Acute manganese administration alters dopamine transporter levels in the non-human primate striatum

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Abstract

We used positron emission tomography (PET) to measure non-invasively the effect of acute systemic administration to manganese sulfate (MnSO₄) on dopamine transporter (DAT) levels in the living non-human primate brain. Baboons received [¹¹C]-WIN 35,428 PET scans to measure DAT levels before and after acute MnSO₄ administration. In one animal, we observed a 46% increase in DAT binding potential (BP), a measure of DAT binding site availability, 1 week after Mn administration. DAT levels returned to baseline values at 4 months and remained constant at 10 months after treatment. A subsequent single MnSO₄ injection to the same animal also resulted in a 57% increase in DAT-BP, 2 days after administration. In a second animal, a 76% increase in DAT-BP relative to baseline was observed at 3 days after Mn injection. In this animal, the DAT-BP returned to baseline levels after 1 month. Using in vitro receptor binding assays, we found that Mn inhibits [³H]-WIN 35,428 binding to rat striatal DAT with an inhibitory constant (K_i) of 2.0 ± 0.3 mM (n = 4). Saturation isotherms and Scatchard analysis of [³H]-WIN 35,428 binding to rat striatal DAT showed a significant decrease (30%, p < 0.001) in the maximal number of binding sites (B_max) in the presence of 2 mM MnSO₄. No significant effect of Mn was found on binding affinity (K_d). We also found that Mn inhibits [³H]-dopamine uptake with an IC₅₀ of 11.4 ± 1.5 mM (n = 4). Kinetic studies and Lineweaver–Burk analysis showed a significant decrease (40%, p < 0.001) in the maximal velocity of uptake (V_max) with 5 mM MnSO₄. No significant effect of Mn was found on Michaelis–Menton constant (K_m). These in vitro findings suggest that the increase in DAT levels in vivo following acute Mn administration may be a compensatory response to its inhibitory action on DAT. These findings provide helpful insights on potential mechanisms of Mn-induced neurotoxicity and indicate that the DAT in the striatum is a target for Mn in the brain.

Keywords: Manganism; Dopamine transporter; Manganese sulfate; Positron emission tomography; Non-human primate; Striatum; Parkinson’s disease

1. Introduction

Manganese (Mn) is an essential trace element for human nutrition but also a toxicant when humans are exposed to high concentrations. Occupational exposures to excess levels of Mn are known to cause a Parkinsonian-like syndrome in humans (Pal et al., 1999). The neurological manifestation of this Mn-induced syndrome include bradykinesia and rigidity which are similar to idiopathic Parkinson’s disease (IPD) (Pal et al., 1999). However, there are clinical distinctions between IPD and Mn-induced parkinsonism. In Mn-induced parkinsonism there is action tremor and absence of resting tremor, more dystonia, and severe gait disturbance “cock-walk” with difficulty in backward walking and no response to levodopa therapy (Kim et al., 2002; Pal et al., 1999). The globus pallidus and striatum are basal ganglia structures that exhibit high levels of Mn accumulation visualized by T₁-weighted magnetic resonance imaging (MRI) following Mn exposure (Eriksson et al., 1992b; Nelson et al., 1993; Newland et al., 1989; Newland and Weiss, 1992). The major pathological findings in manganism include a prominent decrease of myelinated fibers, gliosis and the degeneration of neurons in the globus pallidus and caudate/putamen (Olanow et al., 1996; Yamada et al., 1986). There is also an apparent sparing of the substantia nigra pars compacta...
The brain region that is affected in IPD. Although early reports suggest that the clinical picture and pathophysiology of manganism are distinct from PD, there are case reports of PD patients with a history of Mn exposure (Kim et al., 2002). Further, welders exposed to high levels of Mn have recently been described to have a higher prevalence of PD at an early age (Racette et al., 2001, 2005). These reports suggest that Mn exposure is an important risk factor for PD.

The organomanganese compound methylcyclopentadienyl manganese tricarbonyl (MMT) has been approved in the United States, Canada and other parts of the world as an alternative antiknock additive in gasoline. Upon combustion in an automobile engine, MMT yields a mixture of sulfate, phosphate and dioxide complexes of Mn with the sulfate and phosphate forms being the major emission products (Lynam et al., 1999). Thus, it is likely that extensive use of MMT-containing gasoline may increase Mn exposure levels to the general population. Therefore, a better understanding of the pathophysiology of Mn-induced neurotoxicity and the development of appropriate biomarkers for in vivo detection is needed.

