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UPTAKE AND RELEASE OF MANGANESE BY RAT STRIATAL SLICES*

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Abstract—Accumulation of manganese in rat corpus striatum slices was found to be non-saturable, although relatively strongly temperature dependent, and inhibited by 2,4-dinitrophenol (2,4-DNP). Once incorporated, the metal ion was released by K^+ (55 mM) depolarization in the presence of calcium ions, following a time course of efflux parallel to that of [^{3}H]dopamine ([^{3}H]DA). The release of the metal ion, however, was not induced by tyramine. [^{3}H]DA release was also induced by low concentrations of manganese chloride. The possibility exists that these findings may be related in some way to the functional deficiency of the nigro-striatal dopaminergic system found after manganese poisoning.

Manganese poisoning is characterized by classical symptoms of Parkinson's disease accompanied by a substantial decrease in brain dopamine [1, 2]. Chandra *et al.* [3] have recently reported that monkeys treated chronically have the greatest brain accumulations of manganese in the corpus striatum, and that this accumulation occurs *pari passu* with a decrease in dopamine in the same brain area. Humans intoxicated with manganese have been shown to experience relief with L-dopa treatment in the same way as Parkinsonians [4].

The above findings suggest strongly that the mechanisms underlying the symptoms of manganese poisoning are related in some way to a functional deficiency in the nigro-striatal dopaminergic pathway.

We have reported recently that manganese can be readily taken up by chromaffin granules *in vitro*. Once incorporated, the metal binds to ATP and displaces catecholamines, decreasing the granular catecholamine/ATP ratio [5,6]. Earlier observations had shown that chronic intoxication with manganese could not be alleviated by chelating agents [7], indicating that the metal is firmly bound and inaccessible to these agents.

As a first approach to understanding the relationship between manganese intoxication and a functional deficiency of dopaminergic neurons, we have studied in the present work the characteristics of manganese uptake by rat striatal slices.

MATERIALS AND METHODS

Preparation of rat striatal slices. Striatal slices (0.2 mm) were cut from adult, male Sprague–Dawley rats with a Sorvall tissue sectioner. Routinely, about 10 mg of tissue (four slices) was used for incorporation and release studies.

Accumulation of [^{54}Mn]. Striatal slices were incubated for 30 min at 37° (unless otherwise stated) in 2 ml of Ca^{2+}- and Mg^{2+}-free Krebs–Ringer–Pipes‡ (KRPi) solution (pH 7.2) containing [^{54}Mn] ($MnCl_2$, carrier free, 8.3×10^5 dpm/ml) and various concentrations of $MnCl_2$. At the end of the incubation period the slices were washed three times (10-min period) in 10 ml of the above incubating medium, containing 0.05 mM EDTA, homogenized in 15% trichloroacetic acid (TCA), and counted in a Nuclear Chicago gamma counter (25 per cent efficiency for ^{54}Mn). The homogenate was then centrifuged for 10 min at 12,000 *g* and protein was determined according to Lowry *et al.* [8] using bovine serum albumin as a standard.

Release of [^{54}Mn]. Striatal slices previously incubated for 30 min at 37° with [^{54}Mn] ($MnCl_2$, carrier free, 8.3×10^5 dpm/ml) were transferred to superfusion chambers [9] from which the liberation of the radioactive compound was followed. The slices were superfused (4 ml/min) with continuously oxygenated solutions at 37°. To obtain a constant and steady basal release, an initial superfusion of 10 min with Ca^{2+} and Mg^{2+}-free KRPi plus 0.05 mM EDTA, followed by 23 min with KRPi, was allowed before stimulation. [^{54}Mn] release was induced by superfusing the slices for 1 min with high K^+ (55 mM) KRPi. Samples containing the released material were collected every minute and analyzed for radioactivity. At the end of the superfusion period, the slices were also analyzed for radioactivity.

