Expert Opinion

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The CNS as a target for peptides and peptide-based drugs

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Peptides hold great potential as CNS drugs, but their delivery to the CNS is problematic. However, actual roadblocks to peptide delivery are different from those often perceived. Many peptides cross the blood–brain barrier by saturable and non-saturable mechanisms, and accumulate in brain in amounts sufficient to produce physiological effects. Peripheral factors (e.g., short half-life in blood) can be dominant factors limiting therapeutic use. Production of therapeutics that are enzymatically resistant and have long circulation times, even when the blood–brain barrier penetration is low, can result in substances with significant CNS accumulation. Surprisingly low amounts of peptide in brain can result in CNS effects, and so the dose needed for brain delivery is generally much smaller than for peripheral tissues. Brain-to-blood transporters can greatly limit CNS accumulation of a potential therapeutic. Finally, intranasal and intrathecal routes may be especially useful for substances that are rapidly degraded in blood or are large and hydrophobic, respectively.

Keywords: blood–brain barrier, central nervous system, drug delivery, intranasal, intrathecal, P-glycoprotein

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1. Introduction

Peptides have long been considered to hold great potential as therapeutic agents. This is especially true for diseases of the CNS, as peptides mediate, are involved in, or are affected by so many of the physiological functions of the brain, spinal cord and cranial nerves. Peptides, however, constitute a very small part of the existing pharmacopeia, especially for CNS diseases. There are many issues that complicate the development of peptide drugs for the CNS, such as rapid degradation in blood and difficulty in oral administration. The major perceived difficulty, however, is their low penetration into the CNS; in other words, their low permeability across the blood–brain barrier (BBB). On close examination, the BBB is less insurmountable and constitutes a smaller portion of the difficulties in peptide delivery to the brain than is generally believed. This review discusses some of the common misperceptions and real roadblocks to the successful delivery of peptides to the brain, and examines some potentially successful strategies for developing CNS peptide therapeutics.

2. Philosophical approaches

Box 1 lists some strategies for enhancing peptide delivery to the brain. In the broadest of terms, two philosophical approaches have evolved. One strategy assumes that the BBB is either totally or in practicality impermeable to peptides. This strategy not only ignores any interaction the candidate peptide might have with the BBB, but in its extreme version ignores nearly any other physiological consideration of the BBB. In short, this strategy views the BBB as an obstacle to be overcome, and usually attempts to breach or otherwise disrupt the integrity of the BBB. The other strategy attempts to base delivery strategies on an understanding of the BBB. In its

Box 1. Selected approaches to development of delivery of peptide therapeutics to the CNS.

Chimeric vectors
Lipid solubility
Glycosylation
Neutral polyamines
Cationisation
Nanoparticles
Liposomes
Attach to a substance that crosses (e.g., peptides and penatrins)
Nasal delivery
Intrathecal delivery
BBB disruption (hyperosmolar; bradykinin agonist)
Retroinverso peptides
Viral vectors

BBB: Blood–brain barrier.

extreme version, it would first determine what, if any, interactions occur between the candidate peptide and the BBB, and use such information as a rational basis for drug development. For example, it might be found that a candidate peptide therapeutic can cross the BBB, but that the amount of peptide taken up by the brain is therapeutically ineffectual due to enzymatic degradation. In that case, developing a peptide that is more enzymatically resistant may do more to enhance peptide delivery to the brain than increasing its permeability.

Attempts to produce universal drug delivery systems have taken both approaches. So far, those that attempt to disrupt the BBB have produced unacceptable amounts of toxicity. One reason for this is that disruption allows not only the candidate peptide to enter the CNS, but many endogenous circulating substances as well. Not surprisingly, therefore, two trends in the development of potential universal drug delivery systems are to develop carriers that would only allow penetration of the candidate peptide, and to use strategies based on a rationale that takes advantage of the characteristics of the BBB.

3. Fallacies in peptide delivery to brain

Several fallacies are common that have discouraged or misguided attempts to produce CNS therapeutic peptides. Below, several of these are discussed.

