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Macromolecular permeability across the blood–nerve and blood–brain barriers

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ABSTRACT **The permeability of insulin (Ins), nerve growth factor (NGF), albumin (Alb), transferrin (Trf), and IgG across the blood–nerve barrier (BNB) and blood–brain barrier (BBB) in normal adult rats was quantified by measuring the (permeability coefficient × surface area) product (*PS*) with the i.v. bolus-injection technique in the cannulated brachial vein and artery using radioiodinated proteins. The *PS* values of the BNB for IgG and Alb were low: 0.079 ± 0.029 × 10^{-6} and 0.101 ± 0.088 × 10^{-6} ml·g^{-1}·s^{-1}, ($\bar{x}$ ± SD, respectively). The *PS* values for NGF and Trf were 16.1-fold and 25.5-fold higher than for Alb. The *PS* for Ins across the BNB was 33.190 ± 2.053 × 10^{-6} ml·g^{-1}·s^{-1}—a remarkable 329-fold increase compared with Alb. The *PS* values of the BBB for IgG and Alb in different brain regions were all low, from 0.028 ± 0.017 to 0.151 ± 0.035 × 10^{-6} ml·g^{-1}·s^{-1} ($\bar{x}$ ± SD). NGF and Trf had comparable *PS* values from 13- to 32-fold higher than for Alb, except for the brain stem, where the *PS* for Trf was 66-fold higher than for Alb. The mean *PS* for Ins across the BBB ranged from 15.78 ± 5.45 × 10^{-6} ml·g^{-1}·s^{-1} for the cortex to 22.62 ± 7.50 × 10^{-6} ml·g^{-1}·s^{-1} for the brain stem—again a remarkable 105- to 390-fold increase relative to Alb. Because reliable *PS* measurements were obtained for all proteins tested, the BBB and BNB cannot be considered impermeable to proteins—a concept that has plagued brain- and nerve-barrier research. The low *PS* values for IgG and Alb indicate low rates of transfer; however, Alb, in particular, is the major protein of endoneurial and ventricular fluid, which suggests that these *PS* values may be significant. Ins had the highest *PS* values, which likely reflect the mechanism of transport across the barriers—that is, receptor-mediated transport. Because NGF and Trf had *PS* values 13- to 66-fold higher than for Alb, whether this reflects receptor-mediated uptake, adsorptive-mediated transcytosis, or some other mechanism is unclear. That the *PS* values for NGF and Trf differ from Alb and IgG clearly suggests, however, a different uptake mechanism. Finally, the remarkably high *PS* values for Ins across the BBB and BNB identify this protein and its putative receptor on capillary endothelial cells as a potential target for drug delivery into the central and peripheral nervous systems.**

Our understanding of the transfer of macromolecules across the blood–brain barrier (BBB) and blood–nerve barrier (BNB) has been hampered by a reliance on qualitative approaches and a paucity of quantitative approaches. The former generally suggest that these barriers are impermeable to peptides and proteins. The restriction of movement of these hydrophilic substances from the blood into the central or peripheral nervous systems is the result of a physical limiting barrier consisting of the nonfenestrated, continuous endothelium lining of microvessels with tight intercellular junctions separating the endothelial cells. The transport of these macromolecules across these barriers, therefore, has been previously considered nonexistent and nonphysiological.

The poor or nondetectable penetration of the barriers after peripheral administration is inevitably influenced by the techniques. These techniques have been extensively reviewed (1–4). A general problem in evaluating protein permeability across the barriers is the limitation imposed on choice of the vascular markers. Frequently used vascular markers are ^{51}Cr, [^{3}H]sucrose, [^{3}H]dextran, [^{3}H]inulin, and even ^{111}In-labeled transferrin (Trf) and ^{125}I-labeled albumin (Alb). As pointed out by Van Bree *et al.* (4), the uptake values were of the same order of magnitude as that of the vascular marker and were, therefore, considered negligible, which leads to possible erroneous conclusions that the barrier was impermeable to proteins.

