

# Development of Neuropeptide Drugs that Cross the Blood-Brain Barrier

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**Summary:** In recent years, there have been several important advancements in the development of neuropeptide therapeutics. Nevertheless, the targeting of peptide drugs to the CNS remains a formidable obstacle. Delivery of peptide drugs is limited by their poor bioavailability to the brain due to low metabolic stability, high clearance by the liver, and the presence of the blood brain barrier (BBB). Multiple strategies have been devised in an attempt to improve peptide drug delivery to the brain, with variable results. In this review, we discuss several of

the strategies that have been used to improve both bioavailability and BBB transport, with an emphasis on antibody based vector delivery, useful for large peptides/small proteins, and glycosylation, useful for small peptides. Further development of these delivery methods may finally enable peptide drugs to be useful for the treatment of neurological disease states. **Key Words:** Blood brain barrier peptide transport/antibody based vectors/transferrin receptor/glycopeptides.

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## INTRODUCTION

The role of neuropeptides in disease has become an important area of research over the past two decades. Neuropeptides have been indicated as primary molecules in several neurological disorders including epilepsy and depression (see Table 1). The use of peptides as pharmacological agents is an attractive proposition due to low toxicity of their metabolites and enhanced potency. Despite the growth in understanding of neurological disorders, peptide-based therapeutics are not currently available for treating these clinical problems. This is largely due to inadequate delivery of intact/viable peptides to specific brain regions necessary for neurological disease treatment. The delivery of peptide-based drugs to the brain is limited by two main factors; general bioavailability issues and the presence of the blood brain barrier (BBB).

The bioavailability of pharmaceuticals to the brain can be affected by a number of factors. These include distribution within the cardiovascular space, total volume of distribution, half-life of disappearance, and the ability of the drug to reach the target for biological effects. The

contribution of each of these components is different for each drug, and for peptide neuropharmaceuticals each should be considered a potential problem for drug delivery. The cardiovascular compartment can be split into three fractions: blood cells (predominantly red blood cells), protein fraction, and protein-free fraction (PFF). The PFF contains the peptide drug available at a particular time for transport into the brain. The distribution of peptide within these three compartments is in a dynamic equilibrium. In general the equilibrium is driven to release peptide to the PFF because this pool is constantly depleted by enzymatic metabolism, excretion, and uptake (Figure 1). For peptides, this cardiovascular compartment is of particular importance. Most peptide neuropharmaceuticals are analogs of endogenous peptides and as such can interact with both carrier proteins and endogenous peptidases. It is important to note that levels of these proteins and enzymes can be regulated/alterd by disease, and are also species dependent. Insulin-like growth factor (IGF)-1, a 70-amino acid peptide, has a number of different binding sites within plasma and each has differing binding constants.<sup>1</sup> The level of one IGF binding protein has been shown to be regulated by IGF levels, as well as disease state<sup>2</sup>.

There are a number of endogenous peptidases, both in the serum and on blood vessels that metabolize peptides efficiently (Table 2). Peptidases such as aminopeptidase

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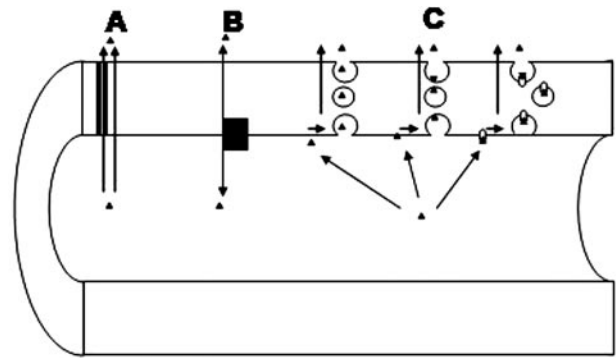
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**TABLE 1.** Potential Targets for Peptide-Based Therapies

Disease	Peptide	Reference
Pain	Opioid peptides	111
	Neurokinins	112,113
Depression	CRH	114,115
	Substance P	116
Epilepsy	Neuropeptide Y	117
	Dynorphins	118
	Somatostatin	119
Learning	Neurotrophins	120,121
Obesity	Leptin	122
	Ghrelin	123
Sleep	CRH	124
Stroke	BDNF	125
Brain cancer	Various growth factors	126,127

CRH = corticotropin-releasing hormone.