3.1 Fallacy 1: peptides do not cross the BBB

The BBB was originally defined based on experiments which showed that peripherally administered basic dyes could not stain the brain. These dyes bind strongly to albumin, which itself does not cross the BBB. Hence, the lack of staining is an indicator of the impermeability of the BBB to circulating proteins such as albumin. Unfortunately, it was assumed with little evidence that peptides would also be excluded by the BBB. This assumption may have been fostered by the erroneous idea that peptides would behave like proteins. Even so, it is still surprising that even peptides smaller than thyroid hormones, which are well known to cross the BBB, were assumed to be unable to cross.

Delta sleep-inducing peptide, a nonapeptide, was the first peptide to be shown to cross the BBB intact [1], and Tyr-MIF-1, a tetrapeptide, the first shown to cross the BBB by a saturable system [2]. Since then, hundreds of endogenous peptides, peptide analogues and regulatory proteins have been shown to cross the BBB in significant amounts [3-7].

3.2 Fallacy 2: lipid solubility plays no role in peptide penetration of the BBB

Most traditional non-peptide small molecules that exert effects on the CNS are lipid-soluble. Such substances can cross the BBB in substantial amounts by the non-saturable mechanism of diffusing across cell membranes [8]. The degree of penetration is related both to the lipid solubility and inversely to the square route of the molecular weight. A misinterpretation of work by Levin [9] has led some to believe that substances with molecular weights greater than ~ 400 Da are unable to cross the BBB by the mechanism of lipid solubility. Although there is a molecular weight penalty, there is no evidence for an absolute molecular weight cutoff for crossing the BBB. The largest substance (7.8 kDa) shown to cross the BBB by transmembrane diffusion is CINC1 [10].

There are other issues with peptides and lipid solubility. Increasing lipid solubility of any substance enhances its sequestration by liver as well as to binding proteins. Therefore, there is a trade-off between increasing penetration of the BBB and decreasing the amount of the substance in the blood and, thus, the amount available to cross the BBB. Substances that are extremely lipid-soluble can partition into the membranes so avidly that they will not repartition into the aqueous environment of the interstitial fluid of the brain.

3.3 Fallacy 3: only a lack of BBB permeability of peptides prevents their use as therapeutic agents

Although BBB permeability is a major issue for peptide delivery to the CNS, it is not the only one. In fact, for many peptides, it is not the major issue, as adequate delivery to the CNS can be achieved by addressing these other factors. Pharmacokinetic and related factors are the major block to effective delivery to the CNS for many peptides [4]. A short half-life in blood due to enzymatic degradation, a large volume of distribution, sequestration by peripheral tissues, or secretion by kidney and liver means that many peptides have a limited exposure to the BBB. Extending the half-life in the blood can, even in the face of a low permeation of the BBB, result in enough peptide (or regulatory protein) entering the CNS to produce an effect on the CNS. Both the small peptide cycloHis-Pro and the large protein erythropoietin have low permeations across the BBB, but are enzymatically resistant and have long half-lifes in the blood [11,12]. As a result, they achieve levels in the CNS within hours of peripheral

administration to reverse ethanol narcosis and reverse ischaemic brain damage, respectively. Binding to circulating proteins classically decreases the penetration of small molecules into the CNS. Protein binding can also affect the penetration of some peptides into the CNS [13].

The BBB can block the accumulation by the CNS of some peptides which are otherwise capable of crossing its cell membranes. The BBB is an enzymatically active tissue and can thus act as an 'enzymatic' barrier [14,15]. The BBB also contains many brain-to-blood saturable transport systems. The most studied of these at present is the P-glycoprotein system, which has many clinically used drugs as substrates [16,17]. Overcoming these barriers can result in significant amounts of drug entering the brain. For example, loperamide is an opiate that ordinarily has no analgesic effects because its CNS accumulation is prevented by P-glycoprotein. In animals that do not express P-glycoprotein, loperamide accumulates in the brain and has analgesic effects [18]. P-glycoprotein has some peptides and proteins as its substrates, such as IL-2 and probably amyloid beta protein [19-21]. Other efflux systems, such as the peptide transport system (PTS) group, predominantly transport peptides. PTS-1, for example, transports Tyr-MIF-1 and the enkephalins from brain to blood [22].

