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Utilization of an endogenous cellular transport system for the delivery of therapeutics across the blood–brain barrier

Phillip M. Friden*

Alkermes, Inc., Cambridge, MA, USA

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Abstract

The delivery of non-lipophilic compounds to the brain is severely limited by the presence of the blood–brain barrier (BBB). This barrier exists at the level of the capillary endothelial cells which form the vascular network within the brain. However, brain capillary endothelial cells do express specific receptors on their surface which are essential for the transport of nutrients and signaling molecules from the bloodstream to the brain. These endogenous transport pathways are potential portals for the transport of therapeutic molecules to the brain. The transferrin receptor is expressed at unusually high levels on the surface of brain capillary endothelial cells and is believed to be the primary, if not only, means for the transport of iron into the brain. It has been shown that antibodies that bind to the transferrin receptor selectively target BBB endothelium due to the high levels of this receptor expressed by these cells. These antibodies are then transported across the capillary endothelial cells into the brain and can function as carriers for the delivery of therapeutic peptides and proteins to the CNS. In particular, this drug delivery system has been used to transport nerve growth factor (NGF) across the BBB in a biologically active form and at levels sufficient to prevent the degeneration of NGF-dependent neurons.

Keywords: Drug delivery; Blood–brain barrier; Transferrin receptor; Antibody conjugate; Neurotrophic factors

1. Introduction

Disorders of the brain are one of the leading causes of illness and death. For example, Alzheimer's disease is currently the third-largest medical problem in the United States, afflicting some four million people at an annual cost of over $50 billion. The discovery and development over the last decade of a number of neuroactive peptides and proteins as possible therapeutics for a range of neurological disorders has generated a great deal of excitement in the neuroscience community. A case in point is the family of neurotrophic factors and their potential use as therapeutics for degenerative conditions such as Alzheimer's and Parkinson's disease. However, the clinical utility of peptide and protein therapeutics for the treatment of neurological disorders is severely limited by their inability to efficiently penetrate the blood–brain barrier (BBB) following systemic administration. Access to the brain by non-lipophilic drugs is blocked by the endothelial cells which comprise the vascular network within the brain (reviewed in [1]). These cells, which are joined together by complex tight intercellular junctions, form a continuous barrier against the passive paracellular movement of substances from the blood to the

*Corresponding author at present address: Periodontix, Inc., 313 Pleasant Street, Watertown, MA 02172, USA. Tel: +1-617-9261980; Fax: +1-617-9264776.

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brain. Transcellular flux across brain capillary endothelial cells is also limited due to the very low levels of fluid-phase endocytosis associated with these cells.

The BBB can be physically circumvented by administering drugs directly into the brain. For peptide and protein therapeutics this can be accomplished by a variety of approaches: (i) intracerebroventricular infusion of the compound into the cerebrospinal fluid, (ii) transplantation into the brain of cells which produce the therapeutic compound, (iii) implantation into the brain of a polymer matrix impregnated with the therapeutic compound, or (iv) delivery of the gene encoding the therapeutic to neuronal cells using viral vectors. However, all of these approaches suffer from the significant disadvantage in that they are invasive procedures which require neurosurgical intervention.

If the drug is to be administered non-invasively via the bloodstream, the available pathways to the brain involve either crossing directly through the capillary endothelial cells (transcellular passage) or passing between the cells by opening the tight junctions which link the endothelial cell membranes (paracellular passage). Paracellular passage of compounds across the BBB can be increased through the intracarotid infusion of hyperosmotic saccharide solutions [2]. This procedure, which has been used for the delivery of therapeutics to the brain [3], is thought to cause a physical shrinkage of the endothelial cells which in turn pulls the tight junctions apart, forming pores in the barrier through which drugs can pass. This method, however, has been associated with neurotoxicity in rats [4–6] and seizures in humans [7].

Increased vascular permeability in the brain has also been achieved using RMP-7, an analog of bradykinin. Intracarotid infusion of RMP-7 in rats increased the uptake into brain tumors of a variety of compounds ranging in molecular mass from 100 to 70 000 Da [8]. Uptake of compounds into some non-tumor areas of brain was increased as well. It has been shown in mice that RMP-7 acts by altering the integrity of tight junctions [9].

A potential drawback to all of the methods that involve increasing the permeability of the BBB is a lack specificity in that all compounds in the circulating blood will also gain access to the brain. Another potential limitation is the size of molecules that can pass the BBB in that the 'openings' formed in the barrier may be size selective and therefore limit passage to low molecular weight compounds.