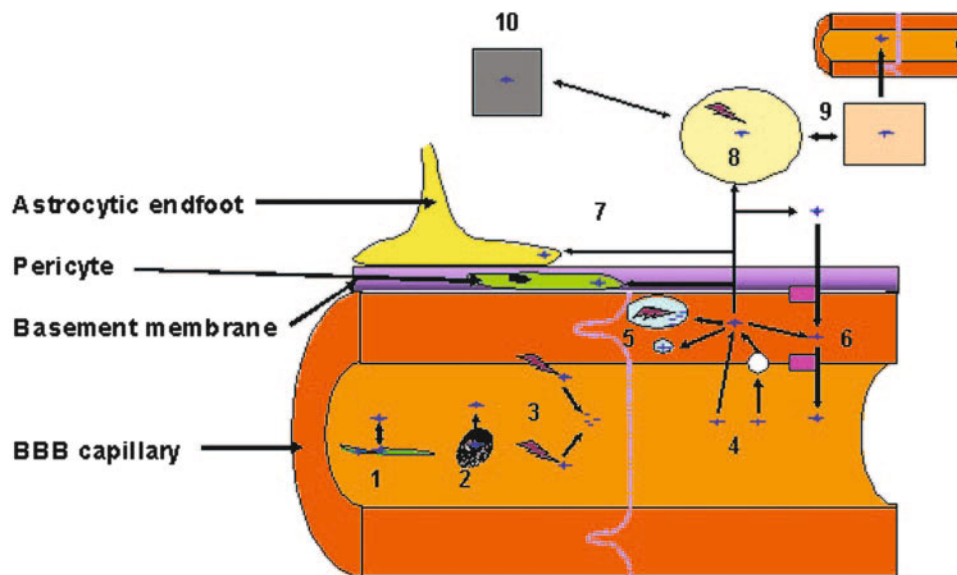
are present in serum<sup>3</sup> and can demonstrate variable concentrations dependant on clinical conditions. For example, aminopeptidase A is at higher levels in pregnant women than in nonpregnant women.<sup>3</sup> To further complicate matters, there is a difference in both the activity and amount of serum proteolytic enzymes dependent on species, resulting in differential metabolism of both peptide and nonpeptide drugs.<sup>4,5</sup> The metabolic stability of bradykinin and [des-Arg9] bradykinin in serum show marked species difference.<sup>6</sup> There is also evidence for



**FIG. 2.** The main types of transport systems that can be targeted for BBB delivery. A = free diffusion; B = carrier-mediated transport; C = receptor-mediated transport (see Table 3).

species differences in the renin-angiotensin enzymatic system.<sup>4</sup>

The BBB also limits the delivery of peptides to the brain. The BBB is situated at the level of the endothelial cells of the brain microvascular capillaries.<sup>7</sup> BBB endothelial cells are connected by tight cellular junctions, which provide a high transendothelial electrical resistance of 2000  $\Omega \cdot \text{cm}^2$ , compared with 3-30  $\Omega \cdot \text{cm}^2$  in peripheral vessels.<sup>8,9</sup> The BBB endothelial cells have a low number of vesicles,<sup>10</sup> indicating reduced vesicular transport. The BBB also lacks fenestrations and is ensheathed by astrocytic end-feet, which provide autocrine