The vascular-space marker chosen must meet several requirements which have been summarized (1). The closer "a vascular volume indicator approaches the biological, physical, and chemical properties of the test substance, the better the former is able to accurately model the intravascular distribution of the latter." In addition, Fenstermacher *et al.* (1) emphasized the need to determine a vascular-space correction for each individual animal rather than having a mean adjustment factor for a separate group of animals.

In our experiments, the residual brain/endoneurial plasma volume (V_p) was determined with the same protein that was radioiodinated with a second isotope of iodine (^{125}I vs. ^{131}I) given 1 to 2 min before the end of the experiment. The same test substance allows an accurate determination of the V_p and also corrects for any nonspecific adherence to capillary walls characteristic of the protein tested. Similarly, this dual isotope approach allows for determination of the vascular space on each individual animal.

The (permeability coefficient × surface area) product (PS) of the BNB and BBB to different radioiodinated proteins was, therefore, corrected for V_p with a second tracer of the same protein. Because the *PS* product is determined without an independent measure of *P* or *S*, the V_p is a useful indicator of *S*, which is not expected to change in normal animals over the time of the experiment. Changes in *PS* with little changes in vascular volume, therefore, permit an assessment of capillary permeability. We use the i.v. bolus-injection method developed by Rapoport and colleagues (5, 6) for determining *PS*. This is a multi-passage/single time-point technique with a two-compartment analysis. This frequently used model has been discussed in detail (2).

MATERIALS AND METHODS

Animals and Reagents. Male Sprague–Dawley rats (24 week old) (460–500 g) were obtained from Bio-Lab (St. Paul)

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Abbreviations: BBB, blood–brain barrier; BNB, blood–nerve barrier; *PS*, (permeability coefficient × surface area) product; V_p, residual brain/endoneurial plasma volume; Alb, albumin; NGF, nerve growth factor; Trf, transferrin; Ins, insulin.

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and used to determine the PS and V_p measurements. All animals were kept a minimum of 3 days under standard housing conditions and feeding schedules before the experiments. Human Alb was isolated from normal human plasma by using CM-Affi-Gel blue (Bio-Rad) and further subjected to boronate affinity chromatography to remove the glycated species (7). Affinity-purified human IgG was obtained from Accurate Chemicals. Ultrapure 2.5 S nerve growth factor (NGF) was obtained from Harlan Bioproducts (Madison, WI). Trf was obtained from Sigma as holo-TRF. Insulin (Ins) was obtained as Humulin RU-100 from Eli Lilly. Carrier-free Na ^{125}I and Na ^{131}I were from Amersham. Protein concentrations were determined by the bicinchoninic acid protein-assay procedure (8) with the Pierce assay kit and bovine serum albumin as the standard.

Protein Radioiodination. Aliquots of the proteins were labeled with ^{125}I and ^{131}I using the chloramine-T method as described (9). Free radioactive iodine was separated from the radiolabeled protein by dialysis against 0.2 M NaI. Purity of the radiolabeled proteins was determined by paper chromatography, as described (10). The radiolabeled protein that stayed at the origin was always >99% of total radioactivity. Radioiodinated proteins were evaluated by SDS/PGE as described (11). No degradative products were found after iodination or after PS/V_p measurements.

***PS* and V_p Measurements of Radioiodinated Proteins.** Experimental details have been described (7); they are based on published methods (5, 6). A bolus of phosphate-buffered saline containing ^{125}I-labeled protein was injected rapidly into the catheterized brachial vein of pentobarbital-anesthetized rats. Blood was sampled during the next 30, 60, or 240 min from the brachial artery, depending on the evaluated protein. Before animal sacrifice, at time periods of 60 or 120 s, the second isotope-labeled protein (^{131}I) was administered i.v. to serve as the V_p indicator.

After the final blood sample was collected, the sciatic nerves were rapidly removed and desheathed. Similarly, the brain and then the meninges were removed. The brain was dissected into the cortex, caudal putamen, hippocampus, thalamus, brain stem, and cerebellum. The tissue was then lyophilized, and the dry weight was determined with a microbalance. These weights were converted to their respective wet weights with wet weight/dry weight ratios previously determined for desheathed rat sciatic nerve and for the various brain regions. Tissue and plasma samples were assayed for ^{125}I and ^{131}I radioactivity in a two-channel γ counter. Radioactivity was corrected for crossover of ^{131}I activity into the ^{125}I channel and background activity. The V_p and PS measurements were calculated as described (7). Statistical evaluations were done with Student's two-tailed paired t test; significance was accepted at the $P < 0.05$ level.

