Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

CNS Drug Delivery: Opioid Peptides and the Blood-Brain Barrier

Submitted: March 30, 2005; Accepted: April 19, 2005; Published: February 24, 2006

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ABSTRACT

Peptides are key regulators in cellular and intercellular physiological responses and possess enormous promise for the treatment of pathological conditions. Opioid peptide activity within the central nervous system (CNS) is of particular interest for the treatment of pain owing to the elevated potency of peptides and the centrally mediated actions of pain processes. Despite this potential, peptides have seen limited use as clinically viable drugs for the treatment of pain. Reasons for the limited use are primarily based in the physiochemical and biochemical nature of peptides. Numerous approaches have been devised in an attempt to improve peptide drug delivery to the brain, with variable results. This review describes different approaches to peptide design/modification and provides examples of the value of these strategies to CNS delivery of peptide drugs. The various modes of modification of therapeutic peptides may be amalgamated, creating more efficacious "hybrid" peptides, with synergistic delivery to the CNS. The ongoing development of these strategies provides promise that peptide drugs may be useful for the treatment of pain and other neurologically-based disease states in the future.

KEYWORDS: DPDPE, biphalin, transport, delivery strategies

INTRODUCTION

In recent years, there have been several important advancements in the development of peptide therapeutics. Nevertheless, the targeting of peptide drugs to the central nervous system (CNS) remains a formidable undertaking. Delivery of efficacious peptide drugs is limited by their poor bioavailability due to low metabolic stability and high clearance by the liver. In addition, peptides are generally water-soluble compounds that will not enter the CNS readily, via passive diffusion, due to the existence of the blood-brain barrier (BBB). The aim of this review is to assess many of the chemical modifications developed to date and to discuss the various delivery-enhancing strategies necessary to produce viable CNS acting opioid peptide drugs.

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Peptide Characterization

The capacity of a peptide to cross the BBB and enter the brain is dependent upon several compositional factors, including size, flexibility, conformation, biochemical properties of amino acids, and amino acid arrangement. Peptide composition also determines, in part, the degree of protein binding, enzymatic stability, cellular sequestration, uptake into nontarget tissue, clearance rate, and affinity for protein carriers. Other aspects independent of peptide composition must also be considered, such as cerebral blood flow, diet, age, sex, species (for experimental studies), dosing route, and effects of existing pathological conditions. Each of these factors must be considered for an appropriated study design strategy.

In this review we focus on opioid peptides with emphasis on 2 unique and well characterized enkephalin analogs, DPDPE and biphalin. Each of these peptides has been the focus of numerous studies to examine novel mechanisms in which to enhance delivery of peptides into the CNS. An additional advantage of using opioid peptides in the examination of new technologies and structural/chemical modifications is the definitive CNS-mediated end point (ie, analgesia) and the well-characterized biochemical nature of the opioid receptors.

Role of the Blood-Brain Barrier in CNS Drug Delivery

The brain is one of the least accessible organs for the delivery of active pharmacological compounds. Despite its relatively high nutrient support and exchange requirements, the uptake of any compound is strictly regulated by the BBB. The surface area of the human BBB is estimated to be 5000 times greater than that of the blood-cerebrospinal fluid barrier, and therefore the BBB is considered to be the primary barrier controlling the uptake of drugs into the brain

parenchyma.[1] To understand why the BBB is such a significant impediment to peptide drug delivery, its characteristics must first be assessed. The BBB is a unique physical and enzymatic barrier that segregates the brain from the systemic circulation. BBB capillary endothelia lack fenestrations and are sealed by tight junctions, which inhibit any significant paracellular transport.[2-4] Specific transporters exist at the BBB that permit nutrients to enter the brain and toxicants/waste products to exit.[5] The BBB further functions as a diffusional restraint, with selective discrimination of substance transcytosis based on lipid solubility, molecular size, and charge.[6] In addition, the BBB has a high concentration of drug-efflux-transporters (ie, P-glycoprotein, multi-drug resistant protein, breast cancer resistant protein) in the luminal membranes of the cerebral capillary endothelium. These efflux transporters actively remove a broad range of drug molecules from the endothelial cell cytoplasm before they cross into the brain parenchyma. Last, the "enzymatic barrier" component of the BBB, capable of rapidly metabolizing peptide drugs and nutrients,[7-10] is also of great significance in regard to peptide viability. As amino acids and their associated linkage are highly susceptible to enzymatic degradation, the nature and concentration of specific enzymes at the BBB can greatly affect the efficacy of a given peptide-based drug.[11,12]