**FIG. 1.** Obstacles for peptide delivery to the brain. Delivery of peptides to the brain can be affected by a number of processes. Peptide drugs can bind to carrier proteins within the blood (1) or can enter the blood cells (2). These processes are in equilibrium, and the release of peptides from these two compartments is dependant on the “off” and “on” rates of the carrier protein. Peptides within these compartments may not be available for delivery to the brain. Another factor which can affect the delivery of peptides is the large number of metabolizing enzymes located in the endothelial cells and also in the plasma (3) (see Table 2). Peptides can enter the endothelial cells of the BBB (4) via simple diffusion or via a number of saturable transport mechanisms (see FIG. 2 and Table 3). Once in the endothelial cell the peptide can cross the cell, enter one of the cell compartments where it is either stored or metabolized (5), or be effluxed from the cell via one of the many energy dependent transporters at the BBB (6) (see FIG. 2). On the brain side of the BBB, the peptide can be taken up by nontarget cells such as pericytes and astrocytes (7), both of which are intimately associated with the endothelial cells. In the brain extracellular fluid (ECF) (8), the peptides can freely diffuse to the CSF and thus be re absorbed or effluxed back into the blood (9). Finally if any of the peptide is left, it can interact with the target cell/receptor (10).

**TABLE 2.** *Enzymes at the BBB*

Enzyme	Example of Action	Reference
Dopa-decarboxylase	L-DOPA to dopamine	128
Monoamine oxidase-B	Inactivates catecholamines	129
Pseudocholinesterase	Deacetylates heroin to morphine	130
Cytochrome P450 (various)	O-Demethylates codeine to morphine	131–133
UDP-glucuronosyltransferase	Metabolizes 1-naphthol	134
Epoxide hydrolase	Reacts with epoxides	134
Renin	Angiotensinogen to angiotensin-1	135
Dipeptidyl dipeptidase	Peptide metabolism	136,137
ACE	Angiotensin conversion	12
Aminopeptidase A	Peptide metabolism	14
Aminopeptidase M	Peptide metabolism	12,138
Glutamyl aminopeptidase	Peptide metabolism	139
Neutral endopeptidase 24.11	Peptide metabolism	12,140
Endopeptidase 24.15	Peptide metabolism	141
$\gamma$ -Glutamyltranspeptidase	Leukotriene C4 to D4	142
Alkaline phosphatase	Purine and pyrimidine metabolism	143

UDP = uridine diphosphate; ACE = angiotensin-converting enzyme.

factors to maintain BBB function.<sup>11</sup> Additionally, the BBB acts as a metabolic barrier possessing a number of proteolytic enzymes<sup>12,13</sup> including aminopeptidase A,<sup>14</sup> aminopeptidase M,<sup>15</sup> and angiotensin-converting enzyme<sup>14</sup> that are known to degrade neuropeptides. Enzymes such as  $\gamma$ -glutamyl transpeptidase, alkaline phos-

phosphatase, and monoamine oxidase have elevated levels in brain microvessels and are either absent or expressed at low levels in peripheral vessels.<sup>16–18</sup>

The BBB plays an important role in brain homeostasis and a number of transport systems are present that enable substances to enter the brain. Specific transport systems

**TABLE 3.** *Main Types of Transport Systems for Targeted Delivery of Peptides across the BBB*

Mechanism	Characteristics	Example	Reference
A			
Diffusion	Flux down an electrochemical gradient Energy independent Flux proportional to concentration Rate independent of action	CTAP (transcellular)	144
B			
Facilitated	Carrier mediated Flux is saturated by increasing concentration Competitive substrates Flux may be asymmetric	Biphalin via LNAA	64
Active carrier mediated	Flux can be against electrochemical gradient Energy dependent (directly or indirectly) Substrate specificity, saturation, competition Flux rate is asymmetric	$\beta$ -Endorphin efflux via P-glycoprotein	53
C			
Endocytosis	Invagination of plasma membrane to form an internalized membrane vesicle Usually energy dependent Flux against gradient		
Fluid phase	Soluble molecules internalized with the vesicle volume Nonsaturable, nonspecific dependent on solubility	Lucifer yellow	145,146
Adsorptive	Solute nonspecifically adsorbs to cell surface proteins/glycoproteins	gp-120	147
Receptor mediated	Can be saturable and show competition, high capacity Highly specific High affinity, saturable, low capacity Asymmetric	Insulin Vectors	148

