Delivery of peptides into the central nervous system

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Research into the delivery of potentially useful peptide therapeutic agents to the CNS has lagged behind the exponential growth in their discovery. There remain many problems in developing effective and safe delivery strategies, but there has been some progress in overcoming this major obstacle. The author provides an overview of those properties of the blood–brain barrier that are specific for peptides and outlines current methods addressing peptide drug delivery to the CNS.

Delivery to the site of action is the most critical aspect of peptide-based therapeutics for diseases affecting the CNS. The lipoidal blood–brain barrier is a major obstacle that must be overcome; peptides generally do not enter the brain and spinal cord from the circulating blood because they are highly polar and lipid-insoluble, metabolically unstable, and do not have receptor-mediated transport systems in this membranous barrier. Thus, the demand emerges for delivery strategies to take therapeutic advantage of their vast biological diversity and high potency.

Peptides and the blood–brain barrier

The etiology of many diseases affecting the CNS involves changes in the synthesis and release of peptides. Hypo- or hypersecretion, alteration in storage, release and catabolism, and post-translational processing could be involved. Native peptides, their agonists or antagonists could be useful in the treatment of various diseases. Screening combinatorial libraries of synthetic peptides now provides many lead candidates for peptide drug discovery[1]. However, peptide pharmacotherapy of CNS diseases has been hampered by the limited ability of these agents to reach the site of action.

Experimental evidence for the existence of a barrier that segregates the blood and brain was supplied in the late 19th century by Ehrlich[2]. In his experiment, intravenously injected dye stained most organs, except the brain and spinal cord. This property of the CNS led to the notion of the blood–brain barrier (BBB). The endothelium of the CNS vasculature shows structural differences compared with that of other organs. Fenestrations between the vascular endothelial cells of most organs, which contribute to the exchange of water, nutrients and metabolites, are not found within the normal mammalian brain. Endocytotic vesicles also are virtually absent in the endothelial cells of the cerebral microvessels[3]. Systemically injected molecules may reach the cellular elements of the tissue in the circumventricular organs (CVOs) of the brain which are perfused by capillaries without endothelial tight junctions. However, there is no evidence of peptide penetration to deeper layers of the CNS. In addition, the surface area of the BBB is about 5,000 times greater than that of the CVOs[4]. Therefore, intracellular or transcellular transport (transport directly through the endothelial cell membrane) is the principal route into and out of the CNS.

Blood–brain barrier structure

The morphological component of the BBB consists of luminal and antiluminal mebranes (two plasma membranes in series) separated by 0.3 μm of endothelial cytosol. The BBB thus behaves as a continuous lipid bilayer, and as such exhibits a low permeability to hydrophilic substances (ions and polar compounds) that do not have specific transport mechanisms. Morphological features of the cerebral microvasculature are shown schematically in Figure 1[5].

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Besides these structural elements, highly active enzymes present in the brain endothelial cells[6–9] and in the cerebral pericytes[10–13] represent a metabolic component that contributes to the homeostatic balance regulated by the BBB. Metabolically unstable substances may, therefore, be rapidly degraded before they reach the brain tissue.

Transport processes

Most naturally occurring neuropeptides are hydrophilic, and thus do not cross the BBB without a specific transport system. Carrier-mediated transport of several dipeptides and tripeptides occurs in the brain[14]. Certain larger peptides (the insulin-like growth factors IGF-I and IGF-II, insulin and transferrin) are known to have receptors on the BBB. These receptors have been identified on the luminal surface of the brain capillaries and are believed to act as transcytosis systems because they are present on both luminal and antiluminal borders[15]. Nonspecific, absorptive-mediated transcytosis, where endocytosis is initiated by the binding of a polycationic substance on the plasma membrane, may also be involved in the BBB transport of peptides and proteins that have multiple positive charges under physiological conditions[5]. However, peptides may be metabolized during transport steps in compartments such as the cytosolic endothelial space, the luminal surface of the BBB, cerebral pericytes, and/or synaptic regions juxtaposed to the brain microvessels[16]. Metabolically stable peptide analogs may exhibit increased resistance to certain peptidases, and a very small proportion of systemically administered peptides that are extremely resistant to peptidase inactivation ([D-Arg^2, Lys^4]dermorphin[17], or the all D-amino acid peptide Ac-Arg-Phe-Trp-Ile-Asn-Lys-NH_2 [Ref. 18]) may reach the CNS by limited passive transport and elicit a pharmacological effect, but their bioavailability is obviously very poor. However, overall protection against the variety of neuropeptide-degrading enzymes is generally not possible until the peptidic nature of the molecule is changed.

