Rapid Brain Uptake of Manganese(II) Across the Blood–Brain Barrier

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Abstract: $^{54}$Mn$^{2+}$ uptake into brain and choroid plexus from the circulation was studied using the in situ rat brain perfusion technique. Initial uptake from blood was linear with time (30 s to 6 min) and extrapolated to zero with an average transfer coefficient of $\sim 6 \times 10^{-5}$ ml/s/g for brain and $\sim 7 \times 10^{-3}$ ml/s/g for choroid plexus. Influx from physiologic saline was three- to fourfold more rapid and exceeded that predicted for passive diffusion by more than one order of magnitude. The lower uptake rate from blood could be explained by plasma protein binding as the free fraction of $^{54}$Mn$^{2+}$ in rat plasma was $\leq$30%. Purified albumin, transferrin, and $\alpha_2$-macroglobulin were each found to bind $^{54}$Mn$^{2+}$ significantly and to restrict brain $^{54}$Mn$^{2+}$ influx. The results demonstrate that $^{54}$Mn$^{2+}$ is readily taken up into the CNS, most likely as the free ion, and that transport is critically affected by plasma protein binding. The results support the hypothesis that Mn$^{2+}$ transport across the blood–brain barrier is facilitated by either an active or a passive mechanism. Key Words: Blood–brain barrier—Manganese—Plasma protein binding—Transport—Neurotoxicity—Parkinson’s disease.


Manganese is an essential trace metal that is required for brain development and function (Smith, 1990). It is found normally in brain in parts-per-million concentrations and serves as a cofactor for numerous brain enzymes including glutamine synthetase, calmodulin-dependent phosphatase, and mitochondrial superoxide dismutase (Prohaska, 1987). Within brain Mn is distributed heterogeneously with highest concentrations in the palladium and putamen and lowest concentrations in the frontal cortex (Larsen et al., 1979). The level of Mn in brain and brain capillaries exceeds that in plasma by $\geq$100-fold (Keen et al., 1984; Tayarani et al., 1989; Murphy et al., 1991a), which implies some form of selective binding or active transport.

Though essential to brain, Mn is also neurotoxic at elevated concentrations and can produce irreversible damage to the CNS (Donaldson, 1987; Aschner and Aschner, 1991). Chronic overexposure results in a syndrome that resembles Parkinson’s disease and responds to L-DOPA treatment (Huang et al., 1989). Toxicity arises in part from disruption of central neurotransmitter systems, especially the dopaminergic system of the basal ganglia (Neff et al., 1969; Bernheimer et al., 1973). The biochemical mechanisms that underlie Mn toxicity are poorly understood but have been related to increased production of free radicals, oxidation of dopamine to cytotoxic substances (e.g., quinones and 6-hydroxydopamine), and direct effects of Mn on neuronal enzymatic, receptor, and signal transduction mechanisms (Graham, 1984; Donaldson, 1987; Aschner and Aschner, 1991).

