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DELIVERY OF TRANSFERRIN AND IMMUNOGLOBULINS TO THE VENTRICULAR SYSTEM OF THE RAT

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and Methods
- *3.1. Animals*
- *3.2. Experimental procedures*
- *3.3. Immunohistochemistry*

4. Results
- *4.1. Adult rat*
  - *4.1.1. Transferrin receptor*
  - *4.1.2. Transferrin*
  - *4.1.3. OX26*
  - *4.1.4. Non-immune IgG*
- *4.2. P7 rat*
  - *4.2.1. Transferrin receptor*
  - *4.2.2. Transferrin*
  - *4.2.3. OX26*
  - *4.2.4. Non-immune IgG*

5. Discussion
- *5.1. Circulation of transferrin and OX26 in the brain*
- *5.2. Cellular uptake of transferrin and OX26*
- *5.3. Is the cerebral ventricle a suitable site for injecting macromolecules*

6. Acknowledgments
7. References

1. ABSTRACT

Human transferrin, non-immune IgG (Ni-IgG), and anti-transferrin receptor IgG2a antibody (OX26) were injected intracerebroventricularly (icv) into the lateral cerebral ventricle of adult and 7-day postnatal (P7) rats. Brain distributions were detected with immunohistochemistry. In adult rats, within 10 min of injecting 1.25 nmol of human transferrin and Ni-IgG, the proteins were detected intracellularly in the vicinity of the ventricular system, in ependymal cells, neurons and glia. Choroid plexus epithelial cells also were labeled. On the pial surface, the proteins were observed in meninges, and intracellularly in leptomeningeal cells. The proteins were detectable in close association with the ventricular wall and the meninges up to approximately 4 hr after the injection. Neuronal human transferrin-immunoreactivity (IR) was observed in cells in close proximity to the ventricular system and subarachnoid space, e.g., neurons of the medial habenular nucleus, hippocampal cortex, and cerebellar cortex. By 24 hr after injection, these proteins were absent. Injection of 0.03 nmol human transferrin, Ni-IgG, or OX26 resulted in labeling of ependymal cells but not periventricular neurons or glia. In P7 rats, prominent labeling was seen irrespective of the injected molecule or dose. Likewise, the labeling of the neurons and glia distant from the ventricle or the pial surface was, however, much higher than in the adult. The proteins were detectable diffusely in the brain parenchyma even 24 hr after injection. The results are discussed with emphasis on whether icv injection of transferrin and OX26 can be used for targeting transferrin receptor-containing neurons.

2. INTRODUCTION

The blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barriers are responsible for preventing simple diffusion into the brain of circulating macromolecules and hydrophilic drugs. Recent studies indicate that cerebrovascular permeability can be improved by trapping such molecules in liposomes, followed by conjugation to monoclonal antibodies generated against peptides expressed by brain capillary endothelial cells (BCECs) (29,30). Examples of antibodies that can be targeted to the BBB are the anti-transferrin receptor IgG2a antibody (OX26), a monoclonal antibody raised against the transferrin receptor, which is present on BCECs (12,15), and the antibody raised against the insulin receptor (9). There is disagreement as to what extent these molecules can pass through the BBB (14). A recent study indicates that OX26 antibodies do not readily cross the BBB, but probably accumulate in BCECs without being transported further into the brain (22). Nonetheless, a significant fraction of OX26 injected intravenously (iv) can be extracted from CSF, suggesting that OX26 crosses the blood-CSF barrier (22). Immunohistochemical studies after OX26 has been injected iv support this: labeling is detectable only in regions with direct access to the CSF,

e.g., hippocampal cortex neurons and cerebellar Purkinje cells (5,20,22).

Expression of transferrin receptors on neurons probably facilitates the uptake of OX26 by these cells. Hence, icv injection of OX26 could be a method by which drugs conjugated to OX26 target neurons. The major objective of the present study was to examine the neuronal uptake of human transferrin and OX26 following (icv) injection. The uptake of these molecules, which both have affinity for the rat transferrin receptor (18,33), was compared with that of non-immune IgG. Postnatal day 7 (P7) and adult rats were examined because CSF turnover is markedly less at P7 (16,17).

