

Delivery of Peptides to the Brain: Emphasis on Therapeutic Development

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Introduction

Many peptides and regulatory proteins cross the blood-brain barrier (BBB) in amounts that are known to affect the functions of the central nervous system (CNS). Such passage constitutes a humorally based form of communication between the CNS and the peripheral tissues [1,2]. Passage can be unidirectional (either in the brain-to-blood or blood-to-brain direction only) or bidirectional, by way of saturable transporters or by non-saturable mechanisms, and saturable transport can be energy dependent (active transport) or non-energy requiring (facilitated diffusion). These pathways are important to understanding peptide physiology and disease states, and can be manipulated for therapeutic purposes. In some cases, altered transport at the BBB results in disease or exacerbation of pathological conditions. For example, impaired transport of the 17 kDa regulatory protein leptin results in peripheral leptin resistance, a hallmark of diet-induced obesity [3]. Another example is failure of LRP-1 in Alzheimer's disease, leading to the accumulation of amyloid beta protein to toxic levels [4,5]

The BBB is also key to treating CNS diseases. Any drug must negotiate the BBB in order to reach the vast majority of CNS tissues. Most major reviews of peptide and regulatory protein drug delivery to the CNS "black box" key aspects of BBB function, producing working models less complex, but also ultimately less useful. Most current workers use a variation of the "Trojan Horse" or "Universal Carrier" approach, where the peptide or protein is inertly packaged and attached to a delivery vehicle. Besides this clever approach, an in-depth evaluation of the BBB suggests other approaches that are either applicable to special cases or more generally. Special case approaches use delivery strategies, such as intrathecal delivery, the extracellular pathways, intranasal delivery, and CNS tissue infusions, which as currently applied are not ideal for most drug delivery conditions, but do fit special situations. For example, large proteins with no brain-to-blood efflux components and anatomical sites of action that contact the CSF are ideal for intrathecal administration [6]. However, a major mechanism which has been essentially ignored has been using the endogenous transporter for the peptide or protein to be delivered to the CNS.

Harnessing Endogenous Transporters

Peptides and proteins were once assumed to be to large to cross the BBB. However, in the absence of an efflux transporter, even large peptides can cross to some degree by way of transmembrane diffusion. The widely quoted, seldom referenced statement that an absolute

molecular weight cutoff of 400 Da exits at the BBB is a misinterpretation of the work of Levin [7]. In reality, those substances did not cross because they are substrates of the p-glycoprotein efflux system. Indeed, many peptides cross the BBB to a degree dictated by their lipid solubility [8-10].

However, the most exciting, but under-explored mechanism for peptide and protein delivery is the use of their endogenous transporters. Many endogenous peptides and proteins with CNS effects and therapeutic potential are transported by saturable systems across the BBB. For example, several workers have tried to develop carrier systems for nerve growth factors, including brain-derived neurotrophic factor (BDNF), stating that these substances do not cross the BBB. In reality BDNF crosses the BBB and does so at a very fast rate [11]. However, BDNF has unfavorable pharmacokinetics, being enzymatically unstable and having a short half-life. Indeed, for some peptides and regulatory proteins, an unfavorable pharmacokinetic profile can be as limiting to their delivery as the BBB itself.

Several peptides and regulatory proteins further exemplify both the challenges and opportunities to use of endogenous transporters. Examples include pituitary adenylate cyclase activating polypeptide (PACAP) which is transported across the BBB by peptide transport system-6 [12], leptin, and interleukin-1, and beta-glucuronidase (GUS). PACAP is an extremely potent neuroprotectant, being active at pmol and fmol levels. Its BBB transporter, peptide transport system-6, delivers small amounts sufficient to reverse ischemic stroke, even when PACAP is begun 24 h after the event [13]. However, the unfavorable pharmacokinetics of PACAP requires that it be delivered by infusion, limiting its use. An enzymatically resistant analog of PACAP still capable of being transported is potentially a major drug for the treatment of stroke. The cytokine interleukin-1 is transported across the BBB with major uptake at the posterior division of the septum [14]. Here, interleukin-1 acts to induce cognitive impairments both directly at its receptor and by inducing additional interleukin-1 release from CNS sources [15]. As discussed above, leptin transport across the BBB allows this large protein to directly relay nutritional information to the brain and failure in transport contributes to obesity. Perhaps the most fascinating insight into the workings of the BBB and the challenges that protein delivery to the brain presents is illustrated by GUS. This is a large (300 kDa) enzyme which when not expressed leads to one of the mucopolysaccharidoses, Sly's syndrome. Given its large size, it would seem that the only approach to for effect

therapeutic delivery to the brain must be by way of a Trojan horse mechanism. However, the neonatal BBB expresses a saturable transporter that incorporates the mannose 6-phosphate receptor (M6PR) and is able to deliver tremendous amounts of enzyme to the brain [16]. As a result, the CNS can be effectively treated with peripherally administered enzyme during this period. Unfortunately, with development, the transporter capacity declines to essentially undetectable levels. This suggests, however, that if the adult BBB could be induced to reexpress the M6PR transporter, effective delivery of enzyme to adults could occur [17].

In conclusion, the complexity of the BBB offers multiple approaches to drug delivery. Some of these approaches will be specialized, whereas others will be more generally applicable. Use of transporters when they exist for the endogenous peptide or protein remains a virtually unexplored approach.

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