Pharmaceutical Research, Vol. 15, No. 3, 1998

Mini-Review

Localized Delivery of Proteins in the Brain: Can Transport Be Customized?

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Received November 4, 1997; accepted December 11, 1997

Certain central nervous system (CNS) diseases are characterized by the degeneration of specific cell populations. One strategy for treating neurodegenerative diseases is long-term, controlled delivery of proteins such as epidermal growth factor (EGF) and nerve growth factor (NGF). Since proteins permeate through brain capillaries very slowly, local administration using polymeric implants, continuous infusion pumps, or transplanted, protein-secreting cells may be required to achieve therapeutic concentrations in the tissue. The efficiency of local distribution, and hence effectiveness of local therapy, depends on the rate of protein migration through tissue. The rate of dispersion of molecules in a quiescent, isotropic medium can be characterized by the molecular diffusion coefficient, D, which can be measured by techniques such as quantitative autoradiography, iontophoresis, and fluorescence photobleaching recovery (FPR). These methods are reviewed, with an emphasis on their application to measurement of D for proteins in the brain. Biophysical techniques yield quantitative descriptions of local protein distribution and may enable discrimination of mechanisms of protein transport in the brain. This capability suggests a new paradigm for design of protein therapies, in which proteins and delivery systems are collectively customized to provide sustained protein availability over predetermined volumes of tissue.

KEY WORDS: controlled release; polymer; diffusion coefficient; autoradiography; fluorescence; FRAP; FPR; iontophoresis; transport.

INTRODUCTION

Neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease afflict more than 5 million people in the U.S. (1,2); annual expenditures attributed to these two diseases total more than 80 billion dollars (1,2). Neurodegenerative diseases are characterized by extensive cell death, often in localized regions of the central nervous system (CNS). In Parkinson's Disease, symptoms are the result of the death of dopaminergic cells in the substantia nigra of the mesencephalon. Drug therapies that increase dopamine levels in the brain reduce symptoms and enhance the quality of life for many patients (3). However, the underlying pathology is progressive, and dopamine-replacement therapies become less useful over time (3). Similarly, symptoms caused by the death of cholinergic cells in Alzheimer's Disease can be reduced by cholinergic agonists (4), but dementia continues to progress.

Treatment strategies that "rescue" cells from death, or encourage cell proliferation, may provide longer-lasting improvement in symptoms. Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) are mitogens which have neurotrophic effects on dopamine-producing, mesencephalic-derived primary cell cultures (5). For example, EGF stimulates dopaminergic neuronal development *in vitro* and protects dopaminergic neurons against MPTP, a neurotoxin that destroys nigrostriatal dopaminergic neurons (6). In fact, elevated levels of EGF are observed in Parkinsonian patients; this may reflect a compensatory reaction to retard further loss of dopaminergic cells (7). EGF, when administered with bFGF, stimulates the proliferation and migration of neural stem cells, which can differentiate into neurons and glia *in vitro* (8,9). Delivery of nerve growth factor (NGF) protects cholinergic cells from injury-induced death (10,11) and enhances the survival of transplanted cholinergic cells (12–14). These studies suggest that long-term delivery of proteins may be beneficial to patients with Parkinson's or Alzheimer's disease.

Systemically administered proteins do not enter the brain. The permeability of brain capillaries is low for most water-soluble compounds; except for proteins that have a specific transport system, the rate of protein permeation through the brain endothelial barrier occurs very slowly (15). Plasma half-life can be extended by protein conjugation to water-soluble polymers such as poly(ethylene glycol) (16), but this modification further reduces permeability across the blood-brain barrier. Several techniques have been developed to provide controlled,

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ABBREVIATIONS: CNS, central nervous system; EGF, epidermal growth factor; NGF, nerve growth factor; FPR, fluorescence photobleaching recovery; bFGF, basic fibroblast growth factor; FRAP, fluorescence recovery after photobleaching; TMA, tetramethylammonium; MRI, magnetic resonance imaging.

0724-8741/98/0300-0377$15.00/0

localized delivery of proteins in the brain: implantation of polymeric delivery systems (17–21), infusion systems (22–24), transplantation of genetically-engineered, protein-secreting cells (13,25), and implantation of cells (26–29). Each mode of delivery offers unique advantages: polymer implants provide the highest local concentrations (19); infusion offers the greatest degree of control; transplantation potentially provides protein delivery for the life of the patient.