Positron emission tomography (PET) is a non-invasive imaging technique that has been widely used to visualize the biochemistry of the living brain. In this study, we used PET with the dopamine transporter (DAT) selective radioligand [11C]-WIN 35,428 (Dannals et al., 1993; Wong et al., 1993) to measure the effect of acute exposure to manganese sulfate (MnSO₄) on DAT levels in the corpus striatum as an index of dopaminergic neurotransmission. Our results show that acute Mn administration produces a transient increase in [11C]-WIN 35,428 binding to DAT in the striatum in vivo. To the best of our knowledge, this is the first report of an increase in striatal DAT binding potential (BP), a measure of binding site availability, from acute Mn administration using in vivo PET imaging. To investigate the molecular mechanisms of this acute effect of Mn-induced dopaminergic dysfunction, we used in vitro assays to measure the effects of Mn on the binding of [3H]-WIN 35,428 to DAT from rat striatal membranes and the uptake kinetics of [3H]-dopamine by DAT from rat striatal synaptosomes. The results indicate that Mn inhibits both the binding of [3H]-WIN 35,428 to DAT in rat striatal membranes and the uptake of [3H]-DA by DAT into rat striatal synaptosomes. These in vitro findings suggest that the increase in DAT levels in vivo following acute Mn administration may be a compensatory response to its inhibitory action on the transporter. We suggest that DAT in striatal dopaminergic terminals is a target for Mn in the brain.

2. Material and method

2.1. Animals and manganese treatment

Two adult male baboons (Papio anubis) with unremarkable histories and weighing 25–30 kg were used in this study. Baboons were single housed in a colony room maintained at 22 ± 1 °C, with free access to food (2050 Protein Primate Diet; Harland Teklad, Madison, WI) and water. Food was supplemented with fruits and other treats. Animals were sedated (8–15 mg/kg ketamine, IM) prior to Mn treatment. Manganese sulfate (10–50 mg/kg in sterile saline) was administered by either subcutaneous or intravenous injection using an infusion pump over a period of 10 min. The injection volume was less than 5 ml for subcutaneous and 10 ml for intravenous. It is important to mention that the subcutaneous administration of MnSO₄ causes severe irritation at the site of injection and this route of exposure should be avoided. We initially performed two subcutaneous injection of MnSO₄ in one baboon referencing previous studies (Eriksson et al., 1987, 1992a) and after experiencing this complication on two occasions, the route of administration was changed to intravenous (i.v.) injection with no complication. All animal protocols (primate and rat) were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions.

2.2. PET imaging acquisition and analysis

Baseline and a series of post Mn treatment PET studies were performed. On the day of the PET study, two intravenous catheters and an arterial catheter were placed for infusion of anesthesia, injection of radiotracer, and arterial blood sampling, respectively. Animals were initially anesthetized intramuscularly with 8–10 mg/kg Saffan®, a formulation that contains 9 mg/ml alfaxalone and 3 mg/ml alfadolone acetate (Arnolds Veterinary Products, Shropshire, UK). Animals were maintained throughout the study by a continuous intravenous infusion drip of 6–9 mg kg⁻¹ h⁻¹ Saffan®. To ensure reproducibility of positioning during the multiple PET studies, a custom-made thermoplastic facemask (Tru Scan Imaging, Annapolis, MD) was fitted to the baboon’s head, which was then attached to the head holder during the PET scans. The laser light in the PET scanner matched markings on the facemask to ensure the exact head position in each study. After baboons were positioned in the PET scanner, a transmission scan was obtained with twin 370 MBq (10 mCi) ⁶⁸Ge pin sources for 10 min and used for attenuation correction of the subsequent emission PET scans. PET scanning started immediately after intravenous injection of 20 mCi of high specific activity [¹¹C]-WIN 35,428 that was synthesized as described previously (Dannals et al., 1993). The average specific activity of the final product calculated at end of synthesis was >2000 mCi/µmol with a radiochemical purity >95%. A GE Advance PET camera (General Electric) with an axial resolution (full width at half maximum) of 5.8 mm and an in-plane resolution of 5.4 mm was used for image acquisition. This scanner acquires 35 simultaneous slices of 4.25-mm thickness, enclosing a total longitudinal field of view of 15 cm. Thirty serial dynamic PET images were acquired during the 90 min after injection of radioligand using the following image sequence: 4 × 15 s frames, 4 × 30 s frames, 3 × 1 min frames, 2 × 2 min frames, 5 × 4 min frames, and 12 × 5 min frames. In all cases, over 32 arterial samples/PET study (for radioactivity) were obtained during the 90 min acquisition time. Arterial samples were also obtained at 5, 10, 15, 30, 60, and 90 min after the injection of the radiotracer for analysis by means of high performance liquid chromatography (HPLC) for the correction of the metabolism of [¹¹C]-WIN 35,428. PET scans were recon-
structured using ramp-filtered back-projection in a 128 × 128 matrix, with a transaxial pixel size of 2 mm × 2 mm. Images were corrected for attenuation and decay. Regions of interest were placed manually over the striatum and the cerebellum, and time–activity curves (TAC) were generated (Fig. 1A and B).