How Mn gains access to brain has never been clearly elucidated. Most Mn in serum is thought to circulate in the 3+ form bound tightly to transferrin ($K_s \sim 10^{10} M^{-1}$) (Aisen et al., 1969; Scheuhammer and Cherian, 1985; Davidsson et al., 1989). Several investigators have suggested that Mn$^{3+}$ is taken up into brain via a transferrin-dependent mechanism (Mena et al., 1974; Aschner and Aschner, 1990, 1991). In support of this, brain capillaries are known to contain high levels of transferrin receptors (Jeffries et al., 1984; Partridge et al., 1987) and Mn$^{3+}$ has been shown to be taken up and internalized in some cells by a transferrin-dependent mechanism (Morris et al., 1987). However, Mn is also reported to exist in plasma in the 2+ oxidation state (Mn$^{2+}$) circulating as the free ion or bound to plasma proteins, such as albumin and $\alpha_2$-macroglobulin (Mildvan and Cohn, 1963; Gibbons et al., 1976), or to low molecular weight solutes (May et al., 1977). These other species may contribute to brain uptake as well. Murphy et al. (1991b) recently reported that Mn$^{2+}$ may be transported into brain by a high-affinity, saturable mechanism. Little is known of the precise distribution of Mn in plasma (Mn$^{3+}$ vs. Mn$^{2+}$) or of the relative contributions of the various pathways.
Previous studies of Mn transport into brain have been complicated by rapid in vivo oxidation of circulating Mn as well as variable plasma protein binding and metal competition (Gibbons et al., 1976; Schehammer and Cherian, 1985; Davidsson et al., 1989; Aschner and Aschner, 1990; Murphy et al., 1991b). Information on the brain uptake of a specific Mn species under defined conditions would greatly aid in the evaluation of the various mechanisms. In the present study, we investigated the role of $^{54}$Mn$^{2+}$ uptake into brain and choroid plexus using an in situ rat brain perfusion technique (Takasato et al., 1984; Smith et al., 1990). The brain perfusion method allows selective exposure of brain to specific Mn species in the absence of peripheral oxidation and at defined concentrations of various chelating agents (e.g., proteins and EDTA). The results suggest that Mn$^{2+}$ rapidly crosses the blood–brain barrier and that uptake is critically influenced by plasma protein binding.

**MATERIALS AND METHODS**

**Perfusion method**

All research was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23) under the supervision of the National Institute of Child Health and Development Animal Care and Use Committee, NIH. $^{54}$Mn transport into brain was measured in pentobarbital-anesthetized (50 mg/kg i.p.) Sprague–Dawley rats (250–350 g) using the in situ brain perfusion technique of Takasato et al. (1984). The following two modifications of the original method were incorporated: (1) The perfusion catheter (PE 50) was placed in the right common carotid artery, instead of the right external carotid artery, to facilitate rapid surgical preparation of animals, and (2) the heart (right and left ventricles) was severed just before perfusion (1–5 s) to eliminate potential flow contributions from the systemic circulation. Perfusion via the common carotid artery was reported previously by Deane and Bradbury (1990) and found to give acceptable values for brain uptake of several nonelectrolytes and amino acids. The heart cut modification was developed by Smith et al. (1990).

Brain uptake was measured using three perfusates, i.e., whole blood, plasma/serum, and saline. Blood for perfusion was collected anaerobically on the morning of the experiment from the descending aorta of pentobarbital-anesthetized donor rats. Samples were placed in heparinized tubes (20 IU of heparin/ml of blood, Upjohn, Kalamazoo, MI, U.S.A.) and filtered (Spectra mesh nylon 41 μm, Spectrum, CA, U.S.A.) and counted for $^{14}$C. Isotope distribution in brain compartments

Processing of samples

Tissue and perfusate samples were placed in tared polypropylene tubes and reweighed. Samples containing $^{54}$Mn were first analyzed for $^{54}$Mn with a gamma counter (Compgamma model 1282, Pharmacia-LKB, Gaithersburg, MD, U.S.A.) and then were digested overnight with 10% piperidine at 50°C. The following morning solutions were diluted with ~10 ml of scintillation cocktail (Ready Gel 1% glacial acetic acid, Beckman Instrument Co., Fullerton, CA, U.S.A.) and counted for $^{14}$C in a Beckman LSC spectrometer (model LS 6800). Counts in the $^{14}$C energy range were corrected for background, quenching, and spill-over from $^{54}$Mn and were expressed as disintegrations per minute via computer analysis. $^{14}$C and $^{13}$C concentrations in non-$^{54}$Mn experiments were determined using standard scintillation methods (Takasato et al., 1984).
tracer-free saline perfusate buffer that had been chilled on ice. After 15 strokes, ~4 ml of a chilled 40% neutral dextran solution was added to obtain a final dextran concentration of ~20%. The solution was then centrifuged at 5,400 g for 15 min (4°C) to obtain a vascular pellet and a supernatant that was essentially blood vessel free. Tracer levels were determined for pellet, homogenate, and supernatant via scintillation and gamma counting. With this method it is assumed that radioactivity in the supernatant represents tracer that has crossed the blood–brain barrier and is not simply bound to or associated with brain capillaries (Trituero et al., 1990).

