

Accumulation of Manganese in Rat Brain Following Intranasal Administration

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Manganese chloride (50–800 μg) was injected unilaterally into the right nostril of rats and its accumulation in the central nervous system (CNS) was monitored. Brain manganese levels were elevated in a dose-dependent, time-dependent, and tissue-dependent manner. Elevated levels of manganese were detected in the right olfactory bulb and olfactory tubercle within 12 hr after instillation and remained elevated for at least 3 days. As little as 100 μg of manganese chloride was sufficient to increase brain manganese levels. No changes were detected on the left side of the brain. The manganese content of the striatum, the target site for manganese neurotoxicity, was unchanged following acute administration, but was elevated when two injections were made 1 week apart. These results suggest that air-borne manganese can be retrogradely transported along olfactory neurons to the CNS and can reach deeper brain structures under appropriate exposure conditions. © 1997 Society of Toxicology.

Many heavy metals are toxic to the CNS, producing a wide variety of neurotoxic effects (LeQuesne, 1992). However, the transport of metals into the CNS is poorly documented. Normally, substances must cross the blood–brain barrier in order to have access to the CNS, but recent evidence indicates that the choroid plexus is able to sequester heavy metals such as cadmium, lead, and mercury and act as a sink to protect the brain from accumulating metals (Zheng *et al.*, 1991).

An alternative route of entry into the CNS has been proposed for air-borne metals, whereby the toxic agent may be taken up by olfactory neurons which arise in the nose and is then retrogradely transported into the CNS (Evans and Hastings, 1992). In support of this hypothesis, intranasally administered cadmium has been located in the olfactory bulb region (Hastings and Evans, 1991), while aluminum has been shown to reach olfactory regions and the cortex and hippocampus when given in very high doses (Perl and Good, 1987). However, the significance of CNS toxicity produced by both cadmium and aluminum is not well established and

comparisons with other routes of administration are lacking. As a result, the significance of the olfactory route as a portal of entry into the CNS is not understood. We chose to further investigate this phenomenon by examining the time course and dose dependency of the CNS uptake of intranasally administered manganese. Manganese was chosen since its toxicity is well documented and is characterized by a slowly progressive deterioration of motor function, with pathological changes primarily localized to deeper CNS structures in the extrapyramidal motor system (Mena *et al.*, 1967; Cotzias *et al.*, 1974). The primary manifestations of manganism are altered gait, limb movements, and tremor (Mena *et al.*, 1967), occurring after months or years of exposure (Rodier *et al.*, 1955). Inhalation of manganese dusts leads to a depletion of the neurotransmitter dopamine in the extrapyramidal brain region of exposed monkeys, and this is accompanied by an increase in brain manganese content (Bird *et al.*, 1984). Thus, the goal of this study was not only to determine whether intranasally administered manganese reaches olfactory regions of the brain, as cadmium and aluminum do, but also to determine if it penetrates to the target site where it produces toxicity.

MATERIALS AND METHODS

Administration of manganese. Adult male rats (250–300 g, Sprague–Dawley obtained from Charles River Farms, Wilmington, MA) were obtained and housed isolated from other species for at least 1 week prior to use. The rats were lightly anesthetized with pentobarbital (40–50 mg/kg, ip) and temporarily restrained in a nose-only inhalation tube and received intranasal injections of manganese essentially as described by St. Clair *et al.* (1990). Manganese chloride (Sigma Chemical, St. Louis, MO) was dissolved in distilled water and 40 μL of the solution was injected through polyethylene tubing attached to a syringe. The tubing was inserted approximately 2.0–2.5 cm into the nasal cavity. The solution reached the olfactory region by gravity. Injections were made unilaterally into the right nostril in a dose range of 50–800 μg manganese chloride/rat (equivalent to 12.5–200 μg manganese/rat). The left side was left uninjected and was used for comparison. Control rats received equal volumes of saline. At experimentally determined times, the rats were decapitated and their brains removed and the olfactory bulb, olfactory tubercle, and striatum were dissected out. In some experiments, trunk blood was collected in colorless polypropylene tubes containing ammonium heparin. The tissues were assayed for the content of manganese as described below.