Polymer-based, infusion, and cellular delivery systems provide protein to the brain via dramatically different mechanisms: diffusion from a polymer matrix, flow through a synthetic channel, and secretion after synthesis by cellular machinery (see (30) for a more complete comparison). These approaches are similar in at least one important regard: after release from the matrix, cannula, or cell, protein molecules move to their cellular sites of action by migrating through the interstitial space of tissue. With some notable exceptions[3], diffusion is the principal mechanism of protein distribution through brain tissue. Diffusion through the tissue interstitium is a slow process (35); substantial metabolism or clearance can occur during the period of migration. As a result, the volume of tissue exposed to protein is relatively small: treatment volumes with a characteristic dimension of 1-mm have been observed after polymer implantation (19) and low-flow infusion (36). This limitation on the penetration of proteins into tissue is valuable, since growth factors often have activities in multiple tissues and, therefore, potential toxicity outside of the treatment region. However, 1 mm is a severe limit on penetration; in clinical situations, much larger treatment regions may be required. One possible solution is to provide sources of protein at multiple sites, which are separated by the 1-mm penetration distance (30). But this solution requires extensive access to the brain, and is viable in limited circumstances. More elegant solutions, with potentially wider capability, are conceivable: for example, delivery systems that can be preprogrammed to treat a desired volume of tissue.

PROTEIN PENETRATION AFTER LOCAL DELIVERY

Design of a delivery system that treats a pre-specified volume of tissue requires manipulation of basic mechanisms of tissue penetration. But can protein migration in brain tissue be customized in a predictable way? The answer requires an awareness of the modes of molecular transport in the brain. To clarify the important features of transport, we will consider the "penetrability" of proteins in tissue. Penetrability is defined as the ability of an agent to migrate through tissue or, more specifically, the ability of molecules deposited within the interstitial space at a specific location to navigate, without loss of activity, to a distant location in the same tissue. Since proteins migrate through the tissue by diffusion and convection and cease migrating when metabolized, internalized by cells, or cleared from the brain, penetrability of an agent depends on the overall rate of dispersion and the overall rate of elimination. Qualitatively, dispersivity enhances penetrability and elimination diminishes penetrability:

$$Penetrability = f[dispersivity/elimination] \quad (1)$$

We expect penetrability to be a function of both the penetrating agent and the penetrated tissue. In previous papers, we described a rational approach for predicting penetrability of agents in the brain, by estimating dispersion and elimination rates for each compound (30,37–39). If elimination kinetics are simple, so that the rate of elimination of the protein from the tissue is proportional to the concentration of protein, elimination can be characterized by k, a first-order overall elimination constant. Dispersivity can be characterized by the diffusion coefficient, the rate of fluid flow in the tissue, and the extent of binding to fixed tissue components, which tends to retard dispersion. In situations for which diffusion is the only mechanism of dispersion, dispersivity can be characterized by the diffusion coefficient, D. The ratio of the relative rates of first-order elimination and diffusional transport, which we call the diffusion/elimination modulus ϕ[4], is defined as $a\sqrt{k/D}$, where a is a characteristic length scale.

Experimentally, penetrability can be assessed by examining the variation of concentration with position in the brain. Concentrations can also be predicted by solving the diffusion equation[5]:

$$C = C_o \exp\left(-\phi\left(\frac{x}{a} - 1\right)\right) \quad (2)$$

where x is the distance from the center of the polymer implant, and a is the characteristic dimension of the delivery vehicle (i.e. the half-thickness of the polymer, radius of the cannula, diameter of the cell or encapsulated cell construct). For large values of ϕ, elimination occurs more rapidly than diffusion and penetrability is severely limited; for small values of ϕ, diffusion is more rapid than elimination, and penetrability is enhanced (Fig. 1). Note that this relationship does not imply that diffusion must be "fast" to achieve good penetrability; it is sufficient that diffusion be "faster" than elimination. The modulus ϕ provides a quantitative measure of penetrability: when ϕ is large, penetrability is poor; when ϕ is small, penetrability is good; and when ϕ is ~1, penetrability is intermediate. The modulus ϕ can be related to the size of the treatment region: for example, if the treated region is assumed to include only the volume of tissue for which the protein concentration is greater than 10% of its maximum value ($C > 0.1C_0$) then the penetration distance is:

$$Penetration\ distance = x - a = \frac{\ln 10}{\sqrt{k/D}} \quad (3)$$

The diffusion/elimination modulus is determined by fitting predicted concentration profiles (such as Equation 2) to experimental data. Experimental concentration profiles can be obtained by any technique that permits spatial mapping of concentrations; quantitative autoradiography and fluorescence microscopy are particularly useful, but both require attachment

[3] One exception is high-flow infusion, in which the extent of migration can be controlled by modulating the rate of flow through the infusion system, and hence the rate of interstitial fluid convection throughout the brain (23). Fluid convection may be important in distributing drugs in other situations (31-34), but in most areas of the CNS, under normal conditions, rates of fluid convection are substantially lower than rates of molecular diffusion.

[4] By analogy to the well-known Thiele modulus, which provides insight into diffusion/reaction coupling in classical chemical engineering process, and was first applied to heterogeneous catalysis by Thiele (40).