3. MATERIALS AND METHODS

3.1. Animals

Male Wistar rats (n = 24) aged 50 days and weighing approximately 180 g (adults), and P7 suckling rats (n = 24), were used for the study. The 180 g rats were fed on a normal diet containing 70 mg iron/kg. Hematocrit measurements were performed to verify that the rats were not anemic. The P7 rats were fed by nursing dams which, like the male rats, had free access to food and water. The various animal procedures as described in this study were approved by the Danish National Council of Animal Welfare.

3.2. Experimental procedures

The adult and P7 rats were anesthetized with tribromoethanol. The adult rats were attached to a stereotactic frame (Stoelting, Illinois) and injected icv with a Hamilton syringe into the body of the left lateral ventricle using the coordinates of Paxinos and Watson (27): 1.3 mm medial-lateral,-0.8 mm anterior-posterior to bregma and 4.0 mm dorsal-ventral from the dura. The P7 rats were injected on coordinates: 1.5 mm medial-lateral, -0.5 mm anterior-posterior to bregma and 3.0 mm dorsal-ventral from the dura using the stereotaxic frame mounted with an adaptor for neonatal animals (Stoelting, Illinois). The medial-lateral coordinates for the P7 rats were used according to the atlas of Paxinos *et al*. (28). The accuracy of these coordinates has been verified previously by injecting an ink solution (20).

Purified human holo-transferrin (T4132, Sigma, mw. 80 kDa) and non-immune IgG (Ni-IgG) (I5381, Sigma, mw. 150 kDa) were dissolved in 0.1M PBS immediately prior to injection icv of 1.25, 0.3, or 0.03 nmol. Monoclonal mouse anti-rat transferrin receptor IgG2a (clone OX26) (MCA155, Serotec, mw. 150 kDa) was injected icv only at 0.03 nmol. The compounds were injected either as a single bolus of 10µl lasting approximately 10 sec or as a continuous injection for 7.5 min where 2.5 µl was injected 4 times at intervals of 2.5 min (0 min; 2.5 min; 5 min; 7.5 min). This longer injection paradigm was used because it has been shown that continuous infusion of solutes into ventricular CSF leads to a pronounced labeling of the brain parenchyma (29). The cannula was left *in situ* in the lateral ventricle for 5 min after the last injection to prevent backward diffusion at the injection site.

At various times (10 min, 4 hr, 24 hr) after a single bolus injection (n = 3 per time point) or a continuous injection (10 min, 30 min) (n = 3 per time point), the rats were deeply anesthetized by an intraperitoneal injection with Brietal (Lilly) and transcardially perfused via the left ventricle, first with heparin (15,000 IE/l) in 0.1 M potassium phosphate-buffered saline (KPBS) (pH 7.4) for half a minute, and then with 4 % paraformaldehyde in 0.1 M KPBS (pH 7.4) for 15 min. The brains were removed, post-fixed for 4 hr at room temperature with 4% paraformaldehyde and immersed in 30 % sucrose-KPBS for 48 hr. The brains were cut by cryostat into serial, coronal 30 µm sections and processed as free-floating sections.

3.3. Immunohistochemistry

The distribution of proteins in the brain was examined using immunohistochemistry. Transferrin was detected using monoclonal mouse anti-human transferrin (Biogenesis, UK) diluted 1:100, followed by biotinylated goat-anti-mouse IgG absorbed with rat immunoglobulins (Sigma) diluted 1:100, followed by stepwise incubation with horseradish peroxidase (HRP)-streptavidin-biotin complex (Vector) and diaminobenzidine (DAB) (19). OX26 and Ni-IgG were also detected by biotinylated goat-anti-mouse IgG absorbed with rat immunoglobulins followed by stepwise incubation with HRP-streptavidin-biotin complex and DAB. The potential cross-reactivity with endogenous (rat) IgG was examined by applying the anti-mouse antibody to sections of uninjected rat brains. To evaluate the extent of non-specific binding of the secondary antibodies, non-immune goat serum was used as a substitute for the primary antibody, and the immunohistochemical procedures were performed as described above. Under such circumstances, immunolabeling was not observed.

The distribution of OX26 in regions in close proximity to the ventricular system and subarachnoid space was correlated with that of the endogenous transferrin receptor, which was also visualized using immunohistochemistry (24).