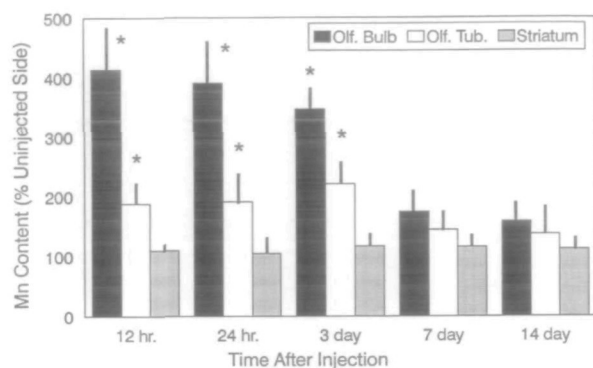


FIG. 1. Time course of manganese accumulation in brain following intranasal administration. Rats received an injection of manganese chloride (800 μg in 40 μL of saline) into the right nostril and the manganese content of the olfactory bulb (solid bar), olfactory tubercle (open bar), and striatum (shaded bar) was measured at various times after injection. The manganese content is expressed as the percentage found in the brain region obtained from the right side of the brain compared with the amount found on the left (control) side ($\pm\text{SE}$, $N = 5-8$, * denotes significantly different from control [100%] at $p < 0.05$).

Measurement of manganese. Brain concentrations of manganese were determined by atomic absorption spectrometry by the method of Paynter (1979) as previously modified (Gianutsos *et al.*, 1985; Clay and Morris, 1989). Briefly, brain tissue was digested in concentrated nitric acid in glass centrifuge tubes for 1 hr at 60°C and diluted 1:10 with distilled deionized water before analysis. A 0.4-ml aliquot of the diluted sample was mixed with 0.2 ml of a 5 mg/ml ultrapure magnesium nitrate solution (used as a matrix modifier) just prior to analysis, transferred to volumetric flasks, and diluted with distilled deionized water [final acid concentration of 10% (v/v)] to bring the analytical measurements into the linear range of operation of the instrument. Blood was diluted with Triton X-100 and 0.04 M HCl.

Analysis of the tissue samples was conducted using a Perkin-Elmer 360 atomic absorption spectrophotometer with an HGA 400 carbon furnace and fitted with a manganese detection lamp. A 20- μL aliquot is injected into the furnace and the furnace programmed as follows (numbers are temperature in °C, ramp in seconds, and hold time in seconds, respectively): dry 150, 15, 100; ash 1400, 10, 60; atomize 2400, 0, 5. The accuracy is tested by analyzing for manganese using a National Bureau of Standards bovine liver standard. The limit of detection in our hands is approximately 2 ppb.

The data were analyzed statistically by ANOVA followed by Dunnett's test with a level of significance of $p < 0.05$.

RESULTS

An intranasal injection of manganese chloride resulted in a time-dependent elevation of brain manganese levels as depicted in Fig. 1. Manganese determinations were made at various times after an injection of 800 μg of manganese chloride (equivalent to 200 μg of free manganese) into the right nostril. It is not known if the intranasal manganese injections induced damage to the nasal tissue. While future histopathological studies would be required to address this issue, St. Clair and co-workers (1990) have shown that the nasal instillation of saline produces no histopathological lesions in the nose. Manganese was maximally elevated (more

than four times the uninjected [left] side) in the olfactory bulb within 12 hr after exposure and remained elevated for at least 3 days before returning to control levels by Day 7. Circulating blood levels of manganese were not changed by this treatment (data not shown). The manganese content of the olfactory tubercle followed a similar time course, although the absolute change in manganese was somewhat less (approximately doubling). The levels of manganese were unchanged in the striatum by this treatment.

The increase in brain manganese content following intranasal administration was also dose-dependent, as shown in Table 1. Manganese was measured in the olfactory bulb and olfactory tubercle 24 hr after a unilateral injection of manganese chloride of 50–800 μg (equivalent to 12.5–200 μg manganese). As little as 100 μg manganese chloride (25 μg manganese) resulted in a significant increase in the manganese content of the olfactory bulb and the olfactory tubercle on the injected side when measured 24 hr after injection. This low dose produced increases in manganese content of 77 and 27% in the olfactory bulb and olfactory tubercle, respectively. Higher doses produced correspondingly larger increases. Unilateral saline injections failed to alter tissue manganese levels.