[5] Assuming steady-state in an isotropic, homogeneous tissue with first-order elimination and negligible binding. See (30,33,39).

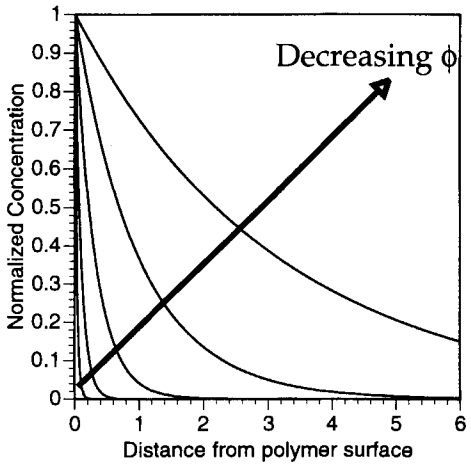


Fig. 1. Concentration versus distance for various values of ϕ. For large values of ϕ, elimination dominates over diffusion and penetration is reduced; as ϕ increases, diffusion is faster than elimination, and penetration is enhanced.

of a radioactive or fluorescent label to the protein. In autoradiography, radiolabeled protein is introduced into the brain and, after various times, the brain is removed, flash-frozen, sliced into thin sections, and exposed to autoradiographic film, which is subsequently scanned for the spatial distribution of exposure. Alternately, fluorescently-labeled protein is administered and the tissue is examined by fluorescence microscopy to obtain a map of fluorescence intensity. By comparison of measured intensities to standards of known concentration, the spatial distribution of labeled protein can be determined. Both techniques have been used to monitor protein concentrations in tissue. For example, autoradiography was used to measure concentration profiles after delivery of NGF by implantation of a polymeric delivery system into the brain of a rat (Fig. 2). Measurements such as these yield penetration distances (Equation 2) of ~1 mm for most agents in brain tissue (see review in (37)).

While the modulus ϕ can be determined by comparing Equation 2 to experimental data, as illustrated in Fig. 2, this approach does not yield absolute rates of diffusion or elimination, since migration and removal are coupled in the overall process of tissue penetration (Equation 1). An independent measurement of either elimination or migration is needed to complete this characterization. For real proteins in tissues, many factors could influence stability and dispersivity, and therefore penetrability; rates of diffusion, convection, elimination, binding, uptake, capillary permeation, and degradation have the potential to modulate the ability of an agent to traverse a tissue barrier (Fig. 3A). An additional independent measurement would be necessary to assess the importance of each mechanism, but designing and implementing experiments that isolate each of these phenomena is extremely difficult. To simplify the treatment, we assume that protein penetration can be adequately described by k and D, and focus on evaluation of penetration dynamics by the measurement or approximation of D.

PROTEIN DIFFUSION IN TISSUES

Certain agents are eliminated from a tissue so slowly that the rate of elimination is negligible compared to the rate of dispersion. These molecules can be used as "tracers" to probe mechanisms of dispersion in the tissue, provided that elimination is negligible during the period of measurement. Frequently used tracers in the brain include sucrose (41,42), iodoantipyrene (43), inulin (41), and size-fractionated dextran (43,44). Since the tracer is not eliminated, the diffusion coefficient is obtained by measuring time-dependent spatial concentration patterns after controlled introduction of the tracer. As described above, quantitative autoradiography and fluorescence microscopy of labeled protein analogs are convenient methods for accomplishing this measurement. Care must be exercised to ensure that elimination is truly negligible and to verify that the fluorescent or radioactive label remains stably associated with the protein.

Consider an application of this approach, in which fluorescent microscopy was used to measure D for proteins in water. Polymer matrices containing fluorescently-labeled protein were "implanted" into capillary tubes filled with buffered water, which were periodically scanned by fluorescent microscopy to obtain concentration profiles (45,46). Since proteins were constrained within the volume of the tube, which was kept absolutely still during the measurement, diffusion was the only mechanism for protein migration in the tube. Proteins were also stable under the conditions of the experiment; therefore, diffusion coefficients for these "tracers" were obtained by fitting an appropriate solution to the diffusion equation to measured concentration profiles. This technique yielded D for proteins in water, which were consistent with diffusion coefficients obtained by established biophysical methods (Fig. 4A). Autoradiographic and fluorescence techniques have been applied in a similar fashion to obtain D for tracers in cultured cell aggregates (47), cytoplasm of cultured neurons (48–51), brain slices (52), and whole animals (53,54) (open symbols in Fig. 4B).