Other approaches for transport of peptides and proteins into the brain involve modification to increase their ability to penetrate the intact BBB via a transcellular route. This can be achieved by increasing the lipophilicity of the protein and thus enhance its ability to penetrate the endothelial cell membrane and cross the BBB. Bodor et al. reported increased delivery to the brain of an enkephalin analog that had been modified to increase both its lipophilicity and resistance to proteolysis [10]. Systemic administration of the modified peptide elicited the expected biological effect in vivo, indicating that intact peptide had crossed the BBB. However, the efficiency of this approach is unclear as less than 0.01% of the injected dose of modified peptide appeared to have penetrated the BBB.

A protein can also be cationized to increase its positive charge density and thus increase its uptake into the brain via absorptive-mediated endocytosis [11]. Cationized immunoglobulin G [12] and rat albumin [13] show enhanced transport into the brain. These two approaches involve fairly non-specific uptake mechanisms and are, therefore, not selective or specific for the BBB. In addition, cationization has been reported to increase the immunogenicity of proteins [14].

An alternative approach to improve uptake and maintain specificity and selectivity for the brain would be to take advantage of a specific transport system present at the BBB. As the brain is physically isolated from the blood, all of the nutrients and other compounds required for normal brain function must be supplied by specific transport mechanisms present on brain capillary endothelial cells. For example, brain capillary endothelial cells have high levels of transporters for D-glucose, amino acids, nucleic acid precursors, short-chain monocarboxylic acids and choline [15]. However, these transport proteins tend to be anchored in the cell membrane and are not involved in the direct transcytosis of their respective ligands; the ligands are internalized via the transporter in the luminal membrane, diffuse across the cytoplasm and are then expelled on the opposite side of the cell by transporters located in the abluminal

membrane. Thus, even if it was possible to modify a protein such that it was internalized via one of these transporters, it is not clear how efficiently it would be transported across the cell and released into the brain.

Receptor-mediated transport systems that normally transport peptides and proteins into the brain could also be exploited for drug delivery to the brain. Receptors present on brain capillary endothelial cells include those for insulin, insulin-like growth factor-I, and II, low density lipoprotein and atrial natriuretic factor [16]. In addition, the transferrin receptor is present in unusually high numbers on brain capillary endothelial cells. The high levels of oxidative metabolism in neuronal cells results in a significant requirement for iron. Iron circulates in the bloodstream as a complex with transferrin and is internalized by cells through the interaction of this complex with transferrin receptors on the cell surface. In regard to the BBB, there is conflicting evidence concerning the transport of transferrin itself across brain capillary endothelial cells, while numerous studies have demonstrated subsequent transport of radiolabeled iron across the BBB [17,18]. While the details of the mechanism remain unclear, these findings indicate that there is a pathway for transporting across the BBB compounds internalized via the transferrin receptor. In contrast to the brain, capillary endothelial cells of non-brain tissues have very low levels of transferrin receptors, presumably because the endothelium in these tissues is sufficiently leaky to allow transferrin to passively enter the extracellular fluid. This uneven distribution of the transferrin receptor could make it an advantageous targeting system for drug delivery to the brain.

The use of transferrin itself as a drug carrier may be limited by the high concentration of transferrin in the blood (on the order of 2–4 mg/ml) which would lead to competition for binding to the receptor. However, some antibody-transferrin fusion proteins have been shown to target the brain and cross the BBB [19]. Alternatively, the transferrin receptor can be targeted for brain drug delivery using antibodies which bind to the receptor. This would avoid some of the potential problems associated with using transferrin itself, especially if the antibodies bind to the receptor at a site distinct from that for transferrin binding. In this scenario, compounds to be delivered to the brain could be linked to the carrier antibody either chemically or, in the case of protein/peptide therapeutics, through genetic engineering to create a bi-functional fusion protein (Fig. 1).

2. Targeting of anti-transferrin receptor antibody to brain vasculature and transport across the BBB

The feasibility of using anti-transferrin receptor (anti-TfR) antibodies for the delivery of drugs to the brain was tested using the anti-rat transferrin receptor antibody OX-26. Immunohistochemical studies were used to confirm that this murine monoclonal antibody preferentially binds to brain capillary endothelial cells following intravenous administration, an observation originally made by Jefferies et al. [20]. This binding in vivo was also found to be a dose-dependent phenomenon [21]. A time course experiment in which animals were sacrificed at various times following i.v. injection of the OX-26 antibody revealed a time-dependent change in the pattern of OX-26 localization: animals sacrificed soon after injection displayed continuous binding of antibody to brain vasculature while animals sacrificed 4–8 h after injection displayed a punctate pattern of antibody localization. These results are consistent with a time-dependent sequestration of the antibody into vesicles within brain capillary endothelial cells, which would indicate internalization of the antibody–receptor complex. The ability of an anti-transferrin receptor antibody to target to brain capillary endothelial cells in vivo and undergo what appears to be receptor-mediated endocytosis was not altered following linkage to diverse 'passenger' molecules such as methotrexate (MTX) or NGF [21,22]. These findings demonstrate that this delivery system can be used to target drug molecules to the brain vasculature. The ability of rat brain capillary endothelial cells to internalize anti-TfR antibodies and antibody-conjugates was confirmed in vitro (M. Moran and P. Friden, unpublished).

