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Barriers to remember: brain-targeting chemical delivery systems and Alzheimer's disease

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Brain-targeted chemical delivery systems (CDSs) represent rational drug design attempts not only to deliver but also to target drugs to their site of action. Using a sequential metabolism approach, the special bidirectional properties of the blood–brain barrier can be exploited to smuggle the precursors of therapeutic compounds across the barrier and lock them inside the brain ready for sustained release of the active drugs. Many potential therapeutic applications can be envisioned for such CDSs; here, the potential of brain-targeted estradiol for the prevention and treatment of Alzheimer's disease is reviewed in detail.

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▼ The brain is a delicate organ with many vital functions, and many formidable mechanisms isolate and protect it from the outside world. Unfortunately, the same mechanisms that prevent intrusive environmental chemicals accessing the brain also prevent the access of therapeutic chemicals. Therefore, many pharmaceuticals are ineffective in treating cerebral diseases because they cannot be efficiently delivered to, or sustained within, the brain.

The blood–brain barrier

It is now well established that the brain is tightly segregated from the circulating blood by a unique membranous barrier, the blood–brain barrier (BBB) [1,2]. Contrary to most capillaries, which have small pores that allow the rapid movement of solutes from the circulation into the corresponding organs, capillaries of the vertebrate brain and spinal cord are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight junctions. These endothelial cells, together with perivascular elements such as astrocytes and pericytes, constitute the BBB. Because of this barrier, exchange must take place transcellularly whereas, in other organs, such exchange is overshadowed by other nonspecific transport mechanisms. Therefore, only molecules that can freely diffuse through this capillary endothelial membrane can passively cross the BBB, and this ability is closely related to their lipid solubility (lipophilicity/hydrophobicity). Not surprisingly, the permeability coefficient of brain capillaries correlates well with the octanol–water partition coefficient, the most commonly used measure of lipophilicity and one of the most informative physicochemical parameters used in medicinal chemistry [3,4]. Practically all drugs currently used to treat brain disorders are lipid-soluble and can readily cross the BBB following oral administration.

The BBB also has an additional, enzymatic aspect: solutes crossing the endothelial cell membrane are subsequently exposed to numerous degrading enzymes within these cells. These cells also contain many mitochondria – metabolically active organelles – and active transport can significantly alter both inward and outward transport for compounds that are substrates of the corresponding transporters. Overall, the BBB is highly efficient and makes the brain practically inaccessible to lipid-insoluble compounds. Brain-delivery of such compounds, therefore, requires a strategy to overcome the BBB. Delivery of compounds such as neuropeptides or oligonucleotides is further complicated by their metabolic lability.

Simple attempts, such as transient osmotic opening of the BBB or biodegradable implants, are seriously limited by their invasive nature and their numerous toxic side effects. Some more recent attempts, such as those based on

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cell-penetrating peptides [5,6] or brain transport vectors [7], focus on exploiting natural transporters. These might provide solutions, but there are still many unresolved issues. Another possible strategy is to smuggle compounds across the BBB in disguise as lipophilic precursors. Heroin, a diacyl derivative of morphine, is a notorious example that crosses the BBB ~100 times more easily than its parent drug just by being more lipophilic. Along similar lines, prodrug approaches can be used to improve the brain uptake of drugs, but simple prodrugs suffer from important limitations such as nonselectivity in target-site delivery.

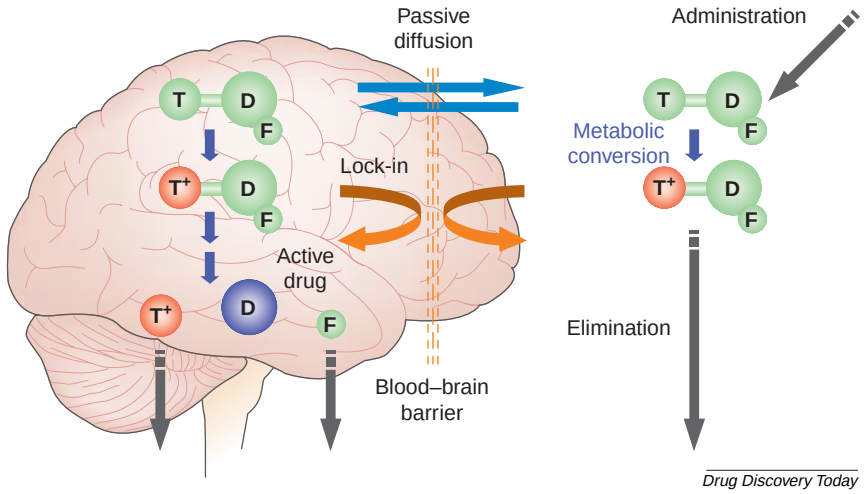

Figure 1. The sequential metabolism used by brain-targeting chemical delivery systems (CDSs) to allow targeted and sustained delivery of the active drug D. An inactive, lipophilic precursor, T-DF (where F represents the modifier, and T represents the targetor moiety), is administered that can passively diffuse through the blood–brain barrier (BBB). Oxidation of T leads to a charged, hydrophilic intermediate (T^+-DF) that can no longer cross-back through the BBB ('lock-in'), but is easily eliminated from the peripheral circulation. Adequately timed enzymatic release of D can provide sustained and brain-specific activity.

Chemical delivery systems

Brain-targeted chemical delivery systems (CDSs) represent a rational drug design approach that exploits sequential metabolism not only to deliver but also to target drugs to their site of action [4,8,9]. By localizing drugs at their desired site of action, one can reduce toxicity and increase treatment efficiency. The CDS concept evolved from the prodrug concept in the early 1980s, but was differentiated by the introduction of targetor moieties and the use of multistep activation [9]. The cunning aspect of these brain-targeted systems is that, in addition to providing access by increasing the lipophilicity, they exploit the specific bidirectional properties of the BBB to 'lock' inactive drug precursors in the brain on arrival, preventing exit back across the BBB (Fig. 1). CDSs are inactive chemical derivatives of a drug, being obtained by one or more chemical modifications. The newly attached moieties are monomolecular units (generally comparable in size to the original molecule) that provide a site-specific or site-enhanced delivery of the drug through multistep enzymatic and/or chemical transformations.

