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Manganese Toxicity: Free amino acids in the striatum and olfactory bulb of the mouse.

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Key words: Manganese poisoning, amino acids, olfactory bulb, GABA, striatum.

Abstract. We studied the levels of twenty two free amino acids in the striatum and olfactory bulb of mice treated during nine weeks with daily intraperitoneal injections of manganese chloride at a concentration of 5.0 mg Mn^{+2}/kg body weight. In the olfactory bulb the contents of alanine, α-amino-n-butyrate, arginine, asparagine, aspartate, citrulline, GABA, glutamate, glycine, isoleucine, leucine, methionine, phenylalanine, serine,threonine, tyrosine, and valine were diminished. No alterations were observed in the concentrations of free amino acids in the striatum of Mn-treated mice. The changes detected in the olfactory bulb merit a thorough evaluation in order to determine its importance on the pathophysiology of manganese poisoning.

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INTRODUCTION

Chronic administration of manganese elicits significant changes in the endogenous levels of some biogenic amines and their metabolites in several brain regions (1,2,6,11,12). The precursors of the neurotransmitters dopamine, serotonin and GABA are the dietary amino acids tyrosine, tryptophan and glutamic acid, respectively. But several other amines and amino acids have also been proposed as neurotransmitters: histidine, glycine, taurine, aspartic acid and glutamic acid. Some of the psychiatric and neurological symptoms of chronic manganese poisoning could be the result of complex interactions and changes in the metabolism of

several of these amino acids neurotransmitters.

We have studied the levels of free amino acids in blood plasma, striatum and frontal cortex of adult rats treated during eight months with manganese chloride added to the drinking water. No significant differences were demonstrated in any of the amino acids analyzed (7). We now report that intraperitoneal injections of manganese chloride cause significant changes in the levels of several free amino acids in olfactory bulb of mice.

MATERIALS AND METHODS

Experiments were carried out on male albino mice (NMRI-IVIC strain from the Venezuelan Institute for Scientific Research) weighing 20-25 g and fed *ad libitum* with rat laboratory chow (Protinal-Maracaibo) containing 70 µg Mn/g dry weight. A group of mice was injected intraperitoneally with manganese chloride (5 mg Mn/kg b.w.) in 0.9% NaCl solution. Control mice were injected with 0.1 ml saline. Both control and Mn-treated mice received one daily injection five days per week. They had unrestrained water access and the quantity of water ingested by each mouse was measured daily. After nine weeks 11 animals of each group were sacrificed by decapitation. The brains were extracted immediately, placed on a Petri dish over ice and the striatum and olfactory bulb dissected. These samples were stored at -80ºC until analyzed.

Spectrophotometric assays: Brain manganese was determined by flameless atomic absorption spectrophotometry (4).

Chromatographic procedure: Amino acids were analized by the procedure of Jones et al. (9) with minor modifications, as previously described (7). A Dupont model 8800 isocratic pump and column compartment was used in conjunction with a Schoeffel FS970 fluorometer, with excitation monochromator set at 330 nm and emission measured with a 418-nm cut-off filter. Buffers were isocratically switched by the use of a Mer Chromatographic 6 position all-Teflon motorized valve. Injections were made with a Rheodyne 7120 valve and a 20µl sample loop. Rainin Instrument Company Microsorb ODS-C18 column (4.6 x 250 mm; 5 µm particle size) fitted with an Upchurch guard column packed with Vydac ODS packing was used. A Perkin-Elmer model 3600 Data Station was used for data acquisition and quantitation by peak heights; 0.05 M sodium acetate buffer, pH 6.05, was used mixed with various quantities of methanol and tetrahydrofuran and used in a five-step isocratic buffer system.

High-purity amino acids, mercaptoethanol, and orthophtaldialdehyde (OPA) were obtained from Sigma Chemical Company, St Louis, Missouri. A standard mixture of amino acids excluding asparagine

and glutamine was made in 0.1N HCl such that each amino acid was 2.0 mM. Ornithine and lysine were, however, 5.0 mM in concentration. A separate 2.0 mM solution in water of a mixture of asparagine and glutamine was prepared. The two stock solutions were frozen at -20 °C and a working standard was prepared by diluting together 500 µl of each solution to 25 ml with 0.1N HCl.

Brain samples were homogenized in 100 volumens of 0.1N $HClO_4$/g wet weight by ultrasonic degradation and centrifuged at 25000 g during 10 min in a Sorvall RC 5-B centrifuge. The OPA derivatizing reagent was prepared as described elsewhere (9), and after exactly 90 sec., 1.6 ml 0.05 M acetate buffer, pH 4.8, were added and 20 µl of the resulting solution were immediately injected.