Protein binding

Plasma protein binding of 54Mn was measured by ultrafiltration using the Centrifuge Micropartition System (Amicon, Danvers, MA, U.S.A.). Filter retention of free 54Mn was assessed via ultrafiltration of protein-free saline or previously ultrafiltered plasma and averaged ~16%. Protein binding was also studied by gel permeation chromatography using a Sephadex G-75 column (1.2 × 30 cm) (Pharmacia, Piscataway, NJ, U.S.A.) that was eluted with sodium phosphate buffer (120 mM NaCl, 20 mM sodium phosphate, pH 7.4) (Aschner and Aschner, 1990; Murphy et al., 1991b). Samples were applied to the column in ~50–100-μl aliquots containing ~0.1–0.5 μCi tracer with blue dextran (molecular mass 2,000 kDa, Sigma) to mark void volume. Elution profiles were based on total recovered tracer with each fraction expressed as a percentage of total recovered radioactivity. The column was calibrated with [14C]sucrose (molecular mass 0.36 kDa), [3H]inulin (molecular mass ~5.4 kDa), and 3H-dextrans (molecular mass 10, 20, 40, and 80 kDa) to identify high-molecular mass and low-molecular mass fractions.

Calculations and statistics

54Mn uptake into the brain or choroid plexus was expressed initially as an apparent “distribution volume” or “uptake space” (ml/g) (Deane and Bradbury, 1990; Trituero et al., 1990) defined as:

\[ V = \frac{Q_{ui}}{C_{p}} \]  

where \( Q_{ui} \) is the quantity of tracer in tissue per gram wet weight, corrected for intravascular (brain) or extracellular (choroid plexus) activity, and \( C_{p} \) is the tracer concentration in perfusate saline or plasma. Residual vascular or extracellular tracer was subtracted from all tissue samples as:

\[ Q_m = Q_{ui} - V \cdot C_{p} \]  

where \( Q_{ui} \) is the total quantity of tracer in tissue sample (vascular + extravascular), \( V \) is tissue vascular (brain) or extracellular (choroid plexus) volume (ml/g), \( C_{p} \) is perfusate tracer concentration, and \( Q_{ui} \) is the corrected quantity of tracer in tissue (cpm or dpm/g). In blood perfusion experiments, care was taken to use the blood tracer concentration for \( C_{p} \) in Eq. 2 and the plasma concentration for \( C_{p} \) in Eq. 1. \( V \) was quantitated in each animal using [14C]sucrose or [3H]inulin. In capillary depletion experiments, the distribution volume was expressed with and without vascular correction.

Blood–brain barrier transfer coefficients (\( K_{in} \)) were calculated, assuming unidirectional influx kinetics, from the total quantity of tracer taken up into brain or choroid plexus (\( Q_{ui} \)) divided by the exposure integral (\( C_{p} \)T) (Takasato et al., 1984):

\[ K_{in} = \frac{Q_{ui}}{C_{p}T} \]  

\( K_{in} \) was also calculated in some experiments using the linear uptake method:

\[ Q_{ui}/C_{p} = K_{in}T + V \]  

where \( V \) corrects for any additional vascular space beyond that measured by [14C]sucrose. Eq. 4 was fit to the data using linear least-square regression.

All values are the mean ± SEM. Values were compared for statistical difference using Student’s t test or analysis of variance with the Bonferroni correction for multiple comparisons.