The influence of brain pathology on the functioning of the BBB must also be integrated into the development of the peptide delivery strategies. The development of new drugs and drug-vectors must also contend with potential pathological conditions of the patient. Several disease states result in enhanced BBB permeability to fluid and/or solutes[13] including hypoxia-ischemia and inflammatory mechanisms involving the BBB in septic encephalopathy, HIV-induced dementia, multiple sclerosis, and Alzheimer disease. The list of factors that may contribute to changes in drug bioavailability (eg, changes in BBB cytoarchitecture, protein binding, receptor site, enzymes) during a pathological state is extensive and must be taken into account for appropriate drug design. Specific changes at the BBB, such as opening/disruption of tight junctions, increased pinocytosis, changes in nutrient transport, and pore formation may enhance/reduce drug uptake. Table 1 lists several potential conditions and factors shown to induce changes at the BBB.

Delivery Strategies to Enhance Peptide Delivery to the CNS

Peptide drug delivery to the brain may be divided into 3 general categories:

1. Invasive procedures, which include transient osmotic opening of the BBB,[73,74] shunts,[75] and biodegradable implants.[76,77] These procedures can be highly traumatic and often have low therapeutic efficiency with substantial side effects.
2. Pharmacologically-based approaches to increase the passage through the BBB by increasing specific biochemical attributes of a compound. This may be accomplished either by chemical modification of the peptide molecule itself, or by the attachment or encapsulation of the peptide in a substance that increases permeability, stability, bioavailability, and/or receptor affinity. In addition, modification of drug structure and/or addition of constituents (eg, lipophilicity enhancers, polymers, antibodies) may enhance drug concentration within the CNS, with a reduced toxic profile.
3. Physiologic-based strategies exploit the various carrier mechanisms at the BBB, which have been characterized in recent years for nutrients, peptide

Table 1. Potential causes of blood-brain barrier alteration*

Paracellular opening:	hyperosmolarity[14]; acidic pH[15]; burn encephalopathy[16]; experimental autoimmune encephalitis[17,18]; multiple sclerosis[19,20]; chemical mediators associated with inflammation: (TNFα,[21] IL-1β,[22] histamine,[23] serotonin,[24] bradykinin,[25] Thrombin,[26] and reactive oxygen species[27]); ischemia[28-30]; lead[31]; aluminum[32]; post-ischemia reperfusion[33,34]; electromagnetic fields[35]; systemic lupus erythematosus[36]
Increased Pinocytosis:	acute hypertension[37,38]; microwave irradiation[39]; ischemia[40,41]; seizures[42]; heat stroke[16]; brain injury[43]; tumors[44]; development[45,46]; hypervolemia[47]; immobilization stress[48]; hypothermia (<16°C)[49]; post-radiation[39,50]; hyperbaric conditions[51]; lead encephalopathy[52]; mercury[53]; Tricyclic antidepressants[54]; meningitis[55,56]; multiple sclerosis[57,58]; lymphostatic encephalopathy[59]
Pore Formation:	Tricyclic antidepressants (chlorpromazine, nortriptyline)[60]; Ischemia[61]
Disease / toxicant transport changes:	Diabetes (GLUT-1)[62,63]; Alzheimer's disease (β-amyloid, RAGE, LRP receptor)[64-66]; Wernickes-Korsakoff syndrome (thiamine)[67,68]; Eating / weight disorders (insulin and leptin)[69-71]; hypertension (choline)[72]

TNFα: tissue necrosis factor-α; IL-1β: interleukin-1β; GLUT-1: glucose transporter type-1; RAGE: receptor for advanced glycation end products; LRP: lipoprotein receptor-related protein (Adapted from Pardridge 1991[11])

and nonpeptide hormones, and transport proteins. These strategies can be combined, dependent of the nature of a given peptide, creating "hybrid" peptides, resulting in synergistic CNS delivery and end-effect.

Peptide drug modification can be broadly divided into several categories: lipidization, structural modification to enhance stability, glycosylation, increasing affinity for nutrient transporters, prodrugs, vector-based, cationization, and polymer conjugation/encapsulation. Table 2 provides a listing of these strategies to increase peptide uptake into the brain, with associated advantages and limitations.

