Perspectives in Medicinal Chemistry

PERSPECTIVE

Low Molecular Weight Opioid Peptide Esters Could be Developed as a New Class of Analgesics

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Abstract: Low molecular weight opioid peptide esters (OPE) could become a class of analgesics with different side effect profiles than current opiates. OPE may have sufficient plasma stability to cross the blood brain barrier (BBB), undergo ester hydrolysis and produce analgesia. OPE of dipeptides, tyr-pro and tyr-gly conjugated to ethanol have a structure similar to the anesthestic agent, etomidate. Based upon the analgesic activity of dipeptide opioids, Lipinski's criteria, and permeability of select GABA esters to cross the BBB, opioid peptides (OP) conjugated to ethanol, cholesterol or 3-glucose are lead recommendations. Preliminary animal data suggests that tyr-pro-ethyl ester crosses the BBB and unexpectedly produces hyperalgesia. Currently, there are no approved OP analgesics available for clinical use. Clinical trials of good manufacturing practice OP administered to patients suffering from chronic pain with indwelling intrathecal pumps could resolve the issue that OP may be superior to opiates and may redirect research.

Keywords: opioid peptided esters, analgesics

Perspectives in Medicinal Chemistry 2011:5 19–26

doi: 10.4137/PMC.S6803

This article is available from http://www.la-press.com.

Hypothesis

It is hypothesized that an ester comprised of a low molecular weight opioid peptide (OP) and a lipophilic moiety that is non toxic to the central nervous system can be parentally absorbed and produce a plasma level sufficient to cross the blood brain barrier (BBB) (in spite of peptidases and esterases in the plasma). The OP with a lipophilic moiety, opioid peptide ester (OPE), can be hydrolyzed by spinal fluid esterase liberating an OP which produces analgesia. The side effect profile of the OPE including tolerance, addiction and opioid hyperalgesia may be distinct from present opiates due to structural differences between the two classes of compounds. The proof of this concept can be obtained by conducting a clinical trial, preceded by animal studies, in which there is intrathecal administration of OP made according to good manufacturing practice (GMP) in patients who have indwelling intrathecal pumps. Although expensive, the results of such a trial could be pivotal for redirecting OP research.

Introduction

For centuries opioids derived from the poppy plant have been the mainstay of therapy for acute pain. In the last few decades, opioid analgesics have become the primary medications for the treatment of intractable chronic pain. As our experience with this therapy increases, we have learned that in some patients the use of chronic opioid therapy has brought on a new set of problems. These include tolerance, addiction, pseudo-addiction, opioid induced hyperalgesia, bowel dysfunction, suppression of testosterone, cognitive impairment, substance abuse and diversion.[1] The most serious complication of chronic opioid therapy is the rising incidence of death from respiratory depression as a consequence of drug overdose of prescribed opioids.[2,3] Some medication specific problems include prolongation of the QT interval from methadone,[4–6] seizures from normeperidine, a metabolite of meperidine,[7,8] convulsions from morphine-3 glucoronide, a metabolite of morphine,[9] and fluctuating bioavailability from use of transdermal fentanyl.[10–12]

Opioid tolerance

The cause of opioid tolerance is not well understood. There are some patients who develop tolerance after minimal exposure, while others may become tolerant over many months and others never develop tolerance to the medication. Although tolerance to short term administration of intrathecal beta endorphin has been reported, it is not known whether tolerance will occur with long term treatment with an OP.[13] The following is support of the proposition that tolerance to OP may be different than tolerance to opiates: (1) Endogenous opioids promote mu receptor internalization through endocystosis which is lacking in narcotic analgesics.[14] Endocytosis closely regulates morphine receptor transduction and inhibits desensitization which leads to tolerance.[14] (2) In vitro studies show that the more efficient an agonist binds to the mu receptor the less likely that tolerance will occur.[15] The OP may bind more efficiently than opiates to the mu receptor because the structure of the OP is different and lacks the piperidine ring found in morphine and its derivatives.[16] (3) Similar to many hormones, OP couple to G protein receptors for activity. After the onset of clinical symptoms, tolerance to the hypersecretory effects of other endogenous hormones that occur in disease states such as Cushing's syndrome and disease, pheochromocytoma, and carcinoid syndrome is exceedingly rare, a mechanism of action that could be shared with OP.[17]