Autoradiography and fluorescence microscopy have several advantages for examination of diffusion in tissues. Low tracer concentrations can be measured (< 100 ng/mL is achievable (19)); in addition, inhomogeneities in diffusion in different anatomical regions of the brain can be observed visually. However, a large number of animals must be examined, particularly when radioactivity is used, because autoradiography requires the use of a single animal for each measurement. These methods are slow, since concentration differences must be measured at spatial separations of ~100 μm (Figure 3B); the characteristic time for diffusion ($t = L^2/D$) in these circumstances is several hours. In addition, autoradiographic images can take weeks to develop. Rapid changes in diffusion rates, such as one might expect to observe in a dynamic environment such as the brain, usually cannot be resolved. Fluorescence microscopy, which has been elegantly applied by Jain and coworkers in their studies of interstitial diffusion (35,55) and lymphatic flow, can be used for real-time measurements within the tissue of a single animal, provided that the tissue can be accessed by light. This access can sometimes be obtained by installing window chambers in the tissue (56). Multiphoton fluorescence imaging, an important new technique pioneered by Webb and colleagues (57–59), promises to broaden the applications of this technique, since quantitative fluorescence imaging can be performed in three-dimensional specimens, even in specimens that scatter light.

Can these techniques be used to measure diffusion coefficients of active proteins in tissues? Most commonly, elimination in the tissue cannot be totally neglected, although it usually occurs over time periods of minutes to hours. Therefore, if rapid measurement techniques are used, elimination can be neglected. Fluorescence

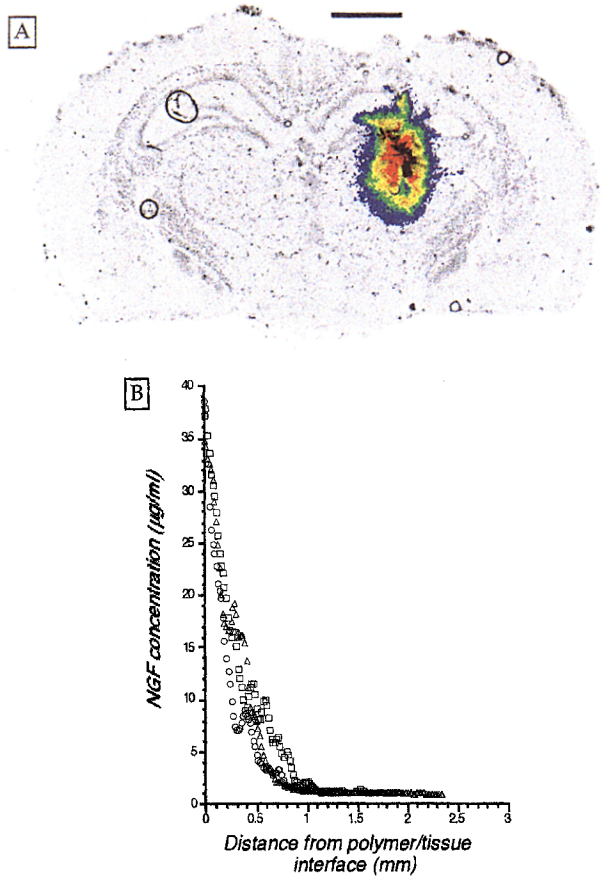
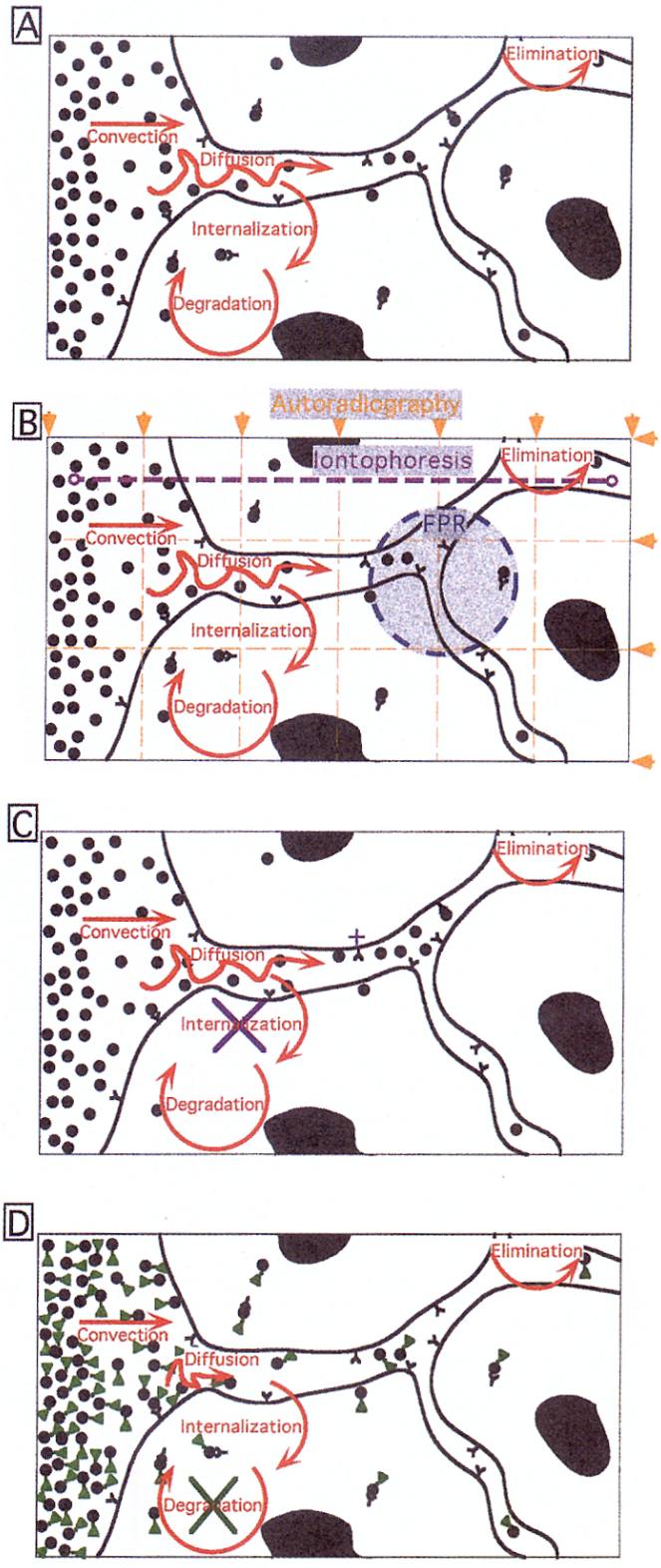