RESULTS

Figure 1 illustrates the time course of 54Mn2+ uptake into cortical brain and lateral ventricle choroid plexus during perfusion with blood or physiologic saline. Uptake for both tissues was linear with time (30 s to 6 min) and extrapolated to zero after vascular correction. Blood–brain barrier transfer coefficients (\( K_{in} \) values), calculated as the slopes of the regression lines (Eq. 4), equaled ~6 × 10^{-3} ml/s/g for brain and 6.9 × 10^{-3} ml/s/g for choroid plexus during blood perfusion (Table 1). Transport in both tissues was three- to fourfold more rapid from protein-free saline (\( p < 0.05 \)). No significant differences were observed in \( K_{in} \) among sampled brain regions for either fluid (Table 1).

Brain 54Mn2+ uptake was not flow limited as increasing saline infusion rate fourfold or more did not appreciably change \( K_{in} \). For example, mean cortical \( K_{in} \) for 54Mn2+ equaled 24 ± 2 × 10^{-5} ml/s/g (n = 5) with an infusion rate of 1.8 ml/min and 28 ± 2 × 10^{-5} ml/s/g (n = 2) with an infusion rate of 7.5–8.0 ml/min (\( T = 120 \) s). Matching cerebral perfusion fluid flow rates at the two infusion values equaled 1.2 ± 0.2 × 10^{-2} ml/s/g and 8.3 ± 0.3 × 10^{-2} ml/s/g (n = 4), respectively. Perfused animals had an intact blood–brain barrier as measured values for \( K_{in} \) to [14C]-thiourea (1.7–3.5 × 10^{-4} ml/s/g, n = 8) and L-[14C]-leucine (1.9–3.6 × 10^{-2} ml/s/g, n = 8) agreed well with those reported in the literature. [14C]Sucrose \( V \) values for blood and saline were within normal limits (0.7–1.5%) as reported for the perfusion method (Takasato et al., 1984). To evaluate whether 54Mn in brain had actually crossed the blood–brain barrier or was simply bound to or trapped within brain endothelial cells, two experiments were performed. In the first, the brain was perfused for 30 s with 54Mn2+ in physiologic saline followed by a 30-s wash with tracer-free saline containing 1 mM EDTA to remove tracer bound to the capillary luminal membrane (Deane and Bradbury, 1990). The results were then compared with animals that did not receive the washout procedure. Calculated brain \( K_{in} \) for 54Mn in EDTA-washed animals
Plasma protein binding of $^{54}$Mn was assayed by two separate methods. Ultrafiltration on Amicon membranes and $\pm 2$% with the vascular pellet (Table 2). The results suggest that the great majority of $^{54}$Mn in brain may have actually crossed the blood–brain barrier and was not simply trapped or bound to the capillary endothelium.

To identify the cause of the three- to fourfold difference in $^{54}$Mn uptake between blood and saline, a series of perfusion experiments were conducted using different fluids. Greater uptake from saline was not the result of poor brain oxygenation, due to absence of red cells in the saline perfusate, as plasma gave low $K_m$ values comparable to those with whole blood ($p > 0.05$) (Fig. 2). Similarly, reduced influx with whole blood was not due to heparin binding as serum gave values virtually identical to plasma (data not shown). Because dialyzed plasma also gave low $^{54}$Mn uptake (Fig. 2), it was suggested that tracer may be binding to high-molecular mass components. The choroid plexus exhibited a comparable pattern of $^{54}$Mn uptake (Fig. 3). Addition of 5 mM EDTA to the perfusate, which tightly complexes Mn$^{2+}$ (Barfart, 1979), essentially abolished $^{54}$Mn uptake into the CNS.