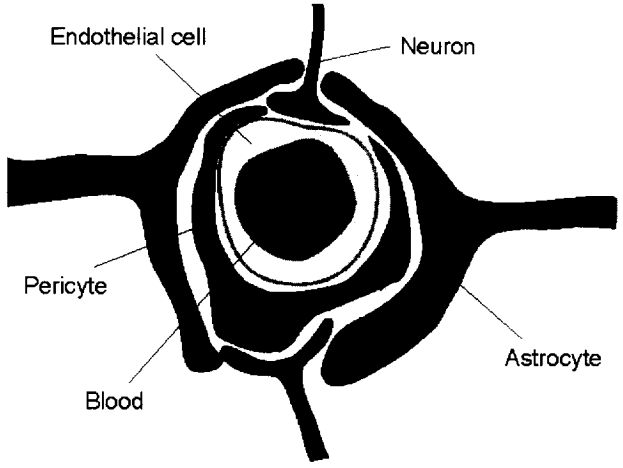

Figure 1. *Model for cerebral microcirculation. The capillary endothelium is endowed with tight junctions, endothelial cells share microvascular basement membrane with pericytes, and more than 90% of capillaries are invested with astrocyte foot processes. Neuronal endings also innervate brain endothelial cells directly. Adapted from Ref. 5 with permission.*

Lipid-soluble substances are generally transported into the CNS by diffusion (passive transport) across the BBB. Size exclusion may limit access of large lipophilic molecules to the brain, and is considered by some researchers[19] to be a restricting factor in the passive transport of substances with M_r > 1,000. However, size exclusion is associated primarily with the molecular volume, determined by the actual geometric size of the molecule, the overall conformation and the heteroatom content that may be involved in inter- and intramolecular hydrogen bonding, and not strictly with the molecular weight. The proposition that size exclusion prevents drugs with M_r > 1,000 from penetrating the BBB independent of their specific molecular structure is unfounded. For conformationally flexible molecules, movement across the membranes should actually be helped by the thermal fluctuation of the membrane lipid, and in practice the point at which size exclusion becomes a limiting factor for passive transport of such molecules across the BBB may be an M_r of 2,000–3,000.

The above discussion highlights the fact that the BBB is the major obstacle to the use of peptides in the pharmacotherapy of diseases affecting the brain and spinal cord. Transport of peptide molecules into the CNS cannot be ruled out, but it is unlikely that they enter the brain in pharmacologically significant amounts. Peptide delivery to the CNS must therefore be addressed in order to exploit the remarkable biological diversity of these compounds and their enormous promise as a future generation of neuropharmaceuticals. Various strategies are available for directing centrally active peptides into the brain. They can be grouped into three categories: invasive procedures, physiologically based approaches, and pharmacologically based strategies[15].

Invasive strategies

Direct delivery to the tissue

For the effective and efficient delivery of peptides to the CNS, the pharmacological effect of the peptide should be

localized and stable for the duration of action, and a sustained and effective dose should be achieved at the site of action. Injection directly into the brain has been suggested for avoiding problems associated with systemic delivery of biologically active substances[20]. Implanted infusion pumps that deliver peptides through an intracerebroventricular or intrathecal catheter have been evaluated[21–23]. Controlled-release polymer implants[24], biodegradable polymer microspheres[25] and multivesicular liposomes[26] have also been used for delivery of peptides direct to the brain. Infusion pumps for intrathecal delivery of thyrotropin-releasing hormone (TRH) were found to be simple to implant and remove under local anesthesia, and were safe, reliable and tolerated well by patients[23]. An infusion rate of 3 mg per 24 h produced predictable steady-state cerebrospinal fluid (CSF) levels of TRH (about 2 μg/ml) without the peaks and troughs associated with parenteral dosing, and also resulted in considerable savings in costs of the drug. However, the same symptoms of shivering, chills and bladder fullness were noted during a 6-h infusion into the CNS as during intravenous infusion of TRH[21]. The rapid appearance of intraventricularly administered peptides in the systemic circulation has also been shown in animal studies[27].