One-tissue and two-tissue compartmental models were used to fit the time–activity curves for cerebellum and striatum, respectively, using metabolite-corrected arterial blood activity as the input function (Fig. 2). Binding potential (BP) (i.e., $B_{\text{max}}/K_d$), which represents the $k_3/k_4$ ratio in striatum was determined in each study using a previously described method (Wong et al., 1993).

2.3. Effect of MnSO$_4$ on [3H]-WIN 35,428 binding to DAT from rat striatal membranes

Adult male Long Evans rats (250–350 g; Charles River, Wilmington, MA, USA) were housed in plastic cages with bedding at 22 ± 2 °C under a 12-h light:12-h dark cycle. Animals were euthanized by decapitation and striatal tissue was dissected and homogenized in 45 volumes of 0.32 M sucrose at 4 °C and centrifuged at 1000 × g for 10 min. The supernatant was centrifuged at 17,000 × g for 20 min, the resulting pellet (P$_2$) was resuspended in 50 mM Tris–HCl buffer (pH 7.4) with 100 mM NaCl and used for the radioligand-binding assay methods as described by Coffey and Reith (1994) with modification. For inhibition studies, 13 different concentrations of manganese sulfate (MnSO$_4$) (from 1 nM to 10 mM) were added to 2.8 nM [3H]-WIN 35,428 (Perkin-Elmer LAS Inc. Shelton, CT, USA). For Scatchard analysis, total binding of [3H]-WIN 35,428 was determined with concentrations ranging from 0.25 to 50 nM with or without 2 mM MnSO$_4$. Non-specific binding was assessed for each [3H]-WIN 35,428 concentration in the presence of 100 μM of the DAT inhibitor, GBR 12909 (Sigma, St. Louis, MO). Binding reaction mixtures were incubated for 120 min at 4 °C in a total volume of 500 μl. The reaction was terminated by vacuum filtration through GF/B filters, followed by three washes with 5 ml of ice-cold buffer. Radioactivity trapped in the filters was measured by liquid scintillation spectrometry. Protein concentrations of tissue homogenates were determined by the Bradford protein assay.

Binding parameters were estimated using the LIGAND in KELL Version 6 program for Windows (Biosoft, Cambridge, UK).

Fig. 1. (A) Representative transaxial images of the distribution of [11C]-WIN 35,428 radioactivity in the baboon brain at the level of the striatum (75–90 min after tracer injection). The bright yellow areas represent a high level of [11C]-WIN 35,428 binding to dopamine transporters (DAT) in the striatum. The activity of [11C]-WIN 35,428 in the striatum increased 1 week after manganese treatment and return to baseline levels after 4 months (data not shown). The color scale on the left represent the levels of [11C]-WIN 35,428 binding to DAT. (B) Brain tissue time–activity curves acquired from PET imaging analysis of the striatum and cerebellum in the same animal before and after acute manganese administration. There is a dramatic increase of TAC in striatum after 1 week of manganese treatment (50 mg/kg × 2, s.c.) compared to baseline level, and the TAC level recovers to baseline after 4 month. There is no change in the TAC in the cerebellum (regions of non-specific binding) after manganese treatment. Thus, the effect of Mn was selective to DAT in the striatum.
2.4. Effect of MnSO\textsubscript{4} on [3H]-dopamine uptake in rat striatal synaptosomes