4. RESULTS

4.1. Adult rat brain

4.1.1. Transferrin receptor

Transferrin receptor-immunoreactivity (IR) in the adult brain was identical to that recently described (23,24). In brief, transferrin receptor-IR was observed in brain capillary endothelium and choroid plexus epithelium regardless of the age of the animals studied. Immunoreactive BCECs were observed throughout the CNS (Figures 1A,C-E), with the exception of those situated within circumventricular organs. Transferrin receptor-IR was never detected in the ependymal cells (Figures 1C-D). Transferrin receptor-IR was not observed in astrocytes, oligodendrocytes or ramified microglial cells. Neuronal

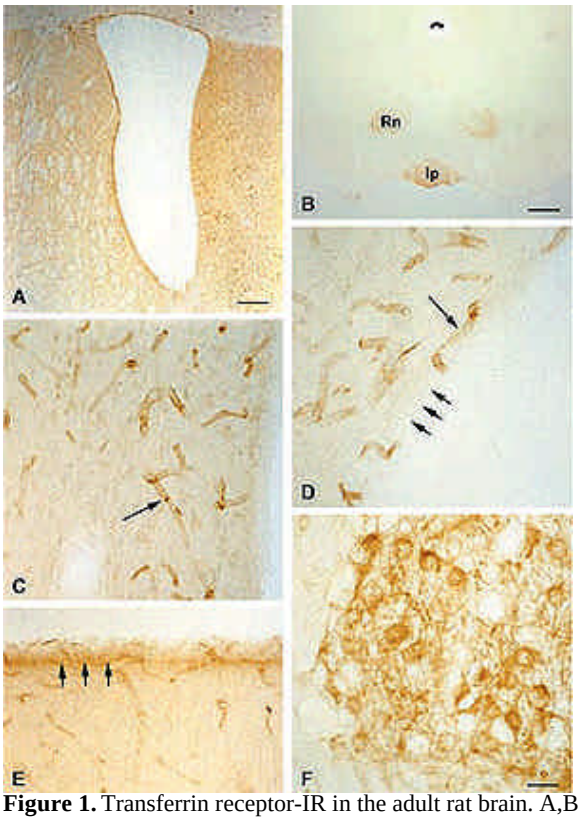

Figure 1. Transferrin receptor-IR in the adult rat brain. A,B Sections of the forebrain at the level of the lateral ventricle (LV) (A) and the mesencephalon (B) shown at low-power magnification. A, Labeling is seen in BCECs of both grey and white matter throughout this section. B, At this magnification, labeling is mainly seen in neuronal nuclei such as the red nucleus (Rn) and interpeduncular nucleus (Ip). The asterisk identifies the cerebral aqueduct. C,D, Labeling of BCECs (large arrows) of the grey matter shown at high magnification. Labeling is not seen in ependymal cells (small arrows). CA, the cerebral aqueduct. E, Labeled BCECs at the cerebral cortex near the pial surface. Labeling is also seen in pial cells (small arrows). F, Labeled cells of the red nucleus with morphology corresponding to neurons. Scale bars: A = 600 mm; B = 1,5 mm; C-F = 40 mm

transferrin receptor-IR was consistently found in grey matter regions of the adult rat brain (Figures 1B & 1F).

4.1.2. Human transferrin

The presence of human transferrin-IR appeared in a dose dependent manner, with the highest dose of transferrin clearly revealing the highest labeling of the brain tissue. The illustrations representing human transferrin-IR were all prepared from sections of brains injected with the highest concentration of transferrin. Following a single bolus injection, human transferrin was detectable in close association with the ventricular wall and the meninges up to approximately 4 hr post-injection (Figure 2). The most pronounced immunoreactivity was observed at 10 min post-injection. Specifically, immunoreactivity was detected intracellularly in cells in the vicinity of the ventricular system, mainly those of the injected lateral ventricle (Figures 2A-D). Cellular accumulation of human transferrin occurred in ependymal cells, neurons and glia. Labeling was also seen in choroid plexus epithelial cells, appearing most strongly in the injected side (Figure 2E). By 4 hr, the number of cells containing human transferrin had diminished and only a few positively labeled cells of the periventricular zone were observed. By 24 hr post-injection, human transferrin-IR was almost completely absent in the brain.