The receptor-mediated transcytosis of blood-borne compounds across the BBB consists of the following steps: (i) binding of the blood-borne molecule to its receptor on the luminal surface of the brain capillary endothelial cell, (ii) internalization of occupied

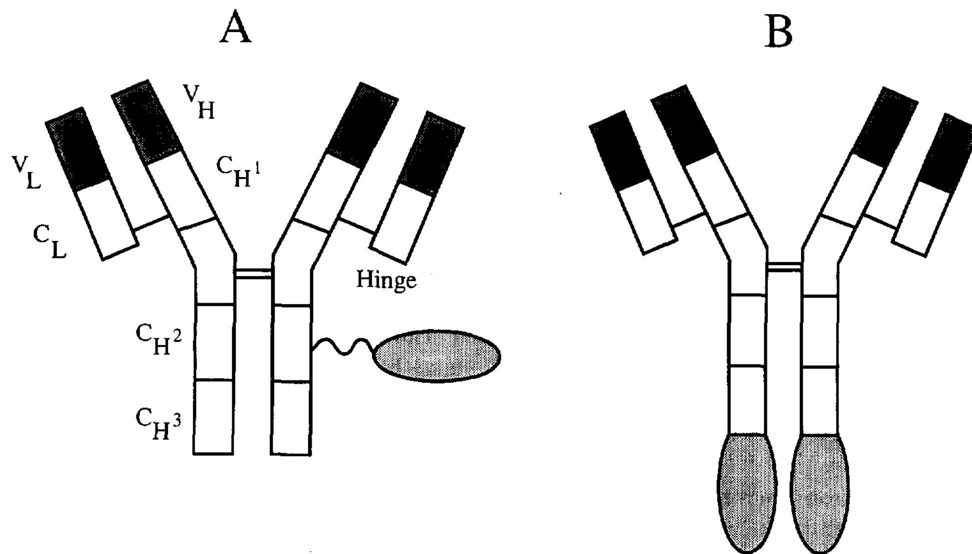

Fig. 1. Strategies for linking therapeutic passenger compounds to carrier antibodies for delivery across the blood–brain barrier. (A) Passenger compounds (stippled ovals) can be chemically linked to the antibody in a random manner through attachment to reactive groups on the protein (e.g. lysine ϵ amino groups) or in a more direct manner through oxidation of the carbohydrate groups present in the C_H2 domain of the IgG molecule. The incorporation of a reducible linkage, such as a disulfide bridge, in the conjugation scheme can allow for the release of the therapeutic compound from the carrier within the brain. (B) Alternatively, peptide and protein therapeutics can potentially be attached to the carrier antibody genetically by linking the DNA sequences encoding the passenger molecule to those encoding the carrier antibody molecule. This chimeric fusion protein would possess both the targeting activity of the antibody and the biological activity of the passenger in a single molecule. (Reproduced from P.M. Friden, Neurosurgery 35 (1994) 294–298. Copyright 1994 by the Congress of Neurological Surgeons).

receptors into endocytic vesicles, (iii) transport of the vesicular contents to the abluminal membrane of the cell, and (iv) fusion of the vesicle with the abluminal endothelial cell membrane and release of the internalized molecules into the brain (Fig. 2; [23]). The immunohistochemistry experiments described above demonstrate that the steps (i) and (ii) occur with the anti-transferrin receptor antibody. To demonstrate that the antibody and antibody-drug conjugates actually cross the BBB, the presence of radiolabeled antibody in the capillary and parenchymal compartments of the brain was followed over time. By examining the temporal distribution of the radiolabeled protein between these fractions of the brain, it is possible to determine whether transcytosis has occurred.

When radiolabeled OX-26 was injected into rats via the tail vein, the majority of the antibody was initially associated with the capillary fraction of the brain, representing protein bound to and internalized by the capillary endothelial cells. Over time, the amount of antibody associated with the capillary fraction of the brain decreased while the amount associated with the parenchyma fraction increased, reaching a peak level of between 0.4 and 0.5% of the injected dose at 24 h after injection (Fig. 3; [21]). This redistribution of radiolabeled antibody as a function of time is consistent with transcytosis of the protein across the BBB. Similar results were obtained with antibody-NGF conjugates in which the 'passenger' compound was specifically radiolabeled ([22]; see Fig. 5).

