Biochemical Pharmacology 83 (2012) 559–566

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Peptide therapeutics for CNS indications

Paul McGonigle *

PsychoGenics Inc., 765 Old Saw Mill River Road, Tarrytown, NY 10591, USA

ARTICLE INFO

Article history:
Received 29 July 2011
Accepted 17 October 2011
Available online 25 October 2011

Keywords:
Peptides
CNS indications
Peptide therapeutics
Oxytocin
Amylin

ABSTRACT

Neuropeptides play a crucial role in the normal function of the central nervous system and peptide receptors hold great promise as therapeutic targets for the treatment of several CNS disorders. In general, the development of peptide therapeutics has been limited by the lack of drug-like properties of peptides and this has made it very difficult to transform them into marketable therapeutic molecules. Some of these challenges include poor in vivo stability, poor solubility, incompatibility with oral administration, shelf stability, cost of manufacture. Recent technical advances have overcome many of these limitations and have led to rapid growth in the development of peptides for a wide range of therapeutic indications such as diabetes, cancer and pain. This review examines the therapeutic potential of peptide agonists for the treatment of major CNS disorders such as schizophrenia, anxiety, depression and autism. Both clinical and preclinical data has been accumulated supporting the potential utility of agonists at central neurotensin, cholecystokinin, neuropeptide Y and oxytocin receptors. Some of the successful approaches that have been developed to increase the stability and longevity of peptides in vivo and improve their delivery are also described and potential strategies for overcoming the major challenge that is unique to CNS therapeutics, penetration of the blood–brain barrier, are discussed.

1. Introduction

It has long been recognized that peptide hormones play a crucial role in the normal function of the central nervous system (CNS) and that peptide receptors hold great promise as therapeutic targets for the treatment of several CNS disorders. To date, technical limitations including in vivo stability, route of administration, blood brain barrier penetration, and complexity of manufacture and cost of manufacture have severely limited the development of peptide therapeutics for these disorders. Recent technological advances in several aspects of the drug development process have resulted in dramatic improvements in some of these properties such as in vivo stability and have overcome limitations by increasing the ease of administration and reducing the cost of manufacture (Fig. 1). These improvements have helped to make protein therapeutics one of the fastest growing segments of the pharmaceutical industry with over 70 therapeutic peptides on the market and over 150 in clinical development. These advances provide a strong rationale for revisiting the potential of peptide therapeutics for CNS disorders. Moreover, the progress in developing safer and more effective drugs for major psychiatric disorders has slowed considerably in recent years and currently marketed drugs for these indications still exhibit serious side effect liabilities and significant efficacy limitations (Table 1).

Peptides have several potential advantages as therapeutic molecules (Table 2). Endogenous peptide hormones have been designed by nature to perform very specific and complex modulatory functions. In general, they exhibit high specificity for their target receptors and are highly potent requiring minimal synthesis of material and have minimal cross-reactivity. Unlike many small molecule therapeutics, peptide hormones do not accumulate in tissues and they are efficiently metabolized by endogenous enzymes. They have a very limited potential for drug–drug interactions and with the exception of some instances of immune reactions are typically free of toxicological complications. As indicated above however, there are a number of technical challenges that must be overcome for peptides to become viable therapeutic molecules. One of the major limitations of peptides as therapeutics is their short in vivo half-life. Many peptides are cleared from the bloodstream within minutes to hours after administration resulting in insufficient exposure in the target tissue. The short half-life typically results from rapid renal clearance and/or from enzymatic digestion by proteases in the blood, kidneys or liver. In general, strategies to increase the size of the peptides to slow clearance and modifying the structure to confer enzymatic resistance have been successfully applied to overcome these challenges. Another practical challenge of peptide therapeutics is the inability to use oral administration because the peptides are poorly absorbed and rapidly degraded by digestive

* Corresponding author. Tel.: +1 914 602 4393; fax: +1 914 593 0645.
E-mail addresses: paul.mcgonigle@psychogenics.com, paulmcgonigle@comcast.net.

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.10.014

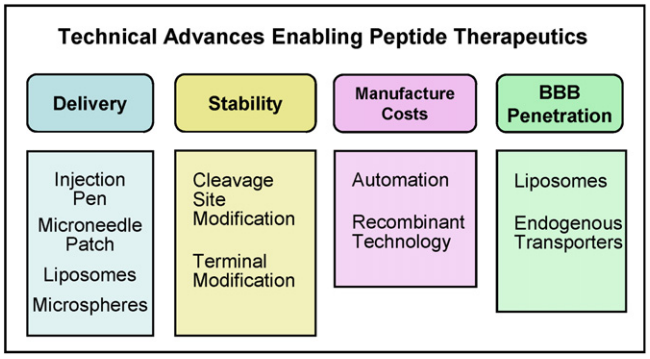

Fig. 1. Technical advances enabling peptide therapeutics.

enzymes. Steady progress in subcutaneous injection technology has significantly reduced the discomfort associated with each injection and reduced the cost of manufacture. For CNS indications, another major challenge is blood–brain barrier (BBB) penetration. In general, peptides do not cross cell membranes, however, there are a number of endogenous peptides such as insulin and leptin that enter the brain via specific transporters and approaches to utilize these transporters to deliver therapeutic peptides to the brain are being developed.

2. Potential targets for CNS indications

Peptide receptors have been the focus of intense drug discovery efforts for multiple CNS indications over the last decade and several small molecule antagonists have advanced to clinical trials. However, the synthesis of small molecule agonists for these targets has proven to be considerably more challenging, presumably due in large part to the steric requirements for agonism at peptide receptors. Evaluation of the therapeutic potential of activation of different peptide receptors will most likely require the use of peptide therapeutics designed to overcome the physicochemical limitations of endogenous peptides. Consequently, this review will focus on peptide targets for which there is evidence that activation of the receptors has therapeutic potential for the treatment of CNS disorders and discuss approaches to overcome the physicochemical limitations of these endogenous peptides.

