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Review

Delivering Peptides to the Central Nervous System: Dilemmas and Strategies

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Peptides have been shown to cross the blood–brain barrier (BBB) as intact molecules so that they can influence the central nervous system. Peptides cross by saturable and nonsaturable mechanisms in the direction of both brain to blood and blood to brain. Passage of peptides, especially by saturable transport, has been shown to be influenced by pharmacological agents and physiological events. These findings support the view that peptides or their analogues could be useful as therapeutic agents for disorders of the central nervous system. They also suggest strategies in approaching therapeutic goals, including manipulating transport rates, targeting diseases due to altered BBB–peptide interactions, and designing analogues capable of taking advantage of such mechanisms of passage as paracellular transmembrane diffusion and brain-to-blood transport.

KEY WORDS: opiates; drug therapy; cell membrane; transport; drug design; blood–brain barrier.

INTRODUCTION

It has long been hoped that peptides could be used as therapeutic agents in the treatment of diseases of the central nervous system (CNS). However, fulfilling this hope has proved formidable, primarily because of both real and perceived difficulties attributed to the blood–brain barrier (BBB). Here, we review work from our laboratory as it relates to five basic questions concerning the passage of peptides across the BBB.

CAN PEPTIDES CROSS THE BBB?

This fundamental question has until recently been very controversial, and there are still respected scientists who would answer negatively. We feel, however, that the evidence is now overwhelming that peptides can cross the BBB. Furthermore, much has been learned about how and to what extent they cross as well as about some of the factors that influence the degree of passage.

The BBB is increasingly recognized as representing a complex system of mechanisms that act in concert to regulate the exchange of fluids and substances between the CNS and blood. In the simplest analysis, it consists of at least two components: the endothelial, or capillary, barrier (often also referred to as the blood–brain barrier) and the ependymal barrier [often referred to as the blood–cerebrospinal fluid (CSF) barrier] found at the circumventricular organs (CVOs) and choroid plexus.

These barriers owe their restrictive abilities to the presence of tight junctions that limit the leakage of fluid between the cells that interface between the CNS and the periphery. Under normal conditions, leakage is all but eliminated throughout most of the CNS. The BBB is, however, not an absolute barrier. In fact, more sensitive methods can measure the entry rates of even those substances classically used as vascular markers. Most substances enter to some extent by a mix of residual leakiness, transmembrane diffusion, and, possibly, transport. Conceptualizing the BBB not anatomically as an absolute barrier but functionally as a regulatory interface is more appropriate. The relevance of a measured permeability, then, depends not only on the degree of penetration, but also on the substance's potency, how it is handled within the CNS, and its peripheral pharmacokinetics.

Much of the controversy about the passage of peptides arose because early studies used insensitive techniques and because the possibility of brain-to-blood passage was not considered. In the last few years, much more sensitive techniques have been developed and applied to peptides. Intravenous injection techniques (1–3) and brain perfusion methods (4–6) for studying blood-to-brain passage, a simple quantitative method for studying brain-to-blood passage (7), and *in vitro* models of the BBB (8) are all now available. These methods are even sensitive enough to determine the much lower rates of penetration for the larger serum proteins. As a result, transport systems are now being described for large proteins such as the cytokines (9,10), transferrin (11,12), immunoglobulins (13), and glycoproteins (14).

Early studies often merely showed that brain tissue contained some intravenously injected peptide. In many cases, the possibility that peptides were present only in the vascular space was not addressed. Later studies included vascular markers or washed out the vascular space and so were able

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to show that the level of peptide exceeded that attributable to the vascular space. However, studies relying on a single time point may not be able to distinguish between actual transport across the endothelial cell of a capillary and reversible binding to receptors on the membrane of the endothelial cell. Such receptor binding can influence CNS function (15) and, if the BBB is considered part of the CNS, might be thought of as the first level at which peptides and the CNS interact. However, other applications of peptides would require that they be able to reach brain tissue.

Evidence showing such entry into the brain is quite abundant. The appearance of peptides in the CSF obviously shows entry into a compartment of the CNS (16,17). Since no barrier exists between the interstitial fluid of the brain and ventricular fluid, this probably means that the peptides also have access to brain cells. A recent study has shown that within minutes after injection into the CSF, peptide can enter the periventricular interstitial space (18). In addition, the methods for measuring unidirectional influx may distinguish between the rapidly reversible binding to vascular receptors and entry (19). Demonstration of passage of a material from the brain to blood is easier, since material appearing in the blood after injection into the lateral ventricles obviously had to cross either the ependymal or the endothelial barriers.

BY WHAT MECHANISM DO PEPTIDES CROSS THE BBB?