Other limitations involved in direct delivery into the brain should also be considered[5,20]. Diffusion in the brain is limited by the decreased extracellular space, by physical barriers such as synaptic regions protected by ensheathing glial processes, by many catabolic enzymes, and high- plus low-affinity uptake sites which reduce extracellular concentrations. The continuous production of CSF also produces a 'sink' for the injected agent. In addition, the large size of peptides leads to low diffusion coefficients, resulting in a steep concentration gradient between the parenchyma and the ependymal surface[5]. Therefore, intrathecal administration of peptides would be expected to deliver the compound only to the surface of the brain[28]. This may be beneficial when target receptors are found on the surface, but is a poor mode of peptide delivery to the brain parenchyma.

Osmotic blood–brain barrier modification

Another invasive method relies on reversible BBB disruption[29]. Intracarotid infusion of hypertonic solutions of mannitol, arabinose, lactamide, saline, urea, glycerol and radiographic contrast agents shrinks the brain capillary endothelial cells, resulting in transient opening of the tight junctions, and thus facilitates the transport of molecules that otherwise cannot cross the BBB. Vascular permeability to small molecules, large biomolecules[30], and even to virus-sized iron oxide particles[31], is increased transiently following infusion of hypertonic mannitol, after which it returns to preinfusion levels within 2 h. Thus far, osmotic modification of the BBB has been studied most commonly as a method for improving brain tumor therapy[32]; delivery of peptides to the brain has not been addressed specifically by this method. The considerable toxic effects of the procedure should be taken into account; it can lead to inflammation, encephalitis and to the incidence of seizures (as high as 20% of the applications).

In conclusion, invasive procedures are only justified for some life-threatening CNS maladies such as brain cancer, and such surgical routes are not preferred for less dramatic illnesses.

Physiologically based strategies

Physiologically based strategies to deliver peptides into the CNS have been well reviewed[5,14,33]. The observed paucity of endocytotic activity of the brain capillary endothelium does not reflect the complete lack of various transcytosis mechanisms but rather their very low capacity. In fact, physiologically based strategies involve the formation of chimeric peptides and rely on the existence of transcytosis systems for the transport of various large peptides and proteins through the BBB. The scheme shown in Figure 2 is based on the

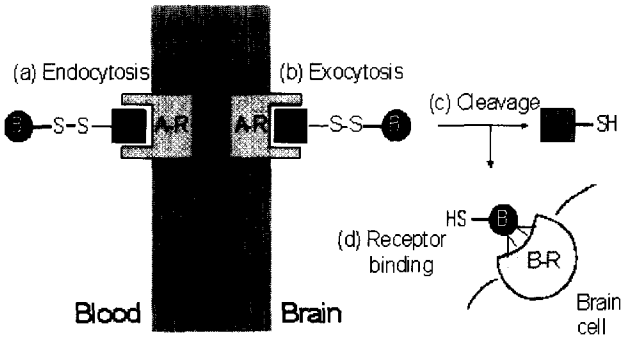

Figure 2. *Hypothesis for delivery of chimeric peptides through the blood–brain barrier (BBB) in four sequential steps: (a) endocytosis of the chimeric peptide at the luminal side of the BBB; (b) exocytosis of the chimeric peptide into brain interstitial fluid; (c) cleavage of the chemical linker joining the therapeutic agent to the transport vector; (d) binding of the peptide to its cognate receptor on brain cells. A, transport vector; B, nontransportable peptide; A-R, receptor for transport vector; B-R, receptor for peptide. Adapted from Ref. 37 with permission.*

covalent coupling (e.g. by a disulfide bond) of a peptide that is not normally transported through the BBB to peptide or protein 'vectors', which undergo receptor-mediated or adsorptive-mediated transcytosis. These so-called chimeric peptides may then be transported via these vectors through the BBB into the brain. After entering the interstitial space of the brain, the active peptide is released and it may then interact with the corresponding receptor to initiate pharmacological action in the brain. Design considerations in the development of effective chimeric peptides include vector specificity for the brain, vector pharmacokinetics, coupling between vector and peptide, cleavability of the vector–peptide linkage, and intrinsic receptor affinity for the peptide released from the transport vector.