RESULTS

Plasma-Clearance Curves for Radioiodinated Proteins. Typical plasma-clearance curves for the five proteins can be seen in Fig. 1. The curves were generated from a standard exponential decay equation using IN PLOT Scientific Graphics (Graph Pad software) (the half-lives for the individual proteins in this experiment are also given in Fig. 1). IgG had the slowest half-life with a mean at 30.20 ± 13.08 min (n = 6),

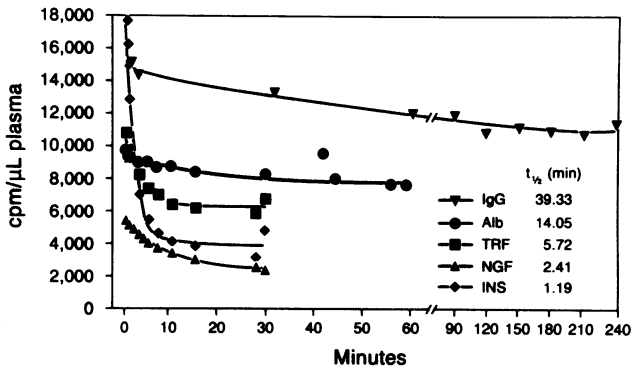

FIG. 1. Plasma-washout curves for radioiodinated IgG, Alb, Trf, NGF, and Ins.

whereas Ins had the fastest half-life (1.18 ± 0.05 min). Based on these half-lives, PS and V_p measurements for IgG were determined with a 120-min period for the first tracer and 2 min for the second tracer. Sixty minutes and 1 min, respectively, were used for the first and second tracers for Alb, which had a half-life of 12.82 ± 5.77 min. The plasma half-lives for NGF and Trf were 5.57 ± 1.01 min and 2.38 ± 0.32 min, respectively. For Ins, Trf, and NGF, the periods for the first and second tracers were 30 min and 1 min, respectively.

***PS* and V_p Measurements of BNB.** The PS and V_p values for IgG, Alb, NGF, Trf, and Ins across the BNB are found in Table 1. In general, the Alb PS measurements were higher than those of IgG; however, the difference was not significant in the nerve. The PS of NGF was 16.6-fold higher than that of Alb ($P < 0.0001$). The PS of Trf was higher than that of NGF and reached a value 25.5-fold greater than that of Alb ($P < 0.0001$). Of all proteins tested, Ins had the remarkably high PS value of 33.19×10^{-6} ml·g^{-1}·s^{-1}. This result was 328.6-fold greater than that of Alb. The V_p values for IgG, Alb, NGF, and Trf did not differ significantly from one another; however, that observed for Ins was 2.6-fold greater than that of Alb ($P = 0.0039$). This increased V_p value for Ins was the result of the 1-min period used for injecting the second tracer, which is comparable to the half-life of the circulating protein (1.18 ± 0.05 min). When the injection time of the second tracer was decreased to 30 s, the V_p value was 2.27 ± 0.55 ml·g^{-1} ($\bar{x}$ ± SD), which did not significantly differ from the V_p for Alb.

***PS* and V_p Measurements of BBB.** Five different brain regions were evaluated for PS and V_p values of the BBB for the five different proteins (Tables 2 and 3). In general, the PS values for Alb exceeded those of IgG, except for the brain stem. None of these values were significantly different, except for the cortex ($P = 0.0086$). In general, the V_p values for IgG were less than those seen for Alb. The PS for NGF in the different brain regions ranged from 1.081×10^{-6} to 2.569×10^{-6} ml·g^{-1}·s^{-1} with the lowest PS value seen in cortex and the highest values seen in the brain stem, a relative increase of 12.7- to 32.5-fold compared with Alb. This relative increase in PS of NGF vs. Alb was highly significant ($P = 0.0002$–0.0115). No significant differences were seen in the V_p values comparing NGF with Alb.