3.4 Fallacy 4: large amounts of peptides must cross the BBB to be effective

This myth is often applied to traditional small molecules as well. In comparison to the uptakes by liver and kidney, or even most other peripheral tissue, uptake of drugs by brain is very low. For example, acetaminophen, which exerts its analgesic effects via the CNS, has an uptake of only ~ 0.2%/g of brain, and morphine has an uptake of < 0.02%/g [23-25]. Many endogenous peptides and regulatory proteins have uptakes that fall within this range and have effects on the CNS after peripheral administration. For these substances, factors other than BBB impermeability can be the major obstacles in therapeutic use.

4. Strategies for effective delivery of peptides and proteins to brain

As the review above indicates, there are several strategies that could result in improved delivery to brain. One approach is to study the native peptide or protein or the parent compound with regards to its ability to cross the BBB, identify the major factor limiting CNS uptake, and to modify the candidate therapeutic accordingly. It may be that the most effective method of increasing therapeutic delivery does not directly involve the BBB, but peripheral factors. Alternatively, there is an increasing number of approaches for 'bypassing' the BBB. Below, examples are considered under three major categories: peripheral factors, BBB factors and bypassing the BBB.

4.1 Peripheral factors

Peptides are especially susceptible to enzymatic degradation. This limits their circulation time and, thereby, their exposure to the BBB and CNS. A large volume of distribution dilutes their concentration in blood and further limits exposure to the BBB and CNS. Regulatory proteins have smaller volumes of distribution, but can also have short half-lifes in the circulation. Production of analogues with smaller volumes of distribution and longer half-lifes will proportionately increase uptake by the CNS. Extreme examples of the effects of improving pharmacokinetics are represented by erythropoietin and IgG antibodies [26]. Neither of these substances crosses the BBB, but gain access to the CNS through the extracellular pathways, that is, by leaking into the brain in the same manner as albumin. However, because of their long residence time in blood, they are able to eventually accumulate in brain [12,27,28].

Many peptides are bound by circulating proteins [13,29]. Protein binding or uptake by circulating cells can decrease the free fraction of a substance in blood [30,31]. This, in turn, can limit access to the BBB, especially for those substances without a saturable transporter located at the BBB.

4.2 BBB factors

The capillary wall which comprises the BBB is a substantial barrier to hydrophobic molecules such as peptides. Increasing the lipid solubility of a peptide can increase its penetration into the brain and enhance its CNS action. Alternatively, much effort has been expended on attaching a peptide to a ligand that is transported across the BBB. Published work indicates that this particular approach is largely unsuccessful, although most workers so far have attached large substances to small ligands. Some attempts have reversed this and used transporters for proteins to deliver smaller substances. A variation that is essentially unexplored is to modify a peptide or protein which is already known to be transported across the BBB. For example, secretin is transported by a saturable system at the choroid plexus, and more enzymatically resistant analogues show therapeutic promise [32].

The BBB can limit accumulation of a substance in the brain through mechanisms besides simply acting as a physical barrier. For example, the BBB can act as an enzymatic barrier, sequester the peptide and thereby prevent it from penetrating beyond the capillary wall, or transport the substance in the brain-to-blood direction. The best studied examples of these is the effect of brain-to-blood transporters on brain uptake of blood-borne substances, including peptides and regulatory proteins. Mu-receptor opiate peptides are ligands for P-glycoprotein, and the delta-receptor enkephalins are ligands for PTS-1 [22,33,34]. Inhibition of efflux pumps can produce a severalfold increase in peptide accumulation by brain [18,34]. For example, pluronic 85 works in part by inhibiting P-glycoprotein [35].

Several seemingly unrelated strategies showing great potential may be using the mechanism of adsorptive endocytosis. This mechanism was first described as the interaction of glycoprotein ligands, such as wheatgerm agglutinin, with glycoproteins on the cell surface [36,37]. This

can induce internalisation into the endothelial cell with one fate being transcytosis of the brain endothelial cell, that is, complete passage across the BBB. The enhanced penetration of peptides seen with glycosylation, attachment of Tat peptides and attachment of protamine sulfate may be by way of adsorptive endocytosis.