Receptor ligands such as insulin, insulin-like growth factor and transferrin are often semi-tissue specific. The affinity of the chimeric peptide for the receptor is invariably 1 to 2 log orders lower than it is for the native peptide, which may result in limited endocytosis of the chimeric peptide[34]. Uptake by nonneural cells or by cells outside the CNS has also been found[35]. The use of a native peptide or protein as a vector has often been limited by its very rapid clearance from the blood stream. To improve vector pharmacokinetics, adsorptive-mediated transcytosis of cationized albumin, whose capacity is far greater than that of receptor-mediated endocytosis[36], has been utilized in the transport of appropriate chimeric peptides through the BBB[37]. Recently, anti-transferrin (OX26)[38] and anti-insulin (Mab83-7 and Mab83-14) receptor antibodies[39] have been proposed as efficient and selective BBB transport vectors. Receptor-specific antibodies are considered to be better delivery vectors than cationic proteins because they have higher BBB permeability (P)–surface (S) products (PS)[33].

Coupling of peptides to transport vectors

The coupling of therapeutic peptides to transport vectors has been achieved by covalent conjugation targeting surface amino, carboxyl, or thiol groups in proteins[36], or by high-affinity noncovalent binding of the biotinylated peptide to a covalent avidin–vector conjugate[40,41]. Covalent conjugation of nerve growth factor (NGF) to a transport vector (OX26) increased the delivery of NGF to the brain parenchyma from about 0.1% of the injected dose when free NGF was administered to about 0.4% after intravenous injection of an equivalent amount of OX26–NGF conjugate[42,43]. Cross-linking reactions may, however, proceed with unsatisfactory (10–15%) yields.

In the avidin–biotin method, first neutral avidin is covalently attached to the vector, for example by a thiol ether linkage to the ε-amino groups of lysine residues of the protein. Avidin is an avian protein that binds biotin with extremely high affinity (a dissociation constant of about 10^{-15} M and a half-life of 89 days)[44]. Then, the biotinylated agent is used to attach the therapeutic molecule to the avidin–vector conjugate. The target therapeutic peptide must be monobiotinylated to prevent the formation of high molecular weight aggregates due to multiple binding sites for biotin in avidin. CNS activity has been demonstrated in rats by the increase of cerebral blood flow and cerebral blood volume after systemic administration (10-min intracarotid infusion) of a biotinylated vasoactive intestinal peptide (VIP) analog conjugated to an OX26–avidin vector[42]. However, the fraction of administered dose of the chimeric peptide entering the brain tissue beyond the BBB was 0.256%/g brain, which was a moderate increase from the 0.096%/g achieved by intracarotid infusion of the avidin complex of biotinylated VIP analog. (Intracarotid infusion, an invasive method of administration used by the authors to show an increase in CNS delivery in these studies, may have caused a relatively high percentage of the systemically administered peptide dose to enter the brain. This method maximizes the exposure of the chimeric peptide to the brain capillary endothelium, thus fully exploiting the low-capacity transport mechanisms. A noninvasive administration such as intravenous injection may result in significantly less efficient delivery.)

Release from the transport vector

Although certain chimeric peptides may retain the full biological activity of the therapeutic agent[40], release of an active peptide from the transport vector to receptor sites on brain cells is usually necessary for CNS activity to be exerted. Opioid peptides, for example, have no pharmacological activity when bound to the transport vector. Although cleavage of a disulfide-based β-endorphin–cationized albumin chimeric peptide was found to yield a product that coeluted with β-endorphin standard on a gel filtration column[37], the biologically active form of the opioid peptide was not obtained. A pharmacologically active, thiolated analog of [Lys^7]dermorphin has also been developed and incorporated into a disulfide-based chimeric peptide[45]. Preliminary studies indicated that the chimeric peptide was stable in plasma *in vivo* and in brain endothelial cells *in vitro*, and was cleaved in rat brain *in vivo*. Unfortunately, the technique used for characterization of the released peptide(s) was unable to distinguish the pharmacologically active dermorphin analog from its inactive metabolites.