On the pial surface, human transferrin-IR was observed in meninges and intracellularly in leptomeningeal cells until 4 hr post-injection (Figure 2F). The underlying brain parenchyma displayed only weak immunoreactivity, indicating that most of the human transferrin in the subarachnoid space was prevented from accessing the brain parenchyma by the pia-arachnoid barrier (Figure 2F). Human transferrin-IR at the pial surface was found in association with major vessels of the brain (Figure 2F). By contrast, human transferrin was not observed in BCECs.

Neuronal human transferrin-IR was observed in cells in close proximity to the ventricular system and subarachnoid space, e.g., neurons of the medial habenular nucleus (Figure 3A), hippocampal cortex, and cerebellar cortex (Figure 3B). In these regions, human transferrin-IR was also seen outside the neuronal perikarya in the region near the ventricular surface and subarachnoid space, whereas it was virtually absent further away from these surfaces (Figures 2A, 2C, 3A). In the cerebral cortex, where the extracellular fluid does not directly communicate with the ventricular system (Morgan and Moos, 2002), human transferrin-IR was observed in virtually all neurons of the cortical layers in the injected hemisphere, most prominently in those layers near the ventricle (Figure 3C). Neuronal nuclei with a high expression of transferrin receptors, e.g., the red nucleus and cranial nerve nuclei, did not contain human transferrin-IR. In circumventricular organs, human transferrin-IR was not observed in BCECs. There was, however, prominent human transferrin-IR labeling in neuronal fibers projecting to these organs (Figure 3D). After the continuous injection, no differences were detected when using different concentrations of human transferrin as compared to when the single bolus injection paradigm was employed (not shown).

4.1.3. OX26

The OX26 antibody was used only at the concentration provided by the vendor (0.03 nmol). At this concentration, it labeled the periventricular regions in a pattern similar to that of human transferrin injected at the same concentration. The OX26 antibody labeled ependymal cells (Figure 4A-B) but not neurons and glia in the periventricular region and superficial regions of the brain near the subarachnoid space. No differences were observed between rats injected with a single bolus or a continuous infusion.

4.1.4. Ni-IgG2a

Ni-IgG2a labeled the ependyma, neurons and glia in a pattern similar to that of human transferrin or OX26

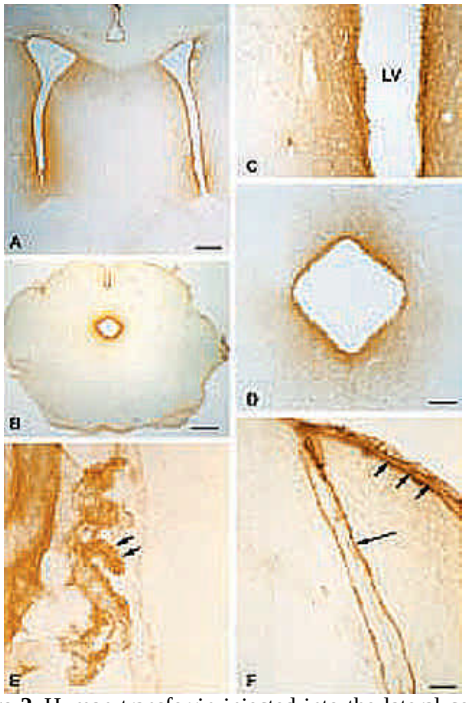

Figure 2. Human transferrin injected into the lateral cerebral ventricle of the adult rat brain. Single injection and post-injection interval at 10 min. A,B, Sections of the forebrain at the level of the lateral ventricle (A) and the mesencephalon (B) shown at low-power magnification. A, Labeling is mainly seen in the periventricular region of the injected ventricle marked with an asterisk. B, In the mesencephalon, labeling is seen around the cerebral aqueduct and at the pial surface. Note the virtual absence of human transferrin-IR in regions further away from the ventricular system or the subarachnoid space. C,D, The injected ventricle (LV) (C) and the cerebral aqueduct (D) shown at higher magnification. Transferrin-IR is prominent in cells of the ependyma, and neurons and glia in the vicinity of the injected ventricle or cerebral aqueduct. E, Section showing the lateral ventricle of the non-injected side. Transferrin is seen in epithelial cells of the choroid plexus (small arrows). F, The pial surface at the level of the superior colliculus. Labeling is seen in cells of the meninges (small arrows), perivascularly (large arrow), and weakly in the remaining parenchyma. Scale bars: A = 300 mm; B = 1,5 mm; C, E-F = 80 mm; D = 200 mm

(not shown). Differences were not observed between rats injected with a single bolus or continuous infusion.