The introduced bioremovable moieties can be classified into two categories. A targetor (T) moiety is responsible for targeting, site-specificity and lock-in; whereas modifier functions (F_1...F_n) serve as lipophilizers, protect certain functions or fine-tune the necessary molecular properties to prevent premature, unwanted, metabolic conversions. Depending on the structure of the drug to be delivered, use of modifier functions (F) might not be required. The CDS is designed to undergo sequential metabolic conversions, disengaging the modifier function(s) and finally the targetor, after the moiety has fulfilled its site- or organ-targeting role (Fig. 1).

Brain-targeting CDSs exploit the fact that if a lipophilic compound enters the brain and is then converted into a lipophobic molecule, it will no longer be able to exit: it will be 'locked-in'. In principle, many targetor moieties are possible for a general system of this kind, but the one based on the 1,4-dihydrotrigonelline ↔ trigonelline (coffearine) system [where the lipophilic 1,4-dihydro form (T) is converted *in vivo* to the hydrophilic quaternary form (T^+)] proved the most useful (structures are shown in Fig. 2). This conversion takes place easily everywhere in the body because it is closely related to that of the ubiquitous NAD(P)H ↔ $NAD(P)^+$ coenzyme system. Because oxidation occurs with direct hydride transfer and without generating highly active or reactive radical intermediates, it provides a non-toxic targetor system [10].

Although the charged intermediate T^+-D consisting of the quaternary targetor (T^+) and drug (D) complex is locked behind the BBB in the brain, it is easily eliminated from the body because of the acquired positive charge, which enhances water solubility. After a relatively short time, the delivered drug D is present only in the brain (as the inactive, locked-in T^+-D), providing sustained and brain-specific release of the active drug. It has to be emphasized that the system not only achieves delivery to the brain, but also provides preferential delivery; that is, brain targeting. Furthermore, CDSs can be used not only to deliver compounds that otherwise have no access to the brain, but also to retain lipophilic compounds within the brain, as has