Statistical analysis: Statistical analysis of data was done by a two-way Analysis of Variance (15). After significant effects due to treatment were determined by ANOVA, the Student's t-test was used to determine the means that were significantly different from each other. P values lower than 0.05 were considered significant.

RESULTS

The growth rate of manganese intoxicated mice was normal (5). The mean ± S.E. daily water intake during the nine weeks for each mouse was: 7.99 ± 1.32 ml for controls, and 7.35 ± 1.68 for the Mn-treated. The difference was not statistically significant ($p > 0.05$). Manganese concentrations increased significantly in the two brain regions studied. The mean ± S.E. of the Mn content (µg/g dry weight) at the ninth week were: a) striatum: 2.65± 0.71 in controls and 8.33 ± 1.09 in Mn-treated mice; b) olfactory bulb: 2.50 ± 0.56 and 9.38 ± 1.45, respectively.

As shown in Table I no alterations were observed in the levels of free amino acids in the striatum of Mn-treated mice. However, in the olfactory bulb the contents of alanine, α-amino-n-butyrate, arginine, asparagine, aspartate, citrulline, GABA, glutamine, glutamate, glycine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tyrosine, and valine were significantly diminished.

Although the range varied from 31.0% (GABA) to 68.9% (α-amino-n-butyric) the largest decrease in concentrations was observed in five neutral (α-amino-n-butyric, glycine, serine, threonine, and valine) and two basic amino acids (arginine and citrulline). However, no difference was detected between these two groups. In general, essential and nonessential amino acids diminished to the same degree.

DISCUSSION

The rate-limiting step in the movement of amino acids from

TABLE I

FREE AMINO ACIDS IN THE BRAIN OF MANGANESE TREATED MICE

	Striatum		Olfactory Bulb	
Amino Acid	Control	Mn-treated	Control	Mn-treated
Alanine	1355.8 ± 85.0	1344.4 ± 45.2	1844.8 ± 92.6	898.4 ± 92.0*
α-amino-n-butyric	142.7 ± 18.9	130.2 ± 4.2	277.6 ± 51.9	86.4 ± 14.0*
Arginine	476.6 ± 46.1	446.6 ± 48.3	586.5 ± 80.1	197.4 ± 22.2*
Asparagine	238.8 ± 25.7	303.0 ± 27.9	136.2 ± 17.6	66.6 ± 4.0*
Aspartate	7107.2 ± 387.4	6394.3 ± 476.8	5464.5 ± 253.7	2603.9 ± 333.7*
Citruline	298.7 ± 14.9	262.0 ± 23.7	287.7 ± 47.5	94.2 ± 10.5*
GABA	1546.8 ± 111.3	1518.0 ± 84.0	2340.3 ± 105.8	1614.3 ± 226.2*
Glutamine	4781.6 ± 208.9	5294.0 ± 425.1	4828.9 ± 207.4	2216.6 ± 181.1*
Glutamate	8179.0 ± 131.4	7945.2 ± 259.8	3605.7 ± 223.0	2191.3 ± 257.2*
Glycine	615.3 ± 31.3	710.4 ± 67.5	3430.6 ± 484.6	1369.0 ± 157.7*
Isoleucine	201.8 ± 16.0	236.8 ± 33.1	149.1 ± 22.7	84.1 ± 10.7*
Leucine	474.3 ± 46.2	509.8 ± 25.1	276.7 ± 44.4	153.4 ± 10.2*
Lysine	158.4 ± 25.4	147.3 ± 29.5	70.9 ± 18.3	45.6 ± 5.1
Methionine	138.4 ± 25.9	170.4 ± 18.1	126.5 ± 21.6	63.4 ± 7.1*
Ornithine	287.3 ± 79.0	275.6 ± 43.7	173.9 ± 45.2	109.0 ± 20.9
Phenylalanine	180.1 ± 18.3	201.6 ± 26.8	155.4 ± 23.3	86.5 ± 10.4*
Serine	2315.5 ± 216.6	2386.4 ± 196.4	1361.6 ± 174.8	527.1 ± 59.7*
Taurine	8630.6 ± 682.7	8297.9 ± 516.7	3066.0 ± 263.6	3162.2± 169.7
Threonine	731.4 ± 52.9	657.6 ± 73.3	541.6 ± 74.9	198.8 ± 23.1*
Tryptophan	312.6 ± 33.0	376.4 ± 41.1	97.9 ± 26.8	94.1 ± 11.4
Tyrosine	323.2 ± 18.6	335.0 ± 17.2	244.8 ± 38.1	102.9 ± 6.2*
Valine	198.7 ± 11.8	194.0 ± 13.7	270.4 ± 31.9	96.6 ± 11.7*

* Significantly different from respective controls ($p < 0.05$).