Lipidization

Lipid solubility is a key factor in determining the rate at which a drug passively crosses the BBB. The presence of hydroxyl groups on peptides tends to promote hydrogen bonding with water leading to a concomitant decrease in the partition coefficient (ie, lipophilicity) and resultant decrease in membrane permeability.[78] Peptide drugs generally contain polar functional groups that impart a degree of dipolarity and hydrogen bonding. The amino acids that compose a peptide determine the respective polarity. The overall balance of polar to nonpolar groups within a drug molecule can be reduced either by removal of a polar group or addition of a nonpolar group.[6] In addition, the relative positioning of polar to nonpolar amino acids (ie, whether polar/nonpolar groups are at the center or ends of a given peptide) will also determine the capability of a peptide to transport across a biological membrane.[6]

Methylation has been shown to reduce the overall hydrogen bond potential. Dimethylation of the tyrosine on the synthetic opioid peptide DPDPE has been shown to significantly enhance analgesia with a concomitant enhancement of bioavailability.[79] This structural modification resulted in a 10-fold increase in the potency over the nonmethylated DPDPE at the delta opioid receptor and a 35-fold increase in potency at the μ-opioid receptor, while substantial delta receptor selectivity was maintained. In another study, the placement of 3 methyl groups on the phenylalanine of DPDPE induced changes in lipophilicity and BBB permeability.[80] These alterations were contingent upon the specific conformation of the respective methyl groups on the phenylalanine. The trimethylated DPDPE was also shown to alter efflux (ie, reduced P-glycoprotein affinity), metabolism, and analgesia, based on the diastereoisomer configuration of the nonaromatic methyl group.[80] Thus, during formulation, the stereoselectivity of the peptide drug must be assessed with preservation of optimal bioavailability and receptor binding affinity.

Halogenation of peptides can also enhance lipophilicity and BBB permeability. Halogenation of peptides such as DPDPE,[81,82] DPLPE,[83] and biphalin[84] have shown to significantly enhance BBB permeability in a manner dependent upon the conjugated halogen (Cl, Br, F, I). Addition of chlorine on the Phe4 residue of DPDPE led to a significant increase in permeability in both in vivo[81] and in vitro studies.[82] Addition of 2 chlorine atoms onto DPDPE further increased BBB permeability, beyond that of the single chlorine. An identical trend was observed for chlorinated biphalin, with an increased BBB permeability both in situ and in vitro.[84] However, when fluorine was added to DPDPE, permeability did not increase, while fluorine addition to biphalin greatly diminished BBB permeability. Another method to increase lipophilicity is through acylation or alkylation of the N-terminal amino acid.[85] Acylation of peptides and proteins has consistently proven to be an effective means to increase membrane permeability with limited interference in receptor binding. Acyl derivatives of DADLE,[86,87] DPDPE,[88] thyrotropin-releasing hormone (TRH),[88,89] and insulin[90] have also shown improved absorption through artificial and biological membranes and enhanced enzymatic stability, while retaining pharmacological activity.

There are many strategies designed to enhance lipid solubility of drugs, but lipidization does have several limitations. Since highly lipid-soluble drugs may be extensively plasma protein bound, there is the potential for a reduction in the amount of free or exchangeable drug in the plasma, thereby compromising brain uptake.[91] The site of modification or attachment of substances/molecules to increase lipophilicity must also be taken into account, as receptor binding affinity may be diminished if alterations are within the pharmacophore region, thus reducing biological activity. Last, enhancement of lipophilicity alone may not necessarily improve BBB transport. Factors such as size, stability, intracellular sequestration, nontarget organ uptake, efflux rates, and P-glycoprotein (P-gp) efflux affinity must also be considered.