In addition to the quantitative studies described above, electron microscopic analysis has revealed ultrastructural evidence that conjugates of OX-26 with horseradish peroxidase (HRP) or colloidal gold cross the blood–brain barrier [24,25]. Both of these studies have found the anti-TfR antibody conjugates in endocytic vesicles within the endothelial cells as well as beyond the abluminal membrane, indicating release from these vesicles to allow penetration into the surrounding brain tissue. It is not clear whether

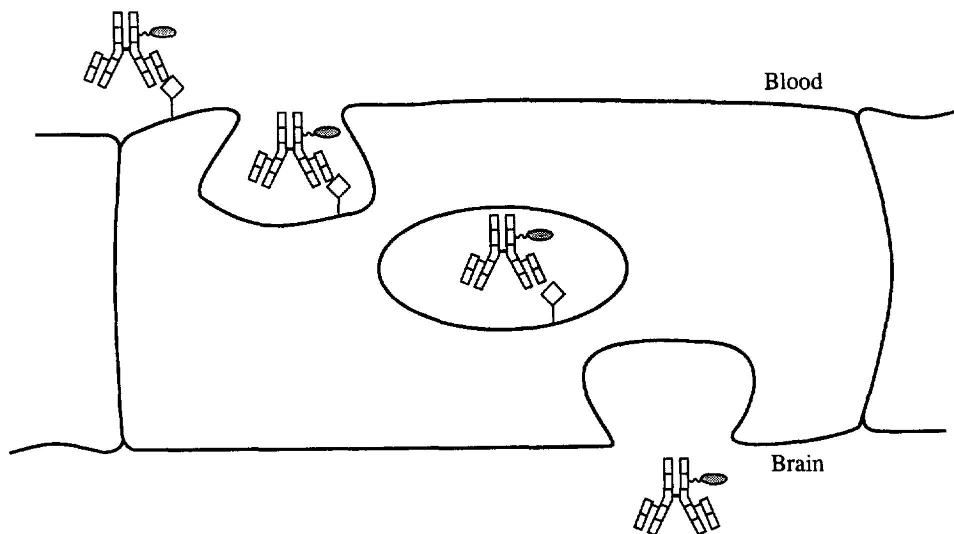

Fig. 2. Transport of anti-transferrin receptor antibody conjugates across the BBB. The antibody conjugates bind to transferrin receptors which are present on the luminal membrane of brain capillary endothelial cells. Through the process of receptor-mediated endocytosis, the antibody conjugates are internalized into vesicular structures within the endothelial cells. The antibody conjugates are eventually transported to and released from the abluminal surface of the capillary endothelial cell and, once released into the brain, diffuse into the parenchyma. (Reproduced from P.M. Friden, Neurosurgery 35 (1994) 294–298. Copyright 1994 by the Congress of Neurological Surgeons).

the conjugates dissociate from the transferrin receptor within the endothelial cell or upon fusion of the vesicles with the abluminal membrane.

3. Preparation and characterization of conjugates with nerve growth factor

While the experiments described above indicate that anti-TfR antibodies can deliver proteins across the BBB, it was critical to show that sufficient levels of active protein could be delivered to the brain to elicit a biological response. Nerve growth factor (NGF), a 26 000 Da homodimer, was chosen as the test molecule for these experiments. This protein is the most thoroughly studied member of a growing family of neurotrophic factors which are required for the differentiation and survival of various populations of neuronal cells [26]. Within the CNS, NGF is required for the survival of cholinergic neurons. When deprived of target-derived NGF, these neurons will degenerate, shrinking in size and losing expression of certain phenotypic markers. This process can be prevented by supplying the neurons with exogenous NGF either through intracerebroventricular infusion or intracranial implantation of NGF-producing cells [27,28]. Degeneration of cholinergic neurons is one of the principle pathologies associated with Alzheimer's disease, suggesting a possible therapeutic role for this neurotrophic factor in the treatment of this disorder [29,30]. The delivery to the brain of this molecule is currently limited to invasive techniques, such as intracerebroventricular administration [31]. The ability to administer this protein systemically would greatly increase its therapeutic potential.

NGF was modified on carboxyl groups and linked to the carrier antibody OX-26, which had been modified on lysine amines, to generate a disulfide-bridged conjugate [22]. The presence of the disulfide bond, which is susceptible to reduction, allows for the potential release of NGF from the carrier within the brain. The biological activity of the NGF following conjugation was measured by examining its ability to stimulate both the differentiation of PC-12 cells from an endocrine to a neuronal phenotype (Fig. 4; [22]) and the out-growth of fibers from explanted sympathetic ganglion [32]. In both assays the biological activity of the NGF was shown to be