Disadvantages of physiologically based strategies

Some serious drawbacks may arise from a physiologically based approach. The poor stoichiometry of the neuropeptide to the carrier molecule limits the mass transport of the target peptide. For a 430 μg/kg dose of the avidin–vector conjugate, the peptide (biotinylated VIP analog) dose in a chimeric peptide was only 12 μg/kg[41]. On the basis of the data reported, less than 2 pmol of the approximately 500 pmol injected dose reached the brain in rats, and this amount could not be substantially increased by injecting larger doses.

Carrier- or receptor-mediated cellular transport has physiologically limited transport capacity (saturable) which might prevent pharmacologically significant amounts of most peptides from entering the brain. The low amount delivered to the CNS stresses the need for extremely potent therapeutic peptides, a need which has not been met by specifically developed, CNS-cleavable chimeric peptides with pharmacological activity at or above 100 pmol on intracerebroventricular injection[45]. Other strict requirements for coupling of a peptide to an avidin–antibody vector, such as monobiotinylation, *in vivo* cleavability of the linker and retention of biological activity after release, make this method very restrictive. Already discovered and potentially useful peptides may have to be radically redesigned to enable them to be incorporated into chimeric peptides for potential delivery to the CNS by a physiologically based strategy.

Peptide prodrugs and chemical delivery systems

Hydrophobic substances, because of their high lipid solubility, can generally diffuse freely across the BBB. The objective of the pharmacologically based strategies is to turn water-soluble substances into lipid-soluble ones, thereby increasing their passive transport across biological membranes. Encapsulating the peptide in liposomes, which were shown to be taken up by cells lining the reticuloendothelial systems of liver and spleen, did not result in any measurable transport across the BBB[41]. Peptide lipidization by forming cyclic derivatives or diketopiperazines that show increased lipid solubility has been applicable only to small peptides such as the C-terminal dipeptide of TRH[42].

Prodrug approach

In the prodrug approach shown in Figure 3, a lipophilic peptide derivative is synthesized[48,49]. Because of its increased lipid solubility, the peptide prodrug may penetrate biological membranes, including the BBB, and may reach organs such as the CNS that are otherwise inaccessible to the unmanipulated compound. Enzymatic or chemical transformation may then convert the inactive prodrug to the pharmacologically active peptide in the CNS. Peptide prodrugs are often designed to limit metabolism of the parent molecule[50].

Efforts to show the utility of prodrugs in improving the delivery of peptides into the CNS have been few. The 1-adamantyl ester of [D-Ala^2]-[Leu^5]enkephalin, a synthetic analog of the naturally occurring opioid pentapeptide, has shown a more than 100-fold increase in lipid solulility compared with the parent peptide based on the partition coefficient in n-octanol/water, and intravenous injection of high doses (5–50 mg/kg) resulted in CNS-mediated analgesia that could be reversed by naloxone, an opioid antagonist[51]. Similar doses of the unmodified enkephalin analog and its potential prodrug forms, protected at the N-terminal Tyr residue, showed no anti-nociception. The CNS activity may have been due, in part, to the conversion of the adamantyl ester to the free carboxylic acid, because the derivative exhibited reduced opioid-binding affinity on isolated guinea-pig ileum. Although the attachment of a lauroyl moiety to the N-terminal pyro-glutamyl residue of TRH (pGlu-His-Pro-NH_2), a peptide rapidly deactivated in plasma, brain and other tissues[52], caused a moderate (19%) decrease in CNS activity, a more

