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ALTERATION OF CEREBRAL NEUROTRANSMITTER RECEPTOR FUNCTION BY EXPOSURE OF RATS TO MANGANESE

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SUMMARY

Adult male rats were treated on 15 successive days with i.p. injection of manganese chloride at 10 or 15 mg/kg body weight. 24 h after the last dose, the binding of the dopaminergic antagonist [^{3}H]spiroperidol to striatal membranes was elevated significantly. At the lower dose, no other high-affinity binding site measured was altered. However, at the 15 mg/kg body weight dose, cerebellar GABA, frontal cortical serotonin, and striatal muscarinic binding were all depressed. Neither dose level altered striatal levels of enkephalin, substance P, dopamine, serotonin, or dihydroxyphenylacetic acid (DOPAC). Receptor binding measurements may be a sensitive means of detecting disturbances of specific neural circuits.

INTRODUCTION

Excessive exposure to manganese (Mn) during mining and processing operations [10, 21] and production of ferromanganese alloy [31, 32] may lead to chronic disabling disease. The clinical symptoms resemble Parkinsonism [11, 12, 21] although other behavioral disturbances also occur [24]. Decrease of dopamine levels in the brain of experimental animals [11, 22, 23] and improvement of some neurological symptoms in Mn poisoning cases following high doses of L-DOPA, the natural precursur of dopamine, suggests an effect of the metal on synaptic function of dopamine [8, 25]. Decreases in brain norepinephrine [22] and serotonin [19] indicates the involvement of other monoamines as well.

In the present study an attempt has been made to see whether Mn exerts its neurotoxic effects mainly by affecting the dopamine system. A survey of high-

Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; QNB, DL-[benzilic-4,4′-^{3}H]quinuclidinyl benzilate.

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affinity binding sites of various neurotransmitters was used as an objective means of detecting damage to a specific neuronal circuit [4]. Since the corpus striatum appears to be the chief site of injury, we have estimated the levels of dopamine and serotonin and their metabolites in this brain region. Levels of [met^5]enkephalin and substance P, which may interact with the nigro-striatal dopamine system [15, 16] were also measured to see if Mn has any effect on neuroactive peptides.

METHODS

(a) Manganese administration

Male Fischer rats 6–7 weeks old were injected i.p. daily for 15 days with distilled water or manganese chloride ($MnCl_2$) solution at 2 levels (10 and 15 mg/kg body weight). This dose range has previously been found to cause enzymic and histological changes in rodent brain [27, 30]. 24 h after the last injection rats were decapitated and brain regions dissected out [17] and frozen at $-20°C$.

(b) High-affinity binding assay

A crude membrane fraction was prepared from brain regions by homogenization of tissue in 19 vols. of 0.32 M sucrose followed by centrifugation ($50\,000 \times g$, 10 min). The precipitate was homogenized in distilled water pH 7.4 and recentrifuged. The final pellet was suspended in 40 mM Tris–HCl pH 7.4 buffer at a concentration corresponding to 50 mg original tissue/ml.

Binding incubations were carried out in triplicate in 1 ml of medium containing 40 mM Tris–HCl pH 7.4 and appropriate labeled and unlabeled pharmacological agents. The incubation mixture used in the assay of serotonin included 10^{-5}M pargyline, $4 \cdot 10^{-3}$M $CaCl_2$ and $5.7 \cdot 10^{-3}$M ascorbic acid. The amount of tissue used per tube corresponded to 5–10 mg original wet weight and contained 300–400 μg protein as determined by the method of Lowry et al. [20]. At the end of a 15 min incubation at 37°C samples were filtered on glass fiber discs (25 mm diameter, 0.3 μ pore size, Gelman Inc., Ann Arbor, MI) and washed twice rapidly with 5 ml Tris buffer. Filter discs were then dried and counted in 5 ml of a scintillation mixture using a scintillation counter at an efficiency of 38–43%. Control incubations were carried out simultaneously containing no unlabeled competing ligand in order to determine the extent of non-specific binding. The final concentration of unlabeled competing compounds in control incubations was 10^{-6}M. The assay of the dopamine receptor was performed using 10^{-9}M [1-phenyl-4 ^{3}H]spiroperidol (23 Ci/mmol) as the binding ligand and haloperidol as the competing compound in control tubes. In a parallel manner 10^{-9}M QNB (29 Ci/mmol) was used to measure muscarinic sites with atropine as a competitor. Benzodiazepine sites were estimated with $0.7 \cdot 10^{-9}$M [methyl-^{3}H]diazepam (73 Ci/mmol) and the unlabeled compound

as a competitor. $8.0 \cdot 10^{-9}$M [methylene-^{3}H(N)]muscimol (7.3 Ci/mmol) and unlabeled GABA were used in GABA-binding site assays. $3.1 \cdot 10^{-9}$M [1,2-^{3}H(N)]serotonin (30 Ci/mmol) was used together with unlabeled serotonin in the assay of this receptor. $6 \cdot 10^{-9}$M [G-^{3}H]strychnine sulphate (13 Ci/mmol) and 10^{-5}M unlabeled strychnine were used to assay the glycine receptor. The method used was essentially similar to other filtration binding methods. However, we felt it necessary to establish basic binding characteristics prior to studies on animals treated with manganese. These included delineation of saturability, specificity, reversibility, and regional distribution [4].