3. Neurotensin

Neurotensin is a tridecapeptide found in the gastrointestinal, cardiovascular and central nervous system that is implicated in the control of blood pressure, pain, appetite and body temperature [1] (Table 3). Neurotensin is found throughout the CNS, with the highest concentrations in the amygdala, lateral septum, substantia nigra and ventral tegmental area [2]. There are three cloned receptors for neurotensin, NTS1 and NTS2, which are G-protein coupled and NTS3, which is a member of the LDL receptor family. Both NTS1 and NTS2 receptors are heterogeneously distributed throughout the CNS with relatively high levels of both receptors expressed in the substantial nigra and ventral tegmental area [3]. There is a close association between the neurotensin system and mesocortical and nigrostriatal dopamine systems and 80% of dopaminergic neurons in the ventral tegmental area and the substantia nigra express neurotensin receptors [3]. This association has provided the basis for exploration of the role of neurotensin in psychiatric disorders. Preclinical studies have demonstrated a striking similarity between the behavioral effects of centrally administered neurotensin and peripherally administered antipsychotics in several tests designed to detect therapeutic potential. For example, neurotensin inhibits both amphetamine and PCP-induced hyperlocomotion, decreases conditioned avoidance without increasing escape responding, restores pharmacologically induced as well as some endogenous sensorimotor gating deficits and inhibits numerous dopamine agonist-induced behaviors including yawning, rearing, stereotyped sniffing, licking, biting and climbing [4]. Typical and atypical antipsychotic drugs increase neurotensin mRNA expression, neurotensin peptide concentration and neurotensin release in the nucleus accumbens after both acute and chronic administration [4]. These results have led to speculation that neurotensin acts as an endogenous antipsychotic and the development of neurotensin agonists for the treatment of schizophrenia would be a viable therapeutic strategy. This hypothesis is supported by clinical studies in which low cerebrospinal levels of neurotensin were observed in a subset of drug-free schizophrenic patients relative to normal volunteers and patients with other psychiatric disorders [1]. In this subset of patients, low levels of neurotensin were correlated with the severity of numerous symptoms including thought disorder, disorganized behavior and impaired functioning and clinical improvement was associated with normalization of neurotensin levels in the CSF [5]. No clinical trials have been conducted to test this hypothesis primarily due to the lack of neurotensin agonists with adequate drug-like properties.

Table 2
Advantages and disadvantages of peptide therapeutics.

Advantages	Disadvantages
Highly specific	Chemical stability
Potent	Route of administration
Minimal cross-reactivity	Poor BBB penetration
No tissue accumulation	Complexity of manufacturing
Efficient metabolism	Poor shelf stability
Limited drug–drug interaction	Size of molecules
Minimal side effects	Cost of manufacture
Low immunogenicity	
Can be synthesized	

Table 1
Limitations of current medications for psychiatric disorders.

Drug class	Side effects	Efficacy
Antipsychotics		
(Atypical)	Weight gain, metabolic syndrome rapid heartbeat, skin rash, drowsiness	Ineffective against cognitive symptoms
(Typical)	Rigidity, muscle spasms tremors, restlessness	Ineffective against cognitive symptoms
Antidepressants	Headache, agitation, nausea, sleeplessness, sexual dysfunction	Delayed onset of action Treatment resistance
Anxiolytics		
(Benzodiazepine)	Nausea, headaches, confusion drowsiness, nightmares	Abuse liability
(SSRI)	Headache, agitation, nausea, sleeplessness, sexual dysfunction	Delayed onset of action

Table 3
Amino acid sequences of key endogenous peptides.

Neurotensin	pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
Amylin	Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH_2
Neuropeptide Y	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr -Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
Cholecystokinin-8	Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2

4. Cholecystokinin

Cholecystokinin is a 33 amino acid peptide that is found in the gastrointestinal, peripheral and central nervous systems and has been shown to be an endogenous satiety signal that causes gastric emptying, gallbladder contraction, pancreatic enzyme release and suppression of appetite. There are several biologically active forms of CCK that all share a common N-terminal sequence with the most prevalent form in the brain being CCK-8 [6]. High levels of CCK are found in several brain regions including the cortex, hippocampus, amygdala, nucleus accumbens, striatum and substantia nigra [7]. Moreover, CCK mRNA is colocalized with tyrosine hydroxylase in dopaminergic neurons in the substantial nigra and ventral tegmental area [8]. There are 2 cloned G-protein coupled receptors for CCK, CCKR1 and CCKR2. CCKR1 is the main peripheral receptor and is expressed in the pancreas, gall bladder and intestine as well as in the brain [9]. CCKR2 is widely expressed in the brain with the highest levels observed in cerebral cortex, nucleus accumbens, caudate nucleus, hippocampus, amygdala, substantia nigra and ventral tegmental area (see Crawley and Corwin [9]). Consistent with the neuronal colocalization and extensive overlap of expression between CCK and the dopaminergic system, CCK peptides have significant effects on dopamine mediated behaviors. Administration of CCK peptides exhibit many of the behavioral characteristics of antipsychotics including inhibition of conditioned avoidance responding [10], inhibition of apomorphine-induced stereotypic behavior [11], and inhibition of amphetamine-induced hyperlocomotion [12]. The neuroanatomical and pharmacological basis of these responses appear to be quite complex. Microinjection of CCK into the anterior nucleus accumbens inhibits dopamine release, inhibits dopamine-mediated behaviors and is blocked by a CCKR2 antagonist whereas injection into the posterior nucleus accumbens has the opposite effects and these effects are mediated by CCKR1 receptors [13,14]. More recently, the nonselective CCK agonist caerulean was shown to produce reversal of amphetamine-induced pre-pulse inhibition, which could be blocked by a CCKR1 but not a CCKR2 antagonist [15]. Coincidently, polymorphisms in the gene and promoter sequences for CCKR1 but not CCKR2 were found to be associated with schizophrenia [16]. Based on the behavioral data and neuroanatomical overlap, the effect of CCK-8, CCK-33 and the nonselective decapeptide agonist caerulein on schizophrenic patients was evaluated in more than 20 clinical studies. The initial single blind or open label studies reported promising results with relief of symptoms lasting for several weeks in some cases [17] but the majority of subsequent double blind studies reported no difference from placebo [18]. The peptides were administered systemically in all of the studies and doses were limited by the emergence of acute peripheral side effects. CSF levels were not measured in these studies and it is likely that exposure levels in the brain were not sufficient to stimulate central CCK receptors. In retrospect, it appears that although over 500 patients have been treated with a CCK agonist, the therapeutic potential of this mechanism has not been adequately tested. It is also important to note that selective CCKR2 agonists that cross the blood brain barrier such as pentagastrin and CCK-4 are used to induce panic attacks in clinical studies. Consequently, a CCK agonist for schizophrenia would need to be either nonselective or CCKR1 selective. There are orally bioavailable small molecule agonists selective for CCKR1 receptors but it is not known whether they cross the blood brain barrier.