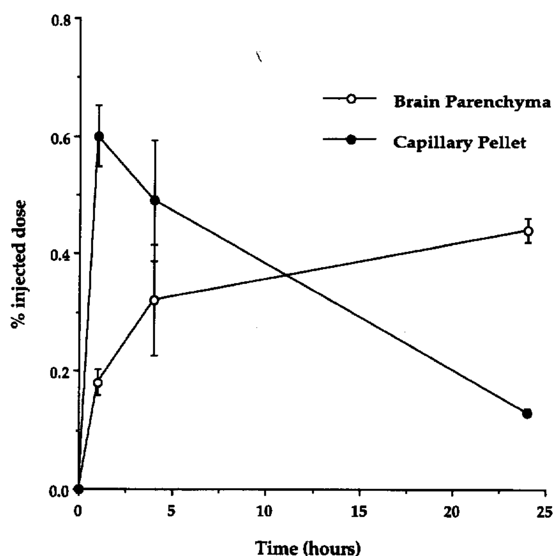

Fig. 3. Time-dependent changes in the disposition of ^{3}H-labeled OX-26 between brain parenchyma and vasculature. Capillary depletion was performed on homogenates prepared from brains taken from animals at 1, 4 and 24 h post-injection of radiolabeled OX-26. The percent injected dose per brain of antibody in the parenchyma fraction and vascular pellet are shown. Similar results were obtained using ^{14}C-labeled OX-26 (data not shown). The values shown are means±S.E.M. (n=3 rats per time point). These results are representative of other studies that have been done. (Reproduced from Friden et al. [21]).

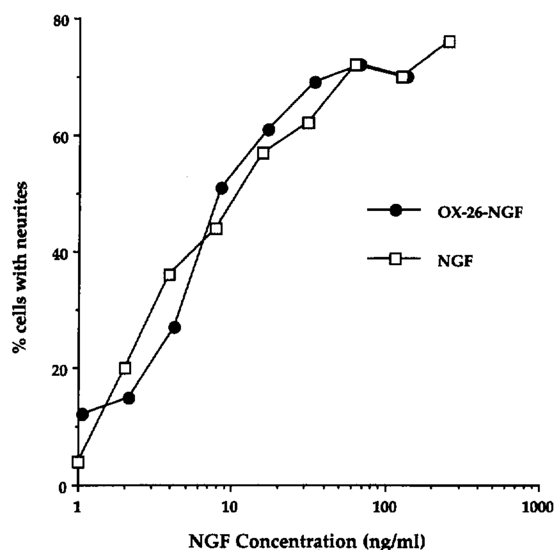

Fig. 4. In vitro bioactivity of antibody NGF conjugates. The neurite outgrowth response of PC-12 cells treated with either unmodified NGF (□) or OX-26-NGF conjugate (●) was examined over a range of doses. The response was expressed as percent of cells extending neurites as a function of NGF dose. (Reproduced from Friden et al. [22]. Copyright 1993 by the AAAS.)

maintained at levels equivalent to that for unmodified NGF.

Capillary depletion experiments were performed using conjugate prepared using radiolabeled NGF [22]. As shown in Fig. 5, the radiolabeled NGF conjugate initially accumulates in the capillaries, followed by accumulation in the neural tissue which is coincident with a loss of material from the capillaries. This temporal redistribution of the labeled NGF is similar to that observed with the OX-26 antibody alone and demonstrates delivery of conjugated NGF across the BBB. In contrast, unconjugated NGF does not accumulate in the capillaries although some material does appear to accumulate in the parenchyma. This could represent uptake of degraded NGF into the brain as the integrity of the labeled molecule was not determined following uptake. Passage of radiolabeled NGF across the BBB has also been reported by Poduslo et al. [33].

4. In vivo efficacy studies with NGF conjugates: Ocular implant model

The ocular implant model was chosen to initially test the ability of anti-TfR antibodies to deliver physiologically relevant levels of NGF across the BBB. This model involves implanting medial septal nucleus tissue, which is rich in NGF-responsive cholinergic neurons, from fetal brain into the anterior chamber of the eye of an adult host [34]. This model allows a study of the effects of NGF on the developing cholinergic neurons in the septum in isolation from other CNS influences, including endogenous NGF. Moreover, the ability to non-invasively monitor the size of the tissue grafts, which is indicative of cell loss or survival, by observations through the eye of the host animal is advantageous in that it allows multiple measurements to be made over time from a single animal.

For these experiments, 2 mm^3 pieces of medial septal nucleus were dissected from rat fetuses at gestational day E18 and bilaterally grafted to the anterior chamber of the eye of adult rats [22].