CTAP = D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Per, Thr-NH<sub>2</sub>.

have been reported for amino acids,<sup>19</sup> glucose,<sup>20</sup> and iron transferrin.<sup>21</sup> Several saturable transport systems have also been reported for peptides, both influx and efflux. Influx (blood-to-brain) systems include leu-enkephalin,<sup>22,23</sup> arginine vasopressin,<sup>24</sup> and [D-penicillamine-2,5] enkephalin.<sup>25</sup> Several efflux systems (brain-to-blood) have also been characterized, including a carrier-mediated efflux system for small N-Tyr peptides,<sup>26</sup> the ATP binding cassette efflux pump P-glycoprotein (P-gp),<sup>27,28</sup> multidrug resistance proteins,<sup>29</sup> breast cancer resistance protein,<sup>30,31</sup> organic anion transporters (OAT),<sup>32</sup> organic anion-transporting polypeptides (OATP)<sup>33</sup> and novel organic cation-transporters.<sup>34</sup> These efflux transporters each have a large range of substrates, including various peptides that they can actively transport.

### STRATEGIES TO IMPROVE PEPTIDE DRUG BIOAVAILABILITY TO THE BRAIN

A number of strategies have been used to improve bioavailability to the brain. Most of these rely on increasing the plasma half-life of the peptide, either by improving the metabolic stability or by reducing clearance from the plasma and brain. Metabolic stability and clearance can be improved by inhibiting proteolytic enzymes via masking enzyme cleavage sites, or by masking the peptides from active efflux transporters in the liver.

An example of using enzyme inhibition to improve brain delivery of a drug is seen with the use of L-amino acid decarboxylase inhibitors to improve L-DOPA delivery to the brain. L-DOPA, a precursor of dopamine used in the treatment of Parkinson's disease, uses the large neutral amino acid transporter (LNAA) to cross the BBB.<sup>35-37</sup> Once in the brain it is converted to dopamine by ADD. However, ADD is in high concentration in the periphery as well, resulting in the rapid peripheral conversion of L-DOPA to dopamine, reducing CNS delivery and drug efficacy and substantially increasing side effects. However, coadministration of an ADD inhibitor that does not cross the BBB, promotes plasma half-life and delivery to the brain. Though a highly effective method for L-DOPA delivery, enzyme inhibition is not practical for most peptides, due to the large number of potential enzyme systems involved in degradation of each peptide drug candidate. Instead the focus has been on masking enzyme cleavage sites, via modification or addition of side chains that interfere with enzyme activity. A classic example of this strategy is the development of a family of enzymatically stable opioid analgesics based on the endogenous amino acid sequence for met-enkephalin (Tyr-Gly-Gly-Phe-Met). The endogenous neuropeptide met-enkephalin, is rapidly enzymatically degraded in both brain and plasma<sup>38</sup> ( $T_{1/2} < 1$  min). A number of strategies have been used to enhance the stability and thus the bioavailability of this peptide, in-