Simultaneous release of [^{54}Mn] *and* [^{3}H]*dopamine.* In experiments in which the simultaneous release of [^{54}Mn] and [^{3}H]DA was studied,

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‡ Abbreviations: Pipes, piperazine *N,N*-bis-2 ethanesulphonic acid; KRPi, Krebs–Ringer–Pipes; 2,4-DNP, 2,4-dinitrophenol; DA, dopamine; and TCA, trichloroacetic acid.

the slices were incubated for 30 min at 37° in 2 ml of Ca^{2+}- and Mg^{2+}-free KRPi with [^{54}Mn] ($MnCl_2$, carrier free, 8.3×10^5 dpm/ml) plus [^{3}H]DA (3×10^{-7} M; sp. act. 7.5 Ci/mmole) and then were transferred to superfusion chambers. When the release of the radioactive compounds was induced by K^+ (55 mM) depolarization, there was an initial superfusion of 10 min with Ca^{2+}- and Mg^{2+}-free KRPi plus 0.05 mM EDTA, followed by 23 min with KRPi before stimulation. When release was induced by tyramine (5×10^{-4} M), however, there was an initial superfusion of 10 min with Ca^{2+}- and Mg^{2+}-free KRPi plus 0.05 mM EDTA, followed by 23 min with Ca^{2+}-free KRPI before stimulation. Samples were collected every minute and analyzed for tritium (50 per cent efficiency, Nuclear Chicago Scintillation Counter) and [^{54}Mn]. At the end of the superfusion period, the slices were homogenized in 15% TCA and centrifuged for 10 min at 12,000 *g*. The clear supernatant fractions were also analyzed for radioactivity.

Release of [^{3}H]*dopamine induced by manganese.* Striatal slices were incubated for 30 min at 37° in 2 ml of KRPi containing [^{3}H]DA (3×10^{-7} M; sp. act. 7.5 Ci/mmole) and then were transferred to superfusion chambers. An initial superfusion of 13 min with Ca^{2+}-free KRPi was allowed before stimulation. [^{3}H]DA release was induced by superfusing the slices for 5 min with $MnCl_2$ (5×10^{-6} M) in Ca^{2+}-free KRPi. Samples containing the released material were collected every minute and analyzed for radioactivity. At the end of the superfusion period, the slices were homogenized in 15% TCA and centrifuged at 12,000 *g* for 10 min, and the clear supernatant fraction was analyzed for radioactivity.

Release of [^{3}H]DA or [^{54}Mn] is expressed as the percentage of the total radioactivity found in the tissue and in different collecting tubes at the end of the superfusion period. Samples analyzed for [^{54}Mn]-gamma radiation were counted at least up to 10,000 cpm to minimize the statistical error.

Solutions and chemicals. The Krebs–Ringer–Pipes (KRPi) solution used had the following composition: 128 mM NaCl, 4.8 mM KCl, 0.75 mM $CaCl_2$, 1.20 mM $MgSO_4$, 16 mM glucose and 5 mM Pipes at a pH of 7.2. High K^+ KRPi was made by replacing proportions of NaCl with equimolar amounts of KCl.

^{54}Manganese ($MnCl_2$, carrier free, sp. act. $>100\ \mu$Ci/μg Mn) was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England; [^{3}H]dopamine (3,4-dihydroxyphenylethylamine ethyl-2-[^{3}H-(N)], sp. act. 7.5 Ci/mmole) from the New England Nuclear Corp, Boston, MA, U.S.A.; and 2,4-dinitrophenol, tyramine (4-hydroxyphenylethylamine) and Pipes from the Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of the highest purity available.

RESULTS

Incorporation of [^{54}Mn] *into striatal slices.* The time course of the incorporation of manganese is shown in Fig. 1. At 37° the accumulation of the metal ion was linear for up to 30 min and, then, it levelled off. It can be seen that the incorporation was reduced significantly both at low temperature (4°) and in the presence of 2,4-dinitrophenol (10^{-4} M).

Figure 2 shows the rate of accumulation of manganese as a function of the concentration of the metal (10^{-9} M to 10^{-3} M) in the incubation medium. No saturation of the system can be seen at concentrations up to 1 mM. Interestingly, under the above conditions, incubation of striatal slices with 1×10^{-6} M $MnCl_2$ resulted in an accumulation of about 3 μg Mn/g tissue (results not shown), a value which is