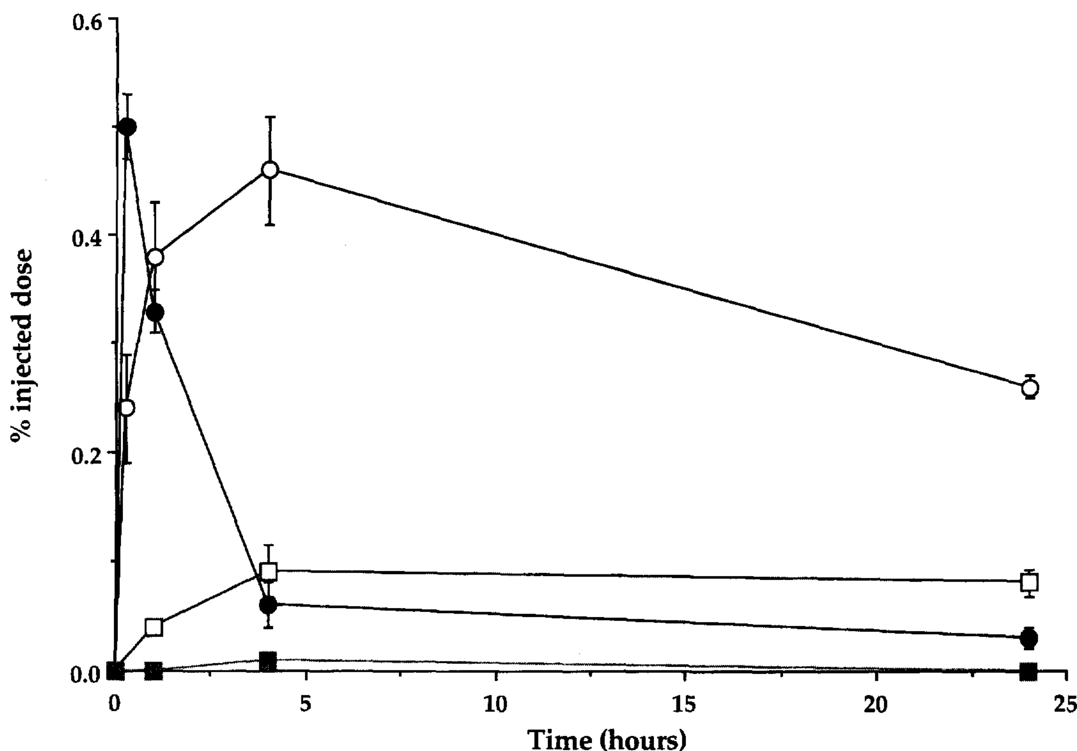

Fig. 5. Enhanced delivery of conjugated NGF across the blood–brain barrier. Radiolabeled NGF, either as an OX-26-NGF conjugate or as free protein, was injected into rats intravenously. Capillary depletion was performed on brains taken from animals at various times post injection. Results are expressed as the percent of the injected dose per brain in either the parenchyma or capillary fraction and shown as means±S.E.M. ($n=3$ animals per time point). ○, OX-26-NGF in brain parenchyma; ●, OX-26-NGF in brain capillaries; □, NGF in brain parenchyma; ■, NGF in brain capillaries. (Reproduced from Friden et al. [22]. Copyright 1993 by the AAAS.)

Treatment was not begun until 2 weeks after grafting to allow vascularization of the implants and reformation of the BBB. The integrity of the barrier was confirmed at this time by dye exclusion studies. The initial dosage regimen involved biweekly i.v. bolus injections of conjugate or controls (dose equivalent to ~6 μg NGF) over a period of 6–8 weeks. During this time period, the effect of the conjugate-derived NGF on the growth of the tissue implants was monitored.

Administration i.v. of antibody alone, NGF alone or saline had no effect on the survival of the ocular implants as determined by changes in graft size (Fig. 6; [22]). However, i.v. administration of the OX-26-NGF conjugate had a significant effect on the growth of the septal transplants, suggesting that the NGF was delivered intact across the BBB of the implant and stimulated the survival of cholinergic neurons. The effect of the conjugate on the septal grafts was also found to be dose dependent [35]. That this result was due to a stimulatory effect of NGF on the target cholinergic neurons was confirmed using choline acetyltransferase (ChAT)-immunostaining to identify cholinergic cells in sections prepared from the transplants at the completion of the dosing period. In these experiments, very few ChAT-positive cells were detected in the control-treated grafts while many ChAT-immunoreactive neurons were observed in the conjugate-treated grafts (ratio of ~1:4). In addition, the effect of the conjugate-delivered NGF on graft size and cholinergic neuron survival was found to be persistent in that the observed increase in graft size and number of ChAT-immunoreactive neurons was maintained 5 months following the cessation of conjugate treatment [32].