Respiratory depression

It has been shown that endomorphins 1 and 2, which are potent OP that have high affinity for the mu receptor, produce less respiratory depression than comparable doses of morphine.[18] Also OP may have a higher threshold for producing respiratory depression than opiates.[18]

Opioid hyperalgesia, addiction and bowel dysfunction

Because the creation of an OPE which can cross the BBB would be a novel class of medications it is not known and there is little data even to speculate whether chronic treatment with an OP will have adverse effects similar to opiates and whether OP induced hyperalgesia and addiction will occur.[19,20] In animal models, endomorphins inhibit gastric emptying time and decrease intestinal propulsion suggesting bowel dysfunction may occur. Therefore, it is unlikely that the use of an OPE will eliminate all the current side effects of opiate therapy.

Obstacles for development of OPE

The development of synthetic opioids, such as, fentanyl, meperidine, and methadone has improved the bioavailability, potency, and to some degree the side effects profile of analgesics. However, OP analgesics have not been developed for clinical use, even though their discovery occurred in the 1970s.[21] One of the major obstacles in the development of an OPE has been developing a stable medication that can cross the BBB.[22,23] More specifically some of the impediments can be listed:

1. Identifying lead compounds
2. Synthesis
3. Bioavailability
4. Stability from plasma peptidases and esterases
5. Crossing the BBB
6. Hydrolysis in the cerebral spinal fluid (CSF) to liberate an active opioid peptide
7. Efflux from the central nervous system (CNS)

1. Identifying Lead Compounds

Molecular size is a significant factor in determining whether a medication will cross the BBB. Medications that have a size greater than 400–600 Daltons are unlikely to cross the BBB.[24,25] Although in the past, the literature showed that tetrapeptides were the smallest active OP[26] newer literature suggests that smaller OP containing two amino acids are analgesics. There is experimental evidence that the two dipeptides, tyr-pro and tyr-gly can produce dose related analgesia that is reversed by naloxone.[27,28] The structure of tyr-pro and try-gly could assume an active conformation of an aromatic and virtual hetereocylic (piperazine like) ring which in theory may be related to analgesia.[16]

Ethanol, cholesterol and 3-glucose are possible conjugates which can be used to form the lead OPE. OPE of ethanol are particularly attractive because of the structural resemblance to etomidate which rapidly enters the CNS after intravenous administration (Fig. 1, 2). All of these compounds are "familiar" within the CNS. Although D amino acids have been substituted in many OP because of increased stability and potency (DAMGO, DALDA) these introduce a metabolite which is foreign to the central nervous system. Cholesterol esters of the neurotransmitter GABA penetrate the BBB with ease and one can expect

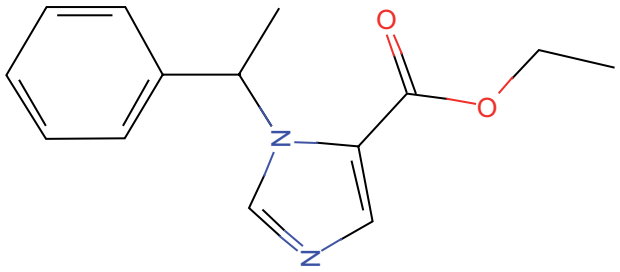

Figure 1. Etomidate.

the same with small OPE made with cholesterol.[29] Another way which can facilitate transport through the BBB would be by using the glucose transport system. An example is the 3-glucose ester of GABA that is not nearly as lipophilic as the cholesterol ester but is still able to have a high brain penetration index (BPI) because of probable transport by GLUT-1 transporter.[30] OPE of ethanol, cholesterol and 3-glucose may theoretically be reasonable lead compounds, however, the gold standard for BBB permeability still requires an in vivo bioassay.

