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FREE AMINO ACIDS IN PLASMA AND BRAIN AFTER CHRONIC MANGANESE INTAKE IN RATS

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SUMMARY

We studied the levels of several 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 difference could be demonstrated in any of the amino acids analyzed.

INTRODUCTION

Chronic administration of manganese has been shown to produce significant decreases in the endogenous levels of some biogenic amines and their metabolites in different brain regions. We have found that after 7 months of feeding 5 mg $MnCl_2$/ml drinking water a decrease in the concentrations of dopamine and homovanillic acid in whole cerebral hemispheres was produced (2). In rats treated with $MnCl_2$ intraperitoneally for a period of 4 months a decrease in dopamine levels and turnover was produced in the striatum (1).

Dopamine is a neurotransmitter whose precursor is the dietary amino acid tyrosine. But several other amino acids have also been proposed as neurotransmitters: histidine, glycine, taurine, aspartic acid and glutamic acid. The present study was conducted to determine the changes, if any, in the concentrations of free amino acids, including those putative neurotransmitters, in plasma and brain after chronic manganese intake.

MATERIAL AND METHODS

Experiments were carried out on male Sprague-Dawley rats weighing 200-250 g and fed ad libitum with rat laboratory chow (Protinal-Maracaibo) containing 70 ug Mn/g dry weight. One mg of Mn as $MnCl_2.4H_2O$ was added per ml of drinking water

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to the treated group. The control group received manganese-free water. Both groups had unrestrained water access and the quantity of water ingested by each rat was daily measured. At eighth month, 11 animals of each group were sacrificed by decapitation. The brains were extracted immediately and placed at 4°C to dissect the striatum and frontal cortex. Prior to the sacrifice a blood sample was obtained from each rat by cardiac puncture. Plasma was separated and stored at –80°C until analyzed.

The manganese concentration in brain regions was measured by flameless atomic absorption spectrophotometry (3).

A Dupont model 8800 isocratic pump and column compartment was used in conjunction with a Schoeffel FS 970 fluorometer, with excitation monocromator set at 330 nm and emission measured with a 418 nm cut off filter. The fluorometer sensitivity setting was 590 and that of range 1.0 uA. Buffers were isocratically switched by the use of a Mer Chromatographic 6 position all teflon motorized valve. Injections were performed with a Rheodyne 7120 valve and a 20 ul sample loop. Rainin Instrument Company Microsorb ODS-C18 column (46 x 250 mm; 5u 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. The procedure followed is a slight modification of that of Jones et al (4).

High purity amino acids, mercaptoethanol and orthophthaldialdehyde (OPA) were obtained from Sigma Chemical Co., USA. A standard mixture of amino acids excluding glutamine were made in 0.1 N HCl such that each amino acid was 2.0 mM. Lysine was however 5.0 mM in concentration. A separate 2 mM solution in water of glutamine was prepared. The two stock solutions were frozen at –20°C and a working standard was prepared by diluting together 500 ul of each solution to 25 ml with 0.1 N HCl.

Tissues were homogenized in 100 vols of 0.1 N HCl/g wet weight by ultrasonic degradation and centrifuged at 25000 x g in a Sorvall RC 5-B centrifuge. The supernatants were frozen at –80°C till they were analyzed.

The OPA derivatizing was prepared as previously described (4). 200 ul of sample or standard solution was mixed with 200 ul of the OPA reagent and exactly after 90 seconds 1.6 ml of 0.05 M acetate buffer pH 4.8 was added and 20 ul of the resulting solution was immediately injected.

Statistical analysis of data was done by a two-way Analysis of Variance performed with region and treatment as the two main factors (9). The Student's t-test was used to determine the means that were significantly different from each other. p-values less than 0.05 were considered significant.

RESULTS

The growth rate of manganese intoxicated rats was normal. Brain manganese concentration increased significantly. The mean ± S.E. of the manganese content (expressed in ug/g dry weight) at the eight month were: a) frontal cortex: 1.7 ± 0.3 in controls and 5.1 ± 0.4 in Mn-loaded rats; b) striatum: 1.8 ± 0.1 and 3.2 ± 0.2.

The mean ± S.E. daily water intake during the eighth month for each rat was: 60.1 ± 0.7 ml for controls, and 54.0 ± 0.5 for the Mn-treated rats. The difference was significant ($P < 0.01$) (5).

As shown in Table I no changes were obtained in the levels of plasma amino acids in manganese treated rats. Similar results were observed in the striatum and frontal cortex (Table II).

TABLE I

FREE AMINO ACIDS IN PLASMA OF MANGANESE TREATED RATS

Amino acid	Control	Mn-treated
Alanine	201.8 ± 3.4*	199.0 ± 4.4
Argine	162.1 ± 19.4	130.2 ± 16.3
Aspartate	47.2 ± 12.0	28.5 ± 6.2
Glutamate	159.0 ± 13.4	139.1 ± 14.4
Glutamine	366.5 ± 5.6	365.2 ± 3.7
Glycine	366.3 ± 5.8	337.0 ± 15.4
Isoleucine	101.0 ± 1.8	99.8 ± 1.0
Leucine	115.0 ± 2.1	114.5 ± 0.8
Lysine	224.1 ± 30.2	211.5 ± 15.6
Methionine	59.5 ± 5.5	52.0 ± 2.7
Phenylalanine	76.1 ± 7.9	70.0 ± 4.7
Serine	535.8 ± 58.8	458.6 ± 51.1
Taurine	142.5 ± 2.9	139.5 ± 3.1
Threonine	367.9 ± 35.5	351.7 ± 30.8
Tryptophan	86.3 ± 6.6	86.0 ± 4.8
Tyrosine	87.9 ± 4.4	91.3 ± 4.6
Valine	159.7 ± 5.2	162.8 ± 2.3
Citruline	75.2 ± 7.0	68.6 ± 3.4

* Values represent the means and SE from 11 rats and are expressed as nmol/ml of plasma.