Peptides cross the BBB by both saturable and nonsaturable means. Examples of peptides that have been shown to cross largely by a nonsaturable mechanism are thyrotropin releasing hormone (TRH) (6), α-melanocyte stimulating hormone (20), and delta sleep-inducing peptide (DSIP) (21), although a saturable component for the latter peptide appears to be present in the guinea pig (22). Of 18 radioiodinated peptides tested, the degree of blood-to-brain passage for the majority could be explained largely by their lipid solubility (23). Other factors that may influence entry are molecular weight, charge, degree of protein binding in the serum, and peptide aggregation, although these seem to play a lesser role than lipophilicity. Recently, binding to lipoproteins has been postulated to modulate the entry of cyclosporin into the brain (24). The term transmembrane diffusion has been coined to distinguish this mode of nonsaturable passage from that of capillary leakage. Others have used the term "paracellular transport" to describe findings from an *in vitro* model of blood-to-brain passage in which molecular weight was a predictor of the degree of permeability, suggesting an aqueous pore model of capillary leakage (25). Molecular weight also appears to be a significant factor in the clearance of substances from the CSF (26). The unidirectional influx rate (K_i) to date for most peptides entering the brain by transmembrane diffusion is in the range of 10^{-3} to 10^{-4} ml/g-min, or about 10–100 times faster than for albumin (Table I). Thus, the pathways that allow limited access to the CNS for serum proteins such as albumin cannot account for the rate of entry for peptides.

Saturable systems also exist that can transport peptides across the BBB (27). We have described several peptide transport systems (PTS), and other laboratories have described additional ones (Tables I and II). The K_i for peptides transported in the blood-to-brain direction is usually in the range of 10^{-2} to 10^{-3} ml/g-min, about 10 times faster than predicted from their lipid solubilities. These systems seem to be restricted to transporting a limited number of structurally related peptides. For example, PTS-1 transports Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide), methionine enkephalin, and a few other closely related peptides, but not D-Tyr-MIF-1 or opiate peptides such as β-endorphin, kyotorphin, and dynorphin$_{1-17}$ (28). PTS-2 transports arginine vasopressin, but not oxytocin (29). The underlying requirements for transport by a given system are not always obvious. For example, oxytocin is transported by PTS-1, although not as rapidly as Tyr-MIF-1 or methionine enkephalin (30). Leucine enkephalin is transported across the BBB (16,31), but not all of that transport is accounted for by PTS-1 (32). This and other

Table I. Blood-to-Brain Unidirectional Influx Rates (K_i) for Peptides

Peptide	Transport system[a]	$(K_i)10^3$ (ml/g-min)
Tyr-MIF-1	None	4.4–2.62
D-Tyr-MIF-1	None	2.42
[D-Ala^1]-Peptide T-Amide	PTS-3	1.3–2.5
LHRH	PTS-4	12.5
RC-160[b]	None	0.0923
RC-160[b,c]	None	20.3
RC-121[b]	None	0.0401
RC-161[b]	None	0.203
Cyclosporin A	ND[d]	0.43–2.53
Leucine enkephalin	NN[e]	3.62
TRH	None	1.14–1.22
AVP	NN	1.95–2.89
DSIP	NN	0.93–1.66
Opiate peptide analogues	ND	19.6–54.6[f]

[a] For blood-to-brain transport.
[b] Analogues of somatostatin.
[c] Value when repeated by a serum-free perfusion method.
[d] Saturability not determined.
[e] Saturation demonstrated but not named.
[f] PA values from reference 70.
$(K_i)10^3$ for albumin (no saturable transport) as measured by this method (Ref. 1) is 0.0097–0.065 ml/g-min.

Table II. Brain-to-Blood Passage of Peptides: Half-Time Disappearances After Intraventricular Injection in Mice

Peptide	Transport system[a]	$t_{1/2}$ (min)
Tyr-MIF-1	PTS-1	12.8–14.1
D-Tyr-MIF-1	None	30.7
Met-enkephalin	PTS-1	10.3
Oxytocin	PTS-1	19.1
Arginine vasopressin	PTS-2	12.4
LHRH	PTS-4	15.0
RC-160[b]	PTS-5	24.0
RC-121[b]	PTS-5	14.4
RC-161[b]	None	53.0

[a] For brain-to-blood transport.
[b] Analogues of somatostatin.
$t_{1/2}$ for albumin (no saturable transport) as measured in mice by this method (Ref. 7) is 30–45 min.
See Ref. 26 for additional substances tested by the ventriculocisternal perfusion method in rabbits.

evidence recently reviewed (33) suggests that a family of interrelated systems may transport opiate-like peptides across the BBB.