5. Oxytocin

Oxytocin is a highly conserved cyclic nonapeptide that is involved in parturition, lactation and numerous social and non-social behaviors. It is primarily synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and transported to the posterior pituitary where it is released into the circulation to produce its peripheral effects. Synthetic oxytocin (Pitocin) is used to induce labor and promote milk production. The paraventricular nuclei also deliver oxytocin to several forebrain nuclei including the amygdala, hypothalamus, hippocampus and nucleus accumbens where it is presumed to have its behavioral effects. A single G-protein coupled receptor has been identified for oxytocin, which is broadly expressed throughout the CNS but the specific pattern can vary with species and gender. In humans, the highest levels of oxytocin receptors are found in the diagonal band of Broca, the lateral septum, the hypothalamus and the substantia nigra [19]. The oxytocin system is involved in numerous central functions including affiliative, maternal, and sexual behaviors, social memory and cognition. Several animal studies have shown that infusion of oxytocin stimulates maternal behavior whereas the administration of antagonists inhibits this behavior [20]. A key role for oxytocin in pair bonding has been elucidated in a series of studies in Prairie and Montane voles. Prairie voles form long term monogamous pairs and are bi-parental whereas Montane voles do not form such affiliations [21]. Central administration of oxytocin to non-mating Prairie voles produces a partner preference whereas antagonist administration disrupts pre-formed bonds [21]. In contrast, central administration of oxytocin to Montane voles does not produce partner preference. This lack of effect is thought to result in part from a significantly different pattern of oxytocin receptor expression in the CNS of Montane voles compared to Prairie voles [22]. Consistent with this hypothesis, overexpression of the oxytocin receptor in the nucleus accumbens of female prairie voles facilitates partner preference formation [23].

The oxytocin system has also been shown to influence the formation of social memory in laboratory animals and humans. Infusion of an oxytocin agonist into the lateral ventricles enhances social recognition in rats and this response is blocked by an oxytocin antagonist [24]. Oxytocin KO mice fail to develop social memory on both the habituation and social recognition tests [25] and oxytocin receptor KO mice show a similar deficit in the social recognition test [26]. In a recent series of studies, intranasal administration of oxytocin to human subjects has been shown to increase recognition memory for faces but not non-social stimuli [27].

Oxytocin also appears to have a dampening effect on the stress response by direct and indirect modulation of the HPA axis and decreases the release of stress hormone in rodents [28] and humans [29]. Oxytocin and oxytocin agonists are active in a number of preclinical tests for anxiolytic activity including the open field, elevated zero maze, four-plate test and stress-induced hyperthermia and these responses are blocked by oxytocin antagonists [30,31]. Intranasal oxytocin reduces activation of the amygdala in response to fearful or threatening scenes in humans [32] and blunts the social stress of public speaking [33]. Oxytocin may also be involved in the regulation of affect in humans since

lower plasma levels have been reported in patients with major depression [34]. Consistent with this result is the observation that oxytocin agonists are active in preclinical tests of antidepressant activity such as the tail suspension test [31].

It has been reported that schizophrenic patients exhibit a lowered oxytocin level in their cerebrospinal fluid and that treatment with the antipsychotic clozapine increases plasma oxytocin [35]. In rodents, oxytocin agonists restore prepulse inhibition that is disrupted by NMDA antagonists or dopamine agonists [31,36]. Recently, intranasal administration of oxytocin for 3 weeks to schizophrenic patients who were already receiving antipsychotic treatment was shown to reduce both positive and negative symptom scores [37], suggesting that oxytocin may be useful as an adjunctive treatment in this disorder.

The oxytocin system has attracted considerable attention based on both preclinical and clinical studies as a potential therapeutic target for autism spectrum disorders. Marked reductions in the circulating level of oxytocin have been reported in autistic children [38] and several single nucleotide polymorphisms in the oxytocin and oxytocin receptor genes have been linked to this disorder [39]. Moreover, administration of oxytocin to autistic patients has been reported to improve speech comprehension, improve social recognition [40] and reduce repetitive behaviors [41]. Combined with the positive effects that oxytocin has on affiliative behaviors described above, oxytocin agonists should receive serious consideration for the treatment of this complex and debilitating disorder.

6. Neuropeptide Y

Neuropeptide Y (NPY) is a highly conserved 36 amino acid peptide that is one of the most abundant peptides in the brain and involved in numerous physiological processes such as food intake, cognition, seizure activity, learning, stress sensitivity and mood. High levels of NPY are found in the locus coeruleus, hypothalamus, amygdala, hippocampus, nucleus accumbens and cortex [42] and it co-localizes with norepinephrine, GABA, somatostatin and agouti-related peptide [43]. It is also abundantly expressed in the periphery and the primary source of circulating NPY is the adrenal medulla. Seven G-protein coupled receptors for NPY have been identified and Y1, Y2, Y4 and Y5 are most abundant in the brain and have discrete patterns of expression. The Y2 receptor can act as a presynaptic autoreceptor and blockade of this site increases the release of NPY. There is extensive preclinical data indicating that NPY plays an important role in the response to stress and expression of anxiety. In rodents, NPY is expressed and released following exposure to stressful stimuli and attenuates many of the behavioral consequences of stress [44]. ICV administration of NPY increases time spent in the open arm in the elevated plus maze [45]. It produces anxiolytic-like activity in the Geller–Seifter and Vogel conflict tests that are particularly sensitive to the effects of benzodiazepines [45]. It has also been shown to be active in the light–dark box, open field and the fear potentiated startle assays [46]. These anxiolytic effects appear to be primarily mediated by stimulation of Y1 receptors since some of these responses are blocked by selective Y1 antagonists [47]. Moreover, NPY may function as an endogenous anxiolytic since administration of a selective Y1 antagonist has been shown to produce anxiogenic responses. The Y5 receptor may also play a role in the anxiolytic effects of NPY since Y5 receptors have been shown to mediate the anxiolytic effect produced by NPY 3–36 when it is administered directly into the amygdala.