Fig. 2. A) Digitized autoradiographic image of radiolabeled NGF released from a polymer implant in a rat brain reproduced from (73). Black is the highest concentration of NGF and blue is the lowest. B) The resulting concentration profile, reprinted from Krewson, C. E., M. L. Klarman, and W. M. Saltzman, Distribution of nerve growth factor following direct delivery to brain interstitium. *Brain Research*, 1995. 680: p. 196-206 with kind permission of Elsevier Science - NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.

photobleaching recovery (FPR), also known as fluorescence recovery after photobleaching (FRAP), is an alternate technique for measuring dispersion of fluorescent molecules within a microscopic tissue volume. A focused laser is used to photobleach the fluorescent molecule in a small volume of tissue; fluorescent molecules from adjacent regions of tissue subsequently diffuse into the bleached volume, causing the fluorescence intensity to increase with time (Fig. 5). The time interval for recovery depends on the diffusion coefficient and the size of the bleached region ($t = L^2/D$); the size is usually small (~1 μm) and adjusted so that recovery occurs within a fraction of a minutes. Over this short interval, elimination can be neglected and D is obtained by adjusting appropriate solutions to the diffusion equation to fit the measured intensity recovery curve. FPR measurements are rapid, so dynamic processes are examined in real time. Its main limitations are interference from out-of-focus light and photodamage of the diffusing molecule or the medium due to the high intensity during the photobleach (60). An improvement upon conventional FPR uses two photons—with twice the wavelength and thus one-half the energy—for excitation (57,58). This modification reduces photodamage outside of the focal region. Further, only fluorescently- labeled molecules inside the focal volume receive enough energy to fluoresce, which reduces scatter from out-of-focus light and improves signal-to-noise. FPR has been used to measure diffusion coefficients in water (61), agarose gels (62), cell suspensions (63), cells in culture (64), and animals (55) (half-filled symbols in Fig. 4B).

An alternate method for determining diffusion coefficients employs ion-selective microelectrodes; this technique has been pioneered by Nicholson (65). A marker ion is ejected from a