Plasma protein binding of $^{54}$Mn was assayed by two separate methods. Ultrafiltration on Amicon membranes indicated that >70% of serum $^{54}$Mn was bound to plasma proteins (Table 3). On the other hand, column chromatography on Sephadex suggested that >90% of plasma $^{54}$Mn was free or associated with low-molecular mass components (Fig. 4). Testing appeared to validate the ultrafiltration method as $\sim 85%$ of $^{54}$Mn in protein-free saline readily passed through the membrane and reformation of ultrafiltered plasma provided a free fraction of $\sim 100%$. Based on these results it was suggested that column chromatography may underestimate Mn binding due to tracer dissociation, similar to that pre-

were very similar to those in control animals, suggesting that vascular-bound tracer did not contribute significantly to calculated uptake ($p > 0.05$). In the second experiment, distribution in the brain capillary and parenchymal compartments was measured after perfusion using the "capillary depletion" method of Triguero et al. (1990). By this method, $\sim 70%$ of the brain $^{54}$Mn was associated with the parenchymal frac-
TABLE 2. Distribution of $^{54}$Mn and $^{14}$C-sucrose in vascular pellet and supernatant fractions after 120 s of brain perfusion with physiologic saline

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Distribution volume (ml/g of brain)</th>
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<tr>
<td></td>
<td>Brain homogenate</td>
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<tr>
<td>$^{54}$Mn (total)</td>
<td>0.0351 ± 0.0085</td>
</tr>
<tr>
<td>$^{14}$C-Sucrose</td>
<td>0.0084 ± 0.0007</td>
</tr>
<tr>
<td>$^{54}$Mn (corrected)</td>
<td>0.0266 ± 0.0088</td>
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Values are the mean ± SEM (n = 3). Brains were perfused for 120 s at 1.8 ml/min with physiologic saline containing $^{54}$Mn$^{2+}$ and $^{14}$C-sucrose. At the end of perfusion, brains were removed and tracer distributions were determined using the capillary depletion method of Triguero et al. (1990). Values are expressed as total distribution volumes ($Q_{d}/G$) = (total cpm or dpm per compartment/g of brain tissue weight)/(dpm or cpm/ml of perfusate). $^{54}$Mn (corrected) indicates values that were adjusted for residual intravascular tracer as $^{54}$Mn (ml/g) - $^{14}$C-sucrose (ml/g).

Values are the mean ± SEM (n = 3). Brains were perfused for 120 s at 1.8 ml/min with physiologic saline containing $^{54}$Mn$^{2+}$ and $^{14}$C-sucrose. At the end of perfusion, brains were removed and tracer distributions were determined using the capillary depletion method of Triguero et al. (1990). Values are expressed as total distribution volumes ($Q_{d}/G$) = (total cpm or dpm per compartment/g of brain tissue weight)/(dpm or cpm/ml of perfusate). $^{54}$Mn (corrected) indicates values that were adjusted for residual intravascular tracer as $^{54}$Mn (ml/g) - $^{14}$C-sucrose (ml/g).

Previously noted for assessment of Mn binding by electrophoresis (Gibbons et al., 1976).

To determine whether column chromatography measured Mn$^{3+}$ bound tightly to transferrin ($K_{d}$ ~ $10^{-10} M^{-1}$) (Morris et al., 1987), $^{54}$Mn$^{2+}$ was first oxidized to $^{54}$Mn$^{3+}$ using permanganate (Gibbons et al., 1976) and then added to physiologic saline containing 3 g/L of apotransferrin. With this procedure, chromatography demonstrated that >90% of the tracer was associated with the high-molecular mass fraction (Fig. 4C). In contrast, <5% of the tracer distributed in the high-molecular mass zone when tracer was not oxidized before addition to transferrin saline (Fig. 4D). Rechromatography of the high-molecular mass $^{54}$Mn complex demonstrated almost complete recovery in the high-molecular mass zone (>95%). Similar results were obtained using air oxidation of tracer to form $^{54}$Mn$^{3+}$ (data not shown). Thus, column chromatography may provide a measure of $^{54}$Mn$^{3+}$-transferrin in perfusion fluid. Routine chromatography of perfusate $^{54}$Mn after addition of transferrin (3 g/L) demonstrated that the putative $^{54}$Mn$^{3+}$ fraction constituted ≤5% of total tracer in almost all experiments.