Saturable transport rates are modulated by various factors. For example, leucine acts like an allosteric regulator of PTS-1, and aluminum acts like a noncompetitive inhibitor (33). In general, PTS-1 is not influenced by a large number of hormones, drugs, or peptides tested. Serotonin (32), however, does appear to regulate the transport rate of PTS-1 through a site similar to the 5-hydroxytryptamine (5HT)-1 receptor subtype (32).

Passage across the BBB can occur in the direction of either blood to brain (Table I) or brain to blood (Table II). In either direction, passage can occur by saturable or by nonsaturable mechanisms. For most peptides studied, a saturable component to passage, if it exists, is usually in only one direction (34,35), but for luteinizing hormone releasing hormone (LHRH) (36,37), a saturable component has been found for passage from both blood to brain and brain to blood. Leucine enkephalin is also transported in both directions, but at least two separate systems are known to be involved, since only part of the brain-to-blood (32) and none of the blood-to-brain (5) transport can be accounted for by PTS-1.

Transport of peptides can occur at either the ependymal (38) or the endothelial (22) components of the BBB. The choroid plexus, in particular, is capable of transporting many substances (39) into or out of the CNS (40), is enzymatically active (41), and can sequester and possibly transport peptides (42,43).

The therapeutic implications for a blood-to-brain transport system are obvious. However, as discussed below, the existence of a brain-to-blood saturable transport system may be no less important and even offer some novel approaches to therapeutic intervention.

DO PEPTIDES CROSS THE BBB AS INTACT PEPTIDES?

Only a few early studies characterized the material crossing the BBB (e.g., Ref. 44), raising the possibility that inactive metabolites could have accounted for the supposed entry for some of the other studies. However, in numerous experiments the material crossing the BBB has been recovered and characterized. The early studies relied on Sephadex chromatography used in conjunction with highly specific radioimmunoassays. Later, high-performance liquid chromatography (HPLC) became available for more precise characterization of the material crossing the BBB.

For peptides crossing by a saturable process, fragments can be very useful in characterizing the transport system. For example, the transport by PTS-1 of radioactive Tyr-MIF-1 (Tyr-Pro-Leu-Gly-amide) labeled at the tyrosine is inhibited by the unlabeled intact molecule, but not by Tyr-Pro-Leu, Try-Pro, Tyr, or ^{127}I, demonstrating that the entire molecule is needed for transport (28). Such competition studies have the advantage over HPLC identification of transported material of further defining the requirements of the transport system.

Many studies now have shown that peptides can cross the BBB in either direction as intact molecules. It is also clear, however, that many peptide fragments can both cross the BBB and exert biological effects (45). Future studies may need to consider less the question of passage of intact vs degradation products and more the question of biologically active vs nonactive moieties.

CAN PASSAGE ACCOUNT FOR THE EFFECTS OF PEPTIDES ON THE CNS?

The possibility that peptides could cross the BBB was first suggested to explain their effect on behavior and other events related to the CNS after peripheral administration. However, at least some of those CNS effects are mediated through peripheral pathways, making it unnecessary for peptides to cross the BBB. The consideration that the rate of entry of peptides may be too low to influence the CNS has also been raised, although it must be remembered that small amounts of peptides can exert very powerful effects. Estimates of the amount of peptide entering the brain have been in the range of 10^{-1} to 10^{-3}% of the amount injected (45–49). By analogy, the entry rate for morphine as measured by the brain uptake index is about the same as for peptides (50), yet it is able to exert powerful effects on the CNS. Therefore, entry rate and potency must be considered together. Nevertheless, determination of the relevance of passage for peptides is a critical issue.

One matter that has caused some confusion relates to the CVOs. The CVOs are areas of the brain with a capillary bed that does not participate in the BBB. Therefore, peptides found in the blood have ready access to these areas that have neural connections to other parts of the brain. Many effects can be explained by peptides working directly at the CVOs. However, the CVOs are delimited from the CSF and the interstitial fluid of adjacent brain tissue by an ependymal layer of cells forming part of the BBB at this level (51,52). This means not only that peptides in the CVOs have to cross the BBB at the ependymal barrier in the blood-to-brain direction to reach deeper areas of the brain, but also that peptides found in the CSF must cross in the brain-to-blood direction to reach the CVOs. Therefore, it is possible that some peripherally administered peptides affecting the CNS might work at the CVOs and so do not have to cross the BBB to produce their effects. However, an effect mediated through the CVOs after injection of a peptide into the CNS would require passage across the BBB in the brain-to-blood direction. Conversely, a peptide working at a CNS site with a BBB would have to cross the BBB if given peripherally but not if given centrally.

