brain of animals exposed to Mn [13], [14]. The present study indicates a slight decrease in the striatal DA after long term exposure to Mn along with a significant decrease in DBH. Reports of initial elevation of both DA and NE have indicated that the effect of Mn on striatal content of catecholamines

changes with the duration of exposure. In this experiment the rats were exposed to Mn for a period of 90 days which show a three fold increase in the Mn levels in the striatum with an associated decrease in DBH activity. No significant decrease in DA was noted. Chandra and Shukla [13] in their study showed no change in striatal DA after exposure to Mn for 120 days, though there was an increase in the initial stages. In the present study the activity of striatal MAO and 5-HT are not affected even after a three fold increase in the Mn content. Chandra and Shukla [13], however have reported increase in striatal MAO activity in rats in the initial period of exposure, but no change has been reported after 120 days of exposure. In another study by Chandra et al [14], decreased DA with no change in 5HT levels in striatum of monkeys after prolonged exposure to Mn has been reported. Similar results have been reported by Neff et al [4]. The effect of prolonged exposure to Mn has been reported to be variable. It does not appear to be related to the Mn level in that region. Thus it is difficult to establish the critical levels of Mn in the brain which is responsible for these changes. The increase in DA in the initial stages and a decline in later stages, as reported by other studies [13], might explain the development of signs and symptoms in both acute and chronic Mn poisoning. The present investigation, however, demonstrates that exposure to Mn for 90 days results in a significant decline in DBH activity along with a slight decrease of DA in rat striatum. Exposure of rats to Mn for still longer periods might answer the symptoms seen in chronic Mn poisoning cases. Whole brain dopamine levels have been reported to be decreased in rabbits [3] and rats [15] after chronic exposure to Mn. No significant change in 5-HT levels was found either in whole brain of rat after long term exposure [16] or in striatum of rat after short term exposure to Mn [17]. In the present study also, striatal 5-HT did not show significant change after 90 days of exposure to Mn. the decrease in DBH activity might be due to inhibitory effect of Mn on the enzyme whereas the decrease in DA might be due to the neuronal damage caused by excess Mn in the brain or due to the inhibition of other enzymes like tyrosine hydroxylase [18] or dopamine decarboxylase [15]. These changes might be more pronounced if the rats are exposed to Mn for a prolonged period.

This study on the influence of Mn on neurotransmitters and enzymes involved in their metabolism suggests the involvement of dopaminergic function in Mn neurotoxicity. It is demonstrated that exposure of rats to Mn results in a significant decrease in striatal DBH and a decrease in DA which might explain the development of signs of Mn neurotoxicity in humans.

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