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CHRONIC MANGANESE POISONING AND STRIATAL ADENYLATE CYCLASE ACTIVITY

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ABSTRACT

Striatal adenylate cyclase activity was markedly decreased in rats ingesting 2.5, 5 and 10 mg Mn/ml of water during 8 months. No stimulation of the enzymatic activity by dopamine was observed in any of the animals whatever the concentration of manganese ingested in the drinking water.

INTRODUCTION

The permanent neurological phase of chronic manganese poisoning in humans (7) and in primates (11) is associated with reduced striatal dopamine. Tyrosine hydroxylase activity has also been found to be decreased in neostriatum of rats fed a high oral load of manganese chloride during eight months (4). Moreover, a marked degeneration of neurons in the zona compacta of the substantia nigra has been demonstrated in a case of human manganese encephalopathy (1). All of the preceeding findings point to a permanent damage of dopaminergic neurons during prolonged manganese neurointoxication.

What would be then the functional state of the striatal dopamine receptors after this decrease in available dopamine? There is evidence indi-

cating that nigral dopaminergic neurons synapse with cholinergic neurons, and possibly, with GABA-ergic neurons in the striatum (10). Apparently, cholinergic neurons are resistant to the toxic effect of manganese since the striatal choline-acetyltransferase activity was not affected after eight months of treatment with manganese chloride (9). On the other hand, GABA metabolism has been studied only during the initial two months of manganese loading when a significant increase in GABA concentration in neostriatum was noted (2). However, after two months of treatment an increase in the activity of tyrosine hydroxylase has also been demonstrated (4). Therefore, we found it necessary to investigate the effect of chronic manganese intoxication on the striatal dopamine receptors intimately associated with a dopamine-sensitive adenylate cyclase (6).

MATERIAL AND METHODS

For these studies male Sprague-Dawley rats weighing 200-250 g and fed ad libitum with rat laboratory chow (73 μg Mn/g dry weight) were divided into 5 groups: an untreated control group and the remaining groups whose drinking water contained 1.0, 2.5, 5.0, and 10.0 mg Mn/ml as $MnCl_2$, respectively. Distilled-demineralized water was used for both control and experimental groups.

At the eight month 9 animals of each group were sacrificed by decapitation. The striatum was dissected out immediately and stored frozen at −70°C until analyzed for basal and dopamine-sensitive adenylate cyclase following the method of Clement-Cormier et al (6). The striatum was homogenized in 50 volumes (W/V) of 2 mM tris-(hydroxymethyl) aminomethane-maleate buffer (pH 7.4) - 2 mM EGTA. In a final volume of 0.5 ml the assay system contained (in m mol/liter): tris (hydroxymethyl) aminomethane-maleate, 80.2; ATP, 1.5; MgSO4, 6.0; theophylline, 10.0; EGTA, 0.6 (including the amount introduced with the tissue homogenate) and 0,05 ml of tissue homogenate. Incubation was for 2.5 min at 30°C. The reaction was terminated by boiling, and cyclic-AMP determined by radioimmunoanalysis (New England Nuclear, Boston, MA). For measuring the dopamine-sensitive adenylate cyclase activity 100 μM of dopamine (final concentration) was added to the incubation system.

Striatal Manganese concentration was determined by flameless atomic absorption spectroscopy (3) only in control rats and in those that ingested 10 mg Mn/ml of water.

Some experiments were designed in which basal adenylate cyclase activity was studied after Mn^{2+} was added to the standard incubation medium in final concentrations ranging from 0.01 to 10.0 μM.

Protein was measured by the procedure of Lowry et al (8). Statistical analysis was performed according to the Student's t test.

RESULTS

As shown in Table I, after 8 months of manganese intake the basal activity of adenylate cyclase in striatum was significantly decreased in the group of rats ingesting 2.5, 5, and 10 mg Mn/ml water. Surprisingly, no stimulation by dopamine of the adenylate cyclase activity was observed in any of the groups of rats tested whatever the concentrations of manganese they had ingested in the drinking water.

TABLE I

EFFECT OF CHRONIC MANGANESE POISONING ON STRIATAL ADENYLATE CYCLASE ACTIVITY

Mn concentration in drinking water (mg/ml)	N	Cyclic AMP formed (pmol/mg prot/2.5 min)		Increase in c-AMP (pmoles)	P
		Minus dopamine	Plus dopamine		
0.0	9	237.2 ± 12.8*	286.3 ± 16.8	49.1	<0.05
1.0	8	231.1 ± 12.3	240.3 ± 11.4	9.2	NS
2.5	8	198.5 ± 8.4**	206.7 ± 7.9	8.2	NS
5.0	8	200.0 ± 9.6**	210.0 ± 14.6	10.0	NS
10.0	7	132.4 ± 15.7**	140.7 ± 11.5	8.3	NS

* Values represent means ± S.E.