Striatal tissue was dissected and homogenized in 20 volumes of 0.32 M sucrose, 50 mM Tris–HEPES (pH 7.4) at 4°C and centrifuged at 900 × g for 10 min. The supernatant was centrifuged at 17,000 × g for 20 min. The pellet was resuspended in ice-cold incubation buffer and mixed by vortexing. The incubation buffer consisted of 10 mM Tris–HEPES, pH 7.4 (145 mM NaCl, 5 mM KCl, 1 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 10 mM glucose, 0.25 mM ascorbate and 15 μM pargyline (Barlow et al., 2003). For inhibition studies, 11 different concentrations of MnSO\textsubscript{4} from 1 mM to 50 mM were used. [3H]-DA (Perkin-Elmer LAS Inc.) was used at a concentration of 10 nM. For kinetic analysis, 11 different concentrations (from 50 to 3000 nM) of DA were used as a mixture of 50 nM [3H]-DA with various concentrations of unlabelled DA. The synaptosomal preparation was pre-incubated for 15 min at 37°C with or without 5 mM MnSO\textsubscript{4}. Uptake reactions were triggered by adding [3H]-DA and incubated for 3 min at 37°C in a total volume of 500 μl. Non-specific uptake was determined by incubation at 4°C or adding 100 μM GBR 12909 at 37°C, both produced similar results. The reaction was terminated by adding 1 ml of cold buffer and vacuum filtration through GF/B filters, followed by three washes with 5 ml of ice-cold buffer. Radioactivity trapped in the filters was measured by liquid scintillation spectrometry. The IC\textsubscript{50} value was obtained using LIGAND program. The K\textsubscript{m} and V\textsubscript{max} were determined by Lineweaver–Burk model with linear regression using SigmaPlot 8.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Acute administration of manganese results in a transient increase in [11C]-WIN 35,428 binding to DAT in the striatum

Using PET imaging, we visualized striatal DAT levels in the living non-human primate brain and prospectively assessed the
effect of acute MnSO₄ administration in the same animal (Fig. 1A). Fig. 1B shows time–activity curves in the striatum and cerebellum, a measurement of [¹¹C]-WIN 35,428 binding to DAT, from the PET imaging during the 90 min scan at different stages of MnSO₄ treatment. Using kinetic compartmental modeling (Fig. 2), [¹¹C]-WIN 35,428 binding parameters to DAT were estimated. In one animal, we measured a 46% increase in DAT binding potential (BP) from a baseline of 6.19 to 9.04 1 week after two MnSO₄ injections (50 mg/kg, s.c., 1 week apart) (Fig. 3A). In this animal, striatal DAT levels returned to baseline values at 4 months (BP = 6.75) (Fig. 3A) and remained constant at 10 months (BP = 6.87) after treatment. A subsequent single MnSO₄ injection (10 mg/kg, i.v.) to the same animal after 22 months from the first MnSO₄ treatment also resulted in a 57% increase in DAT-BP from 6.87 to 10.83, 2 days after administration (Fig. 3B). In a second animal, a 76% increase in DAT BP was measured from a baseline value of 3.89 to 6.83 at 3 days after MnSO₄ injection (20 mg/kg, i.v.) (Fig. 3C). In this animal, the DAT-BP returned to baseline levels (BP = 4.38) after 1 month (Fig. 3C). These findings suggest that acute Mn administration result in a transient increase in [¹¹C]-WIN 35,428 binding to DAT in the non-human primate striatum.

3.2. In vitro inhibition of [³H]-WIN 35,428 binding to DAT in the rat striatum by MnSO₄

In order to examine potential mechanism(s) by which acute Mn administration transiently increases DAT levels in vivo, we used in vitro receptor binding assays to measure the effect of Mn on [³H]-WIN 35,428 binding to DAT in rat striatal membranes. Fig. 4A shows that Mn inhibits [³H]-WIN 35,428 binding to rat striatal DAT with an inhibitory constant $K_i$ of 2.0 ± 0.3 mM ($n = 4$). There was no difference on DAT inhibition between MnSO₄ and MnCl₂ in two different experiments (data not shown). In order to determine how Mn inhibits [³H]-WIN 35,428 binding to DAT, we performed [³H]-WIN 35,428 saturation isotherms in the presence or absence of 2 mM MnSO₄ (Fig. 4B). By converting the saturation graphs (Fig. 4B) to linear Scatchard plots (Fig. 4C), we estimated the maximal number of available binding sites ($B_{max}$) and binding affinity represented by the dissociation constant ($K_d$) (Fig. 4D). There was a significant
decrease (30%, $p < 0.001$) in $B_{\text{max}}$ in the presence of 2 mM MnSO$_4$ (1270 ± 127 fmol/mg protein, $n = 4$) relative to controls (1819 ± 161 fmol/mg protein, $n = 4$) (Fig. 4D). No significant effect of Mn on $K_d$ was measured (control 20.0 ± 1.6 nM; MnSO$_4$ 24.0 ± 2.4 nM, $p > 0.05$, $n = 4$) (Fig. 4D). These findings suggest that Mn inhibit $[^3H]$-WIN 35,428 binding to DAT in a non-competitive fashion.