(c) Assay of monoamine content

The tissue concentrations of dopamine, DOPAC, HVA, 5-HT, and 5-HIAA were determined by HPLC with electrochemical detection [18]. Tissues were homogenized in 250 μl of the mobile phase containing 20 ng of the internal standard (*N*-methyl-5-HT) and following centrifugation an aliquot of the resulting supernatant was injected into the LC. The striatal concentrations of the compounds were calculated from the ratio of their peak height relative to the internal standard using the slope and intercept of a standard curve.

(d) Radioimmunoassay of neuropeptides

Tissue was homogenized in 2N acetic acid, boiled for 5 min and centrifuged at $25\,000 \times g$ for 20 min. The supernatant was lyophilized and the residue was reconstituted with H_2O and radioimmunoassayed for met-enkephalin and substance P using [tyrosyl-3,5-^{3}H]met-enkephalin (36 Ci/mmol) and ^{125}I-substance P (original specific activity 65 μCi/μg). Antisera were raised in rabbits using polylysine conjugates. Six injections were given at 2-week intervals before the animals were bled. The assay was carried out in polypropylene tubes. Nonlabeled peptide or brain extract was incubated with antiserum and [^{3}H]met-enkephalin or ^{125}I-substance P in 0.5 ml of 0.2 M Tris buffer, pH 7.4, containing 0.1% albumin and 0.06% dextran. The incubation was carried out at 4°C for 15–24 h. The [^{3}H]met-enkephalin (or ^{125}I-substance P) bound to antibody was separated from the free [^{3}H]met-enkephalin (or ^{125}I-substance P) by adding 0.2 ml of 1.5% charcoal slurry containing 0.15% dextran (suspended in 0.2 M Tris buffer, pH 7.4) and aliquot of supernatant fluid was counted in a liquid scintillation spectrometer. The validation and specificity of this method have been described in detail [14, 16].

(e) Statistics

Differences between groups were assessed using Fisher's Least Significant Difference Test after a one-way analysis of variance. The accepted level of

significance in all cases was $P < 0.05$ using a two-tailed test. Each point represents values derived from 7–8 individual animals.

RESULTS AND DISCUSSION

At the lower dose of Mn (10 mg/kg body weight, daily for 15 days), only one of the six labeled ligand-binding reactions was significantly different from that of the corresponding control value. Spiroperidol binding to striatal membranes was 61% higher in treated animals (Table I). At the higher dose of Mn (15 mg/kg body weight), striatal dopaminergic binding activity was still the only elevated parameter detected. However, striatal muscarinic binding was depressed below control values as was cerebellar GABA and cortical serotonin binding. The cerebral protein content of rats chronically exposed to manganese has been shown to remain unaffected [29].

Striatal levels of [met^5]enkephalin and substance P were not significantly affected by the Mn treatment and were within 10% and 3% of control values respectively (Table II). The striatal content of dopamine, DOPAC, HVA, and serotonin were also unaltered by treatment. However, the level of 5-HIAA was significantly depressed to 75% of the control value in animals treated with 15 successive doses of Mn at 15 mg/kg (Table III).

The binding data suggest that assay of receptor–ligand interactions may be an especially sensitive means of detection of a derangement of a specific neurotransmitter system. A large increase of spiroperidol binding was seen in Mn-treated animals showing unaltered striatal levels of dopamine and its metabolic product, DOPAC and HVA. No changes in striatal neuropeptides thought to be associated with dopaminergic systems [15, 16] were found in rats exposed to Mn.

TABLE I

EFFECT OF MANGANESE TREATMENT ON HIGH AFFINITY BINDING LEVELS IN SEVERAL BRAIN REGIONS

Data expressed as pmol ligand bound/g protein. Means derived from 7 individual animals per group, together with the standard error, are presented. Daily dosing for 15 days.

		Manganese dose (daily, mg/kg)		
Region	Receptor	0	10	15
Striatum	Dopamine	90 ± 5	145 ± 13[a]	122 ± 11[a]
	Muscarinic	1057 ± 65	1162 ± 39	811 ± 56[a]
Frontal cortex	Benzodiazepine	23 ± 1	23 ± 3	26 ± 3
	Serotonin	73 ± 7	62 ± 4	48 ± 5
	Glycine	40 ± 4	46 ± 3	45 ± 2
Cerebellum	GABA	63 ± 6	52 ± 4	46 ± 2[a]

[a]Differs from zero-dose ($P < 0.05$, Fisher's Least Significant Difference Test).