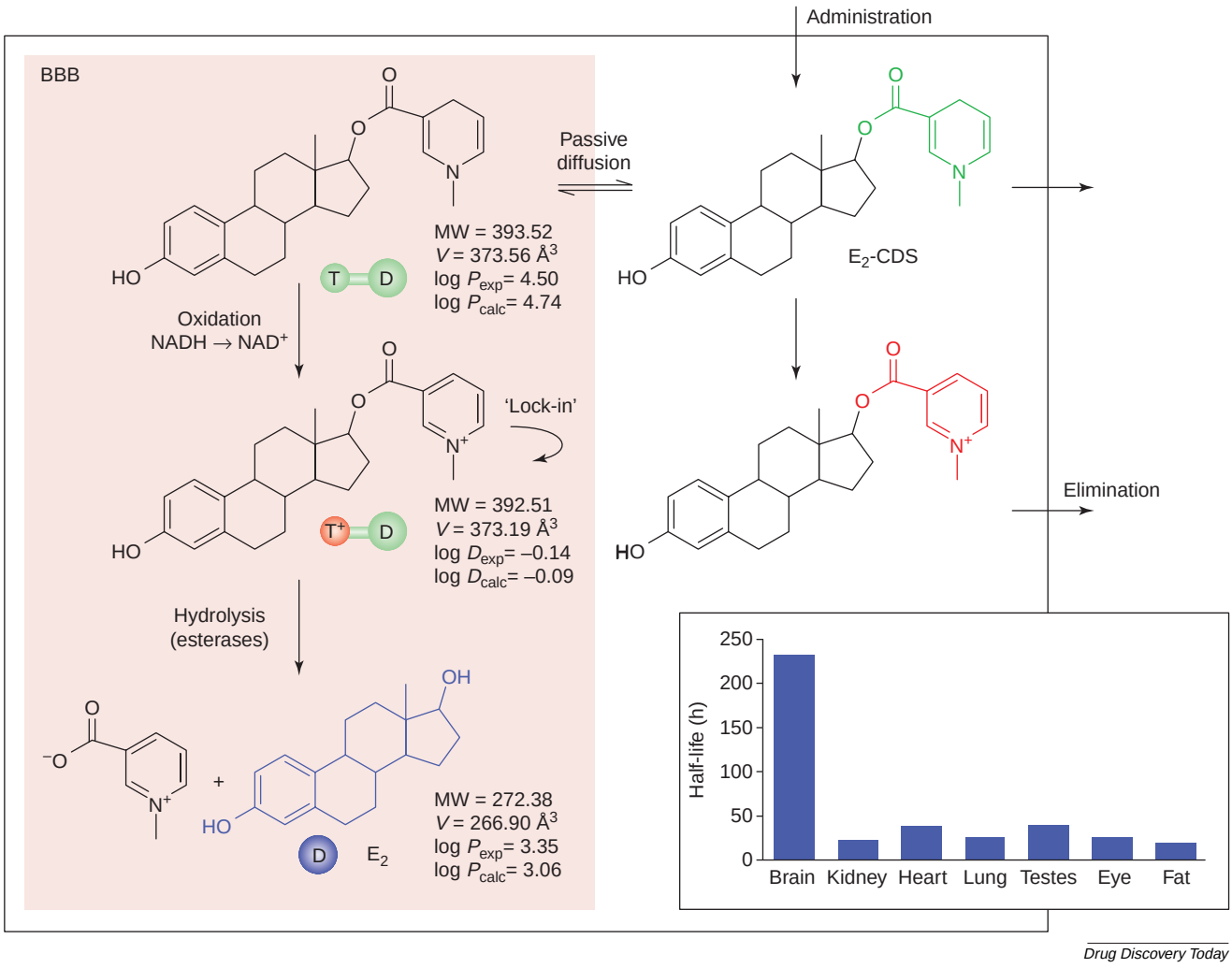

Figure 2. The 'lock-in' mechanism of E_2-CDS provided by the introduction of a targetor moiety that exploits a 1,4-dihydrotrigonelline (green) ↔ trigonelline (red)-type conversion. Experimental and calculated logarithms of the octanol–water partition (log *P*) and distribution (log *D*) coefficients are shown to illustrate the significant changes in partition properties that occur during the sequential metabolism. The inset shows elimination half-lives in various tissues for the T^+–E_2 formed after intravenous administration of E_2-CDS in rats. Because of the 'lock-in' mechanism, elimination from the brain is considerably slower than elimination from other organs. Abbreviations: BBB, blood–brain barrier; CDS, chemical delivery system; D, active drug [in this particular case, estradiol (E_2)]; T, targetor moiety; T-D, the original, neutral targetor-drug complex that forms the CDS; T^+-D, charged intermediate, E_2-CDS in this particular case.

been achieved, for example, with a variety of steroid hormones. During the past decade, the system has been explored with a wide variety of drug classes, and considerably increased brain exposure as well as brain targeting (i.e. brain vs systemic exposure) have been obtained in several cases; for example, 3′-azido-3′-deoxythymidine (AZT)-CDS, ganciclovir-CDS and benzylpenicillin-CDS [4]. For example, area under the curve (AUC) data for the active drug, which is the pharmacokinetically most relevant measurement of drug exposure, show that AZT-CDS administration in rats simultaneously increases brain exposure 32-fold and decreases blood exposure threefold as compared with AZT administration. Among all CDSs, the estradiol chemical delivery system (E_2-CDS) is in the most advanced investigation stage. Following earlier clinical trials (Phase I and II), a new buccal formulation, which is a tablet that should be dissolved in the cheek area of the mouth and not swallowed, has recently completed Phase I and II investigation using circulating luteinizing hormone (LH) as a biomarker.

Estrogens and Alzheimer's disease

In a somewhat ironic twist, estrogen, one of the female hormones often blamed in the past for causing 'raging hormonal imbalances', might have cognitive functions [11]. Estrogen seems to be one of the essential substances for the

maintenance of limbic brain function that regulates memory, emotion, orientation in time and space, motivation, and other cognitive functions. Estrogen might also help delay the process of Alzheimer-type dementia in postmenopausal women.

Alzheimer's disease (AD), which affects ~15 million people worldwide and still has no specific cure, results in progressively worsening symptoms that range from memory loss to declining cognitive ability [12]. It is the leading cause of persistent dementia in late life. Survival for a decade is common, and the prevalence of AD increases from ~3% at the age of 65 years to ~47% at the age of 85 years [12]. AD is second to cancer as the most expensive disease in the USA, with an estimated yearly cost of around US$100 billion if both direct and indirect medical costs are included.

There are approximately twice as many women as men with this disorder and although women, on average, live longer than men, the prevalence rates of AD in women seem to remain higher than in men, even after adjusting for differences in age distribution [13,14]. During the course of illness, women also tend to show greater impairments in naming tasks and other measures of semantic memory [14]. This might be a consequence of the abrupt decline of estrogen production in postmenopausal women. In men, estrogen is maintained at a much more constant level because it is mainly produced by aromatizing testosterone, and testosterone production never ceases, only gradually declines with age. Compared with age-matched women who do not use estrogen, healthy elderly men have significantly higher plasma E_2 levels, and this might have some protective effect on explicit memory [15]. A recent magnetic resonance imaging (MRI)-based study found that the hippocampus (a brain structure involved in memory, which has been shown to shrink in AD patients) was larger in postmenopausal women who were taking estrogen replacement therapy (ERT) than in either postmenopausal women who were not taking estrogen or in a group of elderly men (http://news.ucdmc.ucdavis.edu/estrogen_alzheimers.html).

The connection between sex hormone levels and cognitive functions in men and women is a somewhat controversial area. Testosterone levels, in addition to their well-known connection to aggressive behavior, were positively correlated with visuo-spatial ability and negatively correlated with verbal fluency, both in European men and in Namibian bushmen [16]; however, this might not be a simple, linear relationship. By contrast, there is fairly consistent evidence that estrogen maintains and/or improves verbal memory in postmenopausal women [17]. Furthermore, estrogen seems to have the same effect even in more specialized populations such as, for example, male-to-female transsexuals [18], young girls with Turner syndrome [19], and women with uterine myomas treated with leuprolide acetate depot and showing symptoms of hypoestrogenism [20]. Estrogen is also probably involved in improving the sense of smell. Women's superiority in olfactory capacity arises during puberty, and their sense of smell is keenest around ovulation when estrogen levels are high [21]. Memory and smell sensation are closely linked, and there are indications that AD originates with a decline in olfactory capacity [21].

All these notwithstanding, estrogen produces many effects that might delay the onset of AD, prevent AD or improve the quality of life in AD [22]. Brain areas known to support memory were found to have high densities of estrogen receptors. Estrogen promotes the growth and survival of cholinergic neurons, and it also modulates serotonergic and catecholaminergic neurotransmission. It increases cerebral blood flow and has antioxidant, anti-inflammatory and general neuroprotective activity. The role of estrogen action in the brain seems to extend beyond the confines of sexual differentiation and reproductive neuroendocrine function, and estrogen might act as a neural growth factor, having important influences on the development, survival, plasticity, regeneration and aging of the mammalian brain [11,22,23]. The estrogen receptor might be not only a ligand-induced transcriptional enhancer, but also a mediator of rapid, nongenomic events, such as actions on excitability of neuronal and pituitary cells and modulating of G-protein coupling [22]. Furthermore, the antioxidant and neuroprotective effects of estradiol could involve other receptors and might be independent from estrogenic properties [24].