Values represent mean ± S.E.M. from 11 mice and are expressed as n mol/g/wet weight.

plasma to brain intracellular fluid is the transport across the blood-brain barrier (13,14). The amino acids tryptophan, tyrosine, threonine, leucine, isoleucine, valine, phenylalanine, and methionine freely cross the capillary endothelia that comprise the blood-brain barrier. Small neutral amino acids, such as alanine, glycine, and GABA are restricted in their entry into the brain (14).

Alterations in the rate of movement of the amino acids into the brain of manganese treated mice is an unlikely event since similar changes should have been observed in both brain regions. The decrease of amino acid levels in the olfactory bulb could be due to histopathological changes or to a non-specific insult such as edema produced by the manganese administration in this region.

The present results contrast with the increase in GABA content we have previously reported in rat caudate nucleus after 2 months of manganese intake (10 mg $MnCl_2$/ml water) (3). In that previous work we could not demonstrate any alteration in the enzymes responsible for the synthesis and degradation of GABA. That finding was considered to be due to a decrease in GABA release from GABAergic neurons. In the present study, the absence of changes on the GABA content in the striatum of mice could be due to a lack of effect of the high manganese tissue levels (314% of control values) on striatal GABAergic neurons. It is possible that the latter neurons are as resistant to the toxic effects of manganese as the cholinergic neurons (10). The 31% decrease of GABA levels in the olfactory bulb could be due to an augmented sensitivity to manganese of a different subpopulation of GABAergic neurons. On the other hand, alterations produced in other neuronal types could indirectly alter the activity of the GABAergic neurons located in the olfactory bulb.

The highly significant changes observed in the levels of several free amino acids in olfactory bulb is a finding that merits a thorough evaluation in order to determine its importance on the pathophysiology of manganese poisoning. In miners the first clinical manifestations of manganese intoxication appear as psychiatric symptoms ("manganese madness") characterized by aggressive behavior, irritability, speech disturbance, compulsive actions, and hallucinations.

The human olfactory bulb lies on the cribriform plates. The olfactory primary axons, coming from the olfactory receptor cells in the nasal fossae, form the external layer of the bulb. They synapse with dendrites of mitral cells and tufted cells in the subjacent glomerular layer. The secondary axons originate from mitral and tufted cells and pass posteriorly as the olfactory stalk, enter the base of the hemisphere, and divide into a medial, intermedial, and lateral olfactory stria. The latter interconnects with the corticomedial neuronal group of the amygdala (8).

The projections of the lateral olfactory stria to the amygdala make easily available to the limbic structures of the emotional brain any information coming from the olfactory bulb. Therefore, if the latter represents a primary target for manganese during the early stages of intoxication its dysfunction could be responsible for some of the initial psychiatric symptoms.

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RESUMEN

Toxicidad por manganeso: Aminoácidos libres en el estriado y bulbo olfatorio del ratón. Bonilla, E. (Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad del Zulia, Apartado 1151, Maracaibo, Venezuela), Arrieta, A.; Castro, F.; Dávila, J.O.; Quiroz, I. *Invest Clin 35(4): 175-181, 1994.*

Palabras claves: intoxicación por manganeso, aminoácidos, bulbo olfatorio, GABA, estriado.

Determinamos las concentraciones de veintidós aminoácidos libres en el estriado y bulbo olfatorio de ratones tratados durante nueve semanas con inyecciones intraperitoneales diarias de cloruro de manganeso (5.0 mg Mn^{2+}/kg de peso corporal). En el bulbo olfatorio se observó una disminución significativa del contenido de alanina, α - amino-n-butirato, arginina, asparagina, aspartato, citrulina, GABA, glutamato, glicina, isoleucina, leucina, metionina, fenilalanina, serina, treonina, tirosina y valina. En el estriado no se produjeron alteraciones en las concentraciones de los aminoácidos libres en los ratones tratados con manganeso. Los cambios observados en el bulbo olfatorio deben ser evaluados para determinar su importancia en la fisiopatología de la intoxicación con manganeso.

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