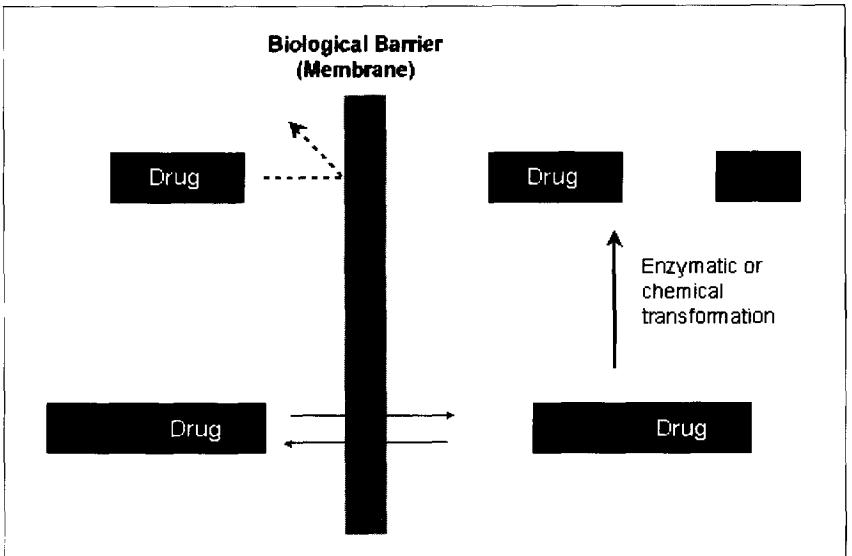

Figure 3. *Prodrug approach. By attachment of a pro moiety to the drug (peptide), the inactive prodrug overcomes the barrier because of its improved physicochemical properties. Once past the barrier, the prodrug reverts to the parent molecule by a postbarrier enzymatic or chemical process.*

pronounced (36%) reduction in endocrine (systemic) activity resulted in an increase of the CNS selectivity[53]. However, it remains unclear whether this derivative was behaving as a prodrug.

Bioreversible derivatization of the imidazole group in TRH with greatly improved lipophilicity and, therefore, better BBB penetration properties has been suggested for possible prodrug development[54]. Conjugation to lipidic amino acids also may improve delivery of peptides such as TRH and luteinizing hormone-releasing hormone to the CNS[55].

Although the acquired lipophilicity of these prodrugs may assure penetration to the BBB (and to other membranes), this is not the sole factor to be considered in the transportability of peptides into the CNS. Enzymatic degradation, and the consequent attenuation or loss of biological activity, should also be prevented during the passage of the substance from the general circulation to the brain tissue. In addition, lipid-soluble peptide prodrugs that can cross the BBB can only sustain active concentrations in the CNS if their blood concentrations are maintained at adequately high levels.

Chemical delivery system

The chemical delivery system (CDS) strategy is distinct from the simple pharmacologically based approach in which a lipophilic peptide prodrug is applied. In the CDS strategy, aptly called 'molecular packaging', the peptide unit appears as a perturbation on a bulky molecule dominated by lipophilic modifying groups, which assure its penetration of the BBB and also prevent its recognition by peptidases[56]. In addition, a specific functional group attached to the target peptide provides its retention in the CNS, and controlled release of the biologically active substance is achieved by predictable sequential metabolism.

In designing a CDS for the CNS, the unique architecture of the BBB is exploited. As with a prodrug, a peptide CDS should be sufficiently lipophilic to allow its uptake by the brain. After this step, the molecule should undergo enzymatic or other conversion to promote its retention within the CNS but at the same time accelerate its peripheral elimination. Finally, the intermediate should be further metabolized to release the active compound in a sustained manner. In molecular packaging, the peptide or its analog is modified to provide increased lipophilicity through biolabile functional groups that are susceptible to easy removal. Among these functional groups, a dihydropyridine-type targetor (T) is included to enhance BBB penetration and which, most importantly, can be converted by enzymatic oxidation to a water-soluble, lipid-insoluble (quaternary pyridinium) salt (T^+).

Upon systemic administration, passive transport partitions the CDS into several body compartments, including the brain, due to its enhanced lipophilicity. At this point, the CDS is simply working as a lipoidal prodrug. The targetor moiety is, however, designed to undergo enzymatically mediated oxidation which converts the membrane-permeable dihydrotrigonellinate into a hydrophilic, membrane-impermeable trigonellinate salt. This conversion occurs ubiquitously, and is analogous to the oxidation of NAD(P)H, a coenzyme that is associated with many oxidoreductases and cellular respiration. The now polar, oxidized targetor–drug conjugate is trapped behind the lipoidal BBB and, in essence, remains 'locked' inside the CNS. Any oxidized salt formed in the periphery will be rapidly lost as it is now polar and an excellent candidate for elimination by the kidney and bile. The conjugate trapped behind the BBB can then undergo biotransformation to release the active species in a slow and sustained manner.