Several recent studies found that estrogen decreased cerebral amyloid deposition and protected against β-amyloid (Aβ)-induced cell death not only in cell cultures [25,26], but also in animal models [27–29]. In guinea pigs, prolonged ovariectomy increased brain Aβ levels on average by 1.5-fold, and E_2 administration significantly reversed this increase [27]. Because an imbalance in Aβ production and clearance is suspected to result in a cascade of events that ultimately lead to neuronal dysfunction and dementia (the amyloid cascade hypothesis of AD) [30], estrogen is expected to have disease-modifying effects in AD.

The actual findings on the effect of estrogen in AD are somewhat conflicting [31]. There have been several small- and large-scale studies suggesting beneficial effects of estrogen in AD [32–40] (Fig. 3) and, to some extent, even in Parkinson's disease [41] or on mental performance in general [17,18]. There is also evidence that women with AD receiving estrogen-replacement therapy (ERT) respond

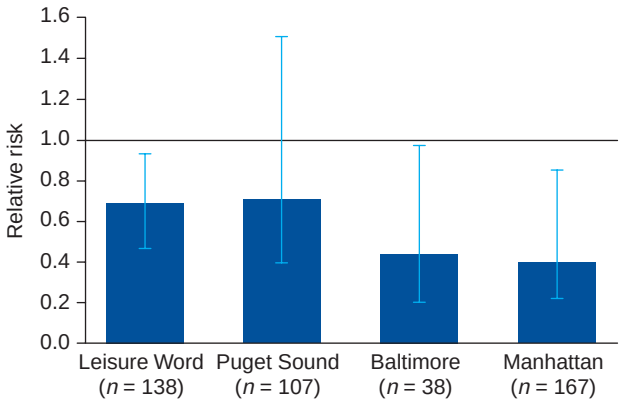

Figure 3. Relative risk-estimates for Alzheimer's disease (AD) associated with postmenopausal use of estrogen-replacement therapy (ERT). Relative risk indicates the odds of getting AD in the ERT group versus the corresponding control group. Therefore, relative risks less than one in all four study groups (shown along the *x*-axis) consistently indicate reduced odds of developing AD in the ERT group. The *n* value for each study group indicates the number of cases of AD, and the Puget Sound study only shows data for oral estrogen. The graphic was prepared using data from four large epidemiological studies [14], and error bars represent the 95% confidence limits.

better to cholinesterase therapy, currently the only FDA-approved therapeutic class for patients with AD, than those not receiving ERT [42]. This confirms the interaction of estrogens with cholinergic mechanisms, which are key to attention processes, learning and memory – domains particularly stricken by AD.

By contrast, a recent large-scale study (Alzheimer's Disease Cooperative Study [13]) found that one year of ERT did not slow disease progression or improve global, cognitive or functional outcomes in women who had already developed mild to moderate AD. However, the study did not address possible preventive effects and was conducted with oral doses of 0.625 or 1.250 mg day^{-1}, which are too low to generate enough estradiol in the brain to affect cognitive processes. Whereas high-dose estradiol (0.10 mg day^{-1} skin patches resulting in plasma levels >100 pg ml^{-1}) improved cognition for women with AD in a randomized clinical study [43], mean serum E_2 levels in all the groups participating in the large-scale trial were considerably below this level. Mean serum levels were 23, 48 and 58 pg ml^{-1} in the placebo, low-dosage E_2 and high-dosage E_2 treatment groups, respectively. These values are at the low end of normal, pre-menopausal levels (which are cycle-dependent and fluctuate over the range of 20–200 pg ml^{-1}) and are close to what are usually considered post-menopausal levels, <40 pg ml^{-1}. It is usually estimated that E_2 levels of at least 40, 70 and 100 pg ml^{-1} are required for osteoporosis prevention, cardiovascular prevention and cognitive enhancement, respectively.

Estrogens are lipophilic endogenous steroids; hence, they can readily penetrate the BBB after peripheral administration. However, they are poorly retained within the brain. Therefore, to maintain therapeutically significant concentrations, frequent or sustained doses have to be administered. Constant peripheral exposure to estrogens has been related to several pathological conditions including cancer, hypertension and altered metabolism [34,44,45]. For example, estrogen is suspected to lead to a 30% increase in the risk of breast cancer, rising to 50% if it is taken for >10 years. Recent results from the Heart and Estrogen/Progestin Replacement Study (HERS) suggest that hormone-replacement therapy (HRT) does not affect the incidence of coronary heart disease one way or the other [46,47]; therefore, there is now less justification for systemic HRT. As the average woman in industrialized nations spends approximately one-third of her life in the post-menopausal stage (menopause occurs at an average age of 51), whether or not to take HRT is an important decision. As the CNS is the target site for many estrogenic actions [48,49], brain-targeted delivery might provide safer and more effective treatment in many cases. With the recent unraveling of the many roles estrogen plays in males [50], therapeutic applications are not only not restricted to females; indeed, there might be more therapeutic possibilities in males than in females.

E_2-CDS

E_2 is the most potent naturally occurring human estrogen. A brain-targeted CDS can be obtained easily by attaching the targetor moiety at the 17-hydroxy function (Fig. 2). This is an especially convenient position to obtain an inactive CDS construct because 17-esters do not interact with estrogen receptors. The resulting E_2-CDS compound has been thoroughly investigated in a variety of animal models.