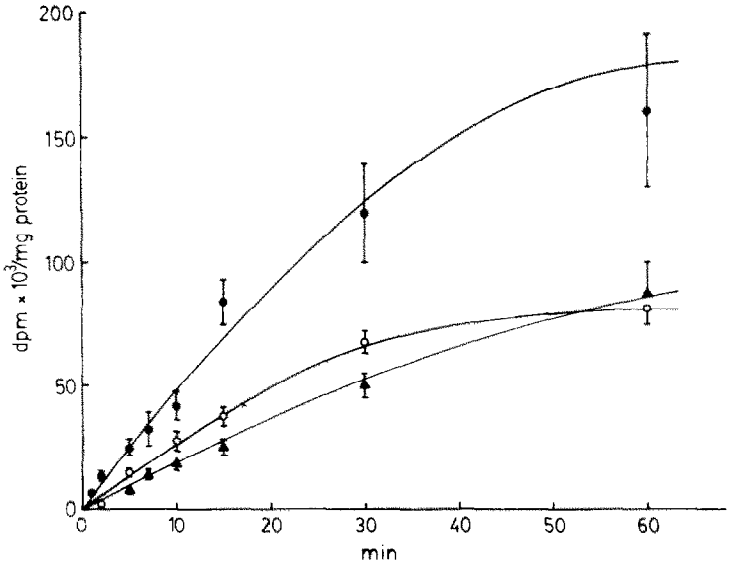

Fig. 1. Time course of the incorporation of manganese into striatal slices. The slices were incubated with [^{54}Mn] (8.3×10^5 dpm/ml; final concentration less than 10^{-9} M) at 37° (●), 4° (▲) or in the presence of 2,4-DNP (10^{-4} M) (○). Each point is the mean ± S.D. of six different experiments.

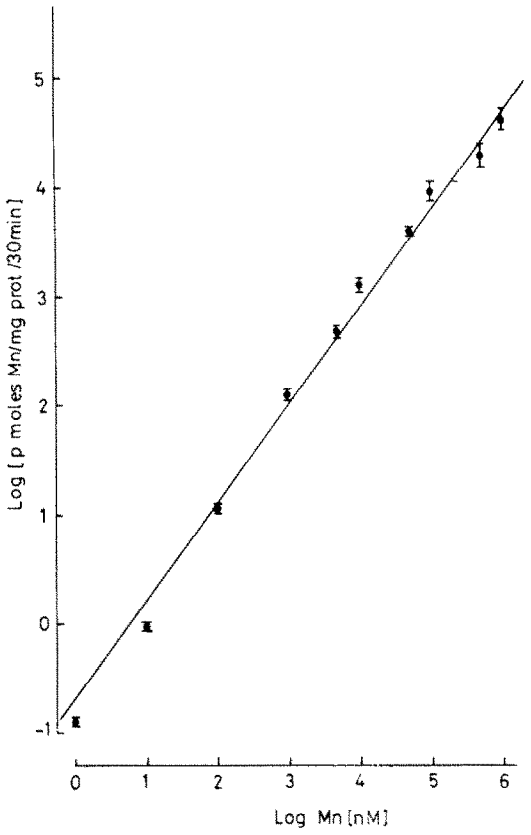

Fig. 2. Concentration dependence of manganese uptake by striatal slices. The slices were incubated at 37° for 30 min in the presence of $MnCl_2$ (10^{-9} M to 10^{-3} M) and [^{54}Mn] (8.3×10^5 dpm/ml) to a known specific activity. Line of best fit was calculated by linear regression analysis ($r = 0.955$); N = 6 for each point; mean ± S.D.