Based on this reasoning, it has often been assumed that if much less peptide is required to evoke an effect after injection directly into the CNS than after iv injection, then the site of action is in a part of the CNS with a BBB. These sorts of studies are numerous and, in general, suggest that passage of peptides across the BBB is required for a great many effects. A logical extension of this approach is to calculate how much peptide is entering the CNS after peripheral administration and to determine whether the observed effect can be replicated when that amount is given directly into the CNS (49). The effects of desglycinamide-arginine vasopressin (DGAVP) on behavior (49) and human interleukin-1α (IL-1α) on temperature (9,53) indicate these compounds cross the BBB to exert their actions.

As reviewed elsewhere (33), more direct approaches have also indicated that some effects of peptides require passage across the BBB. The potencies of DSIP analogues in inducing EEG changes and of molluscan cardioexcitatory neuropeptide (FMRF)-related peptides on analgesia correlate with their abilities to cross the BBB. Some of the cardiovascular effects of TRH are decreased when its entry into the CNS is inhibited. Administration of small doses into the CNS of an antagonist can block the effects of peripherally administered arginine vasopressin on CSF concentrations of β-endorphin. The ability of naloxone or naltrexone, opiate antagonists that cross the BBB, but not of their quaternary salts which do not cross, to block an effect indicates a CNS site of action for an opiate peptide. These are representative of the types of studies (33) that are being done to determine which actions of peptides involve passage across the BBB.

WHAT STRATEGIES COULD BE USED FOR TARGETING PEPTIDES TO THE CNS?

The above information shows that peptides can cross the BBB and that such passage is involved in the ability of peripherally administered peptides to affect CNS function. It also suggests several approaches to enhance the delivery of peptides or their analogues to the CNS.

An obvious first step is to determine how the peptide or analogue in question is handled by the BBB. An analogue designed to take advantage of a blood-to-brain transport system is more likely to affect the CNS, while peptides transported in the brain-to-blood direction might be useful as peripheral agents free of CNS side effects. For peptides that enter by transmembrane diffusion, more lipid soluble analogues can have enhanced CNS activity and less lipid soluble analogues can have reduced CNS activity (54).

Attempts to alter entry rate by the attachment of peptides to other molecules, alteration of their charge, or design of analogues for facilitating entry should first consider how peptides transverse the endothelial cell. One school of thought suggests that receptor-mediated transcytosis is the predominant mechanism of passage (55). This model, originated for serum proteins (56), may not be able to provide a rate of transfer high enough (57) to account for the entry rates of peptides or be able to account for their brain-to-blood transport (58). Another, but by no means mutually exclusive, mechanism suggests that substances might cross the BBB by diffusing through the cell membrane of the endothelial cell without entering the cytoplasm, a process termed paracellular transmembrane diffusion (59). Substances using this pathway may either penetrate directly through the tight junction or pass behind the tight junction (which may involve only the outer leaflet of the cell membrane) by diffusing through the inner leaflet of the cell membrane. Evidence exists for both of these mechanisms as well as others. Which, if any, of these mechanisms predominates would determine the underlying principles that should be used in the rational design of peptides.

Factors other than permeability to the BBB can restrict entry of a peptide into the CNS to a low percentage. For example, serum factors such as protein binding can reduce the permeability of an analogue by several magnitudes (60). A large volume of distribution, rapid clearance, or a high rate of degradation can all result in low concentrations in the blood of peptide and, therefore, a low rate of presentation to the CNS.

Inhibition of a brain-to-blood transport system may be necessary to allow substantial accumulation in the CNS of some peptides. Inhibition of transport alone may be therapeutic, allowing endogenous peptide to accumulate within the CNS rather than being transported out.

Alteration of the rate of transport may be particularly useful if a derangement of the transport system itself was found to underlie the disease process. For example, during withdrawal from ethanol, PTS-1 recovers from its depressed rate of transport in addicted animals (61). This suggests that the decrease in the concentration of enkephalins in the CNS, postulated to be involved in withdrawal (62,63), could be induced by PTS-1. If so, inhibition of this system might affect the enkephalin-sensitive symptoms of ethanol withdrawal. The role of opiate peptides and PTS-1 has also been raised in syndromes of hyperleucinemia such as maple syrup urine disease (64).

Illnesses of the neonate found to be responsive to peptides may be particularly easy to treat. The low enzymatic activity in the gastrointestinal tract of newborns allows the absorption of peptides after oral administration (65). Since peptides administered early in life can have effects persisting into adulthood (66–68), this may represent a therapeutic window.

It is also possible to alter the transmembrane diffusion of peptides across the BBB. Aluminum, for example, can increase transmembrane diffusion (69), but it is probably too toxic to be used therapeutically.

In summary, the ability of peptides to cross the BBB and influence the function of the CNS suggests that they could be useful as therapeutic agents. Strategies for treatment based on the known relationship between peptides and the BBB should be able to increase their therapeutic potential.

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