As a confirmation of the lock-in mechanism, a study in rats found that E_2 released from the quaternary intermediate that is formed after intravenous E_2-CDS administration has an elimination half-life of >200 h (Fig. 2), and brain E_2 levels are elevated 4–5 times longer than after simple E_2 treatment [51]. As a proof of effective targeting, another study found that steroid levels 1–16 days after E_2-CDS treatment were >12-fold higher in brain samples than in plasma samples [52]. A single intravenous administration to ovariectomized rats induced prolonged (3–6 weeks) pharmacological effects as measured by LH suppression, reduced rate of weight gain or, in castrated male rats, re-establishment of copulatory behavior [53]. This last measurement reflects one of the more interesting effects of

brain-targeted estradiol, and is a consequence of the fact that, in many mammals, various aspects of sexual behavior are closely dependent on the local production or presence of estrogens within the brain. Because these effects usually lasted longer than those produced by equimolar estradiol valerate (which is isolipophilic with E_2-CDS), they prove that E_2-CDS is more than a simple prodrug and that it can achieve effective brain-targeting and sustained release. In contrast to E_2-pellet implants, weekly injections of E_2-CDS could significantly attenuate the rise in tail-skin temperature that is associated with administration of naloxone to morphine-dependent, ovariectomized rats – an animal model for menopausal hot flush [54]. E_2-CDS also provided promising neuroprotective effects [55,56].

Phase I and II clinical evaluations in postmenopausal volunteers suggested a potent central effect with only marginal elevations in systemic estrogen levels [57]. For example, a comparison of AUCs for LH suppression and plasma E_2 levels for E_2-CDS and oral Progynova® indicates that, for a certain blood level of E_2, E_2-CDS is significantly more active than Progynova® for both buccal and intravenous administration. A comparison of equivalent doses of intravenous E_2-CDS and E_2 indicates that although they both exert similar effects on LH suppression (70% less suppression), E_2-CDS elevates plasma E_2 levels to only a small fraction (4.4%) of that produced by E_2 administration. Therefore, E_2-CDS holds particular promise as a useful and safe therapy not only for estrogen-dependent cognitive deficits (including AD) but also for menopausal symptoms and possibly neuroprotection.

Molecular packaging: brain delivery of neuropeptides

Neuropeptides, peptides that act on the brain or spinal cord, represent the largest class of transmitter substance and have considerable therapeutic potential for many CNS diseases [58]. The number of identified endogenous peptides is already staggering and is growing continuously. However, delivery of peptides through the BBB is even more challenging than delivery of other drugs, because peptides tend to be rapidly inactivated by the ubiquitous peptidases. Therefore, for a successful delivery, three issues have to be solved simultaneously: enhance passive transport by increasing the lipophilicity, ensure enzymatic stability to prevent premature degradation, and exploit the lock-in mechanism to provide targeting.

Our solution was to extend the CDS approach into a so-called molecular packaging strategy. The peptide unit that has to be delivered is disguised as part of a bulky molecule dominated by lipophilic modifying groups that direct BBB penetration and prevent recognition by peptidases. Successful brain deliveries have already been achieved using this strategy for a Leu-enkephalin analog [59], thyrotropin-releasing hormone (TRH) analogs [60–63] and kyotorphin analogs [64].

Here, we will briefly focus on the delivery of TRH analogs because these compounds can increase extracellular acetylcholine levels, accelerate acetylcholine turnover, improve memory and learning, and reverse the reduction in high affinity choline uptake that is induced by lesions of the medial septal cholineric neurons [65]. Therefore, these peptides are potential agents for treating neurodegenerative disorders and, in particular, AD [66,67]. Although circulating thyroid hormone levels are unaltered by aging and no clear link between thyroid hormones and AD has, as yet, been established, thyroid dysfunction seems to represent a risk factor. Neurological symptoms in hypothyroid patients resemble, at least in part, those in AD. There is also evidence that thyroid hormones, in particular T_3, negatively regulate expression of the β-amyloid precursor protein gene [68]. Even if study results are somewhat inconsistent, they usually suggest an association between hypothyroidism and AD [69–71]. Interestingly, a recent prospective study found that subclinical hyperthyroidism (reduced TSH level) in the elderly considerably increased the risk of dementia and AD [72]. The authors of the study offered as a possible explanation, that this is caused by the reduced levels of TRH in the brain [72]. There is good reason, therefore, to believe that brain-targeted delivery of TRH is of potential therapeutic value in AD.

In our molecular package, the histidine residue was replaced with leucine in the TRH analogs used, so as to dissociate CNS effects from thyrotropin-releasing activity [73]. Correct timing of the sequential metabolic steps is of crucial significance for successful delivery and lock-in with any CDS. It is of particular significance for TRH delivery because the corresponding process might require up to five or six consecutive metabolic steps [60–63] (Fig. 4). Therefore, selection of a suitable spacer moiety, which is inserted between the targetor and peptide units to ensure correct timing for targetor release, proved important for the efficacy of TRH-CDSs. This was measured by the decrease in barbiturate-induced sleeping time in mice, an interesting and well-documented effect of this neuropeptide. Pharmacological effects were determined for the peptide itself (Leu^2-TRH), for various intermediates and building blocks, and for the CDS itself. Whereas the peptide and the various intermediates reduced the sleeping time only slightly (17 ± 7% versus vehicle), the CDS with the best spacers (e.g. proline–proline and proline–alanine) reduced it significantly (e.g. 56 ± 7% versus vehicle), thereby