2. Synthesis

In the literature, there are cited examples of biologically active esters that have been synthesized and these can be used as a guide for developing OPE. In the 1980s, Shashoua synthesized many GABA ester analogs through condensation reactions of the carboxylic acid of GABA and alcohol conjugates in an acidic environment.[30] It would be expected that the NH2 terminal portion of the OP would need to be protected with a t-BOC derivative that would be cleaved after ester synthesis. A more lipophilic compound would more likely be made if the OH of tyrosine were acetylated, similar to the way heroin is made more lipophilic and thus crosses the BBB more easily than morphine. However, the synthesis carries risk of breaking peptide bonds from the energy required to complete the ester condensation(s). The energy required for formation of the of ester condensation(s)

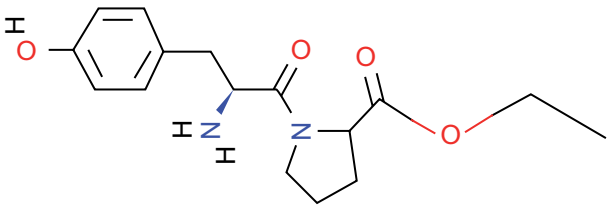

Figure 2. Tyr-pro-ethyl ester.

is lower than that for disruption of various peptide bonds as estimated by Martin,[31] but extreme care may need to be taken when heating the mixture or consider using stabilizers such as cesium salts.[32] Various OP are commercially available and fragments can be obtained using methods described in the literature. This author believes that OPE of ethanol, cholesterol and 3-glucose can be synthesized and purified to conduct biologic trials.

3. Bioavailability

Until a novel enteric coating is developed, it is unlikely that OPE can be absorbed through the gastrointestinal tract because of gastric acidity and digestive enzymes. The formulations of OPE that could be absorbed are parental through transdermal, subcutaneous, intramuscular or intravenous routes.

4. Plasma Stability

Once the OPE enters the plasma, it would be exposed to hydrolysis from plasma esterases and peptidases. The actions of these enzymes would enhance the degradation of the OPE and would be difficult to block. Endomorphin 1 and 2 which are tetrapeptides have been shown to have a very short half-life in plasma with the early degradation occurring between the second and third amino acids. The first two amino acids in the endomorphins are tyr-pro and this peptide bond is somewhat protected from degradation by proline. It takes five hours for aminopeptidase to degrade the dipeptide in vitro.[33,34] This amount of time would seem sufficient for the OPE to cross the BBB.

On the other hand, the ester bond in the OPE would be more likely to break from plasma esterases. However, ester hydrolysis in the plasma does not preclude effective OPE activity, because the rates of hydrolysis may be less than the rate of permeability across the BBB. For example, ester local anesthetics which are rapidly hydrolyzed in the plasma can produce pronounced CNS effects with as little as 60 mg given intravenously in an average size patient and etomidate, an ester of ethanol, is a rapid induction agent.[35]

5. Crossing the BBB

Some of these considerations were discussed in #1 Indentifying lead compounds. Besides molecular size, lipophilic properties, charge and propensity for hydrogen bonding are also important factors restricting a molecule from crossing the BBB.[36] Cholesterol used as a conjugate can increase the lipophilic properties of the OP. The ester formation created with cholesterol will change the OP charge from a zwitterion to that of a single cationic charge on the terminal amine thus favoring penetration through the BBB. Because the amine of tyrosine is a weak base and the CSF (pH ~7.3) is more acidic than the plasma (pH 7.4), ion trapping will occur increasing concentrations of the OPE in the CSF.[16] As previously mentioned, the OH of tyrosine could form hydrogen bonds within the brain capillary endothelium impeding movement across the BBB and therefore esterification may be required to facilitate transfer across the BBB. Preliminary observations with tyr-pro-ethyl ester (See Experimental section) suggest that this may not be the case. Other methods, reported in the literature, to enhance permeation of OP across the BBB include: cyclic prodrugs, viral vectors, fusion proteins, peptide and nutrient transport systems, Trojan horse vectors, and nanoparticles.[18,37–39] Compared to these methods, ester prodrugs are simpler to synthesize and have been demonstrated clinically to cross the BBB.

6. Hydrolysis of OPE in the CNS

Esterases are well known to exists in the CSF and these could beneficially hydrolyze the OPE.[40,41] In comparison, peptidases may not be as plentiful as

Table 1. Molecule weights and potential hydrogen bonding of constituents of possible OPE.