4.2. P7 rat brain

4.2.1. Transferrin receptor

Transferrin receptor-IR in the developing rat brain has been dealt with before (23,24), and the distribution of transferrin receptor-IR obtained in the present study was identical to that previously described. In brief, transferrin receptor-IR was present on BCECs and choroid plexus epithelial cells in a pattern similar to that seen in the adult brain. There was no transferrin receptor-IR in major glia or ependyma. In contrast to the adult brain, transferrin receptors were absent on neurons of the developing rat brain.

4.2.2. Human transferrin

Prominent labeling was seen in the brain parenchyma of P7 rats irrespective of the dose, thereby giving the impression of a more uniform distribution post-icv injection than could be seen in the adult brain (Figures 5A-C). As in the adult brain, labeling of the various nuclei of the brain stem was not significantly higher than that of the nearby parenchyma (Figure 5D). General labeling of brain parenchyma more distant from the ventricle or the pial surface, however, was much more prominent in the P7 brain than in that of adult rats. Human transferrin was detectable diffusely in the P7 brain parenchyma even 24 hr post-injection (Figure 5). Cellular distribution was observed in virtually all cells of the labeled areas.

4.2.3. OX26

The OX26 antibody labeled neurons and glia to the same extent as human transferrin injected at the same concentration. Hence, OX26 injected into the ventricular system penetrated more deeply into the P7 brain than into the adult brain. No differences were observed between rats injected with a single bolus injection or continuous infusion.

4.2.4. Ni-IgG2a

Ni-IgG2a labeled neurons and glia corresponded to those labeled with human transferrin and OX26 in the P7 rat brain. Differences were not observed between rats injected with a single bolus injection or continuous infusion.

5. DISCUSSION

The main findings of this study are that, when injected icv, two large exogenous macromolecules with affinity for the endogenous transferrin receptor, human transferrin and OX-26, significantly labeled cells and their projections in the vicinity of the ventricular system, circumventricular organs, and the subarachnoid space of the adult rat brain. The distribution of these macromolecules did not differ from that of Ni-IgG, which suggests that cellular uptake in these regions is not due to transferrin receptor-mediated uptake. This notion is supported by the fact that human transferrin and OX26 were observed in cells of the ependymal lining and glial cells, which all are devoid of transferrin receptors (24).

Several regions containing transferrin receptor-expressing neurons, e.g. the red nucleus, at the same distance from either ventricular CSF or the pial surface, were unlabeled, suggesting that transferrin and OX26, which are present in the CSF, are unable to enter the deeper parts of the brain. However, regions situated in areas near these surfaces may take up molecules from the CSF and facilitate their transport to deeper areas of the brain; for example, transferrin receptor-containing neurons in the medial habenular nucleus are capable of taking up iron-containing transferrin and transporting iron to the interpeduncular nucleus (20,25). As the only exception to this pattern of uptake, the transferrin receptor, human transferrin and OX26 were detected in cells of the pia mater, which suggests that the transferrin receptor operates to facilitate the uptake of human transferrin and OX26 in