Table 1. PS product and V_p of the BNB for IgG, Alb, Trf, and Ins

	IgG (5)	Alb (10)	NGF (10)	RI* (NGF/Alb)	TRF (12)	RI* (Trf/Alb)	Ins (10)	RI* (Ins/Alb)
PS, ml·g^{-1}·s^{-1} × 10^6	0.079 ± 0.029	0.101 ± 0.088	1.675 ± 0.380	16.6	2.176 ± 0.371	25.5	33.190 ± 2.053	328.6
V_p, μl·g^{-1}	1.843 ± 0.440	1.949 ± 0.589	1.489 ± 0.260	0.8	1.919 ± 0.500	1.0	5.049 ± 0.873	2.6

Data are expressed as $\bar{x}$ ± SD. Numbers in parentheses are n values.
*RI, relative increase; entries are thus a ratio of PS products or V_p.

Table 2. *PS* products of the BBB for IgG, Alb, NGF, Trf, and Ins

	PS ($\bar{x}$ ± SD), ml·g^{-1}·s^{-1} × 10^6							
	IgG (5)	Alb (5)	NGF (5)	RI* (NGF/Alb)	Trf (6)	RI* (Trf/Alb)	Ins (5)	RI* (Ins/Alb)
Cortex	0.054 ± 0.024	0.151 ± 0.035	1.920 ± 0.908	12.7	1.982 ± 0.232	13.1	15.78 ± 5.45	104.5
Caudoputamen	0.028 ± 0.017	0.093 ± 0.069	1.950 ± 0.423	21.0	1.840 ± 0.224	19.8	16.88 ± 4.00	181.5
Hippocampus	0.037 ± 0.018	0.097 ± 0.038	2.490 ± 0.611	25.7	1.877 ± 0.173	19.4	17.16 ± 5.70	176.9
Thalamus	0.059 ± 0.026	0.082 ± 0.041	1.885 ± 0.601	23.0	2.260 ± 0.234	27.6	18.42 ± 5.38	224.6
Brain stem	0.102 ± 0.050	0.058 ± 0.032	1.081 ± 0.434	32.5	3.825 ± 0.495	65.9	22.62 ± 7.50	390.0
Cerebellum	0.093 ± 0.045	0.102 ± 0.056	2.569 ± 0.631	25.2	2.805 ± 0.357	27.5	20.12 ± 6.21	197.3

Numbers in parentheses are *n* values; V_p values for these experiments appear in Table 3.
*RI, relative increase; entries are thus a ratio of *PS* products.

The *PS* values for Trf ranged from 1.84 × 10^{-6} to 3.83 × 10^{-6} ml·g^{-1}·s^{-1} for the different brain regions, which represented a relative increase of 13.1-fold for the cortex to 65.9-fold for the brain stem when compared with Alb. These differences in *PS* values were highly significant ($P = < 0.0001$ to 0.0003). The V_p values for Trf were not significantly different from those of Alb, except for the cortex and hippocampus.

The *PS* values for Ins were all remarkably higher than those of any other proteins tested. *PS* values ranged from a low of 15.78 × 10^{-6} ml·g^{-1}·s^{-1} for the cortex to a high of 22.6 × 10^{-6} ml·g^{-1}·s^{-1} for the brain stem. The relative increase of the *PS* values for Ins versus Alb ranged from 104.5- to 390-fold (*P* values all highly significant). The V_p values for Ins in the different brain regions compared with Alb did not differ significantly, except for cortex ($P = 0.0022$) and the brain stem ($P = 0.0042$).

DISCUSSION

Our data show that the transfer of macromolecules across the BBB and BNB can be reliably quantitated *in vivo* by assessing the *PS* product and the V_p. Although the *PS* values for Alb and IgG were low, such values should not be considered insignificant because Alb has been shown to be the major protein of the endoneurial fluid, (12) and both Alb and IgG are the prominent proteins in the ventricle, cistern, and lumbar spinal fluid (13). Although much of the cerebrospinal fluid protein enters at the choroid plexus, the relative contribution of entry across the cerebrovascular endothelium or the arachnoid membrane, which also compose the BBB, has not been experimentally evaluated. Similarly, the contribution of Alb in the endoneurial fluid from that which crosses the endoneurial vascular endothelium, as compared with that across the inner layers of the perineurium, has not been determined.