	M.W	H bond donor (N+O)H	H bond accept (N+O)
Tyr-gly	238	4	5
Tyr-pro	278	2	4
Tyr-gly-gly	295	5	6
Try-gly-gly-ph	442	6	7
Tyr-gly-gly-phe-leu	556	7	8
Tyr-pro-phe-phe-NH2 (endomorphin-2)	572	5	6
Tyr-pro-trp-phe-NH2 (endomorphin-1)	611	6	6
Ethanol	46	1	1
Cholesterol	387	1	1
3-glucose	180	5	6

Table 2. GABA ester lipid/water partition coefficients and BPI.

	BPI	Kow
Cholesteryl-GABA	25	110
3-Glucosyl-GABA	104	0.21

esterases in the CSF. In animal CSF, endomorphin 1 and 2 have been shown to be remarkably stable.[33,42]

7. Efflux from the CNS

Active transporters have been described that may serve to protect the CNS from toxic substances including metabolites. Two non-synthetic OP (endomorphin 1 and 2) have been shown to be transported out of the brain presumably through an efflux system.[33] However, an OP transporter has not been as yet discovered and at the present time termination of an opioid peptide is primarily thought to occur from redistribution and degradation.

Had the above problems been easy to solve and benefits proven, an OP would likely have been developed decades ago. However, the potential reward of an OP analgesic with improved analgesia and less side effects still remains speculative. All things considered, many in the field (as estimated by the number of publications in this area) believe that resources should be devoted to developing a clinically effective OP.

Physical and chemical properties of potential leads

Low molecular weight opioid peptides are listed in ascending order. Potential hydrogen bond donors (N+O with one or more H) and potential hydrogen bond acceptors (N+O) for each potential OP are listed as well as physical and chemical properties of the possible ester conjugates, glucose and cholesterol.[24] Table 1. The BPI and Kow of GABA conjugated esters of cholesterol and 3-Glucose are listed.[43] Table 2. Possible lead OPE compounds are listed. Table 3. Lipinski's criteria for drug like properties and modifications for CNS penetration are listed. Table 4.

While the above leads compounds do not precisely fit any of the Lipinski's criteria there may be enough interest to stimulate further development. It is worth reiterating:

1. Our experience with ester local anesthetics and etomidate teaches us that significant CNS penetration can occur even with rapid plasma ester hydrolysis. Preliminary animal data suggests that tyr-pro-ethyl ester crosses the BBB and unexpectedly produces hyperalgesia. (See Experimental Section)
2. The tyr-pro is protected and unlikely to be rapidly hydrolyzed in the plasma.
3. The ideal OPE would be comprised of an OP and conjugate that aids crossing the BBB and be acceptable to introduce into the CNS. The above conjugates of ethanol, 3-glucose and cholesterol were chosen because they have been shown in animal models to facilitate crossing of the BBB or they are also "familiar" and relatively non-toxic to the CNS at predicted concentrations. OP conjugates of ethanol look particularly attractive because of similar structure and molecular weight as etomidate which rapidly produces CNS effects after intravenous administration.
4. Glucose is probably actively transported across the BBB so some aspects of the above criteria may not be applicable.

Table 3. Lead OPE compounds to consider for in vivo trials.

	MW	H bonds donor* (N+O)H	H bond acceptor* (N+O)	pKa
1. Ethyl-pro-Tyr	306	3	5	9.1
2. Ethyl-gly-Tyr	266	1	4	9.1
3. 3-Glucosyl-pro-Tyr	440	6	9	9.1, 12.8
4. Cholestryl-pro-Tyr	647	2	4	9.1
5. 3-Glucosyl-gly-Tyr	432	8	10	9.1, 12.8
6. Cholestryl-gly-Tyr	607	4	5	9.1

Note: *Assumes the OH in tyr is not acetylated.

Table 4. Lipinski's criteria for drug properties.

Lipinski's rule of 5[24]	Lipinski's modification for CNS penetration[44]
Kow <5	Kow <5
Molecular weight <500	Molecular weight <400
H-bond donors <5	H-bond donors <3
H bond acceptors <10	H-bond acceptors <7

5. Some other "familiar" conjugates would be dehydroascorbic acid and diethylaminoethanol.[43]
6. It may be reasonable to spend some collective resources on clinical trials of GMP OP in patients suffering from intractable pain rather than totally on laboratory development.