cluding cyclization,<sup>12,39</sup> halogenation,<sup>40</sup> methylation,<sup>41,42</sup> and cationization.<sup>43,44</sup> The met-enkephalin analog, DPDPE, is a cyclized peptide containing D-penicillamine (D-Pen) at positions 2 and 5.<sup>45,46</sup> The two D-Pens, form a disulfide bridge that conformationally constrains the peptide, leading to increased metabolic stability ( $T_{1/2} > 500$  min), bioavailability, and BBB permeability.<sup>47-49</sup> In delivery studies with DPDPE, not only does DPDPE accumulate within the brain, it is also significantly cleared via the hepatic route.<sup>42,47,50</sup> This is often an issue with cyclized peptides. Subsequent studies have shown that DPDPE is a substrate for a number of efflux pumps involved in biliary secretion including Pgp<sup>51-53</sup> and OATP.<sup>54</sup> A number of methods have been used to reduce the specificity of the efflux pumps to DPDPE and thus improve bioavailability. Polyethylene glycol (PEG) conjugation is one method shown to reduce efflux pump affinity of peptide drugs, to enhance peptide drug stability and circulation, while reducing immunogenicity, proteolysis, and clearance.<sup>55,56</sup> Addition of a 2000-Da PEG group to DPDPE significantly enhanced analgesia.<sup>42</sup> The primary reason for the improved analgesia was shown to be a reduction in plasma clearance for the pegylated DPDPE, which results in a higher brain uptake over time.<sup>42</sup> Pegylation also led to a shift away from hepatic clearance.<sup>42</sup> The changes in DPDPE pharmacokinetics by pegylation were shown in part to be due to the masking of DPDPE from Pgp binding.<sup>42</sup> This study demonstrates that increasing the bioavailability of a peptide can increase brain delivery, despite the reduction in blood-to-brain transport properties.

Although specific transport mechanisms are perhaps the best targets to focus CNS delivery strategies, the most simplistic route of enhancing the passive diffusion of peptides (i.e., increasing the lipophilicity) remains a viable method for increasing brain uptake. Lipophilicity has been shown to be a major determinant for the ability of a drug to diffuse across a membrane and remains one of the better tests for *in vivo* peptide permeability,<sup>57</sup> although this is highly dependent on the peptides studied.<sup>58,59</sup> Lipophilicity can be increased by reduction of hydrogen bonding potential and/or addition of lipophilic groups. Reduction of hydrogen bonding potential has been shown to increase BBB transport for a number of substances, including small peptides.<sup>60</sup> Methylation can reduce the overall hydrogen bonding potential of peptides and increases membrane diffusion by enhancing lipophilicity. Dimethylation of the N-terminal Tyr of DPDPE lead to a significant increase in analgesia.<sup>61</sup> In contrast, trimethylation of the Phe group of DPDPE significantly increased transport, without increasing analgesia.<sup>62</sup> Interestingly, this study of four different isomers of [Trimethyl-Phe<sup>4</sup>] DPDPE showed differential effects on bioavailability, ligand-binding, and analgesia based solely on the methyl group position on the Phe benzene

ring; only one isomer showed an increased transport, whereas the others had no effect, or decreased transport.<sup>62</sup> This dramatically illustrates an important point for peptide modifications, that the site and type of modification are vital for both the improvement of delivery and maintenance of biological activity. It is also important (when considering larger peptides) to understand the intramolecular hydrogen bonding involved in the tertiary molecular structure. For example, biphalin, an opioid peptide containing two enkephalin sequences, has numerous potential hydrogen bonds, several of which are critical for maintaining the folding of the peptide within a given solution.<sup>63</sup> An alteration in hydrogen bonding potential could alter the tertiary structure of the peptide and thus reduce its ability to interact with the opioid receptor. This could partially explain the loss of binding shown with the methylation of DPDPE.<sup>62</sup> Despite multiple strategies to enhance lipophilicity, there are obvious limitations. Highly lipophilic drugs tend to be extensively plasma bound, may increase affinity for efflux transporters at the BBB, resulting in intraendothelial sequestration and are readily taken up in the periphery.

More recent studies have focused on targeting specific transport mechanisms either via site-directed vectors or synthetic routes. Some transport systems/sites that have been targeted include the LNAA,<sup>64</sup> receptor-mediated endocytosis/transcytosis,<sup>65–68</sup> and adsorptive endocytosis/transcytosis.<sup>69,70</sup> For the purpose of this review, we will focus on two favored methods for improving brain delivery, the use of vectors and glycosylation.