Acute injections failed to alter manganese content in the striatum, the site where it produces toxicity. Since manganese produces a slowly progressive toxicity (see introduction), the effect of repeated intranasal manganese chloride injections on brain manganese content was also tested. The results are illustrated in Fig. 2. Two injections of manganese chloride (800 μg) were made into the right nostril at weekly intervals and measurements were made 24 hr after the second injection (these time points were chosen since manganese is elevated in the brain 24 hr after injection, but had returned to control levels by 1 week in the acute study). As in the case of the acute injection, an increase in manganese content was observed in the olfactory bulb and olfactory tubercle. However, the repeated treatment also resulted in an increase in the manganese content of the striatum. A fourfold elevation was observed in the olfactory bulb, while a doubling occurred in the olfactory tubercle and striatum. Equivalent injections of saline vehicle did not alter manganese content in any brain region (data not shown). The increase due to the manganese injection was also restricted to the right side of the brain, corresponding to the side receiving the intranasal injection. No gross behavioral changes were observed in the treated rats.

DISCUSSION

Intranasal injections of manganese chloride produce a time- and dose-dependent increase in the manganese content of the brain. These results are in agreement with previous findings with cadmium (Hastings and Evans, 1991) and alu-

TABLE 1
Dose-Dependent Accumulation of Manganese in the CNS Following Intranasal Injection of Manganese Chloride

Dose of MnCl ₂ (μ g)	Manganese content (μ g/g \pm SE)			
	Olfactory bulb		Olfactory tubercle	
	Left side	Right side	Left side	Right side
0	0.48 \pm 0.07	0.49 \pm 0.06	0.51 \pm 0.09	0.52 \pm 0.06
50	0.45 \pm 0.04	0.54 \pm 0.04	0.49 \pm 0.03	0.47 \pm 0.03
100	0.40 \pm 0.02	0.71 \pm 0.10*	0.41 \pm 0.02	0.52 \pm 0.03*
200	0.44 \pm 0.03	1.44 \pm 0.17	0.46 \pm 0.04	0.79 \pm 0.10
400	0.47 \pm 0.02	1.54 \pm 0.48	0.49 \pm 0.04	0.82 \pm 0.10
800	0.55 \pm 0.02	2.13 \pm 0.35	0.59 \pm 0.10	1.05 \pm 0.22

Note. Rats received an injection of different doses of manganese chloride, in a total volume of 40 μ L, into the right nostril and brain levels of manganese were measured 24 hr later. Separate determinations were made in the brain regions on each side of the brain. $N = 6$. * refers to the lowest dose where manganese levels were significantly different from uninjected (left) side at $p < 0.05$.

minum (Perl and Good, 1987) which suggested that the nose may serve as a portal of entry of metals into the CNS.

Hastings and Evans (1991) proposed that the entry of cadmium into the olfactory bulb following intranasal injection was via a retrograde transport along the olfactory neuron. Our results are consistent with this hypothesis, since the appearance of manganese in the brain was restricted to the right side of the brain, corresponding to the nostril receiving the manganese injection. A mechanism based upon absorption and transport through the systemic circulation is

inconsistent with this pattern of unilateral distribution into the CNS. In addition, blood levels of manganese are unaltered by this treatment; this is in contrast to the very large increases in blood manganese levels produced by systemic manganese injections which were associated with increased brain manganese levels (Gianutsos *et al.*, 1985). Furthermore, the pattern of brain distribution is consistent with a localized transport mechanism, since the earliest and largest changes were observed in the olfactory bulb where the olfactory neurons terminate, while a smaller effect was observed in the more rostral olfactory tubercle.

