Published in final edited form as:

Biopolymers. 2013 April ; 100(2): 127–131. doi:10.1002/bip.22179.

Adventures in Peptides and Science With Students! The Joys of Research

Victor J. Hruby¹

¹University of Arizona, Tucson, 85721, USA

Introduction

It is a great honor to receive the Murray Goodman Award for Scientific Excellence and Mentorship. I believe it is primarily a reflection of the tremendous scientific abilities, productivities, and accomplishments of my Graduate Students and Postdoctoral Associates both while in my laboratory and since. It also is tremendous to be associated in this way with Murray Goodman who was a good friend and colleague. He gave me much good advice in numerous aspects of my career. We also shared some important adventures as consultants for the United Nation's Development Program, and the in the development of the American Peptide Symposium and American Peptide Society.

It is very daunting to reflect on my life as a Professor and mentor, since I have had so many outstanding graduate students and postdoctoral associates including 20 Masters students, 65 Ph.D. students and over 90 Postdoctoral Associates, most of who have published multiple papers with me. How can I summarize their tremendous efforts and accomplishments when associated with me and my laboratory? I have tried to solve this problem by simply illustrating how our thoughts and sciences have evolved on a few joint accomplishments we have had in the 42 plus years I have been a mentor. Hopefully they will illustrate my philosophy as a teacher and mentor (Figure 1).

1. Every person is unique; help them find their uniqueness.
2. Everyone needs to take ownership of their research. Make the most of your ideas.
3. Collaboration within the group and with our biological colleagues is encouraged and often necessary for excellence.
4. Good ideas and creativity can come from everyone, but generally by choosing to solve a difficult problem.

In so doing there is much I will not talk about including: 1) use of ²H, ¹³C, etc. NMR to examine peptide-protein interactions; 2) computational chemistry and peptide drug design; 3) asymmetric synthesis of novel amino acids and β -turn mimetics; 4) peptide synthesis; 5) oxytocin conformation-activity relationships; 6) development of combinatorial chemistry and the selectide process; 7) glucagon - development of pure antagonists, inverse agonists and multiple signaling pathways; 8) substance P, CCK, melanocyte concentrating hormone and other neurotransmitters; and 9) conversion of somatostatin to a highly selective mu opioid antagonist.

Results and Discussion

Melanotropin Peptides and Mimetics, Melanocortin Receptors and Biological Activities

Our initial efforts in studying the melanotropin peptides (α -MSH, β -MSH, γ -MSH) was to obtain an analogue of α -MSH that was more stable for *in vivo* studies. Based on chemical,

biochemical and pharmacological studies done primarily by Tomi Sawyer in my lab and Chris Hewart in Mac Hadley's lab, we designed [Nle⁴, D-Phe⁷] α -MSH (NDP- α -MSH) (MT-I) which was highly potent, stable *in vivo* (hours vs minutes for α -MSH) and had extraordinary prolonged activity (days to weeks) [1, 2]. Modeling building based on the Ramachandran plot led to the concept of a β -turn in the vicinity of the D-Phe⁷ residue, and then to the concept that cyclization of a linear peptide. c[Cys⁴, Cys¹⁰] α -MSH [3] was designed which also was a potent analog with high *in vivo* stability. Extensive structure-activity studies and NMR analysis by several graduate students and postdocs led Fahad Al-Obeidi to do extensive computational studies which led to the design of the truncated cyclic lactam analogue of α -MSH Ac-Nle⁴-c[Asp⁵, D-Phe⁷, Lys¹⁰]- α -MSH(4-10)-NH₂ (MT-II) [4, 5]. These two peptides along with the first discovered melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R) antagonist Ac-Nle⁴-c[Asp⁵, D-Nal(2')⁷, Lys¹⁰]- α -MSH(4-10)-NH₂ (SHU-9119) [6] have been the cornerstone peptides for very extensive and exciting studies on the chemistry, biology, and medical applications by hundreds of academic and industrial laboratories worldwide. The discoveries in the 1990's as part of the human genome project, that there were 5 melanocortin receptors, four of which utilized the basic pharmacophore His-Phe-Arg-Trp [7] were transformational. These receptors were found to be involved in most of the key behaviors and biological process related to survival in animals including feeding behavior, sexual behavior, response to stress, pigmentation, fear-flight learning behavior, immune response, etc. It was also found that they were involved in numerous diseases including obesity, anorexia, metabolic syndrome, pigmentary disorders, erectile dysfunction, cancer, premature labor and many others.