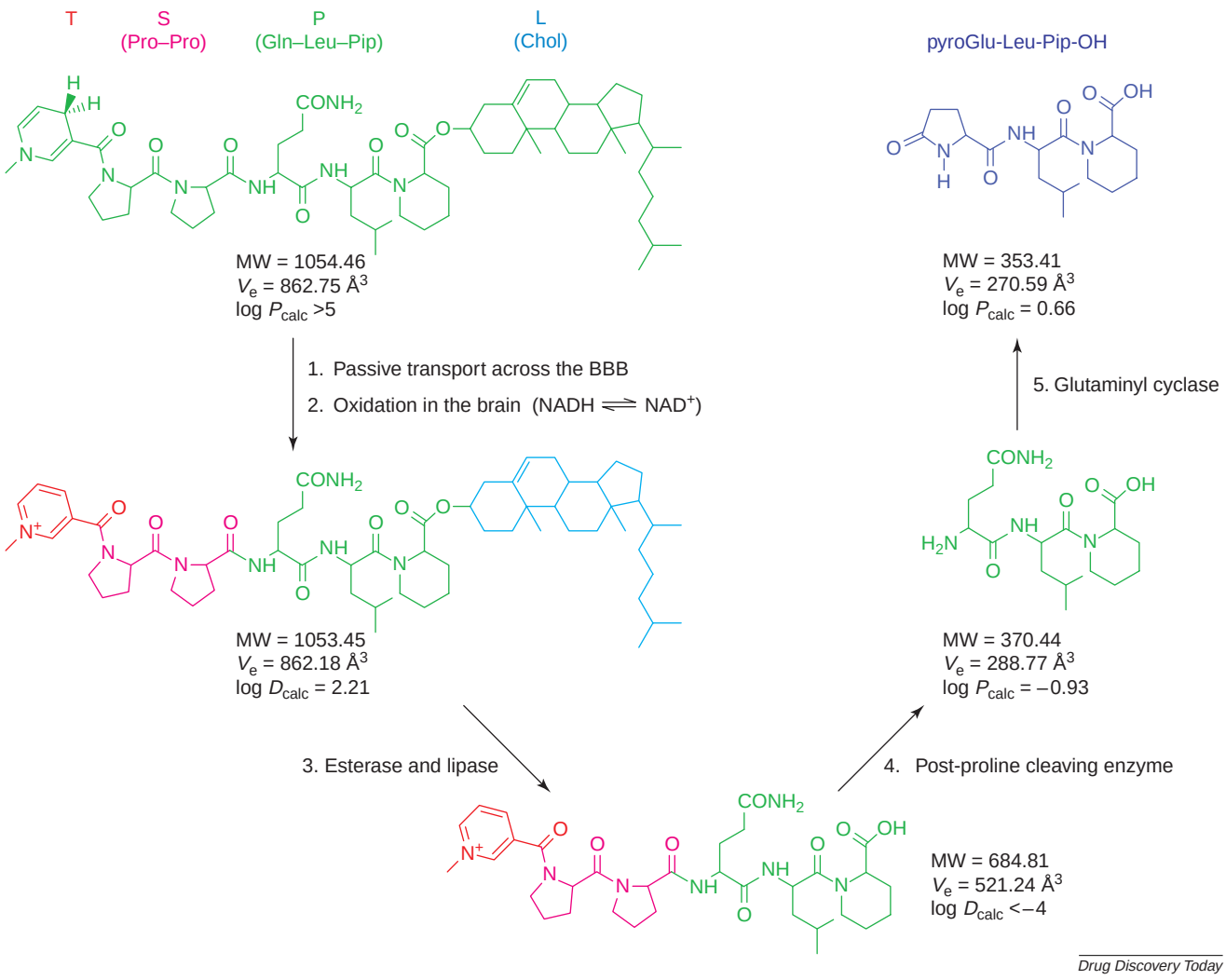

Figure 4. Sequential metabolism of a CDS for a TRH analog with a pipecolic acid terminal. In addition to the peptide (P) to be delivered, the overall package includes a targetor (T), a spacer (S) and a lipophilic (L) moiety. The original molecular package is lipophilic enough to cross the blood–brain barrier (BBB; step 1). Oxidation of the targetor moiety (step 2) and enzymatic cleavage (step 3) of the lipophilic cholesterol moiety, which provides the required lipophilicity and serves to disguise the peptide nature of the package, results in intermediates that can no longer cross back through the BBB (lock-in). Removal of the targetor-spacer complex by post-proline cleaving enzyme (step 4) and cyclization of glutamine by glutaminyl cyclase (step 5) results in formation of the final active molecule. Abbreviations: CDS, chemical delivery system; TRH, thyrotropin-releasing hormone.

confirming successful delivery [62]. Treatment with a TRH-CDS also significantly improved memory-related behavior in a passive avoidance paradigm in rats bearing bilateral fimbrial lesions, without altering thyroid function [61].

Conclusion

Chemical delivery systems can provide noninvasive brain-targeted delivery and sustained release for a variety of neuropharmaceuticals, including neuropeptides. Because the corresponding design principles are general, systematic and sufficiently flexible, they can be applied to almost any lead compound. Substantially increased and prolonged brain exposure has been demonstrated in several cases, and E_2-CDS holds particular promise for several applications, including the treatment of AD and menopausal hot flushes.