Structural modification to enhance stability

Structural design to reduce enzymatic degradation is another approach used to enhance peptide bioavailability to the CNS. These modifications require extensive analytical investigation to define the site of enzymatic cleavage and the proteolytic enzymes that act upon a specific peptide.[11] Enzyme masking strategies include modification of the amino acid terminus, with N-acylation or pyroglutamyl residues, to reduce aminopeptidase M activity.[85,92] However, such modifications may result in reduced biological activity; for example, opioid peptides require the amino terminus to be free for effective receptor binding.[93,94] An alternative

Table 2. Modes of Peptide Modification to Enhance CNS Delivery*

Strategy	Advantages	Limitations
Lipidization	Increases membrane permeability	Increases plasma protein binding Intracellular sequestration Increased hepato-biliary elimination Non-specific targeting Receptor interference Increase size of molecule may counter membrane permeability enhancement
Structural modification to enhance stability	Increases stability Potential increase in membrane transport Potential increase in receptor affinity	Potential decrease in membrane transport Potential decrease in receptor affinity
Glycosylation	Increases membrane permeability Increases stability Increases serum half-life	Nonspecific targeting Receptor interference
Nutrient Transport	Drug entry not dependent upon passive diffusion Potentially specific targeting	Limited capacity of carriers Potential interference with endogenous substrate of carrier Specificity of carrier Similarity of carrier mechanisms throughout body may reduce specificity of targeting
Prodrug	Increases membrane permeability and/or pharmacokinetics Potential target specificity based on design of cleavable linker and/or enhancer moiety "Redox" prodrugs lock drug in tissue	Kinetics of drug release must be precise Requires optimization of linker and drug attachment Charged "Redox" prodrugs are rapidly eliminated from body
Vector-based	Specific membrane targeting Linker strategies may be incorporated to enhance stability and detachment of vector portion	Delivery to brain is directly limited to the transporter concentration Potential downregulation of transporters with continual dosing Potential interference with endogenous substrate of carrier/ transporter Requires optimization of linker and drug attachment Altered pharmacokinetic profile Significant cost in design and production
Cationization	Increases membrane permeability Increases serum half-life	Increases plasma protein binding Immune complex formation Non-specific targeting Rapid elimination of charged moiety
Polymer conjugation	Increases stability Increases serum-half life Decreases elimination rate Decreases immunogenicity/toxicity Decreases protein binding Potential for control-release design	Hydrophobic polymers reduce membrane permeability Decrease receptor binding with improper selection or placement of polymer Nonspecific targeting

* Adapted for Witt et al, 2001[12]

modification for opioids, to reduce aminopeptidase activity, is to substitute the Glycine^2 (Gly^2) residue with a D-Alanine^2 (D-Ala^2) residue. Substitution of D-Ala^2 for L-Gly^2 at the N-terminal of met-enkephalin, and amidation of its C-terminus, produce greater enzymatic stability.[95] In addition, the substitution of D-Ala^2 and D-Leucine^5 (D-Leu^5) DADLE has been shown to significantly increase half-life by reducing enzymatic proteolysis.[96] Modifications of amino

acids or attachment of secondary structures onto peptides within these regions may also reduce enzyme degradation.

Other modes to reduce enzymatic degradation and increase membrane permeability of peptides include the introduction of conformational constraints and altering chirality of amino acids. Cyclization of peptides may reduce hydrogen bonding, increase lipophilicity, and reduce the hydrodynamic radius in solution, thereby enhancing membrane permeability.[97-100] This conversion of linear peptides into cyclic conformationally constrained peptides, via a disulfide-bridge, has met with considerable success. The use of disulfide-bridge constrained peptide analogs has been shown to significantly reduce enzymatic degradation,[81,101] with the potential advantage of enhanced specificity for receptor subtypes.[102] In the case of linear met-enkephalin, the conversion into the cyclic analog (DPDPE) (Figure 1) resulted in a δ-opioid-specific peptide[103] with a saturable mode of transport at the BBB.[104-106] Due to the incorporation of 2 D-penicillamine residues and conformational restriction by a disulfide bridge, DPDPE is enzymatically stable.[101,107] Another variation on conformational bridging is shown with the opioid peptide biphalin (Figure 2), a unique analog containing 2 enkephalin sequences linked by a hydrazide bridge. Thus, biphalin has 2 biologically active pharmacophores, with affinity for both μ- and δ-opioid receptors.[108-110] Administered intracerebroventricularly in mice, biphalin was 6.7- and 257-fold more potent than etorphine or morphine, respectively, in eliciting analgesia.[111] Biphalin is protected from aminopeptidase activity by D-Ala residues in the 2 and 2′ positions and is protected from carboxy peptidase activity by the hydrazide bridge.