The *PS* values obtained for Alb in our study are comparable to reported values by Rapoport and colleagues. Shimon-Hophy *et al.* (14) reported a *PS* for the cortex that ranged from 0.5 to 0.8 × 10^{-6} ml·g^{-1}·s^{-1} for native bovine serum Alb, which is 3.3- to 5.3-fold greater than reported for the cortex here. Wadhwani *et al.* (15) reported a mean *PS* for the BNB of 1.7 × 10^{-6} ml·g^{-1}·s^{-1}, 16.8-fold greater than reported here. Although the methods are similar, the V_p reported in these two studies was determined by ^{51}Cr injection to estimate the vascular tissue volumes in separate animal groups. In addition, the femoral artery and vein were catheterized instead of the brachial artery and vein, and calculations were based on wet weight instead of dry weight, as done here. In our previous studies, we determined a *PS* for human Alb across the BNB that was ≈0.3 × 10^{-6} ml·g^{-1}·s^{-1} (7) and even higher in an earlier study (0.6 × 10^{-6} ml·g^{-1}·s^{-1}) (16). This variability occurred with different sources of human Alb, and because we have demonstrated increased permeability across the BNB with glycated Alb (7), we determined that this variability was due, at least in part, to differences in glycation of the endogenous Alb isolated from normal individuals (J.F.P. and G.L.C., unpublished observation). In the present experiment, the purified human Alb was subjected to boronate affinity chromatography to remove any glycated species; this procedure resulted in more consistent *PS* values for both the BNB and BBB, as reported here. In a separate review, Banks *et al.* (17) reported a blood-to-brain unidirectional influx rate (K_i), as measured by described methods (18) for Alb of 0.0097 to 0.065 × 10^{-3} ml·g^{-1}·min^{-1}, which is similar to that reported here for the cortex.

Knowledge of the mechanisms by which peptides and proteins are transported across the BBB and BNB is still limited; several recent reviews (4, 17, 19–21) have been published. At least five methods by which peptides and proteins can permeate the BBB and BNB have been postulated: (*i*) transmembrane diffusion, (*ii*) carrier-mediated transport, (*iii*) fluid-phase endocytosis, (*iv*) nonspecific adsorptive endocytosis, and (*v*) specific or receptor-mediated adsorptive endocytosis.

The simplest route of transport across endothelial cells that compose the BBB or BNB is by passive diffusion of peptides and proteins down a concentration gradient. Although passive diffusion of such molecules can occur through either inter- or intracellular pathways, the size of peptides and proteins often precludes diffusion through intercellular pathways due to the tight intercellular junctions that separate brain and nerve capillary endothelial cells. Passive diffusion across these barriers is nonsaturable, temperature and energy

Table 3. V_p values of the BBB for IgG, Alb, NGF, Trf, and INS

	V_p ($\bar{x}$ ± SD), μl/g							
	IgG	Alb	NGF	RI* (NGF/Alb)	Trf	RI* (Trf/Alb)	Ins	RI* (Ins/Alb)
Cortex	3.932 ± 0.464	9.492 ± 1.550	5.964 ± 1.935	0.6	5.141 ± 0.597	0.5	6.438 ± 1.103	0.8
Caudoputamen	4.178 ± 0.458	7.974 ± 3.133	5.886 ± 0.517	0.7	4.791 ± 1.032	0.6	6.080 ± 0.672	0.8
Hippocampus	4.435 ± 0.488	8.395 ± 1.267	7.046 ± 0.664	0.8	4.973 ± 0.668	0.6	7.043 ± 1.513	0.8
Thalamus	5.776 ± 0.544	7.846 ± 1.061	6.225 ± 0.938	0.8	6.764 ± 0.778	0.9	7.599 ± 1.500	1.0
Brain Stem	7.204 ± 1.581	7.283 ± 2.371	4.314 ± 1.319	0.6	11.155 ± 3.878	1.5	12.352 ± 2.210	1.7
Cerebellum	7.544 ± 1.318	12.149 ± 2.276	9.369 ± 1.059	0.8	9.561 ± 0.969	0.8	10.559 ± 2.136	0.9