There is also considerable preclinical data supporting a role for NPY in depression. ICV administration of NPY produces an antidepressant-like effect in the learned helplessness paradigm as well as in mouse and rat forced swim tests [48]. All of these responses can be blocked by selective Y1 antagonists suggesting that they are mediated by Y1 receptors. An antidepressant-like increase in swimming time has also been observed in the Flinders Sensitive rat line, a genetic model of depression. NPY also exhibits antidepressant-like inhibition of hyperactivity in olfactory bulbectomized rats, a surgical model of depression [49]. Chronic treatment with traditional antidepressants such as desipramine and citalopram, repeated exposure to electroconvulsive shock and chronic administration of lithium all have been shown to increase NPY levels in the brain [50].

There are several published reports that the level of NPY is decreased in the plasma or CSF in patients suffering from major depression but these findings are not universal. However, a positive correlation has been observed between chronic treatment of depressed patients with citalopram and increased levels of NPY in CSF [51]. Significant increases in NPY have also been detected in the CSF of refractory depressed patients who received electroconvulsive therapy [52]. The therapeutic potential of NPY or agonist analogs has not been evaluated in humans due to the lack of brain penetrant peptides. It is important to note that NPY is a potent appetite enhancer and a peptide agonist developed for the treatment of anxiety or depression would need the correct balance of selectivity at the NPY receptor subtypes to minimize undesirable side effects.

7. Amylin

Amylin is a 37-amino acid peptide hormone that is co-secreted with insulin from pancreatic β cells and whose primary metabolic role is to reduce the rate of glucose entry into the bloodstream. It has been shown to decrease food intake, slow the rate of gastric emptying and reduce postprandial release of glucagon [53]. Its effects are thought to be mediated by interaction with the calcitonin receptor, a GPCR, which partners with individual receptor-modifying proteins or RAMPs to confer different specificity and functionality. These amylin-specific receptors are expressed in the nucleus accumbens, dorsal raphe nucleus and the area postrema. While amylin is not thought to cross the blood brain barrier, activation of a dense population of receptors in the area postrema, which is outside the blood brain barrier has been shown to activate a well defined neuronal pathway in the CNS. This pathway involves reciprocal projections between the area postrema, the nucleus of the solitary tract, and the parabrachial nucleus, which in turn projects to the central nucleus of the amygdala. Focal lesions and measurement of cfos activation have been used to show that this pathway is stimulated sequentially to mediate the anorexigenic effects of amylin [54]. Preclinical studies suggest that activation of this pathway may also have therapeutic potential for the treatment of psychiatric disorders that involve stress. For example, long term stress has been shown to trigger an increase in palatable feeding and peripheral administration of amylin has been shown to inhibit stress-induced sucrose consumption [53]. Amylin treatment has also been shown to reduce stress-induced activation of c-fos in the CNS. More recently, peripheral administration of amylin has been shown to be active in several behavioral tests for antidepressant or anxiolytic activity, increasing marble burying, increasing the number of crossings in the 4-plate test, reducing immobility in the forced swim test and inhibiting the hyperthermic response to restraint stress [53]. The last response was blocked by lesioning the area postrema, providing support for the key role of this nucleus. Although the data supporting the therapeutic potential of amylin for psychiatric disorders is clearly preliminary, it is intriguing because of the comorbidity of psychiatric and metabolic disorders and it does not require penetration of the blood brain barrier. Moreover, pramlintide, an analog of amylin with superior drug-like properties, is already on the market for the treatment of diabetes.

8. Increasing stability and duration of action

One of the major challenges historically associated with the development of peptide therapeutics is their limited stability and short duration of action due to their susceptibility to serum and tissue proteases in vivo and their rapid clearance from the circulation. However, several new approaches designed to address these issues have shown great promise. In particular, targeted modification of the peptide by altering its length and replacing L-amino acids with unnatural D-amino acids can significantly enhance enzyme resistance. For example, the half-life of the somatostatin analog octreotide was increased by an order of magnitude by reducing it to 8 AA and substituting D-amino acids. For larger peptides, it is sometimes possible to identify the cleavage sites that are vulnerable to common proteolytic enzymes in the molecule and make targeted substitutions with unnatural amino acids to reduce or eliminate degradation. The substitution of D-amino acids at position 6 and 10 of several luteinizing hormone releasing hormone analogs resulted in increases in biological activity as well as plasma half-life in the marketed peptides triptorelin, leuprolide and buserelin [55].

Another approach to prolonging plasma stability is terminal modification by either N-acetylation or C-amidation. For example, N-pyroglutamylation of GlP-17–36 led to significant improvement in enzymatic stability [56]. Similarly, covalent attachment of fatty acids or PEG can protect a peptide from enzymatic degradation. The C-terminal PEGylation of glucose-dependent insulinotropic polypeptide (GIP1–30) conferred a high level of dipeptidyl peptidase resistance while maintaining full biological activity [57]. Head to tail cyclization of peptides can also be used to prevent degradation by exopeptidases. Clearly, alteration of the shape of the molecule can lead to a loss of activity, but Marastoni et al. [58] demonstrated that head to tail cyclic analogs of peptide T remained bioactive and were highly resistant to degradation.