The clinical view of OPE

It remains speculation whether OP will offer any advantages over opiates for the treatment of chronic pain. Experience delivering OP for chronic pain to humans and animals is limited. Many papers have been published assuming and speculating that discovering a bioavailable central acting OP is a worthy goal. However, the capacity to answer this question is within our reach if OP, preferably natural products familiar to the CNS, such as leu-enkephalin, and endomorphin 1 and 2 were manufactured according to GMP so that, after appropriate animal studies, clinicians could conduct clinical trials in patients suffering intractable pain

Table 5. Cold immersion experiment.

Pre-dosing

Date: 6/30/2011

Animal ID	Trial 1		Trial 2		Trial 3		Mean	SD
	Time	Latency	Time	Latency	Time	Latency		
1	1408	17.8	1419	18	1434	16.8	17.5	0.6
2	1405	7.4	1417	5.6	1432	8.4	7.1	1.4
3	1409	14.2	1420	9.8	1435	9.7	11.2	2.6

Eliminating the longest latency period, means are as follows

Animal ID	Mean	SD
1	17.3	0.7
2	6.5	1.3
3	9.8	0.1

Date: 7/1/2011

Animal ID	Dosage (1 ml)	Time of dosing
1	1 mg	1333
2	2 mg	1334
3	0.5 mg	1331

Post-dosing

Date: 7/1/2011

Animal ID	Trial 1		Trial 2		Trial 3		Mean	SD
	Time	Latency	Time	Latency	Time	Latency		
1	1358	13.2	1409	22.1	1421	11.5	15.6	5.693
2	1400	4.7	1411	4.7	1422	12.2	7.2	4.3301
3	1357	6.45	1408	6.2	1423	9.0	7.2167	1.5495

Eliminating the longest latency period, means are as follows

Animal ID	Mean	SD
1	12.4	1.2
2	4.7	0.0
3	6.3	2.0

who have indwelling intrathecal pumps and thus the BBB is bypassed.

Experimental section

This investigation was approved by the Institutional Animal Care and Use Committee of the Durham Veterans Affairs Medical Center and was conducted by Dr. Jem Scott-Emuakpor of the Durham Veterans Affairs Medical Center.

Methods

Three male Wistar rats at 8 weeks of age (Taconic Farms, Germantown, NY) were utilized in the experiment. Each rat was grasped firmly, while the distal half of its tail was immersed in a liquid bath at a temperature of –20 °C. Latency periods (in seconds) were measured from the time that tails were immersed to the time that the rat removed its tail from the liquid. Prior to dosing, rats were given three trials, approximately ten minutes apart. On the following day, rats were given i.p. doses (0.5 mg, 1 mg, 2 mg) of Tyr-pro-ethyl ester (Genscript, Piscataway, NJ) and, after thirty minutes, another three trials were conducted. Post-dose trials were also, approximately ten minutes apart. Animals were examined for acute adverse effects to both the cold liquid bath and the test article.

Results (Table 5)

Interpretation

Data suggests that, at the doses given, Tyr-pro-ethyl ester may have some neurological effects on rats and that it may, in fact, increase sensitivity to noxious stimulus. However, given the limited data that can be collected from so few test subjects, it is difficult to say whether the rats experienced greater sensitivity to cold or whether they were more sensitive to handling.

Conclusion

Historically, many attempts to improve present opiates for long term treatment of intractable pain have failed. Morphine and methadone continue to remain the most commonly prescribe opioids in chronic pain clinics. An OP, targeting the mu receptor for long term control of pain, has not been accomplished due a number of difficulties particularly developing a compound that can cross the BBB. A number of the roadblocks such as plasma and CNS stability have been discussed. However, as minimal size opioids have been discovered that can be ester conjugated to lipophilic groups, the possibility of a successful opioid peptide analgesic is increased. Preliminary data in rats suggest that tyr-pro-ethyl ester crosses the BBB and may produce hyperalgesia and larger OPE may be pharmacologically active. Once the active OPE is developed, the next step would be to determine if it has more favorable qualities than opiates. Clinical trials of OP manufactured according to GMP in patients with indwelling intrathecal pumps could provide the answer to this very important question.