*RI, relative increase; entries are thus a ratio of V_p values.

independent, and dependent upon the lipophilicity and molecular size of the peptide or protein. Carrier-mediated transport usually is restricted to the transport of nutrients, such as glucose and amino acids, but can also occur for relatively small peptides. Carrier-mediated transport is characterized by saturable, specific binding that is often stereospecific for a given amino acid or sugar. Several peptides have been reported to use specific carrier-mediated transport systems in the BBB, including those with N-terminal tyrosine residues (22), such as Tyr-MIF-1(methionine enkephalin) and antiopioid peptide (23), [Met^5]enkephalin (24), [Arg^8]vasopressin (24, 25), D-Ala-peptide T-amide (26), and somatostatin (27), to name a few.

The endocytotic transport process can be either fluid-phase or adsorptive endocytosis. The former process involves the internalization of a small portion of the extracellular fluid environment and subsequent transport of soluble macromolecules along with the extracellular fluid in a nonsaturable, noncompetitive, and both temperature- and energy-dependent process. Alb transport across brain endothelial cell monolayers has been reported to occur in this way (28). Adsorptive endocytosis is either nonspecific or specific. Nonspecific adsorptive endocytosis is a saturable energy-requiring process that depends upon predominantly electrostatic interactions between the transported molecule and the endothelial-cell surface. An example of this is the transport of histone (29) and wheat germ agglutinin conjugated to horseradish peroxidase (30) across the BBB, as well as that for cationized Alb (28, 31, 32) and cationized IgG (33). Specific or receptor-mediated adsorptive endocytosis involves an energy- and temperature-dependent, saturable transport process, which depends upon binding of the macromolecule to specific receptor sites on the endothelial cell. Specific examples include Ins (34–37), atrial natriuretic factor (38, 39), Ins growth factors (35, 40), and Trf (41–43). More extensive examples of peptides and proteins that can be transported by each of these methods can be found in ref. 44.

It is reasonable to hypothesize that these different mechanisms of transport could be manifested in different *PS* values measured for individual proteins. While the *PS* values for Alb and IgG were low, such values are consistent with a fluid-phase endocytosis mechanism (28). Controversy exists with regard to whether or not there are receptors for Alb, particularly in other tissues (45–54). Studies (55) argued against a classical receptor for Alb on brain endothelial cells but could not rule out possible chemical or electrostatic interactions. Ultrastructural studies (56) in cultured sheep brain microvascular endothelium provide evidence for fluid-phase endocytosis of Alb involving transfer from apical to basolateral surfaces. Using an avidin–biotin cytochemical detection system, Seitz *et al.* (57) found IgG in endoneurial connective tissue of peripheral nerves only after 4 days of daily i.p. injection of biotinylated IgG, whereas brain, spinal cords, and spinal roots were free of IgG. Zlokovic *et al.* (58) provided data, however, that suggest a specific and saturable transport system for IgG across the BBB, using a vascular brain-perfusion method. The low *PS* values for IgG for both the BBB and BNB in our study would argue against a specific transport system.