To examine the effect of the NGF conjugate on

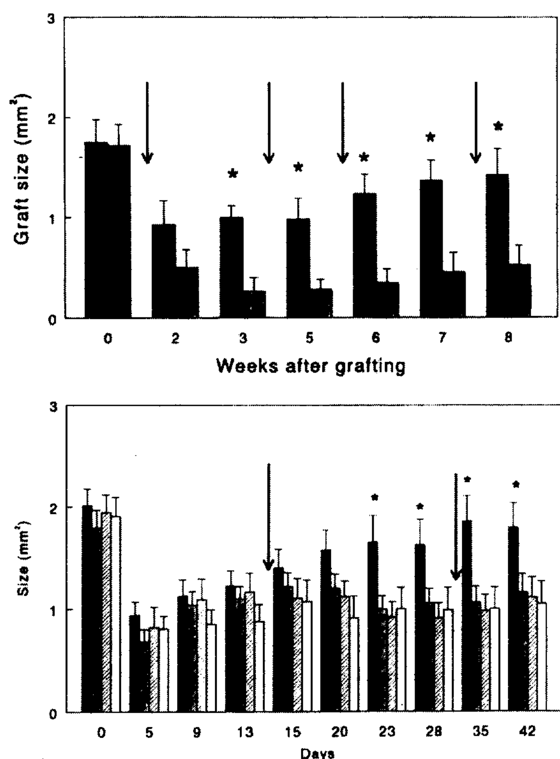

Fig. 6. The intraocular growth of medial forebrain transplants from time of transplantation (T=0). The upper graph shows the sizes of the grafts in the first experimental series and the lower graph shows the sizes of the grafts in the second series. OX-26-NGF conjugate treatment is shown by the solid bar and OX-26 administration alone is shown by the narrow crosshatch bar in both series. Peripheral injection of an equivalent dose of NGF and peripheral injection of an equivalent volume of saline are shown by wide crosshatch and stippled bars, respectively, in the second series, *P<0.05. (Reproduced from Friden et al. [22]. Copyright 1993 by the AAAS.)

mature brain tissue, fetal transplants were allowed to mature for 16 months following transplantation before treatment. The NGF conjugate or OX-26 antibody alone was then administered once every 2 weeks until the transplants were 24 months of age [35]. Analysis of the transplants following sacrifice of the animals revealed that the cholinergic neurons in the NGF conjugate-treated animals were significantly larger, had more processes and stained more intensely for ChAT than the controls. This result demonstrates that both fetal and mature cholinergic neurons respond to the conjugate-delivered NGF and has implications for the treatment of neurodegenerative disorders such as Alzheimer's disease.

5. In vivo efficacy studies with NGF conjugates: excitotoxic lesion model

To follow-up on the studies using transplanted brain tissue, a model was sought in which degeneration of neurons could be created in situ with minimal perturbation to the brain tissue and BBB. Several studies have demonstrated that intracerebral administration of NGF prevents the degeneration of cholinergic striatal interneurons following intrastriatal injections of excitotoxins such as quinolinic acid [36–39]. Direct administration of quinolinic acid to the striatum causes a pattern of cellular degeneration that is similar to that seen in Huntington's disease. To determine if NGF delivered non-invasively could also prevent neuronal cell loss following treatment with quinolinic acid, the anti-TfR antibody carrier was used to deliver NGF to the brains of animals with striatal excitotoxic lesions.

Quinolinic acid was administered unilaterally directly into the striatum of adult rats. These animals were treated for 2 consecutive days prior to the lesion, the day of the lesion and then every other day thereafter for 2 weeks [40]. The treatment groups consisted of OX-26-NGF conjugate (20 μg NGF/injection), a mixture of OX-26 and NGF identical in composition to the conjugate and vehicle.

Stained brain sections revealed that all animals displayed a discernible excitotoxic lesion localized in the striatum. The mean percent difference of NGF responsive ChAT-positive cells between injected and non-injected striata (i.e. loss of cells) was 38% for vehicle, 43% for the co-mix control and 24% for the OX-26-NGF conjugate. The NGF conjugate did not protect somatostatin- and NADPH-diaphorase-containing neurons from the striatal quinolinic acid injections (Table 1). Rats treated with the OX-26-NGF conjugate displayed a 50% loss of somatostatin-immunoreactive neurons while control rats in the mixture and vehicle groups displayed 47% and 43% losses, respectively. Similarly, rats treated with the OX-26-NGF conjugate displayed a 43% loss of NADPH-diaphorase-containing neurons, while control rats in the mixture and vehicle groups displayed

Table 1
Striatal neuron loss following quinolinic acid lesions: effect of OX26-NGF conjugate

Neuron type	OX26-NGF conjugate			OX26/NGF mixture			Vehicle		
	No. of neurons		%Loss	No. of neurons		%Loss	No. of neurons		%Loss
	Lesion	Intact		Lesion	Intact		Lesion	Intact	
ChAT	540.7±53.9	714.3±74.5	24.3**	388.2±62.5	679.1±83.4	42.8	351.4±67.1	573.3±87.4	38.7
Somatostain	312.7±24.6	625.6±29.8	50.0	343.2±26.3	640.0±29.7	46.4	336.3±18.5	586.8±17.7	42.7
NADPH	465.3±43.3	802.2±34.9	42.0	411.1±73.7	825.3±56.5	49.8	438.8±20.8	742.9±47.9	41.1

Values are mean number of neurons±S.E.M.; % loss is relative to the intact side.
$^{**}P<0.01$ relative to OX26/NGF mixture or vehicle control groups.
Reproduced from Kordower et al. [40].