Acknowledgement

The author would like to express great appreciation to Dr. Jem Scott-Emuakpor and her staff of the Durham Veterans Affairs Medical Center who conducted the animal trials with tyr-pro-ethyl ester.

The author would like to thank Dr. Pari Azari and Dr. Thomas Buchheit for proof reading this manuscript and Julie Rosato of Duke University for illustrations.

Disclosure

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

References

1. Furlan AD, Sandoval JA, Mailis-Gagnon A, Tunks E. Opioids for chronic noncancer pain: a meta-analysis of effectiveness and side effects. *CMAJ*. May 23, 2006;174(11):1589–94.
2. Shah NG, Lathrop SL, Reichard RR, Landen MG. Unintentional drug overdose death trends in New Mexico, USA, 1990–2005: combinations of heroin, cocaine, prescription opioids and alcohol. *Addiction*. January 2008; 103(1):126–36.
3. Overdose deaths involving prescription opioids among Medicaid enrollees—Washington, 2004–7. *MMWR Morb Mortal Wkly Rep*. October 30, 2009;58(42):1171–5.

4. Pearson EC, Woosley RL. QT prolongation and torsades de pointes among methadone users: reports to the FDA spontaneous reporting system. *Pharmacoepidemiol Drug Saf*. November 2005;14(11):747–53.
5. Krantz MJ, Kutinsky IB, Robertson AD, Mehler PS. Dose-related effects of methadone on QT prolongation in a series of patients with torsade de pointes. *Pharmacotherapy*. Junuary 2003;23(6):802–5.
6. Huh B, Park CH. Retrospective analysis of low-dose methadone and QTc prolongation in chronic pain patients. *Korean J Anesthesiol*. April 2010;58(4):338–43.
7. Hagmeyer KO, Mauro LS, Mauro VF. Meperidine-related seizures associated with patient-controlled analgesia pumps. *Ann Pharmacother*. January 1993; 27(1):29–32.
8. Marinella MA. Meperidine-induced generalized seizures with normal renal function. *South Med J*. May 1997;90(5):556–8.
9. Smith MT. Neuroexcitatory effects of morphine and hydromorphone: evidence implicating the 3-glucuronide metabolites. *Clin Exp Pharmacol Physiol*. July 2000;27(7):524–8.
10. In brief: heat and transdermal fentanyl. *Med Lett Drugs Ther*. August 10, 2009;51(1318):64.
11. Heiskanen T, Matzke S, Haakana S, Gergov M, Vuori E, Kalso E. Transdermal fentanyl in cachectic cancer patients. *Pain*. July 2009;144(1–2):218–22.
12. Jumbelic MI. Deaths with transdermal fentanyl patches. *Am J Forensic Med Pathol*. March 2010;31(1):18–21.
13. Hosobuchi Y, Meglio M, Adams JE, Li CH. beta-Endorphin: development of tolerance and its reversal by 5-hydroxytryptophan in cats. *Proc Natl Acad Sci U S A*. Sepetmber 1977;74(9):4017–9.
14. Berger AC, Whistler JL. How to design an opioid drug that causes reduced tolerance and dependence. *Ann Neurol*. May 2010;67(5):559–69.
15. Pawar M, Kumar P, Sunkaraneni S, Sirohi S, Walker EA, Yoburn BC. Opioid agonist efficacy predicts the magnitude of tolerance and the regulation of mu-opioid receptors and dynamin-2. *Eur J Pharmacol*. Junuary 1, 2007;563(1–3):92–101.
16. Goldberg JS. Stereochemical basis for a unified structure activity theory of aromatic and heterocyclic rings in selected opioids and opioid peptides. *Perspect Medicin Chem*. 2010;4:1–10.
17. Personal communication.
18. Pan W, Kastin AJ. Polypeptide delivery across the blood-brain barrier. *Curr Drug Targets CNS Neurol Disord*. April 2004;3(2):131–6.
19. Janecka A, Perlikowska R, Gach K, Wyrebska A, Fichna J. Development of opioid peptide analogs for pain relief. *Curr Pharm Des*. 2010;16(9): 1126–35.