3.3. MnSO$_4$ alters the function of the DAT by decreasing the maximal velocity of dopamine uptake ($V_{\text{max}}$) with no change in substrate affinity ($K_m$)

To further investigate the effect of Mn on DAT function, we performed $[^3H]$-dopamine (DA) uptake assays in rat striatal synaptosomal preparations. High concentrations of Mn inhibited $[^3H]$-DA uptake of rat striatal synaptosomes with an IC$_{50}$ value of 11.4 ± 1.5 mM (n = 4) (Fig. 5A). Kinetic analysis of $[^3H]$-DA uptake in the presence or absence of 5 mM MnSO$_4$ resulted in a significant decrease (40%) in $V_{\text{max}}$ (control: 1.86 ± 0.31E−7 mmol/min/mg protein; MnSO$_4$: 1.05 ± 0.31E−7 mmol/min/mg protein, $p = 0.003$, $n = 4$). No significant change on $K_m$ (control: 158.9 ± 29.8 nM; MnSO$_4$: 148.0 ± 64.3 nM, $p = 0.73$, $n = 4$) (Fig. 5D) was observed.

4. Discussion

The present study shows that acute administration of Mn results in a transient increase (46–76%) in $[^11C]$-WIN 35,428 binding to DAT in the living non-human primate striatum. These acute effects of Mn on DAT are different from previous reports using chronic Mn treatment and may represent an early event on the effects of Mn on the dopaminergic system. Previous PET studies in Mn-exposed non-human primates show a 60% reduction of $[^11C]$-nomifensine binding to DAT after chronic administration of MnO$_2$ (12 injections of 0.4 g over 16 months with total accumulation around 1200 mg/kg) (Eriksson et al., 1992b). Single photon emission computed tomography (SPECT) studies in humans exposed to Mn show 6–9 or 57–72% reductions of DAT level using $[^99mTc]$TRO-DAT-1 or $[^{123}I]$-β-CIT, respectively (Huang et al., 2003; Kim et al., 2002). Other studies also show decreased DAT level (75%) after chronic Mn treatment using $[^3H]$-mazindol quantitative autoradiography in postmortem monkey brain (Eriksson et al., 1992a). In contrast, the present findings demonstrate that acute Mn administration causes a transient increased in DAT levels, an effect that is different from those produced by chronic Mn administration.
There are several possible scenarios that can explain the transient increase in \([^{11}C]\)-WIN 35,428 binding in the non-human primate striatum after Mn administration. First, acute administration of Mn may decrease synaptic dopamine (DA) concentrations. A Mn-induced decrease in synaptic DA concentrations reduces the ability of endogenous DA to compete with \([^{11}C]\)-WIN 35,428 for binding to DAT resulting in an apparent increase in \([^{11}C]\)-WIN 35,428 binding potential. There are reports indicating that chronic Mn administration to non-human primates decreases the level of DA in the caudate and putamen (Eriksson et al., 1987; Neff et al., 1969) and in rat striatum (Ingersoll et al., 1999). However, there is no evidence for the same effect using acute Mn administration.

Secondly, Mn may cause an up-regulation of DAT protein in the pre-synaptic terminal. Increased DAT protein provides a greater number of binding sites for \([^{11}C]\)-WIN 35,428 binding and may represent a dynamic response of DAT to Mn inhibition. The later possibility is more likely to explain the in vivo findings because our in vitro studies indicate that Mn can inhibit the binding of \([^{3}H]\)-WIN 35,428 to DAT (Fig. 4) and the uptake of \([^{3}H]\)-DA in striatal synaptosomes (Fig. 5). Studies with DAT blockers such as cocaine have shown that it can produce a rapid upregulation of DAT both in vivo and in vitro (Gulley and Zahniser, 2003; Mortensen and Amara, 2003). Thus, it is possible that the inhibitory effects of Mn on transporter function measured in the present study may explain the in vivo effects of acute Mn administration as a compensatory upregulation of DAT levels.

An important question raised by the in vivo studies is whether the acute doses of Mn administered to the animal are relevant to environmental or occupational exposures. The lowest dose given to one of the animals was 10 mg MnSO4/kg body weight as a compensatory upregulation of DAT levels. A Mn-induced decrease in synaptic DA concentrations may explain the in vivo effects of acute Mn that the inhibitory effects of Mn on transporter function measured in the present study may explain the in vivo effects of acute Mn administration in the striatum of non-human primates may represent an early event in Mn neurotoxicity that may predispose the dopaminergic neuron to damage by the increased uptake of dopamine or other toxins. These preliminary findings provide helpful insights on potential mechanisms of Mn-induced neurotoxicity and indicate that the DAT in striatal dopaminergic terminals is a target for Mn in the primate brain.

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**References**