Finally, to confirm the effect of plasma protein binding on brain $^{54}$Mn$^{2+}$ uptake, purified proteins that were reported to bind Mn (i.e., transferrin, albumin, and α2-macroglobulin) (Mildvan and Cohn, 1963; Gibbons et al., 1976; Scheuhammer and Cherian, 1985; Davidsson et al., 1989) were obtained and
Numerous studies support the hypothesis that plasma protein binding can influence brain Mn uptake. The exact form and species of Mn that is transported in the brain is uncertain due to oxidation and binding by plasma high-molecular mass and low-molecular mass compounds. The present study is the first to examine brain Mn uptake in vivo in the absence of oxidation and solute binding. The results demonstrate that Mn is readily taken up into brain from protein-free saline with a transfer coefficient of \( \sim 20 \times 10^{-5} \text{ ml/s/g} \). The measured \( K_m \) for Mn exceeds that for Na\(^+\), Cl\(^-\), and Ca\(^2+\) by 10–30-fold (Smith and Rapoport, 1986; Murphy et al., 1988) and is greater than that expected for passive diffusion (Takasato et al., 1984). Such rapid uptake suggests facilitated blood–brain barrier transport by a special channel or carrier mechanism. This finding is consistent with the recent report by Murphy et al. (1991b) of saturable Mn transport at the blood–brain barrier endothelium.

The exact species of Mn that is transported at the blood–brain barrier is uncertain, but it is possible that Mn gains access primarily as the free ion. May et al. (1977) estimated that the majority of non-protein bound Mn exists in plasma as the free ion (60%). Lesser amounts are complexed with HCO\(_3\), citrate (10%), and other low-molecular mass solutes, primarily phosphate and oxalate (5%). Although the exact contribution of each species has not been determined, preliminary experiments suggest that uptake is not mediated predominantly as the HCO\(_3\) complex, as a comparable \( K_a \) value can be obtained for Mn during perfusion with HCO\(_3\)-free buffer (O. Rabin and Q. Smith, unpublished observations). Similarly, the contributions of the citrate and oxalate complexes are likely minimal, at least in the saline perfusion experiments, as these solutes were not added to the saline perfusate buffer.

Several transport systems have been reported that accept Mn as a ligand, including Ca channels (Drapeau and Nachshen, 1984; Narita et al., 1990), the Na/Ca exchanger (Frame and Milanick, 1991), the active Ca uniporter (Gavin et al., 1990), and the Na/Mg antiporter (Gunther et al., 1990). In addition, specific Mn transport systems have been identified in a number of cells, including hepatocytes (Schramm and Brandt, 1986), glia (Wedler et al., 1989; Aschner et al., 1992), and certain bacterial and fungal species (Auling, 1989). Recently, Murphy et al. (1991b) presented evidence for saturable Mn transport at the blood–brain barrier and estimated a \( K_m \) of \( \sim 1 \mu M \), which agrees with values reported for hepatocyte, astrocyte, and bacterial membranes. If such a system exists at the blood–brain barrier, it may also facilitate the brain uptake of other metals. Interestingly, the \( K_m \) for Mn approximates that reported by Bradbury and Deane (1986, 1988; Deane and Bradbury, 1990) for free lead (Pb\(^{2+}\)), which suggests that brain uptake of both metals may be "facilitated." If so, it would be interesting to determine if the same mechanism mediates transfer of both compounds.