The continuing creative efforts of many students in my research group led to several critical contributions to this still exciting and ongoing area of research of which a few examples follow. Work of Shubh Sharma led to one of the first demonstrations that multivalent conjugated ligands could be used for the microscopic visualization and characterization of melanoma, of melanocortin receptor trafficking including patching, internalization, and recycling of these receptors [8]. Many biologists, endocrinologists, and physiologists were particularly interested in determining the underlying mechanism(s) of the prolonged actions of NDP- α -MSH (MT-I) and MT-II, and we provided numerous laboratories with the peptides and in some cases, the animals or tissues, for their studies. Though we were able to demonstrate that these were cAMP and Ca²⁺ dependent bioactivities, further insights into the mechanism of the prolonged bioactivity (as if the ligand had made a "constitutively active" receptor; a novel hypothesis) have not been forthcoming, and so with Carrie Haskell-Luevano, Lackmal Boteju and others in my research group we decided to see if we could determine the chemical (conformational and topographical structure) basis for this novel biological function. For this purpose, we turned to our concepts of topographic space as it relates to biological activity (for an earlier review see [9]). We first examined the use of the 4 isomers β -methyl-phenylalanine in the D-Phe⁷ position since the D-Phe⁷ residue was so critical to bioactivity, but no new insights were obtained. However, when we turned to use the 4 isomers of β -MeTrp [10] exciting new insights were obtained. All four isomers of β -MeTrp [(2S,23) (2S,3R), (2R,3S) and (2R, 3R)] were incorporated into MT-II in the 9 position (for Trp⁹). Evaluation of prolonged bioactivity of all 4 diastereoisomers of MT-II demonstrated that the [(2R,3R)- β -MeTrp⁹]-MT-II analogue was as prolonged acting as MT-II, the (2S,3S)- β -MeTrp derivative was intermediate and the other 2 isomers had no prolatingatin. Extensive NMR studies demonstrated that the [(2R, 3R)- β -MeTrp⁹]MT-II had unique topographic relationships for the 4 key pharmacophore side chain residues which could account for its prolonged biological activity [11].

From a biological/medical perspective, we were the first to demonstrate, in collaboration with Roger Cone, using the superagonist MT-II and the MC3R/MC4R antagonist SHU-9119, that a single dose of MT-II could dramatically reduce food intake in both normal

mice and obese mice and the effect was completely reversible with the antagonist SHU-9119 [12]. Prolonged application of MT-II led to weight loss. In collaboration with our biological and medical colleagues, we have performed several clinical trials in humans on the biological effects of MT-I and MT-II. Among other findings, we demonstrated that MT-I (and later MT-II) led to pigmentation throughout the body without sun [13, 14] and that this hormone-induced pigment could reduce U.V. radiation-induced DNA damage in humans. Equally exciting was the finding that MT-II, in a double blind, placebo controlled, clinical trial could lead to erectile function in men with non-organic erectile dysfunction [15] when given peripherally. These compounds may soon be in clinical medicine.

Conformational and Topographical Considerations in the Design of Novel Ligands for Pain and Addiction

Another area of research which has been of great interest to us for many years is that of pain and addiction, and many outstanding students and postdocs have made significant contributions in this area. Here we will discuss a few key discoveries along the way.