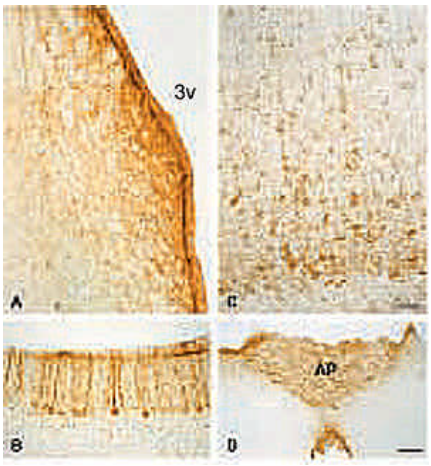

Figure 3. Human transferrin injected into the lateral cerebral ventricle of the adult rat brain. Single injection and post-injection interval of 10 min. A, Section of the diencephalon showing the medial habenular nucleus (MHB), which is found near the third ventricle (3V). Neurons contain human transferrin-IR. The insert demonstrates the neuronal accumulation of human transferrin at a higher magnification. Note the virtual absence of human transferrin-IR in regions further away from the third ventricle. B, The cerebellar cortex with accumulations of human transferrin in Purkinje cells and various cells of the molecular layer, and only little in the internal granular layer. C, Labeled neurons of the neocortex. Such accumulation is only seen in neurons of the injected side suggesting that these neurons take up human transferrin from the lateral ventricle. D, Human transferrin in the area postrema (AP). The accumulation of human transferrin in circumventricular organs is confined to neuronal projections and not the vascular compartment.Scale bars:A =300mm;B-D=50 mm

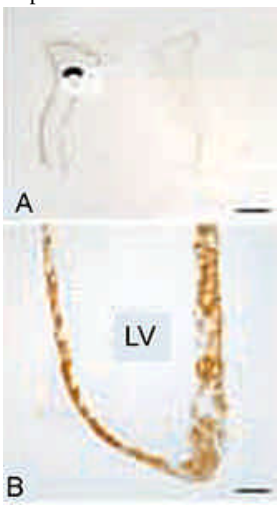

Figure 4. OX26 injected into the lateral cerebral ventricle of the adult rat brain. Continuous infusion and post-injection interval at 10 min. A, Sections of the forebrain at the level of the lateral ventricle shown at low-power magnification. Labeling is mainly seen in the periventricular region of the injected ventricle marked with an asterisk. Note the virtual absence of OX26-IR in regions further away from the ventricular system. B, The injected ventricle (LV) shown at higher magnification. Labeling is confined to the ependymal cell lining. Scale bars: A = 300 mm; B = 80 mm

these cells. These cells also take up iron *in vivo* (20), which they may use for secreting iron-containing transferrin (26).

The brains of P7 rats were much more highly labeled than the adult brains, probably due to a lower rate of CSF turnover at this developmental age which enables transferrin and OX26 to diffuse more deeply into the brain parenchyma (24). In contrast to the adult brain, neurons of the P7 brain have no transferrin receptors (23,24), suggesting that neurons take up human transferrin and OX26 by a non-receptor mediated transport mechanism. Supporting this notion, Ni-IgG revealed a cellular distribution similar to those of human transferrin and OX26.

5.1. Circulation of transferrin and OX26 in the brain

The CSF is thought to consist of brain interstitial fluid mixed with fluid transported through the blood-CSF barrier (6). The brain interstitial fluid contains transferrin that may derive from secretion by oligodendrocytes or transport through the BBB, although the evidence for the latter is weak (21). The choroid plexus is capable of transporting molecules, including transferrin, through the epithelial cells due to the presence of transferrin receptors on their surface (24). The choroid plexuses of the lateral and third ventricles also secrete transferrin, which is synthesized within the epithelial cells (4). The presence of transferrin receptors on choroid plexus epithelial cells (24) also suggests that OX26 can enter the CSF after facilitated uptake, which may contribute to the higher Vd values for OX26 than those of non-immune IgG in CSF after iv injection (22). Hence, transferrin and OX26 injected iv can be found in the CSF, which may be explained by transport across either the BBB or the blood-CSF barriers (11,22). However, the fact that OX26 is detected only on neurons close to the ventricular system and not on neurons in close proximity to BCECs elsewhere in the brain clearly suggests that transport across the blood-CSF barrier is the major source of OX26 in CSF.

The high turnover of transferrin in CSF in the adult brain suggests that transferrin is rapidly washed out of the brain (2,20). The fact that human transferrin remained virtually confined to cells near the ventricular system supports this. Consequently, transferrin in ventricular CSF probably is capable of donating iron only to cells situated in close proximity to the ventricular walls, circumventricular organs, and to meningeal cells of the pia-arachnoid, whereas regions further from the ventricles and the pial surface do not receive iron from CSF (except when iron is transported in projecting neurons as mentioned above). Transferrin in CSF may also be important for neutralization and export out of the brain of divalent cations other than iron.