Glycosylation

The addition of carbohydrate moieties to a peptide (glycopeptide) produces changes in the molecular structure that, in turn, can have dramatic effects on the pharmacodynamic and/or pharmacokinetic properties.[112] Glycosylation has proven to enhance biodistribution of multiple substances to the brain.[113,114] Attachment of simple sugars to enkephalins increases their penetration of the BBB and allows the resulting glycopeptide analogs to function effectively as drugs. Several different sugar moieties have been investigated, including glucose and xylose, with respective sugars exhibiting variations in BBB permeability.[115] The improved analgesia exhibited by glycosylated opioids may be due to increased bioavailability of glycopeptides via higher metabolic stability,[116] reduced clearance,[117] and improved BBB transport.[115] Improved analgesia has also been reported for glycosylated deltorphin,[118,119] cyclized methionine enkephalin analogs,[115,120] and linear leucine enkephalin analogs.[121] The δ-selective glycosylated leu-enkephalin amide, H_2N-Tyr-D-Thr-Gly-Phe-Leu-Ser(βD-Glc)-$CONH_2$, produces analgesic effects similar to morphine, even when administered peripherally, yet possesses reduced dependence liability as indicated by naloxone-precipitated withdrawal studies.[115] Although the mode of enhanced transport at the BBB has yet to be fully elucidated for glycosylated peptides, the improved BBB transport is not related to increased lipophilicity, given that octanol/saline distribution studies of glycosylated peptides indicate that the addition of glucose significantly reduced lipophilicity, thereby reducing passive diffusion.[115] In addition, endocytotic mechanisms have been shown to transport glycosylated peptides.[122-125]

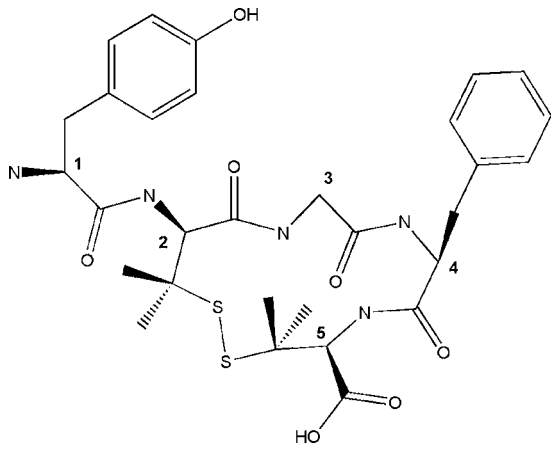

Figure 1. Structure of DPDPE[106] NH2-Tyr1-D-Pen2-Gly3-Phe4-D-Pen5-OH

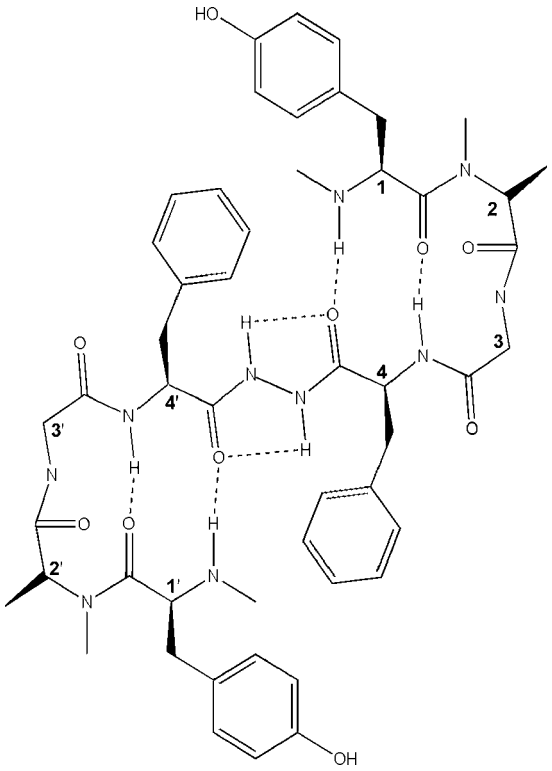

Figure 2. Structure of Biphalin[106] NH_2-Tyr[1]-D-Ala[2]-Gly[3]-Phe[4]-NH-NH-Phe[4′]-Gly[3′]-D-Ala[2′]-Tyr[1′]