The discovery of the peptide enkephalin (H-Tyr-Gly-Gly-Phe-Leu(Met)-OH) is the natural endogenous opioid in animals, opened a new era in pain research, as did the discovery of three opioid receptors μ , δ , and κ . Since the non-peptide plant natural product, morphine (an alkaloid) was shown to be selective for the μ opioid receptor, the search for delta selective ligands has been a key aspect of opioid research. Our efforts were again based on conformational considerations which lead Hank Mosberg and Robin Hurst in my group, informed by our considerations of cyclic melanotropins to the design and synthesis of the cyclic, conformationally constrained 14-membered cyclic analogue of enkephalin c[D-Pen², D-Pen⁵] Enkepalin (Tyr-c[D-Pen-Gly-Phe-D-Pen] (DPDPE) which was a highly selective agonist for the delta opioid receptor [16] using penacillamine residues (β,β -dimethyl-cystine) as conformational constraints due to gliminal dimethyl transannular effect in medium sized rings. Subsequently extensive NMR, Computational and eventually X-ray crystal structure analysis of DPDPE established that DPDPE had similar conformation in aqueous and DMSO solutions and the crystal [e.g. 17]. However, still this left unknown the topographical require of the exocyclic Tyr¹ residue, a key pharmacophore for the opioid receptor. To examine this problem led Xinhua Qian, Ding Jiao, K.C. Russell and Mark Shenderovich in my group to the synthesis of all 4 isomers of [2S3S, 2S3R, 2R3S, 2R3R] β -methyl-3', 5'-dimethyltyrosine (TMT)[18] which are highly biased in chi-1 conformation (g(-), g(+) or trans) depending upon the isomer. All 4 isomers were incorporated into DPDPE and their conformational and biological properties examined with the expectation that if the delta opioid receptor preferred a particular topography only one of four isomers would mimic DPDPE in its potency and selectivity. Indeed it was found that only the [2S,3R]TMT¹-DPDPE analogue had both high affinity and high selectivity for the delta opioid receptor[19, 20]. Interestingly, this same analogue turned out to be an antagonist at the mu opioid receptor albeit a weak antagonist. Though we tried to publish it all in a single paper in the J. Am. Chem. Soc. (synthesis, conformational analysis, biological properties), the JACS editor and a referee made us take out much of the biological data. Though we vigorously objected that this was an excellent example addressing how chemical thinking could explain biology, we did not prevail. The myopia of chemists, even chemical leaders, is unfortunate and has greatly limited progress in both chemistry and biology. In any case, with these results in hand with Mark Shenderovich, Subo Liao and Jose Alfaro-Lopez, we decided we could utilize these results to design *de novo* a non-peptide peptide mimetic of DPDPE. This involved conformational and topographic evaluation of key pharmacophores in 3D vector space in conjunction with analysis of simple organic templates as a possible scaffold for the key pharamacophore moieties. This led to the design of SL-3111 as shown in Figure 2 [21]

which had binding and second messenger activity properties very similar to DPDPE but not as other similar piperazine analogues in the literature.

Recent New Directions in Peptide Structure Based Drug Design

Though we have had many other exciting adventures in peptide chemistry, biophysics, biology and medicine, I will end my brief discussion the exciting journey I have taken with my students the past 40 plus years, with a brief outline of our more recent adventures that are still in progress and for which we are very excited.

In our view, current methods of drug design and development for many of our most prevalent diseases are inadequate or ill advised. Modern genomics, proteomics, etc. have demonstrated that many degenerative diseases result from multiple changes in the expressed genome. Thus we must re-examine our methods of drug design to address and eventually cure the disease state. In most cases, our analyses indicate that ligands with multiple bioactivities are needed.

In this regard, our efforts to address the problem of prolonged and neuropathic pain for which medications for acute pain such as morphine are either relatively ineffective, or actually counter indicated are very relevant. These pain states involve up regulation of a number of stimulatory neuropeptides and their receptors in pain pathways. Thus we have suggested [e.g. 22] that drug design for these pain states should require design and development of multivalent ligands. To address this hypothesis, a number of design approaches are possible including: design of multivalent ligands with overlapping pharmacophores; adjacent pharmacophores; or template separated pharmacophores for the various receptors. In our many studies to date, we have designed bifunctional ligand [e.g. 23], trifunctional [e.g. 24, 25] and tetrafunctional ligands [e.g. 26] and thus far have obtained a number of ligands that have unique biological profiles against pain states in which opiates and other current drugs for pain have minimal or no efficacy, using standard doses, and with little or no toxicity [e.g. 27]. Some of these peptides and peptidomimetics are stable against proteolytic breakdown and can cross the blood-brain barrier. We are vigorously pursuing these compounds or enhanced analogues toward clinical trials.

Another major new area of research is toward the detection and treatment of cancer utilizing multivalency. Since cancer involves major changes in the expressed genome, our major goal is to develop a scaffold which can have multivalent ligands that will allow the synthetic construct to distinguish normal from cancer cells [e.g. 28, 29]. Both heteromultivalent and homomultivalent ligands may turn out to be useful, though it seems that heteromultivalency will be the most likely to be successful in vivo.

Finally, we have been developing ligands that can interact with allosteric sites on G Protein Coupled Receptors (GPCRs). It has been postulated that development of such ligands (instead of orthosteric ligands) should have greater selectivity of biological activity and fewer side effects. We have several very good leads especially for the melanocortin receptors [e.g. 30] and we plan to pursue these vigorously into the future.