52% and 40% losses, respectively. This result is consistent with the fact that cholinergic striatal interneurons express the high affinity NGF receptor (trkA) and therefore respond to NGF while somatostatin and NADPH-diaphorase-containing neurons do not [41,42]. Taken together, these results provide the first evidence that intravenous administration of the OX-26-NGF conjugate supports the viability of degenerating neurons in a rodent model of Huntington's disease.

6. Quantitation of the delivery of intact NGF to the brain

To specifically measure the amount of NGF delivered to the brain via the OX-26 carrier antibody, NGF molecules were chemically tagged with biotin prior to conjugation so that they could be distinguished from endogenous NGF in brain tissue extracts using an avidin/biotin/anti-NGF antibody-based detection method [40]. In order to get a positive signal in this assay, the NGF epitope recognized by the anti-NGF antibody must be intact and physically linked to the biotin molecule. As a result, this approach allows degraded NGF to be distinguished from intact NGF, a major limitation of the radiolabeled tracer experiments described earlier. A dose-dependent increase in the amount of biotinylated NGF recovered in the brain was seen following injection of an OX-26-biotinylated NGF conjugate to normal rats (Fig. 7; [40]). In contrast, negligible levels of biotinylated NGF were recovered from the brain following intravenous administration of unconjugated biotinylated NGF. This difference was greater than 20-fold at the 10 and 20 μg doses, clearly demonstrating enhanced uptake into the brain of NGF linked to the carrier antibody.

7. In vivo efficacy studies with vasoactive intestinal peptide

The ability of an anti-transferrin receptor antibody

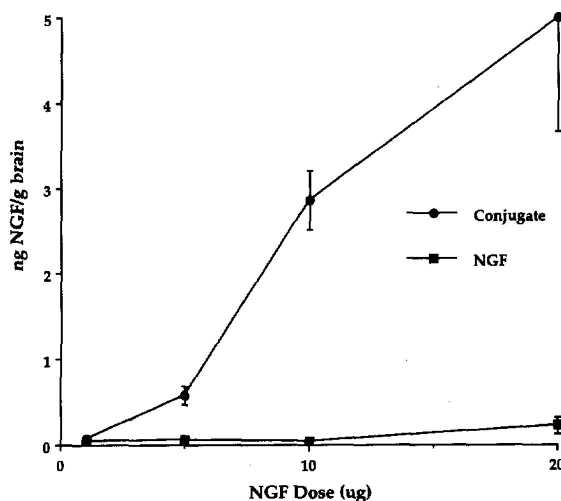

Fig. 7. Quantitation of biotinylated NGF in the brain. Biotin-tagged NGF, either as a conjugate with OX-26 or as free protein, was injected intravenously into rats. The amount of biotin-NGF in brain tissue extracts was quantitated by a luminescent immunoassay (LIA). Results (means±S.E.M.) are expressed as ng NGF recovered per gram of brain tissue as function of NGF dose injected ($n\geq2$ for each NGF dose and $n\geq4$ for each NGF conjugtae dose). ●, OX-26-biotin-NGF conjugate; ■, biotin-NGF. (Reproduced from Kordower et al. [40].)

to deliver to the brain an efficacious dose of a neuroactive peptide has also been demonstrated in vivo using an OX-26-vasoactive intestinal peptide (VIP) conjugate [43]. This conjugate was prepared by first chemically linking avidin to the carrier antibody and biotinylating the peptide. An antibody-VIP conjugate was then formed through the high affinity avidin–biotin interaction. Following delivery across the BBB, VIP would be expected to increase cerebral blood flow through interaction with VIP receptors located on the vascular smooth muscle cells. Indeed, intravenous administration of the OX-26-VIP conjugate lead to a 65% increase in brain blood flow. This effect was not observed with unconjugated VIP, indicating that active peptide was only delivered across the BBB to the target cells when it was linked to the anti-transferrin receptor antibody.

8. Conclusions

In conclusion, the results summarized herein demonstrate that an endogenous cerebrovascular transport system can be exploited to deliver therapeutic compounds, including neuroactive peptides and proteins, from the bloodstream to the brain. It also appears that this approach to the delivery of therapeutics across the BBB is not limited to the rat. Studies in non-human primates with an anti-human/primate transferrin receptor antibody designated 128.1 [44], indicate that this antibody also targets brain vasculature and can be used to deliver macromolecules across the BBB [45]. This technology could have a tremendous impact on the clinical utility of therapeutic agents, such as the neurotrophic factors, which show great potential in treating disorders of the CNS, but whose administration is currently limited to invasive neurosurgical techniques.

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