Of interest, recent studies indicate that transport through the BBB may hinge on the amphipathic nature of the glycopeptides.[126] That is, the amphipathic glycopeptides possess 2 conflicting solubility states: one state that is completely water soluble, and another at water-membrane phase boundaries. However, simply producing highly amphipathic sequences is not enough to promote systemic delivery and penetration of the BBB. The glycopeptide sequence must be capable of assuming a water-soluble random coil conformation (ie, "biousian" activity), comparable to micelle formation, and the barrier between the 2 states must be low enough for rapid interconversion between the 2 states.[127] In addition, it was proposed that some glycopeptides promote a negative membrane curvature at the endothelial cell wall, with the negative curvature leading to increased rates of endocytosis, which in turn result in enhanced BBB transport.[127]

A caveat in regard to opioid peptide glycosylation is that a reduction in affinity for the μ-opioid receptor occurs upon glycosylation. Yet, there is virtually no effect on δ binding or efficacy.[128] This finding is consistent with Schwyzer's membrane compartment theory,[129] which suggests that the μ-receptor binding site resides in a more hydrophilic environment than the binding site for the δ-opioid receptor. This is important because, the primary analgesic action of opioid-based drugs is theorized to be regulated through the μ-opioid receptor.

Nutrient Transporters

Peptide drug design may also incorporate specific molecular characteristics that enable the drug to be transported by one or more of the inwardly directed nutrient carriers. The BBB expresses several transport systems for nutrients and endogenous compounds.[130,131] Utilization of these transport systems is a potential strategy for controlling the delivery of drugs into the brain. These drugs must have a molecular structure mimicking the endogenous nutrient. The prototypical example is levodopa, a lipid-insoluble precursor of dopamine that has been used for the treatment of Parkinson's disease because it contains the carboxyl and α-amino groups that allow it to compete for transport across the BBB by the large neutral amino acid carrier.[132] Biphalin, a potent opioid analgesic,[111,133] is a peptide drug that has been shown to use the neutral amino acid carrier system to gain entry into the brain.[134] Chemical groups could be designed with the ability to attach to specific drugs rendering them substrates for carriers, or drugs specifically designed for a carrier mechanism. The hexose and large neutral amino acid carriers have the highest capacity and presently are the best candidates for delivery of substrates to the brain. Lower capacity carriers may also be utilized for highly potent peptide drugs. Peptides, which generally require low concentrations to induce effect, are most appropriate for this type of targeting design. Yet, the complexity in designing a peptide to target specific nutrient transporters requires a great deal of knowledge of both peptide and transporter. Nevertheless, these transport mechanisms may also be advantageous targets for prodrug and vector-mediated approaches to enhance peptide delivery to the brain.

Prodrugs

Prodrugs contain a pharmacologically active moiety that is either conjugated to a molecule with a known transporter or to a lipophilicity enhancer, which is cleaved at or near the site of action, allowing drug to induce its effect. The rationale for prodrug design is that the structural requirements necessary to elicit a desired pharmacological action and those necessary to provide optimal delivery to the target receptor site may not be the same. The ideal prodrug is enzymatically stable in the blood, but rapidly degraded to the active parent compound once it is within the target tissue. Esters have shown particular promise in the area of prodrug design for brain delivery, owing to the abundance of endogenous esterases in the CNS. Esterification or amidation of amino, hydroxyl, or carboxylic acid-containing drugs may greatly enhance lipid solubility, and thus brain entry.[135,136] Once in the CNS, hydrolysis of the modifying group releases the active compound. Both aromatic benzoyl esters[135] and branched chain tertiary butyl esters[137] have shown stability in plasma, while still remaining adequately cleaved within the CNS. Lipophilic amino acids, such as phenylalanine (Phe), can be used as the cleavable unit. The addition of a Phe group to DPDPE at the amino terminal resulted in enhanced permeability at the BBB.[138] Chains of nonpolar amino acids could further enhance lipophilicity, albeit the balance between molecular size and degree of lipophilic enhancement must be assessed individually.

Another prodrug design focuses on the redox system,[139,140] in which a lipophilic attachment (eg, 1,4-dihydro) is converted to the hydrophilic quaternary form, effectively "locking" the drug in the tissue.[141] When a drug is conjugated to a methyldihydropyridine carrier and subsequently oxidized by NADH-linked dehydrogenases in the brain, it results in a quaternary ammonium salt, which does not cross back through the BBB endothelium.[142] Similar design has been explored with a wide variety of drugs, such as steroids, antivirals, neurotransmitters, anticonvulsants, and peptides (leucine enkephalin analog and TRH analog).[141,143] The primary difficulties with the redox design are that any tissue may take up the lipophilic moiety, and rapid elimination of the charged salt form occurs.