### VECTOR-MEDIATED TRANSPORT

Vectorial transport involves the conjugation of a peptide-drug to a targeting molecule/substance, which has affinity for characteristics or receptors of the target tissue, most often resulting in absorptive or receptor-mediated endocytosis. Vectors that have been used include cationized albumin,<sup>71</sup> liposomes,<sup>72</sup> nanoparticles<sup>73</sup> and conjugation with monoclonal antibodies to receptors such as transferrin.<sup>67</sup>

Of these approaches, the use of the transferrin receptor has shown the most promise and has been used to transport several different classes of peptides across the BBB. The transferrin receptor is constitutively expressed at the BBB<sup>21</sup> at higher levels than other capillary beds<sup>74</sup> and is involved in the transport of iron into the brain. The murine monoclonal antibody to the rat transferrin receptor, OX26, has been successfully used to increase brain uptake of proteins and peptides in a receptor-mediated manner. Friden and colleagues<sup>74</sup> demonstrated that they could target the brain delivery of methotrexate using an OX26-methotrexate conjugate. Although OX26 is a non-peptide, this study was an important first proof of the principle. The specific targeting of OX26 to the brain

was confirmed by Partridge<sup>67</sup> showing that OX26 accumulated in the brain 18-fold higher than nonspecific IgG. Because the initial studies with OX26-methotrexate conjugates, several peptides have been investigated, including vasoactive intestinal peptide (VIP),<sup>75</sup> BDNF<sup>76</sup> and epidermal growth factor (EGF).<sup>76</sup> Each investigation resulted in increased brain uptake of the peptide in question. Interestingly, this methodology has also been used for immunoliposomes and gene delivery.<sup>77–79</sup> The first peptide that showed good penetration of the BBB with antibody conjugation was VIP. Bickel et al.<sup>75</sup> showed that administration of OX26 linked to VIP via a biotin-avidin linker raised cerebral blood flow, while the biotin-VIP alone did not. Unfortunately, peripheral administration of the OX26-VIP was only efficacious when given by intra carotid administration. The original avidin linkers resulted in rapid elimination due to their highly cationic nature. In a subsequent study the linker was changed to streptavidin, which is biologically neutral in nature.<sup>80</sup> VIP-biotin conjugated to OX26-streptavidin increased blood flow after intravenous administration with a reduction in unwanted side effects in peripheral organs.<sup>80</sup> An issue that can be a problem for delivery of peptides using antitransferrin receptor antibodies is the potential steric inhibition of the peptide binding to the receptor or even the peptide interfering with the antibody binding. This issue has been addressed by the addition of polyethylene-glycol linkers.<sup>81</sup>

Antibody-peptide vectors have shown significant advantages in the treatment of acute disorders. In animal models of stroke an increase in the levels of BDNF mRNA is observed, starting approximately two hours after stroke commencement.<sup>82</sup> The mRNA was observed predominantly in cells which have normal morphological appearance.<sup>82</sup> Subsequent studies showed that administration of BDNF via an osmotic pump directly into the infarct following middle cerebral artery occlusion (MCAO), could lead to a significant reduction in infarct size.<sup>83</sup> Studies in single allele BDNF knockout mice confirmed these findings.<sup>84</sup> Like many other peptides, BDNF does not cross the BBB, which makes it an ideal candidate for conjugation to OX26. Initial studies with a BDNF-OX26 conjugate showed that there was indeed an increased delivery to the brain of intact BDNF. To test the potential of the conjugate for stroke treatment, the effect of BDNF-OX26 on a transient MCAO model was investigated.<sup>66</sup> The administration of BDNF-OX26 directly after the ischemic incident followed by 6 further daily injections, prevented significant neuronal damage. In contrast, BDNF alone or OX26 alone did not produce any significant protection.<sup>66</sup> These data indicate that the protective effect of the BDNF-OX26 conjugate is directly correlated to its ability to cross the BBB. Clinically, the time course of this study may not be relevant, as most stroke victims have a significant delay between