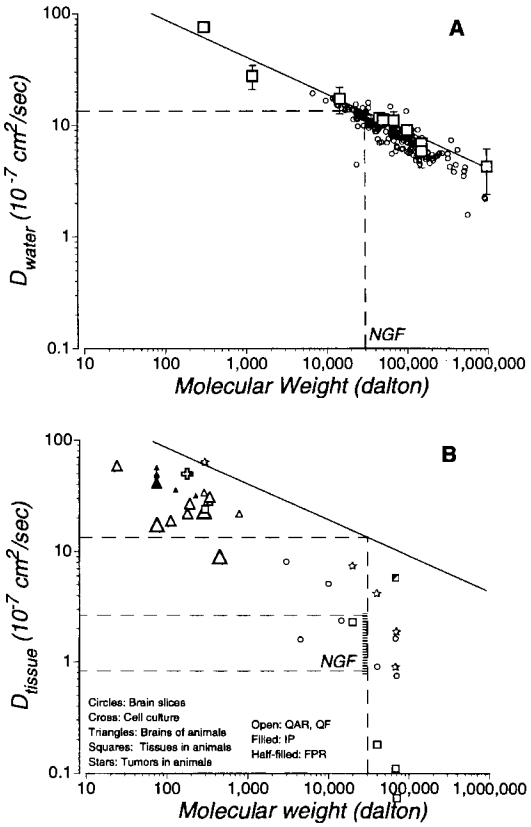


Fig. 4. A) Diffusion coefficients of molecules in water as a function of molecular weight, reproduced with permission from Saltzman *et al.* (45). The dashed lines represent values of the diffusion coefficient and molecular weight of NGF (13 x 10^{-7} cm^2/s and 28,000 Daltons, respectively). B) Diffusion coefficients of molecules in tissue versus of molecular weight. The hashed line is the range of diffusion coefficients for NGF measured in tissue; the dashed lines represent the diffusion coefficient of NGF in water. The elimination constant, *k*, can be estimated from *a*, ϕ, and *D*, and yields a value between 0.8 to 2 x 10^{-4} per second. QAR, quantitative autoradiography; QF, quantitative fluorescence; IP, ionophoresis.

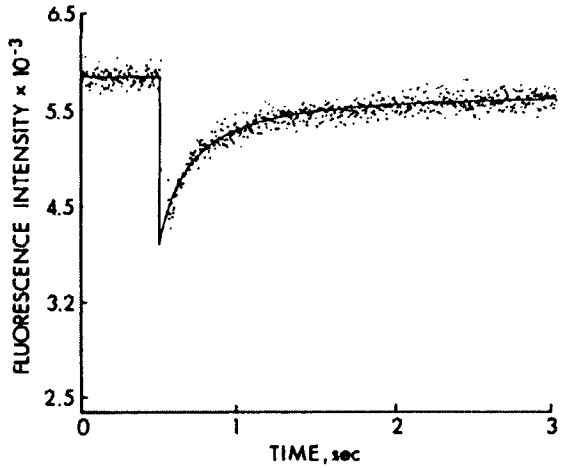


Fig. 5. A representative intensity profile obtained from FPR, reproduced with permission from Arakawa *et al.*(91).

micropipette using either an iontophoretic current or pressure injection (Fig. 6A). The ion migrates away from the source; concentration is measured by an ion-selective microelectrode positioned 50–150 μm away. Concentration-time profiles are plotted and a suitable diffusion equation, which neglects elimination and assumes the tissue is homogeneous and isotropic, is adjusted to best represent the data: volume of distribution and diffusion coefficient are obtained in this procedure (66). Iontophoretic measurements are rapid, so sudden changes in the tissue dynamics, such as occur during anoxia (67), can be resolved. This technique has been used to measure the diffusion coefficients of ions, most frequently tetramethylammonium (TMA) (Fig. 6B), in agarose gels (67), living brain slices (68), and animals (66,67) (filled symbols in Fig. 4B). Iontophoresis is sensitive to ions such as dopamine (69) (~ 20 ng/mL), but interference from other ions can be a problem. A key drawback of iontophoresis is the limited number of relevant ions that can be detected and the difficulty of using this technique for studying polypeptides.

In summary, several biophysical techniques are available to measure rates of diffusion in water (Fig. 4A) or hindered diffusion in cells, brain slices, or animals (Fig. 4B). Each method has characteristic strengths and weaknesses (Table 1). Other methods are available, but less extensively tested: for example, magnetic resonance imaging (MRI) is non-invasive, so migration of agent in a single animal can be monitored over time, provided the agent contains a paramagnetic label, such as gadolinium (70).

PROTEIN ELIMINATION FROM TISSUES

These experimental tools permit examination of protein distribution in tissue after application of novel delivery systems. For example, autoradiography of brain sections demonstrated that protein distributed through a region ~1 mm in any direction from the edge of an NGF-releasing polymer matrix (Fig. 2); this pattern persisted for the duration of protein release, up to two months after implantation (19,71).

What mechanisms of transport and elimination are consistent with this pattern of distribution? The experimental data can be used to test the suitability of mechanisms (Fig. 2). By comparing the measured concentrations to Equation 2, ϕ was estimated to be ~1.3. To estimate the relative contributions of elimination and diffusion, an independent estimate of either *k*

Fig. 3. *(opposite)* A) The modes of transport of molecules (•) through tissue: convection of fluid, diffusion, elimination in the extracellular space, receptor-based and non-receptor-based internalization, and intracellular degradation. B) Illustration of the spatial dimensions of measurement in the three major methods reviewed here. Note: the scale of the three methods of measurement are correct with respect to each other, e.g., autoradiography provides a measurement of concentration in a 10 μm by 10 μm pixel, FPR can bleach about a 15 μm diameter volume, and iontophoretic electrodes can be as close as 50 μm from each other. However, the extracellular space and molecules are not on the same scale as the measurement techniques for illustrative purposes. C) To enhance transport, receptors can be modified to become internalization-deficient (purple receptors), leading to a higher effective concentration of diffusing molecule through the extracellular space. Molecules can still become internalized through non-receptor mediated events, however. D) Another method of enhancing transport is conjugation of the molecule to a more-slowly degraded molecule (green triangles), which also serves to increase the effective concentration and boost penetration. Notice that conjugation also reduces the transport due to diffusion.