In general, small peptides (<5 kDa) that are not bound to plasma proteins are rapidly excreted via filtration through the kidneys whereas peptides with molecular mass larger than 50 kDa are not excreted by this route. Consequently, the plasma residence time of small peptides can be significantly prolonged by increasing their size with the addition of larger molecules like PEG or polysialic acids. PEG possesses many pharmaceutically desirable properties including high water solubility, high mobility in solution, lack of toxicity, lack of immunogenicity and ready clearance from the body. Depending on the size of the peptide, some of these properties, such as reduced immunogenicity are transferred to the PEGylated peptide. In addition, PEGylation may also improve stability by protecting vulnerable termini from endopeptidases and provide steric hindrance to proteolytic enzymes. There are several PEGylated peptides currently on the market, including PEG-intron, a PEGylated interferon for the treatment of hepatitis C that has an elimination half-life of 50 h compared to the 5 h half-life of native α-interferon. The PEGylated peptide erythropoietin agonist Hematide has been reported to be well tolerated and maintain efficacy with administration every 4 weeks [59]. Amgen has recently described a "peptibody" approach where the peptide is fused to the human antibody Fc domain to increase stability and has completed successful Ph III clinical trials with a thrombopoietin agonist AMG 531 that uses this approach [60].

Prolongation of plasma half-life without chemical modification can also be accomplished by utilizing sustained delivery systems. Liposomes are widely used as drug carriers and incorporation of streptokinase in liposomes produces a 16-fold increase in plasma half-life [61]. In another study, levels of insulin-like growth factor (IGF-1) administered in lisosomes were sustained for 5–7 days compared with less than 1 day for the free form [62].

9. Advances in delivery technology

Most peptide and protein therapeutics are currently administered subcutaneously using injection pens that use fine gauge needles and are minimally invasive. While these devices continue to improve in terms of convenience and minimal pain production, they do not address the stability and rapid clearance that can undermine the therapeutic potential of peptides. However, considerable progress has been made on delivery technologies that are specifically designed to prolong the efficacy of biological based therapeutics. In general, these technologies are designed to control the release of the drug into the circulation and enable a long lasting and relatively constant level of exposure, thus avoiding bursts of exposure that are likely to produce unwanted side effects and prolong the effect of a single administration of the drug.

Encapsulation of drugs in liposomes to prolong their biological activity has been one of the more common approaches and Depofoam represent one of the most successful examples of this technology. Depofoam is an aqueous suspension of multivesicular liposomes each of which is a honeycomb-like structure of non-concentric aqueous chambers surrounded by a lipid membrane barrier. Drugs are gradually released from the aqueous chambers through reorganization of the lipids in vivo and breakdown of the particles. The rate of release is controlled primarily by the composition of the lipid layer. For example, long chain triglycerides prolong the duration of release relative to shorter triglycerides. Release rates range from days to weeks. The system is biodegradable and biocompatible and is one of the best tolerated sustained release injectable formulations available. The particles are small and can be easily injected through a fine gauge needle. Depofoam-encapsulated interferon alpha-2b increased therapeutic plasma levels from 1 day to 1 week [63].

Another evolving approach involves the production of injectable microspheres that are composed of a biodegradable polymer that is similar to the materials used in dissolvable sutures, a solvent and formulated peptide particles. This depot preparation is injected subcutaneously or intramuscularly. Early stage drug release is predominantly diffusion out of the system while at later stages polymer degradation controls the release of the drug. The use of microspheres to deliver the LHRH agonist leuprolide was shown to produce stable and therapeutic levels of drug for over 3 months in preclinical studies [64].

Transdermal delivery can represent an attractive alternative to hypodermic injection and even oral delivery, however, only a limited number of drugs are amenable to standard transdermal delivery due to restrictions of molecular size, hydrophilicity and potency. Many of these restrictions have recently been overcome by the introduction of microneedle technology to transdermal patch formulation. Microneedles painlessly pierce the skin and increase skin permeability by creating micron-scale pathways for peptides and proteins from an extended-release patch. Proteins such as parathyroid hormone and vaccines against influenza and hepatitis B have been successfully delivered using microneedle patches [65].

10. Increasing brain penetration

One of the major remaining hurdles in the development of peptide therapeutics for CNS disorders is overcoming the blood brain barrier and delivering them to their site of action. This impermeability primarily results from tight junctions formed between capillary endothelial cells that are formed by cell adhesion molecules. These cells express high levels of efflux transport proteins, such as p-glycoprotein and possess few alternative transport pathways. This problem is not unique to peptides as it is estimated that 98% of therapeutic molecules, both

large and small, do not penetrate the BBB [66]. Consequently, considerable effort has been focused on approaches to overcome this barrier and significant progress has been made in understanding how the small and large endogenous molecules, such as glucose and insulin, required for normal brain function cross the BBB.

Endogenous BBB transporters are grouped into three categories: carrier mediated transport, active efflux transport and receptor-mediated transport [66]. Of these, receptor mediated transport is considered to be the most promising mechanism for delivering peptides to the brain. These transporters use a process called transcytosis to deliver large essential molecules such as insulin and transferrin to the brain. Receptor-mediated transport involves the following steps: endocytosis of the compound at the luminal side of the epithelium followed by movement through the cytoplasm and finally exocytosis of the compound to the basolateral (brain) side of the epithelium. Several receptor-mediated transporters have been identified including those for insulin, transferrin, insulin-like growth factor, leptin and Fc fragments [66]. By coupling a peptide to a specific ligand or antibody for these transporters, it is possible to utilize them to carry the peptide across the BBB.

In addition to endogenous ligands, it is possible to make monoclonal antibodies that undergo receptor mediated transport across the BBB using endogenous transporters. The antibody binds to an exposed epitope that is spatially removed from the binding site of the endogenous ligand and this binding allows the antibody to "piggy back" across the BBB via the endogenous receptor transporter system. Such antibodies have been developed to facilitate brain penetration in several species including antibodies to the mouse and rat transferrin transporter and antibodies to the primate insulin transporter [67,68]. For example, conjugation of vasoactive intestinal peptide to an antibody to the transferrin transporter using avidin-biotin technology produced a significant increase in cerebral blood flow [69]. Similarly, conjugation of the growth factor BDNF to this antibody provided complete neuroprotection to pyramidal cells in the CA1 region of the hippocampus in rats subject to transient forebrain ischemia [70]. Other peptides and proteins that have been successfully transported across the BBB using this approach include: FGF-2, EGF, beta amyloid and beta-galactosidase [71]. Alternatively, genetic engineering can be used to create a fusion protein. In this case, the peptide is fused to the carboxyl or amino terminus of either the heavy or light chain of the transporter directed monoclonal antibody. This approach has been successfully used to deliver neurotrophin to the brain using an antibody directed against the insulin transporter [72].