4.3 Bypassing the BBB

Two strategies, intrathecal and intranasal delivery, will be discussed here. Intrathecal administration is not effective for traditional, lipid-soluble molecules, as they easily cross the BBB in the brain-to-blood direction. Thus, the same principles that underlie their effective delivery to brain after peripheral administration also explain their poor delivery to the brain after intrathecal delivery [38]. However, proteins delivered by intrathecal administration can reach the CNS [39-41]. Just as large, water-soluble molecules cross the BBB in the blood-to-brain direction poorly, so they also cross the BBB in the brain-to-blood direction with difficulty. Thus, they are 'trapped' in the cerebrospinal fluid, and can ascend the spinal column and enter the cranial CSF.

Some peptides have also been shown to be taken up into the brain when given by intranasal administration [42-45]. Uptake is usually very high in the olfactory bulbs, with much lower uptake by other brain regions. However, even if uptake by intranasal administration is no better than uptake after intravenous administration, intranasal administration has an advantage of producing very low levels in the blood, thereby minimising systemic delivery of the peptide and any associated unwanted actions. The peptide could, in theory, enter the blood after draining to the cervical lymphatics, but this did not occur to a measurable degree for a glucagon-like-peptide-1 analogue [46]. The peptide can enter the blood with the reabsorption of the cerebrospinal fluid. Therefore, the ideal candidate for intranasal delivery may be one that has a characteristic which is a disadvantage for peripheral administration: that of rapid degradation in blood.

5. Conclusion

Delivery of peptide therapeutics to the brain has been hampered by both real obstacles as well as misperceptions about BBB/peptide interactions. Many peptides and regulatory proteins do cross the BBB by saturable and non-saturable mechanisms in amounts sufficient to affect CNS function. For these substances, therapeutic potential can be enhanced by addressing non-BBB issues, such as pharmacokinetics in blood. Brain-to-blood transporters are a major cause preventing many substances from crossing, and drugs that could selectively inhibit such transporters would be very useful therapeutically. Delivery of peptides and regulatory proteins by intranasal and intrathecal routes also shows promise.

6. Expert opinion

The belief that peptides will be useful CNS therapeutics once delivered to the brain will continue to drive the search for delivery mechanisms. The drive will be accelerated by the realisations that many diseases are produced by peptides and regulatory proteins themselves and that alterations in how the BBB handles such peptides and regulatory proteins can also produce disease. The latter realisation will make the BBB itself a therapeutic target. Delivery of peptide- and protein-based antagonists, including antibodies, will also be of interest. Development of a universal carrier will probably dominate industrial efforts, both because such a carrier could be adapted to delivery of so many compounds and also because it allows investigators to 'black box' the BBB. The biggest deterrents to the development and delivery of drugs of any sort to the brain (but especially of peptides and proteins) is the relative paucity of workers trained in an understanding of the BBB and the tendency of industry to 'black box' or oversimplify the BBB. Eventually, a host of approaches will complement or replace the universal delivery system as individual peptides become useful therapeutics. These will probably include delivery systems that are restricted to specific classes of peptide or protein, use of endogenous transporters, including those transporting peptides and proteins, and manipulation of the BBB transporters themselves. 'Bypass' approaches, such as intranasal and intrathecal delivery and use of the extracellular pathways, may prove very useful, especially for selected problems. No single approach is likely to be a complete answer, as the diseases of the CNS are likely to present such diverse problems that a pallette of approaches to delivery will be required. Specifically, a characteristic of a delivery strategy may confer great advantage in the treatment of one type of CNS disease, but produce unacceptable outcomes for another. Soon after effective delivery across the BBB of a handful of peptide therapeutics is established, targeting specific areas of the brain or populations of neurons with the therapeutic will quickly be perceived as the new major problem in brain delivery. Finally, probably well beyond a 10-year horizon, interest may develop in controlling the ability of the BBB to secrete peptides and proteins; this approach offers the possibility of treating patients with their own, endogenous peptides.

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