References

1 Goldstein, G.W. and Betz, A.L. (1986) The blood–brain barrier. *Sci. Am.* 255, 74–83
2 Begley, D.J. (1996) The blood–brain barrier: principles for targeting peptides and drugs to the central nervous system. *J. Pharm. Pharmacol.* 48, 136–146
3 Buchwald, P. and Bodor, N. (1998) Octanol–water partition: searching for predictive models. *Curr. Med. Chem.* 5, 353–380

4 Bodor, N. and Buchwald, P. (1999) Recent advances in the brain targeting of neuropharmaceuticals by chemical delivery systems. *Adv. Drug Deliv. Rev.* 36, 229–254

5 Lindgren, M. *et al.* (2000) Cell-penetrating peptides. *Trends Pharmacol. Sci.* 21, 99–103

6 Rousselle, C. *et al.* (2000) New advances in the transport of doxorubicin through the blood–brain barrier by a peptide vector-mediated strategy. *Mol. Pharmacol.* 57, 679–686

7 Pardridge, W.M. (2002) Drug and gene targeting to the brain with molecular Trojan Horses. *Nat. Rev. Drug Discov.* 1, 131–139

8 Bodor, N. and Brewster, M.E. (1982) Problems of delivery of drugs to the brain. *Pharmacol. Ther.* 19, 337–386

9 Bodor, N. and Brewster, M.E. (1991) Chemical delivery systems. In *Targeted Drug Delivery* (Juliano, R.L., ed.), pp. 231–284, Springer, Berlin

10 Brewster, M.E. *et al.* (1988) A dihydropyridine conjugate which generates high and sustained levels of the corresponding pyridinium salt in the brain does not exhibit neurotoxicity in cynomolgus monkeys. *Neurosci. Lett.* 87, 277–282

11 Wickelgren, I. (1997) Estrogen stakes claim to cognition. *Science* 276, 675–678

12 Mayeux, R. and Sano, M. (1999) Treatment of Alzheimer's disease. *N. Engl. J. Med.* 341, 1670–1679

13 Mulnard, R.A. *et al.* (2000) Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. *J. Am. Med. Assoc.* 283, 1007–1015

14 Henderson, V.W. (1997) The epidemiology of estrogen replacement therapy and Alzheimer's disease. *Neurology* 48, S27–S35

15 Carlson, L.E. and Sherwin, B.B. (2000) Higher levels of plasma estradiol and testosterone in healthy elderly men compared with age-matched women may protect aspects of explicit memory. *Menopause* 7, 168–177

16 Christiansen, K. (1993) Sex hormone-related variations of cognitive performance in !Kung San hunter-gatherers of Namibia. *Neuropsychobiology* 27, 97–107

17 Sherwin, B.B. (1999) Can estrogen keep you smart? Evidence from clinical studies. *J. Psychiatry Neurosci.* 24, 315–321

18 Miles, C. *et al.* (1998) Estrogen and memory in a transsexual population. *Horm. Behav.* 34, 199–208

19 Ross, J.L. *et al.* (2000) Use of estrogen in young girls with Turner syndrome: effects on memory. *Neurology* 54, 164–170

20 Sherwin, B.B. and Tulandi, T. (1996) 'Add-back' estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *J. Clin. Endocrinol. Metab.* 81, 2545–2549

21 Vroon, P. *et al.* (1997) *Smell: The Secret Seducer*, Farrar, Straus and Giroux, New York

22 McEwen, B.S. (2001) Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. *J. Appl. Physiol.* 91, 2785–2801

23 Toran-Allerand, C.D. *et al.* (1999) Novel mechanisms of estrogen action in the brain: new players in an old story. *Front. Neuroendocrinol.* 20, 97–121

24 Behl, C. and Manthey, D. (2000) Neuroprotective activities of estrogen: an update. *J. Neurocytol.* 29, 351–358

25 Hosoda, T. *et al.* (2001) Estrogen protects neuronal cells from amyloid β-induced apoptotic cell death. *Neuroreport* 12, 1965–1970

26 Zhang, L. *et al.* (2001) Estrogen protects against β-amyloid-induced neurotoxicity in rat hippocampal neurons by activation of Akt. *Neuroreport* 12, 1919–1923

27 Petanceska, S.S. *et al.* (2000) Ovariectomy and 17β-estradiol modulate the levels of Alzheimer's amyloid β peptides in brain. *Neurology* 54, 2212–2217

28 Smith, J.D. and Levin, J.A. (2002) Estrogen and ApoE: drug discovery implications. In *Drug Discovery and Development for Alzheimer's Disease 2000* (Fillit, H.M. and O'Connell, A.W., eds), pp. 129–136, Springer, New York

29 Zheng, H. *et al.* (2002) Modulation of Aβ peptides by estrogen in mouse models. *J. Neurochem.* 80, 191–196

30 Scorer, C.A. (2001) Preclinical and clinical challenges in the development of disease-modifying therapies for Alzheimer's disease. *Drug Discov. Today* 6, 1207–1219

31 Haskell, S.G. *et al.* (1997) The effect of estrogen replacement therapy on cognitive function in women: a critical review of the literature. *J. Clin. Epidemiol.* 50, 1249–1264

32 García-Segura, L.M. *et al.* (1994) Gonadal hormones as promoters of structural synaptic plasticity: cellular mechanisms. *Prog. Neurobiol.* 44, 279–307

33 Ohkura, T. *et al.* (1994) Evaluation of estrogen-treatment in female-patients with dementia of the Alzheimer-type. *Endocr. J.* 41, 361–371

34 Lobo, R.A. (1995) Benefits and risks of estrogen replacement therapy. *Am. J. Obstet. Gynecol.* 173, 982–989

35 Tang, M-X. *et al.* (1996) Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* 348, 429–432

36 Kawas, C. *et al.* (1997) A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology* 48, 1517–1521

37 Jacobs, D.M. *et al.* (1998) Cognitive function in nondemented older women who took estrogen after menopause. *Neurology* 50, 368–373

38 Yaffe, K. *et al.* (1998) Estrogen therapy in postmenopausal women – effects on cognitive function and dementia. *J. Am. Med. Assoc.* 279, 688–695

39 Asthana, S. *et al.* (1999) Cognitive and neuroendocrine response to transdermal estrogen in postmenopausal women with Alzheimer's disease: results of a placebo-controlled, double-blind, pilot study. *Psychoneuroendocrinology* 24, 657–677

40 Costa, M.M. *et al.* (1999) Estrogen replacement therapy and cognitive decline in memory-impaired post-menopausal women. *Biol. Psychiatry* 46, 182–188