It should be noted that the precise mechanism responsible for the entry of manganese into the CNS described in this report is not known, but could involve active or passive transport mechanisms or a combination of several factors. Furthermore, the effect on manganese entry of nasal damage, if it is shown to occur in future studies, cannot be adequately predicted at this time. However, the results strongly suggest that manganese, like other metals studied, is capable of directly entering the CNS after nasal exposure. Finally, it is noted that this study used the water-soluble chloride salt of manganese. Once deposited on the mucous lining layer, an inhaled manganese chloride aerosol would be expected to dissolve, due to its high solubility, suggesting that instillation may mimic *in vivo* cellular exposure conditions. This may not be identical in the case of insoluble manganese salts.

In the earlier cadmium studies (Hastings and Evans, 1991), transport of cadmium beyond the olfactory bulb was not observed. In our study, increased manganese was also observed in the deeper striatal region, although repeated injections were necessary to affect this region. This may be particularly significant in light of the known neurotoxicity associated with manganese. Manganese produces a selective effect on extrapyramidal regions responsible for motor regulation following inhalation exposure (Bird *et al.*, 1984),

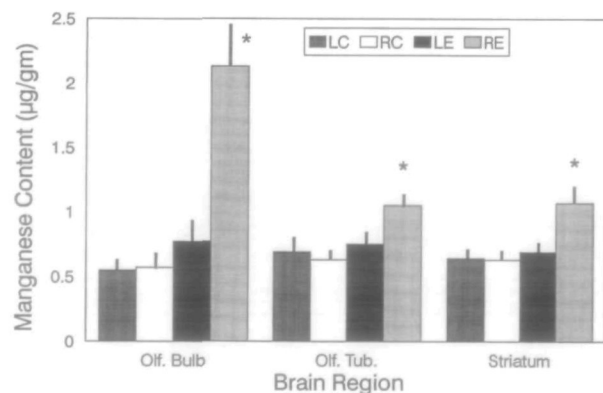


FIG. 2. Distribution of manganese in the brain following repeated intranasal administration. Rats received two injections of manganese chloride (800 μ g in 40 μ L of saline, designated E) into the right nostril. Control (C) rats received an equal volume of saline in the right nostril. The injections were given 1 week apart and the manganese content was measured in the olfactory bulb, olfactory tubercle, and striatum 24 hr after the second injection. Data are expressed as the amount of manganese (μ g/g) detected in the tissue sample of the brain region dissected from the left (L, control) side of the brain or the right (R, injected) side of the brain, obtained from the saline-treated (C) or experimental (E) group. Data are expressed as amount of manganese (\pm SE, $N = 6$, * denotes significantly different from corresponding left [control] side).

where it interferes with or destroys neurons utilizing the neurotransmitter dopamine (Cotzias *et al.*, 1974), possibly by enhancing the autooxidation of dopamine and generating damaging oxidative free radicals (Graham, 1984). Our results indicate that metals not only may have access to brain regions in intimate contact with the olfactory nerves, such as the olfactory bulb, but also may penetrate into deeper brain regions. Indeed, the elevation in the manganese content of the striatum, one of the suspected targets for toxicity, occurred only when intranasal injections were repeated. Interestingly, manganese neurotoxicity is a delayed event, requiring long-term exposure, suggesting that a slow, but persistent delivery of manganese to critical target sites may, at least in part, underlie the latent toxicity.

These results suggest that airborne metals may have access to the CNS through multiple mechanisms. While passage into the lungs and absorption into the systemic circulation may provide one means of delivering metals to the brain, local transport through or along the olfactory nerves may also contribute. This is particularly interesting because of studies which have demonstrated that the blood-brain barrier acts as a sink to exclude metal uptake into the CNS (Zheng *et al.*, 1991). In particular, recent evidence suggests that the choroid plexus may sequester manganese and restrict its penetration into the CSF (Ingersoll *et al.*, 1995). Localized direct transport of manganese through the olfactory nerves may serve to bypass the blood-brain barrier and lead to accumulation of the metal in the brain. In this regard it is noteworthy that comparative studies have shown that manganese toxicity is greater following inhalation exposure than with comparable oral treatments (Morganti *et al.*, 1985). The ability of even very low doses of manganese (25 μg) to elevate brain manganese levels reinforces the potential toxicological significance of this mechanism.

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