Because the design of this system, concentrations of the active drug are very low in the periphery, thus reducing systemic, dose-related toxicity. In addition, the drug in the CNS is present mostly as an inactive conjugate, thus lowering any central toxicity. This approach should provide a more potent compound, because a larger proportion of the administered dose is delivered to its site of action. The redox targetor has been widely applied for brain targeting of a variety of substances, and its attachment alone results in the brain-specific delivery of small molecules such as dopamine[57]. For peptides, the N-terminus of the molecule is available for coupling the targetor to the molecule. However, the attachment of 1,4-dihydrotrigonellyl (T) to the N-terminus alone will not impart a sufficient increase in lipid solubility to a large molecule, and will only protect the peptide against aminopeptidases.

The unmodified C-terminal portion of the molecule will be susceptible to cleavage by numerous exo- and endopeptidases present in the BBB[10]. A bulky and lipophilic protection, such as a cholesteryl moiety[58] attached to the C-terminus of a peptide through an ester bond, greatly increases the lipid solubility and prevents this part of the molecule from being recognized by peptide-degrading enzymes. This part of the molecule is, however, labile towards esterase and/or lipase, which permits its removal after delivery. The final step of the drug delivery is the release of the biologically active peptide from the targetor–peptide conjugate. Attachment of the targetor directly to the N-terminal residue may not yield the target peptide because of the low amidase activity of the brain tissue. Therefore, a spacer function (S), consisting of an

additional amino acid residue or a pair of residues, is used to separate the peptide sequence to be delivered from the targetor part of the CDS. This spacer portion of the molecule is selected on the basis of the peptidolytic activity prevalent at the site of action, so that release of the desired peptide is favored over degradation by other peptidases. With a single alanine residue as a spacer, dipeptidylpeptidase (DP) II (EC 3.4.14.2) and/or DP IV (EC 3.4.14.5) is responsible for the release of the peptide from the conjugate, but the spacer function can be optimized to involve other cleaving enzymes for each target peptide. Essentially, the molecular architecture should conform to the desired attribute that the peptide moiety appears only as a perturbation on a bulky, lipophilic molecule dominated by the lipophilic portion (L) and the targetor (T); this is reflected by the concept of 'molecular packaging'.

Studies of [D-Ala^2]-[D/L-Leu^5]enkephalin CDSs in rats showed that the packaged molecule reached the CNS after systemic administration (20 mg/kg intravenous dose), was rapidly oxidized at the targetor, and the cholesteryl removed to give about 500–700 pmol/g tissue of the T$^+$ | S (= Ala) | peptide conjugate (Figure 4) 15 min after injection[59,60]. This conjugate is a weak opioid; the 50% inhibitory concentration (IC_{50}) in a [^{3}H]diprenorphine-based competitive assay in rat brain pellets was only a fraction (>10^{-7} M) of that of the unmodified enkephalin analog ($IC_{50} \approx 10^{-8}$ M). However, the 'locked-in' [D-Ala^2]-[D-Leu^5]enkephalin conjugate has been shown to release the parent opioid peptide analog slowly, and to provide a steady-state concentration of the biologically active peptide at the intended site of action, the brain[61]. As a result, a sustained and statistically significant increase in supraspinal analgesia was obtained after an intravenous bolus injection of the CDS for [D-Ala^2]-[D-Leu^5]-enkephalin in rats. The animals receiving the parent peptide or peptide prodrugs derivatized either with the targetor or with the lipophilic ester showed no effect. This observation has highlighted the requirement for the presence of all the functional units of the molecular packaging in a CDS to enhance brain delivery. An additional feature of the strategy is that, after distribution into the brain and the rest of the body, elimination of the conjugates followed by oxidation of the CDS is accelerated, while the membrane-impermeable depot forms are retained in the CNS[59].