TABLE II

STRIATAL LEVELS OF ENKEPHALIN AND SUBSTANCE P AFTER MANGANESE TREATMENT

Means derived from 8 individual animals per group are given together with the standard error. Daily dosing for 15 days.

	Peptide level (ng/mg protein)	
Manganese dose (mg/kg, daily)	[met^5]enkephalin	Substance P
0	19.0 ± 0.8	2.22 ± 0.05
10	20.6 ± 0.6	2.27 ± 0.06
15	20.6 ± 1.0	2.23 ± 0.10

TABLE III

STRIATAL CONCENTRATIONS OF DOPAMINE, SEROTONIN, AND THEIR METABOLITES IN MANGANESE-TREATED RATS

Data are expressed as ng/mg protein; daily dosing for 15 days. Means and standard errors derived from data of 8 individual rats per group.

	Manganese dose (daily, mg/kg)		
	0	10	15
Dopamine	90 ± 2	99 ± 4	92 ± 3
DOPAC	16.8 ± 0.7	18.1 ± 0.9	17.1 ± 0.6
HVA	7.9 ± 0.3	8.6 ± 0.5	7.1 ± 0.3
Serotonin	3.4 ± 0.2	3.4 ± 0.2	3.2 ± 0.2
5-HIAA	5.5 ± 0.2	4.9 ± 0.4	4.1[a] ± 0.2

[a]Differs from corresponding control ($P < 0.05$, Fisher's Least Significant Difference Test).

There is evidence that rats are relatively resistant to Mn, as evaluated by alterations in levels of dopamine, serotonin, and their metabolites [28]. Chronic exposure lasting many months may alter dopamine levels [8].

The depression of serotonin and GABA receptor activity seen at the higher (15 mg/kg) Mn dose may represent a less specific toxic effect of this metal. However, Mn has been shown to increase striatal GABA in rats after chronic administration [7] and cerebral serotonin levels are depressed in rats fed a diet containing 2 mg Mn chow [19]. The depressed level of the serotonin metabolite 5-HIAA in animals given the repeated 15 mg/kg Mn dose, also suggests serotoninergic involvement. Since dopaminergic input to the cerebellum is minimal, the decreased GABA receptor binding activity observed in this region in animals treated at the higher Mn level, is unlikely to be a secondary response mediated directly by dopamine. However, in view of the complex interdependence of all neurotransmitter circuits, it may be that alteration in dopaminergic mechanisms underlies many other observed changes. The heightened level of striatal spiroperidol binding suggests a disuse supersensitivity-

like response although we have not been able to detect major changes in dopamine metabolism. Changes may be more readily detected in receptors, largely confined to the synapse, than in the concentrations of neurotransmitters or their metabolites which reflect the state of the entire neuron [4]. Mn treatment has been shown to modify the activity of tyrosine hydroxylase activity in striatum which has may be related to the blockade of dopaminergic receptors [6]. The increase in the [^{3}H]spiroperidol binding to striatum of Mn-exposed animals is consistent with this observation.

300 mg $MnCl_2$ given daily to Sprague–Dawley male rats for 30 days has been reported to increase striatal Mn to approx. $0.5 \cdot 10^{-6}$M [6]. Our dosing was for a shorter period at 1/10 the dose used in the above work. Since 10^{-6}M $MnCl_2$ in vitro had no effect upon striatal spiroperidol binding (P.K. Seth, unpublished result), the effect of $MnCl_2$ dosing upon the striatal dopamine receptor in vivo, reported here is indirect.

No gross behavioral deficits, e.g. tremor, were observed in the treated rats. An earlier study involving 10× the dose level of Mn for up to 8 months also failed to detect any sign of neurological disease [6]. The use of an appropriate pharmacological challenge such as altered responses to apomorphine might demonstrate abnormality of the dopamine system in Mn-treated animals before the onset of overt behavioral changes.

The effect of gestational and neonatal exposure of rat pups to Mn on neurotransmitter receptors is important since very early exposure to Mn has been reported to increase striatal catecholamine levels, an opposite effect to that found in the adult [9]. Pre- and postnatal effects on neurotransmitter-related phenomena are frequently opposite to those found in the adult. For example, prenatal exposure of rats to acrylamide depresses striatal dopaminergic receptors [2]; the opposite effect is found after acrylamide treatment of adult rats [1, 3, 5]. A parallel situation is found with chlordecone treatment except that neonatal exposure elevates striatal dopaminergic receptor binding which is depressed following adult exposure [26]. The striatal dopamine receptor may often be more sensitive to neurotoxicant-induced disturbances than are a variety of other transmitter receptor sites. This may account for the relatively common occurrence of tremor and other Parkinsonian symptoms found as a result of exposure to a variety of agents damaging to the CNS.

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