The neocortex is thought not to drain immediately to ventricular CSF (24). Nonetheless, human transferrin and OX26 were observed in neurons of the neocortex. These proteins accumulated mainly in neurons ipsilateral to the injected side. This implies diffusion from the injected lateral ventricle into the intercellular space of

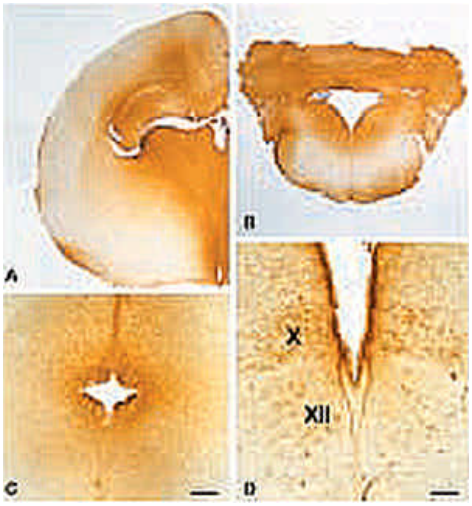

Figure 5. Human transferrin injected into the lateral cerebral ventricle of the P7 rat brain. Single injection and post-injection interval of 24 hr. A,B, Sections of the forebrain at the level of the injected lateral ventricle (A) and the medulla oblongata (B) shown at low-power magnification. A, Labeling protrudes from the ventricle to the over-laying neocortex and many diencephalic regions. B, In the medulla oblongata, labeling extends from the periventricular and subarachnoid surfaces deeper into the brain to include the entire cerebellum and the majority of the medulla oblongata. C,D, The mesencephalon (C) and medulla oblongata (D) of the injected P7 brain shown at high magnification. C, Human transferrin-IR extends from the periventricular zone deeply into the parenchyma at this age (compare with figure 2D that shows the mesencephalon of the adult rat brain injected with an identical dose). D, in the medulla oblongata, labeling is seen in many cranial nerve nuclei. This illustration depicts human transferrin-IR in the dorsal vagal nucleus (X) and the hypoglossal nucleus (XII). Scale bars: A-B = 600 mm; C= 80 mm; D = 50 mm

the overlying corpus callosum, followed by uptake and retrograde axonal transport in neocortical neurons.

5.2. Cellular uptake of transferrin and OX26

Although large macromolecules such as albumin, ferritin, HRP, and human transferrin injected icv penetrate only the lining membranes of ventricles and meningeal surfaces, and diffuse very slowly through the brain parenchyma in the rat (1,7,20, the present study), a similar injection paradigm in the adult cat revealed a much more profound distribution 10 min after injecting 40 mg of HRP icv (29). HRP was distributed through the perivascular space (Virchow Robin space) of large penetrating vessels. It also labeled BCECs widely throughout the brain, including those situated at a distance from CSF. This principal difference in the distribution of HRP injected into rodents and into large animals such as the cat may be caused by stronger pulsation of arteries penetrating into the brain of large animals, creating a driving force that leads to perivascular microcirculation of CSF and interstitial fluid. Ultrastructurally, HRP was confined to the perivascular space of these vessels (29). Although it remains to be proven, it is likely that the basal membranes of the major vessels and BCECs are continuous, which would indicate that HRP spreads from the Virchow Robin spaces of penetrating arteries more deeply into the brain parenchyma, leading to labeling of the perivascular space of BCECs. Despite this profound labeling of brain vasculature, HRP injection did not result in any labeling of neurons or glia. Thus, HRP is either catabolized by the various cells comprising the vasculature, and is exported by the brain to blood transport, or it diffuses into and is dissolved in brain interstitial fluid and goes back to CSF. Macromolecules with an affinity for the transferrin receptor, such as human transferrin and OX26, might be shown in a large animal also to spread inside the perivascular space deep into the brain, but they probably would not target the transferrin receptor-containing neurons.