The results of the present study also show that Mn binds extensively to plasma proteins and that binding restricts Mn uptake into the CNS. In normal rat plasma, \( >70\% \) of Mn is associated with high-molecular mass proteins, as assayed by ultrafiltration. Purified transferrin, albumin, and \( \alpha_2 \)-macroglobulin each bound Mn significantly with free fractions of \( <35\% \). Binding was far weaker than Mn to transferrin (\( K_a \sim 10^{10} \text{ M}^{-1} \)) (Morris et al., 1987), with estimates for the association constant of Mn to albumin being in the range of \( 0.7–3.0 \times 10^4 \text{ M}^{-1} \) (Mildvan and Cohn, 1963; Hirsch-Kolb et al., 1970; Chapman et al., 1973). Calculation of plasma-free Mn fraction based on reported binding constants for albumin predicts a value of \( <30\% \). From the present data it is impossible to tell which proteins contribute significantly in vivo in the presence of other metals and binding agents. However, from the data of Gibbons et al. (1976) and Scheuhammer and Cherian (1985), it may be reasonable to propose \( \alpha_2 \)-macroglobulin as a primary candidate.
FIG. 4. Gel chromatography elution profiles for $^{54}$Mn on a Sephadex G-75 column with phosphate buffer as eluate. $^{54}$Mn was applied to the column in physiologic perfusion saline (A), in rat serum (B), and in perfusion saline containing 3 g/L of transferrin either with (C) or without (D) prior oxidation of $^{54}$Mn$^{2+}$ to $^{54}$Mn$^{3+}$. Low-molecular mass solutes (<10 kDa: $[^{14}$C$]$sucrose, $[^{3}$H$]$inulin) ran in fractions 15–30, whereas high-molecular mass solutes (blue dextran, $[^{3}$H$]$dextran, >40–80 kDa) ran in fractions 0–10. Fraction counting was begun with the start of elution of blue dextran, which was added to every sample. Calculations were based on total recovered radioactivity. See Materials and Methods for exact composition of physiologic perfusion buffer.

Deane, 1986; Smith and Rapoport, 1986; Murphy et al., 1988; Zheng et al., 1991). This difference may reflect in part enhanced binding of Mn$^{2+}$ to choroid plexus epithelial cells and extracellular matrix. In addition, it may reflect enhanced transport and accumulation as the choroid plexus uptake of some metals can be reduced by transport inhibitors (Zheng et al., 1991). Though Mn$^{2+}$ uptake into choroid plexus is quite rapid, it is unlikely that the plexus is a primary route for Mn influx into the CNS as the normal $K_{in}$ for $^{54}$Mn$^{2+}$ transfer across the choroid plexus into CSF is only a fraction of that for brain tissue (Zheng et al., 1991b). The exact role that the choroid plexus plays in the regulation of CNS Mn is uncertain. Murphy et al. (1991b) found no evidence for saturable Mn$^{2+}$ uptake into CSF, yet choroid plexus may still play a protective role by actively sequestering and secreting Mn from the CNS (Friedheim et al., 1983; Zheng et al., 1991).

Finally, this study prepares the way for more detailed investigations of Mn transport across the blood–brain barrier using the perfusion method. Preliminary evidence indicates that brain Mn$^{2+}$ uptake is critically influenced by Mn$^{2+}$ concentration, pH, and the competition of other metal cations (O. Rabin and Q. Smith, unpublished observations). Further, it will be possible to clearly distinguish the separate roles of Mn$^{2+}$ and Mn$^{3+}$ in overall Mn transport into the CNS. Some investigators suggest that Mn$^{3+}$ transferrin is the primary pathway, whereas others suggest that Mn$^{2+}$ has an important role. The results will aid our knowledge of Mn activity and toxicity within the CNS, especially because Mn$^{2+}$ has been found to markedly influence brain function.
FIG. 5. Transfer coefficients for brain uptake of $^{54}Mn^{2+}$ from physiologic saline with added albumin (rat, 28 g/L), transferrin (human, 3 g/L), and/or $\alpha_{2}$-macroglobulin (human, 2 g/L). Concentrations of purified proteins were chosen to approximate the normal plasma level. Bars represent the means ($\pm$ SEM) for $n = 4$–7 animals. All values in protein-containing fluid differed significantly from those in protein-free saline ($p < 0.05$). ALB, albumin; TRANS, transferrin; MACRO, $\alpha_{2}$-macroglobulin; MIX, mixture of the three proteins. Bars represent data for parietal cortex, hippocampus, and caudate nucleus. Similar trends were seen in other brain regions. The choroid plexus was not examined.

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