DISCUSSION

The rate-limiting step in the movement of amino acids from plasma to brain intracellular fluid is the transport across the blood-brain barrier (8). There are three trans-

port systems for amino acids at the cerebral capillaries: one for large neutral amino acids, one for basic amino acids, and one for acidic amino acids (7).

TABLE II

FREE AMINO ACIDS IN THE BRAIN OF MANGANESE TREATED RATS

	Striatum		Frontal Cortex	
Amino acid	Control	Mn-treated	Control	Mn-treated
Alanine	657.7 ± 56.2*	704.7 ± 52.8	686.8 ± 46.5	615.3 ± 58.2
Arginine	175.4 ± 30.5	172.4 ± 28.2	158.2 ± 7.8	138.0 ± 11.2
Aspartate	1576.4 ± 272.4	1604.7 ± 226.5	3292.1 ± 302.2	3201.8 ± 391.3
Glutamate	5405.7 ± 251.5	4682.3 ± 390.1	4606.7 ± 347.6	4955.5 ± 508.1
Glutamine	4285.0 ± 355.5	3893.2 ± 415.4	1787.0 ± 135.8	1876.8 ± 293.2
Glycine	1604.8 ± 133.9	1699.3 ± 173.7	1555.3 ± 104.4	1720.5 ± 339.9
Isoleucine	85.5 ± 13.8	90.1 ± 11.2	50.2 ± 3.4	53.9 ± 14.3
Leucine	151.3 ± 22.6	173.2 ± 20.9	114.8 ± 3.8	134.6 ± 43.3
Lysine	149.4 ± 20.3	160.6 ± 16.9	192.3 ± 23.9	287.5 ± 101.5
Methionine	87.5 ± 16.2	74.1 ± 6.4	50.3 ± 1.7	56.5 ± 17.7
Phenylalanine	106.1 ± 14.0	111.1 ± 14.7	59.7 ± 2.5	72.5 ± 24.3
Serine	627.0 ± 128.5	484.6 ± 66.5	687.5 ± 29.0	653.8 ± 63.2
Taurine	6431.1 ± 147.5	6039.1 ± 359.0	4331.0 ± 536.8	4672.6 ± 868.9
Threonine	283.5 ± 67.4	242.0 ± 48.0	190.4 ± 3.6	220.5 ± 42.1
Tryptophan	157.0 ± 15.4	167.3 ± 23.0	17.0 ± 0.0	19.5 ± 5.2
Tyrosine	63.2 ± 7.9	70.3 ± 15.9	73.7 ± 2.1	73.6 ± 13.0
Valine	153.4 ± 22.6	162.2 ± 19.4	72.5 ± 4.4	79.7 ± 20.6

* Values represent the means and SE from 11 rats and are expressed as nmol/g wet weight.

The amino acids tryptophan, tyrosine, threonine, leucine, valine, isoleucine, phenylalanine and methionine freely cross the capillary endothelia that comprise the blood-brain barrier (8). The entry of circulating trytophan or tyrosine into the brain can be accelerated either by raising plasma tryptophan or tyrosine levels or by lowering plasma levels of other large neutral amino acids (11). Apparently, the total amount of tryptophan in plasma (albumin-bound plus free) determines the amount of the amino acid that is potentially accesible to the brain. Tyrosine is abundant in dietary proteins, and can also be obtained from the phenylalanine contained in protein. At physiologic concentrations of the large neutral amino acids, at least half the tyrosine that passes from the extracellular fluid of the brain to nerve terminals does so via low-affinity mechanism (6). However, although tyrosine is the precursor of catecholamines, the limitation on the synthesis of these neurotransmitters is not set by the concentration of tyrosine, but by the amount of reduced tyrosine hydroxylase produced by pteridine (10). Since in the present work no changes were observed in the levels of the plasma and brain amino acids it is safe to assume that the chronic manganese intake of 1mg Mn/ml water did not affect either the gastrointestinal absorption of the amino acids nor their passage through the blood-brain barrier.

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RESUMEN

Aminoacidos libres en plasma y cerebro de ratas después de la ingestión crónica de manganeso. *Bonilla E. (Instituto de Investigaciones Clínicas. Universidad del Zulia e INBIOMED-FUNDACITE. Maracaibo, Venezuela), Prasad A.L.N., Dávila J.O., Arrieta A., Villalobos R.* **Invest Clín** *29(2): 55-60, 1988.*– El presente trabajo fue realizado con el objeto de determinar la concentración de varios aminoácidos libres en el plasma sanguíneo, el estriado y la corteza frontal de ratas adultas machos tratadas durante 8 meses con cloruro de manganeso agregado al agua de bebida. No se pudo demostrar ninguna diferencia significativa en el contenido de los aminoácidos libres, en las regiones señaladas, después de la ingestión crónica de manganeso.

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