Prodrug design is presently at the forefront of pharmaceutical exploration in terms of CNS targeting. The difficulty with prodrugs lies primarily within the pharmacokinetics.

Precise placement and choice of cleavable moiety must be optimized to obtain the most efficacious pharmacokinetic profile.

Vector-based

Physiologic vector-based strategies involve the use of existing BBB transport properties to enhance brain entry of a specific drug, much the same as prodrugs. The principle of the vector-mediated or chimeric delivery strategy lies in the coupling of a moderately impermeable peptide to a substance that increases the affinity to and transport across a biological membrane via receptor-mediated or absorptive-mediated endocytosis. After entry into the brain, these chimeric drugs release via enzymatic cleavage, allowing the drug to initiate a pharmacological action in the brain. This technology may be adapted for use with peptide pharmaceuticals, nucleic acid therapeutics, and small molecules. Important design considerations of such chimeric peptides include vector specificity for the brain, pharmacokinetics of the vector, and placement and cleavability of the linker between the drug and vector. Multiple concepts for such systems exist,[144-147] with present focus on antibody attachment and chemical linker strategies.

Receptor-mediated vectors for brain delivery must be specific. There are several potential receptors at the BBB that may serve this purpose. The plasma protein transferrin is able to bind and undergo endothelial endocytosis in brain capillaries and has proven to be a suitable vector. A murine monoclonal antibody (OX26) to the transferrin receptor has been successfully used to increase brain uptake of proteins and peptides. An analog of the opioid peptide dermorphin ([Lys7]dermorphin) was conjugated to the OX26 vector, demonstrating analgesia, which was reversed by naloxone.[148] Cationized albumin has also been used as a vector to enhance peptide uptake, via adsorptive-mediated endocytosis. When cationized albumin was conjugated to β-endorphin, it yielded increased uptake into isolated brain endothelial cells, as compared with β-endorphin alone.[149]

Several additional caveats should be considered with use of a receptor-mediated vector design. There exists the potential competition between the vector-drug and the endogenous ligand for the receptor as such endocytotic mechanisms tend to have low capacity in the brain. This phenomenon would result in decreased vector transport and/or decreased concentration of a required nutrient to the brain, resulting in a subsequent pathology. Last, the quantity of drug delivered to the brain is directly limited by transporter concentration. Transporter capacity may become saturated or downregulated over time, decreasing the ability to deliver an adequate and consistent dosage of drug to elicit a pharmacological effect. This possibility is important if the use of a drug requires consistent and repeated doses. Therefore, this approach would likely require extremely potent therapeutic peptides, which may be most effective for acute disease states.

Cationization

Cationization of peptides is a manner of increasing membrane entry via absorptive-mediated endocytosis (AME).[145,150-153] Presence of anionic sites on BBB endothelium brings about attraction of cationized substances to the membrane surface.[154] The dynorphin-like analgesic peptide E-2078, and the adrenocorticotropic hormone (ACTH) analog, ebiratide, are polycationic peptides at physiologic pH shown to internalize into brain capillaries by AME.[155-158] The μ-opioid receptor-selective [D-Arg^2]-dermorphin tetrapeptide analogs H-Tyr-D-Arg-Phe-Sar-OH, and H-Tyr-D-Arg-Phe-β-Ala-OH (TAPA) were developed,[159] showing potent analgesic activity with low physical and psychological dependence.[160] It was reported that TAPA crosses the BBB via AME, which is triggered by binding of the peptides to negatively charged sites on the surface of brain capillary endothelial cells.[152] Yet, when 2 additional [D-Arg^2]-dermorphin analogs were designed (*N*αamidino-Tyr-D-Arg-Phe-β-Ala-OH(ADAB)and*N*α-amidino-Tyr-D-Arg-Phe-MeβAla-OH (ADAMB), the peptides exhibited a slower degree of analgesic onset, attributed to the parallel decrease in AME across the BBB.[153]