20. Jacob JN, Hesse GW, Shashoua VE. gamma-Aminobutyric acid esters. 3. Synthesis, brain uptake, and pharmacological properties of C-18 glyceryl lipid esters of GABA with varying degree of unsaturation. *J Med Chem*. Sepetmber 1987;30(9):1573–6.
21. Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature*. December 18, 1975;258(5536):577–80.
22. Witt KA, Davis TP. CNS drug delivery: opioid peptides and the blood-brain barrier. *AAPS J*. 2006;8(1):E76–88.
23. Gentilucci L. New trends in the development of opioid peptide analogues as advanced remedies for pain relief. *Curr Top Med Chem*. 2004;4(1):19–38.
24. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. March 1, 2001;46(1–3):3–26.
25. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *Neuro Rx*. January 2005;2(1):3–14.
26. Becker KL. *Principles and practice of endocrinology and metabolism*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001.
27. Guzevatykh LS, Voronina TA, Emel'ianova TG, et al. Analgesic activity of dipeptide Tyr-Pro. *Izv Akad Nauk Ser Biol*. January–February 2008;1:61–7.
28. Jaba IM, Vasincu D, Manolidis G, Haulica I, Mungiu OC. Experimental data regarding the implications of certain minimum structure enkephalin-like peptides in nociceptive processing. *Rom J Physiol*. January–June 2004; 41(1–2):119–26.
29. Shashoua VE, Jacob JN, Ridge R, Campbell A, Baldessarini RJ. Gamma-aminobutyric acid esters. 1. Synthesis, brain uptake, and pharmacological studies of aliphatic and steroid esters of gamma-aminobutyric acid. *J Med Chem*. May 1984;27(5):659–64.
30. Shashoua VE. Gaba esters and GABA analog esters. *United States Patent 5,051,448*. 1991.
31. Martin RB. Free energies and equilibria of peptide bond hydrolysis and formation. *Bioplymers*. 1997;45:351–3.
32. Wang SS, Gisin BF, Winter DP, et al. Facile synthesis of amino acid and peptide esters under mild conditions via cesium salts. *J Org Chem*. April 15, 1977;42(8):1286–90.
33. Van Dorpe S, Adriaens A, Polis I, Peremans K, Van Bocxlaer J, De Spiegeleer B. Analytical characterization and comparison of the blood-brain barrier permeability of eight opioid peptides. *Peptides*. July 2010;31(7):1390–9.
34. Janecka A, Staniszewska R, Gach K, Fichna J. Enzymatic degradation of endomorphins. *Peptides*. November 2008;29(11):2066–73.
35. Schnapp M, Mays KS, North WC. Intravenous 2-chloroprocaine in treatment of chronic pain. *Anesth Analg*. November 1981;60(11):844–5.
36. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *Neuro Rx*. October 2005;2(4):541–53.
37. Bodor N. Targeting of drugs to the brain. *Methods Enzymol*. 1985;112: 381–96.
38. Patel MM, Goyal BR, Bhadada SV, Bhatt JS, Amin AF. Getting into the brain: approaches to enhance brain drug delivery. *CNS Drugs*. 2009;23(1):35–58.
39. Egleton RD, Davis TP. Development of neuropeptide drugs that cross the blood-brain barrier. *Neuro Rx*. January 2005;2(1):44–53.
40. Kambam JR, Horton B, Parris WC, Hyman SA, Berman ML, Sastry BV. Pseudocholinesterase activity in human cerebrospinal fluid. *Anesth Analg*. April 1989;68(4):486–8.
41. Sirvio J, Rakonczay Z, Hartikainen P, Kasa P, Riekkinen PJ. The molecular forms of acetylcholinesterase in cerebrospinal fluid of normal subjects–effect of aging. *J Neural Transm Gen Sect*. 1991;86(2):147–50.
42. Kastin AJ, Banks WA, Hahn K, Zadina JE. Extreme stability of Tyr-MIF-1 in CSF. *Neurosci Lett*. Junuary 6, 1994;174(1):26–8.
43. Goldberg JS. Selected gamma aminobutyric acid (GABA) esters may provide analgesia for some central pain conditions. *Perspect Medicin Chem*. 2010;4:23–31.
44. Lipinski CA. Drew university medicinal chemistry special topics course 1999.