41 Marder, K. *et al.* (1998) Postmenopausal estrogen use and Parkinson's disease with and without dementia. *Neurology* 50, 1141–1143

42 Schneider, L.S. *et al.* (1996) Effects of estrogen replacement therapy on response to tacrine in patients with Alzheimer's disease. *Neurology* 46, 1580–1584

43 Asthana, S. *et al.* (2001) High-dose estradiol improves cognition for women with AD: results of a randomized study. *Neurology* 57, 605–612

44 Yager, J.D. and Liehr, J.G. (1996) Molecular mechanism of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 36, 203–232

45 Beral, V. *et al.* (1999) Use of HRT and the subsequent risk of cancer. *J. Epidemiol. Biostat.* 4, 191–210

46 Wells, G. and Herrington, D.M. (1999) The heart and estrogen/progestin replacement study: what have we learned and what questions remain? *Drugs Aging* 15, 419–422

47 Meade, T.W. and Vickers, M.R. (1999) HRT and cardiovascular disease. *J. Epidemiol. Biostat.* 4, 165–190

48 McEwen, B.S. and Alves, S.E. (1999) Estrogen actions in the central nervous system. *Endocr. Rev.* 20, 279–307

49 Cyr, M. *et al.* (2000) Drugs with estrogen-like potency and brain activity: potential therapeutic application for the CNS. *Curr. Pharm. Des.* 6, 1287–1312

50 Sharpe, R.M. (1998) The roles of oestrogen in the male. *Trends Endocrinol. Metabol.* 9, 371–377

51 Mullersman, G. *et al.* (1988) High performance liquid chromatographic assay of a central nervous system (CNS)-directed estradiol chemical delivery system and its application after intravenous administration in rats. *Pharm. Res.* 5, 172–177

52 Sarkar, D.K. *et al.* (1989) Chronic inhibition of hypothalamic–pituitary–ovarian axis and body weight gain by brain-directed delivery of estradiol-17β in female rats. *Neuroendocrinology* 50, 204–210

53 Anderson, W.R. *et al.* (1987) Evidence for the reestablishment of copulatory behavior in castrated male rats with a brain-enhanced estradiol-chemical delivery system. *Pharmacol. Biochem. Behav.* 27, 265–271

54 Rahimy, M.H. *et al.* (1991) Effects of a brain-enhanced estrogen delivery system on tail-skin temperature of the rat: implications for menopausal hot flush. *Maturitas* 13, 51–63

55 Simpkins, J.W. *et al.* (1997) Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J. Neurosurg.* 87, 724–730

56 Rabbani, O. *et al.* (1997) 17β-estradiol attenuates fimbrial lesion-induced decline of ChAT-immunoreactive neurons in the rat medial septum. *Exp. Neurol.* 146, 179–186

57 Estes, K.S. *et al.* (1994) Evaluation of an estradiol chemical delivery system (CDS) designed to provide enhanced and sustained hormone levels in the brain. *Adv. Drug Deliv. Rev.* 14, 167–175

58 Bodor, N. and Buchwald, P. (1998) All in the mind. *Chem. Br.* 34, 36–40

59 Bodor, N. *et al.* (1992) A strategy for delivering peptides into the central nervous system by sequential metabolism. *Science* 257, 1698–1700

60 Prokai, L. *et al.* (1994) Chemical delivery system to transport a pyroglutamyl peptide amide to the central nervous system. *J. Am. Chem. Soc.* 116, 2643–2644

61 Prokai, L. *et al.* (1998) Synthesis and behavioral evaluation of a chemical brain-targeting system for a thyrotropin-releasing hormone analogue. *Eur. J. Med. Chem.* 33, 879–886

62 Prokai, L. *et al.* (1999) Metabolism-based brain-targeting system for a thyrotropin-releasing hormone analogue. *J. Med. Chem.* 42, 4563–4571

63 Yoon, S-H. *et al.* (2000) Brain-targeted chemical delivery of [Leu^2, Pip^3]-TRH: synthesis and biological evaluation. *Bioorg. Med. Chem.* 8, 1059–1063

64 Chen, P. *et al.* (1998) Strategies to target kyotorphin analogues to the brain. *J. Med. Chem.* 41, 3773–3781

65 Itoh, Y. *et al.* (1994) Effect of NS-3, a thyrotropin-releasing hormone analog, on *in vivo* acetylcholine-release in rat-brain – regional differences and its sites of action. *J. Pharmacol. Exp. Ther.* 271, 884–890

66 Kelly, J.A. (1995) Thyrotropin-releasing hormone: basis and potential for its therapeutic use. *Essays Biochem.* 30, 133–149

67 Bennett, G.W. *et al.* (1997) Effect of neuropeptides on cognitive function. *Exp. Gerontol.* 32, 451–469

68 Belandia, B. *et al.* (1998) Thyroid hormone negatively regulates the transcriptional activity of the β-amyloid precursor protein gene. *J. Biol. Chem.* 273, 30366–30371

69 Breteler, M.M.B. *et al.* (1991) Medical history and the risk of Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int. J. Epidemiol.* 20 (Suppl. 2), S36–S42

70 Yoshimasu, F. *et al.* (1991) The association between Alzheimer's disease and thyroid disease in Rochester, Minnesota. *Neurology* 41, 1745–1747

71 Ganguli, M. *et al.* (1996) Association between dementia and elevated TSH: a community-based study. *Biol. Psychiatry* 40, 714–725

72 Kalmijn, S. *et al.* (2000) Subclinical hyperthyroidism and the risk of dementia. The Rotterdam study. *Clin. Endocrinol.* 53, 733–737

73 Szirtes, T. *et al.* (1984) Synthesis of thyrotropin-releasing hormone analogues. 1. Complete dissociation of central nervous system effects from thyrotropin-releasing activity. *J. Med. Chem.* 27, 741–745

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