Cationized albumin attached to beta-endorphin, in a vector-based manner, showed significant increases in membrane uptake by AME.[161] Cationized albumin displayed longer serum half-life and a general selectivity to the brain.[147] In addition, avidin-cationized albumin conjugates have been proposed to affect the delivery of biotinylated, phosphodiesterase, antisense oligodeoxynucleotides to the brain. These conjugates retain function (ie, inactivation of target mRNAs) and impart a degree of stability to serum and cellular enzymes.[162]

Unfortunately the potential toxicity of this strategy limits its clinical use as a therapeutic. Cationized proteins have been shown to induce immune complex formation with membranous nephropathy,[163,164] and increased cerebral and peripheral vascular permeability.[150,151,165] Cationized albumin has been shown to be significantly cleared by the kidney and liver, posing a potential toxicological threat as well.[147,166,167] This approach is also nonspecific as to tissue uptake, unless additionally coupled to a selective vector.

Polymer conjugation/encapsulation

Structural modifications can be obtained by the covalent conjugation of peptides to polymers. Polymer conjugations are used to increase peptide stability, reduce elimination, and reduce immunogenicity.[168-172] Chemical modification

of peptides with macromolecular polymers, such as poly(ethylene glycol) (PEG),[170,172] and poly(styrene maleic acid),[173,174] shows significant promise.

Pegylation is a procedure of growing interest for enhancing the therapeutic and biotechnological potential of peptides and proteins. When PEG is correctly linked to a peptide it will modify many of the pharmacokinetic features, while theoretically maintaining the primary biological activity (ie, enzymatic activity or receptor recognition). PEG chains may contain linear and branched structures, which can be conjugated directly to the peptide drug or linked in a prodrug manner. PEG conjugation masks the peptide's surface and increases the molecular size, thereby reducing immune response, enzymatic degradation, toxicity, and renal ultrafiltration.[171] PEGs may also produce improved physical and thermal stability, as well as increased solubility.[175] The principal disadvantage of pegylation is the potential loss of activity with improper choice of PEG (ie, length, branching, chemical design) or unfavorable choice of attachment site. Another considerable disadvantage to pegylation, in regard to CNS focused drug delivery, is the enhanced hydrophilicity and molecular size that can bring about significant reductions in passive diffusion across the BBB.

When the opioid peptide DPDPE was pegylated, decreased clearance, reduced plasma protein binding, and first-pass elimination was shown, while increases in the analgesic effect following intravenous administration was demonstrated.[176] BBB permeability of the pegylated form was not significantly different when compared with the native form, despite enhanced hydrophilicity. This finding was possibly owing to an association with the saturable mechanism observed at the BBB[104] or a reversal of P-gp affinity[177] of the native compound.

Encapsulation, via nanoparticles and liposomes, may also be an effective manner by which to increase delivery to the brain. Nanoparticles are polymeric particles ranging in size between 10 and 1000 nm, which have a polysorbate overcoating. Drugs can be bound in the form of a solid solution or dispersion, or they can be absorbed to the surface of the particle or chemically attached. Polysorbate-80 nanoparticles have been shown to enhance delivery of the leu-enkephalin analog dalargin.[178] The particles are thought to mimic low density lipoproteins (LDL) and could therefore be taken up into the endothelium of the BBB via the LDL receptor. Enhanced transport of these nanoparticles may also involve tight junction modulation[179] or P-gp inhibition.[180] Liposomes are composed of a phospholipids bilayer that may act as a carrier for both hydrophilic and hydrophobic drugs. The beneficial attributes of liposomes are enhanced plasma half-life, decreased clearance, and decreased toxicity of associated drug.[181] One such liposome formulation is the pluronic copolymer P85, a self-forming micelle preparation that encapsulates a drug. The P85 formulation has been shown to enhance the delivery of digoxin into the brain,[182] and to enhance the analgesic profile of biphalin, DPDPE, and morphine,[183] with the mechanism of action theorized to be the inhibition of P-gp. One of the negatives of the P85 micellular formulations is the observation that P85 may function via depletion of ATP, which would reduce P-gp activity, yet may also result in undesired responses within the endothelium.[184]

Conclusion

Despite significant impediments of brain-directed peptide therapeutics, several delivery strategies show considerable promise for enhancement of CNS uptake. The specific biochemical modifications and delivery enhancers addressed in this review can be combined to create "hybrid" peptides, able to take advantage of multiple components of BBB passage. With this understanding, opioid peptide hybrids, specifically designed for increased brain entry, may provide highly potent treatment for CNS-mediated pain.

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