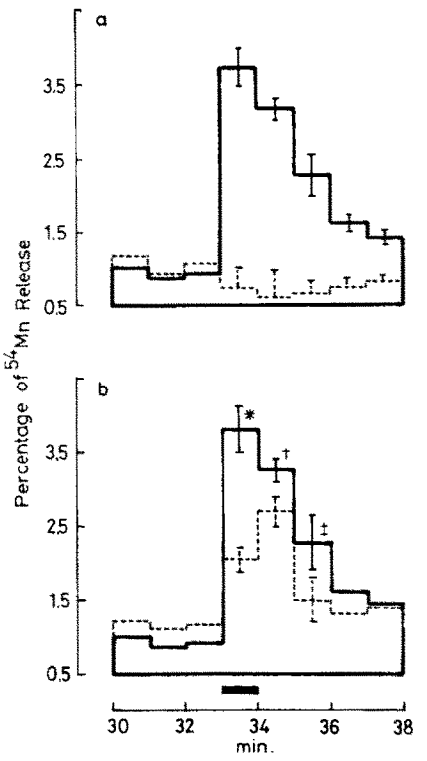

Fig. 3. Potassium-induced release of [^{54}Mn]. Striatal slices were incubated for 30 min at 37° with [^{54}Mn] (8.3×10^5 dpm/ml) and then superfused as described in Materials and Methods. Panel a: K^+ (55 mM) stimulation was carried out for 1 min in KRPi (——) or Ca^{2+}-free KRPI (- - -). Panel b: striatal slices were incubated in the absence (——) or in the presence (- - -) of 2,4-DNP and were stimulated for 1 min with high K^+ KRPi. The black horizontal bar represents the time of stimulation with high potassium. The tissue took up an average of 34,000 ± 3,700 dpm of [^{54}Mn] for controls and 17,500 ± 2,030 dpm of [^{54}Mn] for 2,4-DNP incubated slices. Results are expressed as the percentage of released (mean ± S.D.) with respect to the total accumulated radioactivity (N = 4 for both conditions). Key: (*) $P < 0.001$; (†) $P < 0.02$; and (‡) NS.

very close to that found by Chandra *et al* [3] in the corpus striatum of monkeys exposed to manganese for 18 months.

Release of [^{54}Mn] *evoked by* K^+ *depolarization.* The efflux of [^{54}Mn], previously taken up by striatal slices, increased significantly during K^+ depolarization. This effect was reduced markedly in the absence of calcium ions, indicating a Ca^{2+} dependence for manganese release (Fig. 3a). The K^+-induced release of [^{54}Mn] was also decreased significantly if the slices had taken up the metal in the presence of 2,4-DNP (Fig. 3b).

Simultaneous release of [^{54}Mn] *and* [^{3}H]*dopamine evoked by* K^+ *depolarization or tyramine.* Striatal slices previously incubated in the presence of [^{54}Mn] and [^{3}H]DA showed a parallel release of both the metal ion and the amine when stimulated by K^+ (55 mM) depolarization (Fig. 4a). However, when tyramine (5×10^{-4} M) was used as a releasing agent, only [^{3}H]DA efflux was increased significantly (Fig. 4b).

Release of [^{3}H]*dopamine induced by manganese ions.* Striatal slices previously incubated with [^{3}H]DA showed a significant increase in the efflux of the labeled amine when superfused with low concentrations of $MnCl_2$ (Fig. 5). Under the same conditions, Ca^{2+} did not induce the release of [^{3}H]DA (data not shown).

DISCUSSION

Our results show that manganese was accumulated by rat striatal slices. The process, although it did not show saturation, had a relatively strong temperature dependence and was inhibited by 2,4-DNP. These results probably indicate the presence of multiple binding sites, some of which might depend upon an energy source.

We draw attention, though, to the fact that the accumulated manganese could be released by K^+ depolarization and that this release was abolished in the absence of calcium ions. This indicates that at least part of the manganese taken up by the tissue slices was accumulated in storage vesicles. This interpretation is supported by the simultaneous release of [^{3}H]DA and [^{54}Mn]induced by K^+ depo-