Larger quantities of peptides can potentially be delivered to the brain by using specialized liposomes. In this case, the peptide is encapsulated in the liposome, which is conjugated with strands of polyethylene glycol. The tips of a small subset of the strands of polyethylene glycol are conjugated with the transporter monoclonal antibody. This approach has been used successfully to deliver cDNAs to rodent brain with transferrin antibodies and primate brain with insulin antibodies [73,74].

A new family of peptides derived from proteins that efficiently cross the BBB using the low density lipoprotein related receptor has been designed to facilitate delivery of therapeutics to the brain [75]. Angiopeps are a family of 19 amino acid peptides derived from the kunitz domain of aprotinin that demonstrate a high transcytosis rate [75] using LRP-1 and are applicable to small and large molecules ranging is size from 500 D to 150 kD. The most advanced molecule using this technology is ANG1005, an engineered peptide formed by conjugation of an angiopep with three molecules of paclitaxel. This molecule exhibits 10–100-fold greater uptake into rat brain than paclitaxel alone and is in Phase I clinical trials for the treatment of brain cancers.

11. Summary

Despite tremendous commercial success and decades of progress in the development of drugs for the treatment of major psychiatric disorders, they still have major therapeutic limitations and a multitude of side effects and there is a significant need for safer and more efficacious medications, The therapeutic limitations include inadequate control of the negative symptoms of schizophrenia, treatment resistance of a significant proportion of patients suffering from depression, delayed onset of action of many drugs for the treatment of depression and anxiety and the abuse liability of the benzodiazepine class of anxiolytics. In the case of autism, the need for effective treatments is even more pressing, since there are no drugs approved to treat most of the core symptoms of the disorder and the population of affected individuals continues to grow at a rapid pace. The clinical and preclinical data supporting the therapeutic potential of agonists at several of the peptide receptors described in this review continues to increase and these targets clearly merit a thorough evaluation in the clinic. Given the difficulty that the pharmaceutical industry has had improving upon the current generation of drugs for psychiatric disorders and the technical advances that have occurred in the discovery and development of peptide therapeutics, it might be an opportune time to revisit the therapeutic potential of some of these peptides. For example, development of a brain penetrant version of neurotensin would permit an evaluation of its therapeutic potential in the clinic and synthesis of selective peptide agonists for the NT1 and NT2 receptors could lead to a more specific and safer medication for the treatment of schizophrenia. Likewise, synthesis of a brain penetrant version of a selective peptide agonist for the CCK1 receptor would enable a re-evaluation of therapeutic potential of this target for schizophrenia. In the case of NPY, synthesis of brain penetrant versions of peptides selective for Y1, Y5 or Y1 and Y5 receptors would allow an assessment of these targets for the treatment of anxiety disorders. Nasal administration of ocytocin has been shown to have clinically beneficial effects in several limited clinical studies and formulation of a more brain penetrant and stable version would enable an evaluation of efficacy in more comprehensive clinical trials. However, small molecule oxytocin agonists have recently been described in the literature and depending on their progress the need for a peptide agonist may not be as pressing. In the case of amylin, there is an FDA approved version of this peptide that is currently on the market for the treatment of diabetes that could potentially be used to carry out clinical proof of concept studies for psychiatric disorders such as anxiety and depression. Continued progress in overcoming the technical limitations traditionally associated with peptide therapeutics provides some real hope that the promise of these targets will eventually be realized.

References

[1] Caceda R, Kinkead B, Nemeroff CB. Neurotensin: role in psychiatric and neurological diseases. Peptides 2006;27:2385–404.
[2] Uhl GR. Distribution of neurotensin and its receptor in the central nervous system. Ann N Y Acad Sci 1982;400:132–49.
[3] Fassio A, Evans G, Grisshammer R, Bolam JP, Mimmack M, Emson PC. Distribution of the neurotensin receptor in the rat CNS studied using an amino-terminal directed antibody. Neuropharmacology 2000;39:1430–42.
[4] Binder EB, Kinkead B, Owens MJ, Nemeroff CB. The role of neurotensin in the pathophysiology of schizophrenia and the mechanism of action of antipsychotic drugs. Biol Psychiatry 2001;50:856–72.
[5] Breslin NA, Suddath RL, Bissette G, Nemeroff CB, Lowrimore P, Weinberger DR. CSF concentrations of neurotensin in schizophrenia: an investigation of clinical and biochemical correlates. Schizophr Res 1994;12:35–41.
[6] Hokfelt T, Morino P, Verge V, Castel MN, Broberger C, Zhang X, et al. CCK in cerebral cortex and at the spinal level. Ann N Y Acad Sci 1994;15:7–163.
[7] Lindefors N, Lindén A, Brené S, Sedvall G, Persson H. CCK peptides and mRNA in the human brain. Prog Neurobiol 1993;40:671–90.