** $p < 0.05$ relative to control minus-dopamine values.

The addition of Mn to the incubation medium, at the micromolar range selected, did not produce any change in the basal adenylate cyclase activity.

A striatal concentration of 0.17 ± 0.01 and 0.30 ± 0.02 μg Mn/g wet weight was found in the control and in the ratas ingesting 10 mg Mn/ml water, respectively.

DISCUSSION

It has been suggested that some of the neurotransmitter functions of dopamine may be mediated by cyclic AMP (6) through the stimulation

of adenylate cyclase. On the other hand, it has been shown that Mn^{2+} (1 mM) produced a significant stimulation of basal adenylate cyclase activity in homogenates of rat striatum. In fact, Mn^{2+} alone was found to be superior to Mg^{2+} in supporting adenylate cyclase activity. In addition, inclusion of 6 mM Mg^{2+} appeared to have little effect on the stimulatory action of Mn^{2+} (12).

Considering the striatal content of manganese (0,30 μg/g wet weight) that we have detected in rats ingesting the highest oral load a final concentration of 0.01 μM Mn was obtained in the incubation medium used for the assay of adenylate cyclase activity. Since our in vitro studies showed no changes in enzymatic activities at this Mn concentration the decreased activity observed in ratas ingesting 2.5, 5.0, and 10 mg Mn/ml water would appear to be the result of the striatal damage produced by the manganese poisoning (4).

In the present investigation the observed lack of stimulation of the basal adenylate cyclase activity by dopamine at all concentrations of Mn ingested, suggests the possibility that subtle changes could be induced in this enzyme after prolonged exposure to this metal.

In the study published by Walton and Baldessarini (12) they reported that at Mn^{2+} concentration of 1 mM (in absence of added Mg^{2+}) dopamine (100 uM) produced a significant increase (38%) in the basal enzymatic activity. On the contrary, after using a concentration of Mn^{2+} (6 mM) giving near maximal stimulation of basal adenylate cyclase, dopamine produced a slight but significant inhibition (13%). When dopamine was added to the medium containing Mn^{2+} (1 mM) and Mg^{2+} (6 mM) a small but significant increase (17%) above basal activity was noted. However, if only Mg^{2+} (6 mM) was present the increase was much more pronounced (90%). Therefore, Mn^{2+} added to the incubation medium diminished the activation produced by dopamine in the adenylate cyclase activity when assayed in the presence of Mg^{2+}.

Since the site of interaction of Mn^{2+} with adenylate cyclase appears to be the catalytic rather than the receptive or regulatory subunit (12) it looks as if after in vivo manganese administration some kind of changes are produced by this cation at the catalytic site in order to prevent the stimulation of this enzyme by dopamine, in vitro.

If the failure of dopamine to stimulate the striatal adenylate cyclase activity after prolonged manganese intoxication also occurs in vivo, it should be traduced by changes in the activity of the neurons bearing the dopamine receptors coupled to adenylate cyclase. Since cholinergic neurons do not appear to be affected by the manganese poisoning (9) the need

for more detailed investigations on the activites of other striatal neurons (GABA-ergic, peptidergic, etc.) is clearly indicated. In fact, in a previous work we found that rats receiving 2.2 mg Mn^{2+}/ml water during 7 months developed a decrease in brain dopamine and homovanillic acid content. Therefore, dopaminergic neurons are very sensitive to the toxic effect of the metal (5).

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RESUMEN

Intoxicación crónica con manganeso y actividad de la Adenilato ciclasa del estriado. *Bonilla, E. (Instituto de Investigaciones Clínicas. Facultad de Medicina. Universidad del Zulia. Apartado Postal 1151, Maracaibo 4001-A, Venezuela).* **Invest Clín** *26(1): 45-50, 1985.*— La actividad de la adenilato ciclasa del neoestriado disminuyó significativamente en ratas que ingirieron 2.5, 5.0 y 10.0 mg Mn/ml de agua de bebida durante 8 meses. La dopamina no produjo estimulación de la actividad enzimática en ninguno de los grupos de animales tratados con manganeso.

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