Acknowledgments

I have greatly benefited and tremendously enjoyed being a professor/student working with exceptionally talented students, postdocs and visiting scientists. They are always an inspiration, and the best have challenged me to think anew, to more critically evaluate what we are doing and why, and they keep me young in mind, heart, and spirit. Without their efforts, creativity, and hard work, this award would not have been possible, and I thank them with great joy and humility. I also thank my M.S. (A. William Johnson), Ph.D. (A.T. Blomquist) and Postdoctoral (Vincent du Vigneaud) mentors who sent me on paths I otherwise would have not taken. They gave me the courage to try the difficult, and the hope that I could do it. I also thank grant and manuscript reviewers for their sometimes kind and sometimes harsh critiques of our ideas and accomplishments. I hope they stimulated us to think in creative

and productive ways. In any case, I am still looking and like to think that my group's best ideas are just around the corner.

Over the years we have been generously supported constantly by the U.S. Public Health Service, National Institutes of Health, and for various periods of time by the National Science Foundation and other private foundations and companies.

References

1. Sawyer TK, et al. Proc. Natl. Acad. Sci. U.S.A. 1980; 77:5754. [PubMed: 6777774]
2. Hadley ME, et al. Science. 1981; 213:1025. [PubMed: 6973820]
3. Sawyer TK, et al. Proc. Natl. Acad. Sci. U.S.A. 1982; 79:175. [PubMed: 6798568]
4. Al-Obeidi F, Hadley ME, Pettitt RM, Hruby VJJ. Am. Chem. Soc. 1989; 111:3413.
5. Al-Obeidi F, et al. J. Med. Chem. 1989; 32:2555. [PubMed: 2555512]
6. Hruby VJ, et al. J. Med. Chem. 1995; 38:3454. [PubMed: 7658432]
7. Hruby VJ, et al. J. Med. Chem. 1987; 30:2126. [PubMed: 2822931]
8. Sharma SD, et al. Proc. Natl. Acad. Sci. U.S.A. 1996; 93:13715. [PubMed: 8943000]
9. Hruby VJ, Al-Obeidi F, Kazmierski WM. Biochem. J. 1990; 268:249. [PubMed: 2163604]
10. Boteju L, et al. Tetrahedron. 1997; 50:2391.
11. Haskell-Luevano C, et al. J. Med. Chem. 1995; 38:4720. [PubMed: 7473600]
12. Fan W, et al. Nature. 1997; 384:165. [PubMed: 8990120]
13. Levine N, et al. J. Am. Med. Assoc. 1991; 266:2730.
14. Hadley ME, et al. Ann. N.Y. Acad. Sci. 1993; 680:424. [PubMed: 8390162]
15. Wessells H, et al. J. Urology. 1998; 160:389.
16. Mosberg HI, et al. Proc. Natl. Acad. Sci. U.S.A. 1983; 80:587.
17. Flippen-Anderson JL, et al. J. Am. Chem. Soc. 1994; 116:7523.
18. Qian X, Russell KC, Boteju LW, Hruby VJ. Tetrahedron. 1995; 51:1033.
19. Qian X, et al. J. Am. Chem. Soc. 1996; 118:7280.
20. Bilsky ED, et al. J. Pharm. Exp. Therap. 2000; 293:151.
21. Liao S, et al. J. Med. Chem. 1998; 41:4767. [PubMed: 9822547]
22. Hruby VJ, et al. Am. Assoc. Pharm. Sci. J. 2006; 8:E450–E460.
23. Lee YS, et al. J. Med Chem. 2011; 54:382. [PubMed: 21128594]
24. Hruby VJ, et al. Life Sciences. 2003; 73:699. [PubMed: 12801591]
25. Yamamoto T, et al. J. Med. Chem. 2007; 50:2779. [PubMed: 17516639]
26. Lee YS, et al. Bioorg. Med. Chem. Letters. 2010; 51:748.
27. Largent-Milnes T, et al. Brit. J. Pharmacol. 2010; 161:986. [PubMed: 20977451]
28. Gillies RJ, Hruby VJ. Expert Opin. Ther. Targets. 2003; 7:137. [PubMed: 12667092]
29. Vagner J, et al. Angew. Chem. Int. Ed. 2008; 47:1685.
30. Cain JP, et al. Bioorg. Med. Chem. Letters. 2006; 16:5462.

Figure 1.

Figure 2.
De novo Design of Non-Peptide Peptide mimetic of a δ -Opioid agonist