An important extension of the method to peptides with N-terminal pyroglutamyl and C-terminal carboxyamide functions has also been reported[62]. Based on C-terminal glycine being an amide precursor for proline via peptidylglycine α-amidating monooxygenase (PAM) and glutamine serving as a precursor of the N-terminal pyroglutamyl, molecular packaging of Gln-Leu-Pro-Gly was performed to deliver [Leu^2]TRH to the brain. Delivery of a pharmacologically significant amount of the TRH analog was demonstrated in mice by the marked decrease in barbiturate-induced sleeping time – a measure of the activational effect on cholinergic neurons. Intravenous administration of the unmodified peptide in an equimolar (30 μmol/kg) dose showed only a marginal

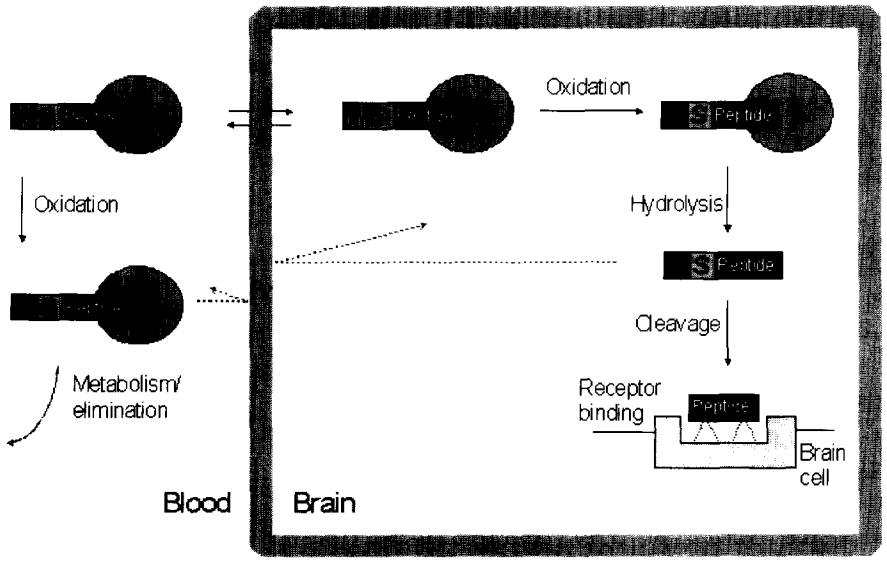

Figure 4. *Peptide delivery into the CNS by molecular packaging and sequential metabolism. The peptide is packaged by covalently attached lipophilic groups including a lipoidal 'blanket' (L) and a 1,4-dihydropyridine 'targetor' (T) which undergoes enzymatic oxidation and forms an ionic, membrane-impermeable pyridinium moiety (T$^+$). After distribution in the body and into the CNS by crossing the BBB, the chemical delivery system is converted to ionic compounds which are retained in the brain tissue, but the ionic conjugates produced in the rest of the body are readily eliminated. The membrane-impermeable conjugates 'locked' into the brain undergo sequential metabolism and yield the therapeutic peptide in the CNS which binds its cognate receptor. A spacer (S) function controls the enzymatic rate of the oxidized targetor (T$^+$) removal.*

decrease in sleeping time, whereas CDS resulted in a statistically significant reduction in sleeping time. The feasibility of using readily cleavable spacer (S) functions, by allowing peptide release to proceed via endopeptidase cleavage, has also been shown.

The further evaluation of the CDS approach will require the selection of therapeutically useful peptides and the design/optimization of molecular strategies intended to achieve brain-enhanced delivery and sustained release at the site of action. The influence of peptide size and/or molecular complexity on the efficacy of transport also needs to be addressed in correlation with related BBB properties.

Conclusions

Research on the delivery of potentially useful peptide therapeutic agents to the CNS has lagged behind the exponential growth in their discovery. Various methods that offer invasive, physiologically and biochemically/pharmacologically based approaches have been conceived to overcome the major obstacle: the blood–brain barrier. Much remains to be learned about these strategies before their promise to deliver peptides safely and efficiently into the CNS is realized. However, progress is steady and the methods discussed above clearly warrant the continuation of exploration and development in this field.

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