[8] Savasta M, Ruberte E, Palacios JM, Mengod G. The colocalization of cholecystokinin and tyrosine hydroxylase mRNAs in mesencephalic dopaminergic neurons in the rat brain examined by in situ hybridization. Neuroscience 1989;29:363–9.
[9] Crawley JN, Corwin RL. Biological actions of cholecystokinin. Peptides 1994;15:731–55.
[10] Cohen SL, Knight M, Tamminga CA, Chase TN. Cholecystokinin-octapeptide effects on conditioned-avoidance behavior, stereotypy and catalepsy. Eur J Pharmacol 1982;83:213–22.
[11] Zetler G. Neuroleptic-like effects of ceruletide and cholecystokinin octapeptide: interactions with apomorphine, methyphenidate and picrotoxin. Eur J Pharmacol 1983;94:261–70.
[12] Schneider LH, Alpert JE, Iversen SD. CCK-8 modulation of mesolimbic dopamine: antagonism of amphetamine-stimulated behaviors. Peptides 1983;4:749–53.
[13] Vaccarino FJ, Rankin J. Nucleus accumbens cholecystokinin (CCK) can either attenuate or potentiate amphetamine-induced locomotor activity: evidence for rostral–caudal differences in accumbens CCK function. Behav Neurosci 1989;103:831–6.
[14] Crawley JN. Subtype-selective cholecystokinin receptor antagonists block cholecystokinin modulation of dopamine-mediated behaviors in the rat mesolimbic pathway. J Neurosci 1992;12:3380–91.
[15] Feifel D, Reza T, Robeck S. Antipsychotic potential of CCK-based treatments. Neuropsychopharmacology 1999;20:141–9.
[16] Tachikawa H, Harada S, Kawanishi Y, Okubo T, Suzuki T. Linkded polymorphisms ($-33G > T$ and $-286A > G$) in the promoter region of the CCK-A receptor gene may be associated with schizophrenia. Psychiatry Res 2001;103:147–55.
[17] Nair NPV, Lal S, Bloom DM. Cholecystokinin peptides, dopamine and schizophrenia—a review. Prog Neuropsychopharmacol Biol Psychiatry 1985;9:515–24.
[18] Montgomery SA, Green MC. The use of cholecystokinin in schizophrenia. Psychol Med 1988;18:593–603.
[19] Loup F, Tribollet E, Dubois-Dauphin M, Dreifuss JJ. Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. Brain Res 1991;555:220–32.
[20] Pedersen CA, Boccia ML. Oxytocin antagonism alters rat dams' oral grooming and upright posturing over pups. Physiol Behav 2003;80:233–41.
[21] Young LJ, Wang Z. The neurobiology of pair bonding. Nat Neurosci 2004;7:1048–54.
[22] Shapiro LE, Insel TR. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Ann NY Acad Sci 1992;652:448–51.
[23] Ross HE, Freeman SM, Spiegel LL, Ren X, Terwilliger EF, Young LJ. Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. J Neurosci 2009;29:1312–8.
[24] Benelli A, Bertolini A, Poggioli R, Menozzi B, Basaglia R, Arletti R. Polymodal dose-response curve for oxytocin in the social recognition test. Neuropeptides 1995;28:251–5.
[25] Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci 2001;21:8278–85.
[26] Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, et al. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc Natl Acad Sci USA 2005;102:16096–101.
[27] Rimmele U, Hediger K, Heinrichs M, Klaver P. Oxytocin makes a face in memory familiar. J Neurosci 2009;29:38–42.
[28] Stachowiak A, Macchi C, Nussdorfer GG, Malendowicz LK. Effect of oxytocin on the function and morphology of the rat adrenal cortex: in vitro and in vivo investigations. Res Exp Med (Berl) 1995;195:265–74.
[29] Legros JJ. Inhibitory effect of oxytocin on corticotrope function in humans: are vasopressin and oxytocin ying-yang neurohormones? Psychoneuroendocrinology 2001;26:6499–555.
[30] Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, Luo B, et al. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology 2006;185:218–25.
[31] Ring RH, Schechter LE, Leonard SK, Dwyer JM, Platt BJ, Graf R, et al. Receptor and behavioral pharmacology of WAY-267464, a non-peptide oxytocin receptor agonist. Neuropharmacology 2010;58:69–77.
[32] Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 2005;25:11489–93.
[33] Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 2003;54:1389–98.
[34] Scantamburlo G, Hansenne M, Fuchs S, Pitchot W, Marechal P, Pequex C, et al. Plasma oxytocin level and anxiety in patients with major depression. Psychoneuroendocrinology 2007;32:407–10.
[35] Uvnas-Moberg K, Alster P, Svensson TH. Amperozide and clozapine but not haloperidol or raclopride increase the secretion of oxytocin in rats. Psychopharmacology 1992;109:473–6.
[36] Feifel D, Reza T. Oxytocin modulates psychotomimetic-induced deficits in sensorimotor gating. Psychopharmacology 1999;141:93–8.
[37] Feifel D, Macdonald K, Nguyen A, Cobb P, Warlan H, Galangue B, et al. Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. Biol Psychiatry 2010;68:678–80.
[38] Modahl C, Green L, Fein D, Morris M, Waterhouse L, Feinstein C, et al. Plasma oxytocin levels in autistic children. Biol Psychiatry 1998;43:270–7.
[39] Yrigollen CM, Han SS, Kochetkova A, Babitz T, Chang JT, Volkmar FR, et al. Genes controlling affiliative behavior as candidate genes for autism. Biol Psychiatry 2008;63:911–6.
[40] Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L, et al. Oxytocin increases retention of social cognition in autism. Biol Psychiatry 2007;61:498–503.
[41] Hollander E, Novotny S, Hanratty M, Yaffe R, DeCaria CM, Aronowitz BR, et al. Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. Neuropsychopharmacology 2003;28:193–8.
[42] Chronwall BM, Dimaggio DA, Massari VJ, Pickel VM, Ruggerio DA, O'Donohue TL. The anatomy of neuropeptide Y-containing neurons in the rat brain. Neurscience 1985;115:9–1181.
[43] Kask A, Harro J, von Horsten S, Redrobe JP, Dumont Y, Quirion R. The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci Biobehav 2002;26:259–83.
[44] Heilig M, Thorsell A. Brain neuropeptide Y (NPY) in stress and alchohol dependence. Rev Neurosci 2002;13:85–94.
[45] Heilig M, Soderpalm B, Engel JA, Widerlov E. Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology 1989;98:524–9.
[46] Karlsson RM, Holmes A, Heilig M, Crawley JN. Anxiolytic-like actions of centrally-administered neuropeptide Y, but not galanin, in C57BL/6J mice. Pharmacol Biochem Behav 2005;80:427–36.
[47] Sajdyk TJ, Vandergriff MG, Gehlert DR. Amygdalar neuropeptide Y Y-1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur J Pharmacol 1999;368:143–7.
[48] Redrobe JP, Dumont Y, Fournier A, Quirion R. The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 2002;26:615–24.
[49] Goyal SN, Upadhya MA, Kokare DM, Bhisikar SM, Subhedar NK. Neuropeptide Y modulates the antidepressant activity of imipramine in olfactory bulbectomized rats: involvement of NPY Y1 receptors. Brain Res 2009;1266:45–53.
[50] Husum H, Mikkelsen JD, Hogg S, Mathé AA, Mørk A. Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. Neuropharmacology 2000;39:1463–73.
[51] Nikisch G, Agren H, Eap CB, Czernik A, Baumann P, Mathe AA. Neuropeptide Y and corticotrophin-releasing hormone in CSF mark response to antidepressive treatment with citalopram. Int J Neuropsychopharmacol 2005;8:403–10.
[52] Nikisch G, Mathe AA. CSF monoamine metabolites and neuropeptides in depressed patients before and after electroconvulsive therapy. Eur Psychiatry 2008;23:356–9.
[53] Roth JD, Maier H, Chen S, Roland BL. Implications of amylin receptor agonism. Arch Neurol 2009;66:306–10.
[54] Lutz TA. The role of amylin in the control of energy homeostasis. Am J Physiol Regul Integr Comp Physiol 2010;298:R1475–84.
[55] Werli M, Bernkop-Schnurch A. Strategies to improve plasma half-life time of peptide and protein drugs. Amino Acids 2006;30:351–67.
[56] Green BD, Mooney MH, Gault VA, Irwin N, Bailey CJ, Harriott PBG, et al. N-terminal His(7)-modification of glucagon-like peptide-1(7–36) amide generates dipeptidyl peptidase IV-stable analogues with potent antihyperglycaemic activity. J Endocrinol 2004;180:379–88.
[57] Salhanick AI, Clairmont KB, Buckholz TM, Pellegrino CM, Ha S, Lumb KJ. Contribution of site-specific PEGylation to the dipeptidyl peptidase IV stability of glucose-dependent insulinotropic polypeptide. Bioorg Med Chem Lett 2005;15:4114–7.
[58] Marastoni M, Salvadori S, Scaranari V, Spisani S, Reali E, Traniello S, et al. Synthesis and activity of new linear and cyclic peptide T derivatives. Arzneimittelforschung 1994;44:1073–6.
[59] Macdougall IC, Rossert J, Casadevall N, Stead RB, Duliege AM, Froissart M, et al. A peptide-based erythropoietin-receptor agonist for pure red-cell aplasia. N Engl J Med 2009;361:1848–55.
[60] Kuter DJ, Bussel JB, Lyons RM, Pullarkat V, Gernsheimer TB, Senecal FM, et al. Efficacy of romiplostim in patients with chronic immunethrombocytopenic purpura: a double blind randomized controlled trial. Lancet 2008;371:362–4.
[61] Kim IS, Choi HG, Choi HS, Kim BK, Kim CK. Prolonged systemic delivery of streptokinase using liposome. Arch Pharm Res 1998;21:248–52.
[62] Katre NV, Asherman J, Schaefer H, Hora M. Multivesicular liposome (DepoFoam) technology for the sustained delivery of insulin-like growth factor-I (IGF-I). J Pharm Sci 1998;87:1341–6.
[63] Willis R, Lipid Foams. Lipid foams: formulation development and characterization of depointerferon alpha. In: Proceedings of the Fine Particle Society Meeting in San Diego; 2006.
[64] Burton KW, Shameem M, Thanoo BC, DeLuca PP. Extended release peptide delivery systems through the use of PLGA microsphere combinations. J Biomater Sci Polym Ed 2000;11:715–29.
[65] Prausnitz MR, Mikszta JA, Cormier M, Andrianov AK. Microneedle-based vaccines. Curr Top Microbiol Immunol 2009;333:369–93.
[66] Pardridge WM. Blood–brain barrier delivery. Drug Discov Today 2007;12:54–61.
[67] Lee HJ, Engelhardt B, Lesley J, Bickel U, Pardridge WM. Targeting rat antimouse transferrin receptor monoclonal antibodies through blood–brain barrier in mouse. J Pharmacol Exp Ther 2000;292:1048–52.