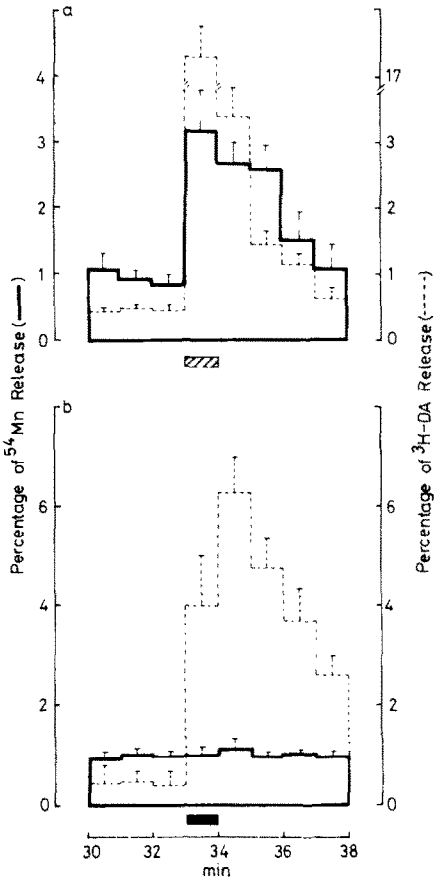

Fig. 4. Release of [^{54}Mn] (——) and [^{3}H]DA (- - -) induced by potassium or tyramine. Striatal slices were incubated for 30 min at 37° in the presence of [^{3}H]DA (3×10^{-7} M) and [^{54}Mn] (8.3×10^{5} dpm/ml) and then superfused as described in Materials and Methods. K^+ (55 mM) stimulation (▨) was carried out for 1 min in KRPi. Tyramine stimulation (■) was carried out for 1 min in Ca^{2+}-free KRPi. The tissue took up an average of 35,000 ± 2,950 dpm of [^{54}Mn] and 1,700,000 ± 83,200 dpm of [^{3}H]DA. Results are expressed as the percentage released (mean ± S.D.) with respect to the total accumulated radioactivity (N = 4 for both conditions).

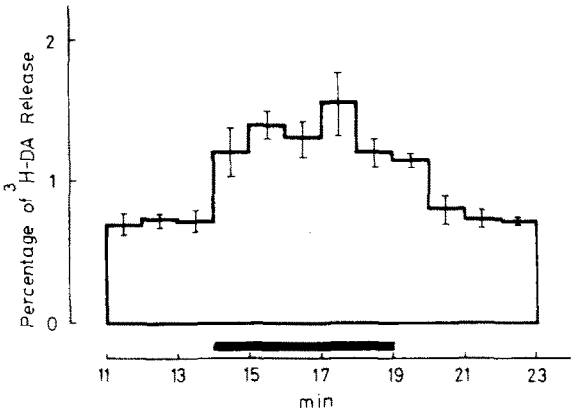

Fig. 5. Release of [^{3}H]DA induced by manganese. Striatal slices were incubated for 30 min at 37° in the presence of [^{3}H]DA (3×10^{-7} M) and then superfused as described in Materials and Methods. Stimulation was carried out for 5 min with $MnCl_2$ (5×10^{-6} M) in Ca^{2+}-free KRPi. The tissue took up an average of 1,850,000 ± 75,300 dpm of [^{3}H]DA. The black horizontal bar represents the time of stimulation with $MnCl_2$. Results are expressed as the percentage released (mean ± S.D.) with respect to the total accumulated radioactivity (N = 4).

larization, because they followed the same time course of efflux. Furthermore, the release of [^{54}Mn] by K^+ depolarization was inhibited significantly when the metal ion had been taken up previously by the slices in the presence of 2,4-DNP. This inhibition did not occur, however, if 2,4-DNP (10^{-4} M) was present only during depolarization (data not shown); hence, a direct effect of this agent on the release process can be discarded. The above finding suggests, instead, that the uncoupler was partially impeding the accumulation of manganese by those compartments that released their contents by K^+ depolarization.

Additional support for an intravesicular accumulation of the metal ion is the fact that manganese induced the release of [^{3}H]DA previously taken up by dopaminergic terminals, an effect that did not occur when Ca^{2+} was used instead of manganese, thus ruling out a simple ionic effect. Manganese ions presumably entered the storage vesicles and therein displaced dopamine from its binding sites. This interpretation correlates well with our previous finding that manganese displaces catecholamines from their storage complex in isolated adrenal chromaffin granules [6] and also in adrenal slices (unpublished observations). It is tempting to suggest that dopamine can be stored in the same way as catecholamines are in the adrenal medulla, by forming a complex with ATP. Manganese would strongly bind to ATP and therefore displace dopamine. Tyramine also induces the release of [^{3}H]DA but not that of [^{54}Mn]. This may indicate that the intravesicular binding site for manganese is much stronger, or otherwise different, than that for dopamine. At the present stage, however, we cannot distinguish between these two possibilities.

In conclusion, our results indicate that manganese was accumulated by rat striatal slices and that a fraction of it was associated with storage vesicles. It may be that this accumulation is responsible, in part, for the functional deficiency in the nigro-striatal dopaminergic pathway, that is observed during manganese poisoning. In support of this, we have observed recently that manganese, taken up previously by striatal slices, markedly depresses [^{14}C]dopamine synthesis from [^{14}C]tyrosine (unpublished observations). We are currently investigating this inhibitory mechanism.

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