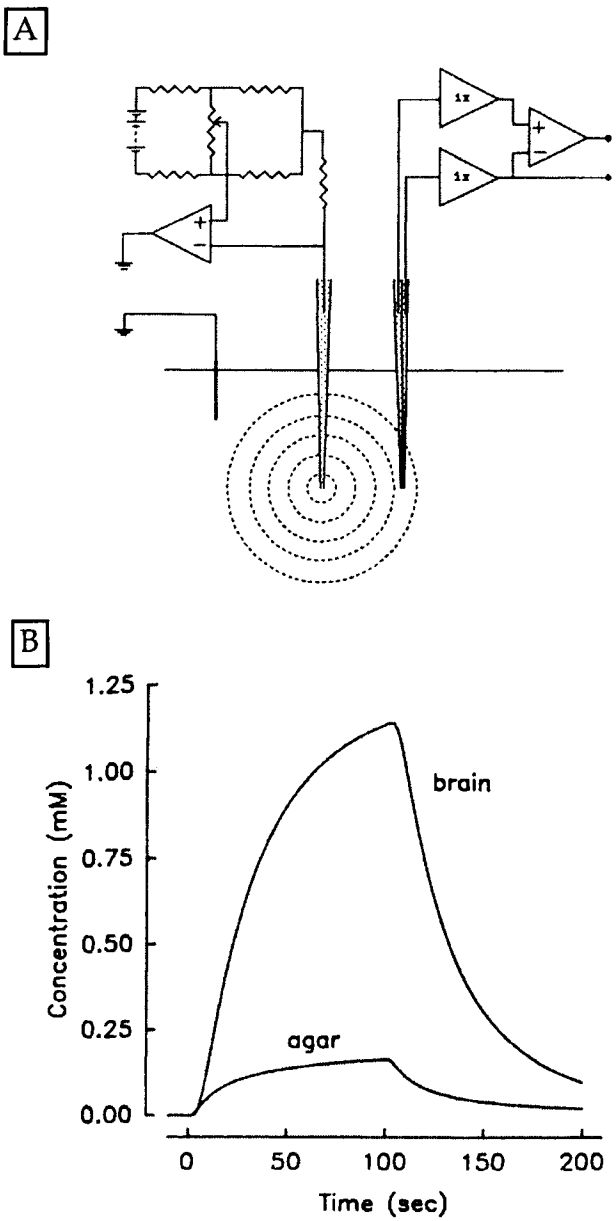


Fig. 6. A) Schematic of the iontophoretic apparatus, reproduced from Lundbaek *et al.* (68). B) The resulting concentration-time profile from iontophoresis in agarose gel and brain tissue. Reproduced with permission from Nicholson *et al.* (92).

and D is required. The D for NGF in buffered water is approximately 13 x 10^{-7} cm^2/s, based on the size of the 28,000 kDa homodimeric protein (dashed lines in Fig. 4A). Because transport in the brain is accompanied by elimination, no direct measurements of NGF diffusion coefficient in the brain are available. As an alternative, the diffusion coefficient for NGF in brain interstitium can be estimated by comparison to diffusion coefficients measured for tracers of comparable size: this procedure yields D in the range 0.8 to 2 x 10^{-7} cm^2/s for NGF in the brain (hatched line in Fig. 4B). The elimination constant, k, can be approximated from a (0.4 mm in this case), ϕ and D. The resulting k is in the range 0.8 to 2 x 10^{-4} per second; this corresponds to a half-life of NGF in brain tissue of 1 to 2 hours. Similar results are obtained with mouse and recombinant human NGF (19,71). The assumptions and limitations of this approach have been discussed (19,71).

The elimination rate estimated from the autoradiographic data is consistent with the half-life of ~1 hr measured following intraventricular injection of [^{125}I]NGF (72). This agreement provides some support for the approach, which follows a justifiable (albeit circuitous) path. Interestingly, the half-life for NGF in brain tissue is substantially longer than the half-life in plasma after i.v. injection, 2.4 minutes (15), and substantially shorter than the half-life due to elimination via permeation through the capillary wall, ~100 hours[6]. So brain-to-blood transcapillary permeation is probably not important in NGF elimination from the brain. The most probable mechanism for elimination is by binding, internalization, and intracellular metabolism: other molecules (e.g. asialoglycoproteins, EGF, and formylated peptides) have similar kinetics of degradation after internalization by cultured cells (73,74).