[68] Pardridge WM, Kang YS, Buciak JL, Yang J. Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries in vitro and rapid transcytosis through the blood–brain barrier in vivo in the primate. Pharm Res 1995;12:807–16.
[69] Wu D, Pardridge WM. Central nervous system pharmacologic effect in conscious rats after intravenous injection of a biotinylated vasoactive intestinal peptide analog coupled to a blood–brain barrier drug delivery system. J Pharmacol Exp Ther 1996;279:77–83.
[70] Wu D, Pardridge WM. Neuroprotection with noninvasive neurotrophin delivery to the brain. Proc Natl Acad Sci USA 1999;96:254–9.
[71] Kurihara A, Pardridge WM. Imaging brain tumors by targeting peptide radiopharmaceuticals through the blood–brain barrier. Cancer Res 1999;59:6159–63.
[72] Boado RJ, Zhang Y, Pardridge WM. Genetic engineering, expression, and activity of a fusion protein of a human neurotrophin and a molecular Trojan horse for delivery across the human blood–brain barrier. Biotechnol Bioeng 2007;97:1376–86.
[73] Y. Zhang Y, Schlachetzki Y, Pardridge WM. Global non-viral gene transfer to the primate brain following intravenous administration. Mol Ther 2003;7:11–8.
[74] Shi N, Zhang Y, Zhu C, Boado RJ, Pardridge WM. Brain-specific expression of an exogenous gene after i.v. administration. Proc Natl Acad Sci USA 2001;98:12754–9.
[75] Demeule M, Currie JC, Bertrand Y, Che C, Nguyen T, Regina A, et al. Involvement of the low-density lipoprotein receptor-related protein in the transcytosis of the brain delivery vector Angiopep-2. J Neurochem 2008;106:1534–44.