With its affinity for the transferrin receptor, OX26 should, in principle, label cells to the same extent as human transferrin when injected in the same dose. The commercially available OX26 used in the present study only labeled the ependymal lining, which was similar to labeling found with human transferrin and non-immune IgG injected in the same doses. In a recent study, OX26, when injected iv, was found to label neurons near the ventricular and subarachnoid spaces in the brain of P15 rats (22). The turnover rate of CSF in the P15 rat is smaller than in the adult; injected OX26 reaches a significantly higher concentration in CSF of the P15 rat (22). CSF concentration of OX26 injected iv is higher than that of non-immune IgG, which is due to receptor-mediated transport through the choroid plexus epithelial cells. This higher concentration may explain a greater occurrence of OX26 than of non-immune IgG in neurons with projections to the CSF (22). However, it cannot be excluded that this higher uptake results from receptor-mediated uptake of OX26. In the present study, human transferrin was detected in the medial habenular nucleus, which is situated in close proximity to the ventricular system. The neurons of this nucleus are among the highest transferrin receptor-expressing neurons in the rat brain, and their receptors may take up the human transferrin and OX26. A recent study investigating the fate of iv injected OX26 conjugated to liposomes containing beta-galactosidase (30) found that neurons of the habenular nucleus expressed beta-galactosidase to a high level, and concluded that the expression was due to receptor-mediated transport across the BBB. CSF was not studied, but beta-galactosidase reached a clearly higher level of expression in periventricular areas than in the rest of the brain. Therefore, it is possible that the claimed BBB transport of OX26 conjugated to liposomes is due to facilitated transport through the blood-CSF barrier.

The high accumulation of macromolecules injected icv in the P7 rats is probably due to a much lower rate of production and turnover of CSF than in the adult, thereby increasing the time available for diffusion of proteins from CSF into the brain parenchyma (23). It may also reflect a greater rate of diffusion of proteins in the brain interstitial fluid in immature than in adult rats. In the young animal, CSF may be a more important source of iron-containing transferrin for the cells of the developing brain since the transfer of transferrin and iron from the

plasma to CSF is more efficient than in the adult (11). In contrast to observations made in the adult rat brain, recent studies in postnatal rat brain failed to detect transferrin receptors on neurons (20,23). Neuronal expression of the transferrin receptor in the developing brain is bi-phasic, with a pronounced expression in the neuroectoderm (10,23) and again from P21 after the growth spurt of the brain terminates (24). The absence of transferrin receptors on neurons of the young postnatal brain is probably due to a down-regulation of the transferrin receptor mRNA because of a ready access to iron at this age (cf. 23).

5.3. Is the cerebral ventricle a suitable site for injecting macromolecules

Even though large macromolecules like HRP, transferrin, and anti-transferrin receptor antibodies may diffuse deeply into the brain of large species, including man, a crucial question is whether injecting such substances into the ventricular system is relevant as the macromolecules probably only distributed to perivascular spaces. The present study does not support the idea of using the ventricular space for injection of macromolecules, except for attempts to target neurons with close relation or direct projection to the CSF or circumventricular organs. When injected icv, the small molecule corticotrophin releasing factor (CRF) having high affinity for its receptor, targets mainly CRF receptor-containing neurons near the ventricular or subarachnoid surfaces (3). Ciliary neurotrophic factor (CNTF) increases survival of motor neurons when introduced iv but not icv, suggesting that the icv route is unsuitable for providing CNTF access to the neurons (13). Other studies which involved injecting various drugs icv also showed that the drugs were mainly confined to the ventricular and subarachnoid surfaces (8,32,34).

The present study does not indicate that an increase in the injection time or concentration would enhance the penetration of macromolecules further into the adult rodent brain, although high doses revealed a more prominent labeling of cells situated near the ventricular system, circumventricular organs or subarachnoid space. In contrast, the developmental age clearly affected the penetration of the macromolecules injected icv.

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Abbreviations: BBB, blood-brain barrier; BCEC, brain capillary endothelial cell; CNTF, ciliary neurotrophic factor; CSF, cerebrospinal fluid; CRF, corticotropin releasing factor; HRP, horseradish peroxidase; icv, intracerebroventricularly; IR, immunoreactivity; iv, intravenously; KPBS, potassium phosphate-buffered saline; P, postnatal; Ni-IgG, non-immune IgG; OX26, anti-transferrin receptor IgG2a

Key Words: Blood-brain barrier, Blood-CSF barrier, Capillary, Choroid plexus, Transferrin receptor, OX26

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