symptom onset and treatment. Also, the model involved clamping the carotid arteries to produce a global "stroke," again not as common a situation clinically in humans. To investigate the effects of delayed treatment with BDNF-OX26 on MCAO, Zhang and Pardridge<sup>85</sup> used the intraluminal thread model of MCAO. This model produces a smaller infarct in the middle cerebral artery region and is a more clinically viable model. The BDNF-OX26 conjugate was given either at the commencement of reperfusion or following 1 h of reperfusion. In both treatment paradigms, a significant neuroprotection was observed.<sup>85</sup> A follow up study showed that the protective effect of BDNF-OX26 was both time (i.e., the sooner the conjugate is given after the infarct the better) and concentration dependent.<sup>86</sup> It is thus apparent that this technology may be a useful method for delivering growth factors to ischemic brain regions during stroke, and could be adapted to other peptides/proteins. Other studies have shown that basic FGF conjugates may also be effective<sup>87</sup> in stroke treatment.

One issue that should be considered when targeting a nutrient carrier such as the transferrin receptor is how this affects the transport of the native substrate. Although OX26 has been shown to bind to a region distant from the receptor binding site,<sup>21</sup> Ueda and colleagues<sup>88</sup> observed that pretreatment of animals with OX26 reduced Fe-transferrin delivery across the BBB by 30-40%. Whether this reduction is prolonged, physiologically relevant or relevant to other anti-transferrin antibodies used has not been fully addressed. It does however offer evidence that this approach should be used with caution in a limited use or with potent peptides that only require limited delivery to the CNS.

## GLYCOSYLATION

Glycosylation has shown significant potential for enhancing biodistribution of peptide-drugs to the CNS. The idea of glycosylation, as a mode of drug delivery, initially started through studies examining diabetes. It was noted that glycosylated proteins, produced by the Maillard reaction, had increased distribution to nervous tissue in both the periphery and brain.<sup>89,90</sup> The chemical attachment of carbohydrate moieties to a protein (glycoprotein) or peptide (glycopeptide) produces changes in the molecular structure that, in turn, can have significant effects on the pharmacodynamic and pharmacokinetic properties of proteins and peptide hormones.<sup>91</sup> Non-specific glycosylation was found to improve metabolic stability and increase activity of gastric inhibitory proteins.<sup>92-94</sup> This strategy has been used to increase the half-life of erythropoietin, another glycoprotein, with at least one variant being approved by the Food and Drug Administration for the treatment of anemia.<sup>95</sup>

The increased stability induced by glycosylation has

made it a focus for peptide drug examination / development. Glycosylation of small peptides has led to increase hydrophilicity, stability, and bioavailability.<sup>96-99</sup> Additionally, glycosylation of opioid peptides has shown to enhance BBB penetration leading to increased pharmacological activity.<sup>97,98,100-102</sup> BBB permeability studies of glycopeptides have indicated up to a three-fold increase in the rate of brain delivery, compared to the unglycosylated parent peptides.<sup>97,101,102</sup> Evidence also suggests that the type of glycosylation (i.e., mono-, di-, tri-glycosylation) can alter tissue distribution patterns,<sup>103-105</sup> BBB permeability,<sup>102</sup> and peptide/receptor interactions.<sup>106,107</sup>