BALANCING TRANSPORT AND ELIMINATION

"Penetrability" of NGF after administration by interstitial drug delivery is consistent with known rates of protein dispersion and elimination in the brain as demonstrated in the preceding example. But can the tissue distribution of locally- administered proteins be customized? The preceding sections described experimental methods for examining local distribution, diffusion, and elimination of proteins in the brain. Once the dynamics of protein dispersion and elimination are known, can protein agents be tailored to satisfy disease-specific requirements? Although the mechanisms of protein distribution in the brain are only partly understood, the studies reviewed above suggest several methods to improve interstitial drug delivery for brain tumors and neurodegenerative diseases. In particular, local transport and elimination rates can be manipulated to obtain agents that distribute over a predetermined volume of tissue.

Dispersion (represented by D) and elimination (represented by k) are both important in determining penetrability of proteins (Equation 1). Therefore, local distribution can be altered by making changes that influence either protein dispersivity or stability in the interstitial space. For example, reduction of the elimination rate (decreases in k) leads to molecules that are retained longer in the interstitial space and, therefore, diffuse greater distances.

For proteins that are eliminated by receptor-mediated internalization and degradation, the rate of elimination can potentially be adjusted at a number of levels (75). For example, changes in receptor number or affinity will alter the rate of protein internalization (76) (Fig. 3C), as will changes in the ratio of the receptor recycle rate to lysosomal degradation rate (77). A recent study of protein permeation in three-dimensional tissue analogs (78), and several studies of protein distribution in developing embryos (79–81), demonstrate that protein interactions with cell surface receptors have a significant influence on the pattern of protein penetration in tissue. Fundamental changes in the molecular machinery of cells are now performed routinely by addition or deletion of genes in cultured cells and

[6] Half-life=$t_{1/2}$ = ln(2)/PS, where PS is the permeability coefficient-surface area product, reported to be ~2 x 10^{-6} l/g-s for blood-to-brain permeation of NGF in the rat (15).

Table 1. Comparison Between the Three Methods of Diffusion Coefficient Measurement Reviewed Here: Autoradiography, Iontophoresis, and FPR

	Advantage	Disadvantage
Quantitative Autoradiography and Fluorescence	Direct observation of inhomogeneities in tissue	Requires stability of the label Requires many animals
Iontophoresis	Fast Can measure real-time changes in conditions	Limited number of suitable ions Difficult to apply to polypeptides
FPR	Fast Can measure real-time changes in conditions	Requires stability of the label

animals. While genetic modification may not be a feasible (or desirable) approach for altering the transport barriers of tissues, further study of molecular mechanisms of receptor-mediated internalization will almost certainly lead to less dramatic, and more easily controllable, approaches. For example, one can imagine the development of agonists, antagonists, or "suicide-substrates" for specific steps in protein transit through cells; these agents—which might operate at the level of the gene or the receptor or any stage in between—could be delivered in concert with the therapeutic protein, providing a navigable, long-distance diffusion pathway.

Penetrability can also be enhanced by modifications in the protein that increase either dispersivity or stability (Fig. 3D). If diffusion is the primary mechanism for dispersion, increases in transport rate will be difficult to secure: a 100-fold decrease in protein molecular weight will produce less than a 5-fold increase in D (Fig. 4A). Alternately, protection of the protein from elimination may yield substantial increases in penetrability. Proteins can often be stabilized by conjugation to inert polymers, such as dextran (82–84) or poly(ethylene glycol) (85, 86). Although conjugation increases molecular weight, and decreases the diffusion coefficient (Fig. 4A), penetrability can be increased provided that the rate of elimination is decreased more than the rate of diffusion. Increased penetrability has been observed after conjugation of NGF to dextran (82).

CONCLUSIONS

Novel drug delivery systems promise new opportunities in the therapy of chronic brain disease. These delivery systems are already impacting clinical practice; implantable polymeric delivery systems for the chemotherapy agent BCNU improve survival in patients with malignant glioma (87) and were approved for use by the U.S. Food and Drug Administration in 1996. Protein delivery to the brain is also possible, but clinical application will require advanced designs based on an understanding of the mechanisms for migration and elimination of proteins in the brain. In addition, the limitations of this approach, which introduces high concentrations of potentially toxic compounds (88) at the polymer surface, have not been fully explored. Biophysical techniques for measuring distribution, diffusion, and elimination have already permitted analysis of important aspects of protein transport, and suggest new opportunities in the design of protein therapeutics.

ACKNOWLEDGMENTS

Our original research on drug delivery systems for the brain was supported by the National Institutes of Health (CA52857), the National Science Foundation (BCS-9123070), and the National Aeronautics and Space Administration (#NAG 9-654). Our work on NGF delivery systems was greatly facilitated by the group in Pharmaceutical Research and Development at Genentech, Inc., who provided encouragement as well as valuable reagents.

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