The remarkably high *PS* values obtained for Ins across the BBB and BNB infer a specific receptor-mediated transport process. Indeed, Ins is generally believed to be transported across the BBB by such a receptor-mediated process. This process has been shown both *in vivo* and *in vitro* and is competitive and temperature dependent (34–37). It is of interest that the *PS* for Ins in the brain ranged from 15.8×10^{-6} ml·g^{-1}·s^{-1} for the cortex to 22.6×10^{-6} ml·g^{-1}·s^{-1} in the brain stem, whereas the *PS* for Ins in nerve was substantially higher (33.2×10^{-6} ml·g^{-1}·s^{-1}). These differences may be related to differences in receptor densities in the various regions. The higher value in normal nerve may also suggest additional functions for Ins, such as an involvement in the maintenance of normal nerve. Although the route of entry of Ins into the brain was earlier believed to be across the blood–cerebrospinal fluid barrier (choroid plexus), more recent data suggest that the primary entry site is through the capillary endothelial cells (59). Transcytosis of Ins has been demonstrated in cardiac muscle *in vivo* (60) and in endothelial-cell cultures (61). A similar pathway is thought to also mediate delivery of Ins across the brain and nerve endothelium, although this hypothesis, surprisingly, has not been rigorously investigated. In any case, the presence of Ins receptors in brain (62) and the recent observations that suggest Ins acts as an afferent central nervous system signal that regulates normal energy balance (59) infer that Ins crosses the BBB intact, by an efficient receptor-mediated process.

The *PS* values for Trf ranged from 8% to 24% those obtained for Ins in brain and ≈7% for those in nerve. Although these *PS* values are lower than for Ins, the mechanism by which Trf is transported across the BBB and BNB is decidedly a receptor-mediated process. Receptors for Trf have been identified, but only on the luminal membrane of brain endothelial cells (41–43, 63–66). Although several of these studies suggest that these receptors mediate transcytosis of Trf from blood to brain, a recent ultrastructural study (67) convincingly shows that the Trf receptor is highly polarized at the BBB, being localized only at the apical membrane. These data support a receptor-mediated endocytic pathway at the luminal membrane of brain capillaries whereby iron-loaded Trf is taken up, and the iron dissociates from Trf in endosomal compartments. The iron is then transcytosed by unknown mechanisms while Trf and its receptor are retroendocytosed, suggesting that neither undergoes significant transcytosis in the brain endothelial cell. Similar findings (68) were obtained after i.v. injection of ^{59}Fe- and ^{125}I-labeled Trf. These observations would argue against use of the Trf receptor for drug delivery across the BBB or BNB.

Interestingly, the *PS* values for NGF resembled those of Trf, except for nerve, brain stem, and thalamus, where the NGF *PS* values were lower than those of Trf, and the hippocampus, in which the value was higher for NGF. Because Trf is transported by a receptor-mediated process, the NGF data also suggest that NGF might be transported similarly. In general, receptors for NGF are widely distributed on nonneuronal cells, such as the Schwann cells and perineurial cells of the peripheral nervous system (69, 70) and in the choroid plexus (71–73), ependymal cells (72, 74), blood vessels (72, 75–77), meninges (72, 76, 78, 79), and cerebral arteries (80) of the central nervous system. It is generally thought that the high-affinity receptors for NGF are localized on NGF-dependent neurons, whereas low-affinity NGF receptors are localized on nonneuronal sites (81–83). The cellular distribution of these receptors, however, remains unknown, as does the mechanism of transport. Their presence on perineurial cells of nerve and on the choroid plexus, ependymal cells, meninges, and cerebral arteries of brain suggest peripheral functions that might regulate these cells rather than the direct modulation of neuronal functions by systemic presentation. Indeed, NGF has been suggested to be involved in the regulation of blood-vessel pressure (84, 85) by inhibiting adrenergic neurotransmission (86). Although NGF was thought to not cross the BBB (87), brain noradrenaline levels increased when NGF was given s.c. in adult animals (88). In any case, *PS* values for NGF clearly differ from those for Alb and IgG. Although a 5-fold molecular weight difference exists between NGF and Alb, the *PS* for NGF was significantly higher, which, in turn, suggests a

different uptake mechanism. The higher *PS* values for NGF in the hippocampus compared with Trf suggest a specific delivery mechanism in this brain region that could be used to supplement endogenous sources of the trophic factor in an area with dense cholinergic innervation.

Finally, the dramatically high *PS* values seen for Ins across the BBB and BNB, its efficient transport by a receptor-mediated process, and its presumed transcytosis as an intact macromolecule to affect central functions infer that Ins would be effective for targeting drug delivery into the central or peripheral nervous systems. This might prove to be a rational approach to efficiently deliver therapeutic drugs into the nervous system for the treatment of a variety of neurological diseases.

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