Peptides investigated to date include recombinant human erythropoietin,<sup>108</sup> leptin,<sup>108</sup> dermorphins<sup>98</sup> and met-enkephalin analogs.<sup>99</sup> The first study on glycopeptide opioids focused on a cyclized met-enkephalin analog, [D-Cys-2,5] enkephalin (DCDCE).<sup>99</sup> In this study the authors added a Ser  $\beta$ -D-glucose at various structural positions of the peptide and investigated receptor binding and analgesia following intravenous administration. The addition of the glyco group within the cyclized region of the peptide led to large decrease in binding to opioid receptors and no *in vivo* activity. In contrast, addition of the glycol group at position six led to only minor changes in receptor binding and a significant increase in analgesia following peripheral administration.<sup>99</sup> The improved analgesia was related to a combination of increased metabolic stability and delivery to the brain potentially via a glucose transporter. Subsequent studies proved that there was indeed increased transport across the BBB, but not via an interaction with the glucose transporter.<sup>102,109</sup> It is currently believed that the enhanced transport is due to adsorptive endocytosis.<sup>69</sup> The studies by Polt and colleagues<sup>99</sup> highlight the importance of location of the glycosylation group on biological activity. Opioid enkephalin peptides require the alignment of the phenol rings of both the Tyr<sup>1</sup> and Phe<sup>4</sup> for maximal opioid receptor binding, placement of a Ser  $\beta$ -D-glucose between these groups reduced the binding to the  $\mu$ -opioid receptor from 30 nM to approximately 50,000 nM. Placement of the Ser  $\beta$ -D-glucose outside of the active region at position 6, lead to  $\mu$ -opioid receptor binding of 53 nM, only a minor change,<sup>99</sup> indicating that the glycosylation did not affect the alignment of the Tyr and Phe groups. This was confirmed by NMR and molecular modeling, which showed that glycosylation at the Ser<sup>6</sup> does not sterically affect the active region of the peptide,<sup>110</sup> which is critically important in maintaining efficacy.

Since the initial studies on glycosylated enkephalin opioids a number of opioid analogs have been tested, the most promising of which are based on linear leu-enkephalin analogs. The monoglycosylated peptide Tyr-D-Thr-Gly-Phe-Leu-Ser ( $\beta$ -D-glucose), produces analgesia after both central and peripheral administration with an

A50 value comparable to that of morphine after IV administration.<sup>97</sup> Surprisingly, it was shown that this peptide was more analgesically potent when given subcutaneously.<sup>97</sup> Subsequent studies revealed that this glycopeptide had a higher metabolic stability in both plasma and serum than the parent peptide,<sup>101</sup> double the brain penetration<sup>101</sup> and the transport of the glycopeptide was reported to be saturable.<sup>101</sup> These studies have therefore shown that glycosylation may be a useful tool for delivering small peptides to the CNS. Recent examinations have shown that opioid glycopeptides seem to be well tolerated, with minimal side effects.<sup>97,100</sup> However, as of yet no chronic pharmacological or toxicological studies have been performed with these glycopeptide analgesics and much more research is required before a clinically viable candidate is available for treatment consideration.

### SUMMARY

Peptides are a relatively untapped and potentially powerful therapeutic for disease prevention and treatment, yet as a group of drugs they suffer from numerous obstacles. Furthermore, the development of peptide-drugs, specific for CNS disorders, has historically been limited due to the BBB. Present technologies are advancing our ability to focus peptide drug delivery in manners previously unforeseen, with such concepts as specific antibody directed vectors and glycosylation, which not only enhance the pharmacological profile of these drugs, but can increase BBB transport into the brain in a regionally specific manner. The use of vector-mediated delivery shows continued promise in targeting peptides, as well as genes, with increasing degrees of success. This approach may prove ideal for chemotherapeutic applications, chronic CNS diseases in specific brain regions (i.e., Parkinson's), and for delivery of neuroprotective agents during acute disorders (i.e., stroke). Glycosylation has proven to be a useful methodology for enhancing biodistribution to the brain, through increased stability, reduced clearance, and improved BBB transport. Improving analgesia of opioid peptides is just one potential use of the glycosylation strategy. This approach may also prove highly useful in situations where prolonged administration of drugs is required, such as depression or chronic pain. The future of CNS focused peptide-drug delivery may likely be a composite of many of such strategies as the only limitation has become the imagination and creativity of the researcher.

**Acknowledgments:** This work was supported by National Institutes of Health Grants RO1 NS 42652, NS 39592, and DA 11271 (to T.P.D.) and RO1 DK 065003 and PO1 DA 006284 (to R.D.E.).

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