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DESIGN OF CYCLIC PEPTIDES WITH BIOLOGICAL ACTIVITIES FROM BIOLOGICALLY ACTIVE PEPTIDES: THE CASE OF PEPTIDE MODULATORS OF MELANOCORTIN RECEPTORS

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Abstract

Design of biologically active peptides is of critical importance for the development of potent, selective, nontoxic bioavailable drugs. A major approach that has been developed to accomplish this is the development of methods for the design and synthesis of a wide variety of cyclic peptides and peptidomimetics. In this short and general review, we outline the methods that have been developed for cyclization of peptides and how these have been used for peptide and peptidomimetic design using the melanotropin peptides and melanocortin receptors MC1R, MC3R, MC4R and MC5R to illustrate aspects of this approach.

Introduction

Most human peptide hormones and neurotransmitters are linear peptides that are conformationally flexible and subject to degradation by proteases. Some have cyclic disulfide structures (e.g. oxytocin, vasopressin, insulin, etc.) but even they are inactivated by protease activity. Nonetheless, they often naturally have to travel long distances in the body to go from where they are released to where their target receptors are located. At our current state of knowledge and drug development, these intrinsic properties of numerous peptide hormones and neurotransmitters have proved difficult for the design of peptide and peptide mimetic analogues and derivatives that might be useful agonist or antagonist drugs that are relatively stable and can cross membrane barriers. In this short overview, we will briefly but systematically discuss how we and others (e.g. 1-3) have developed strategies of conformation and topographic structural constraints that are both powerful and predictive in peptide drug development. We will discuss some general principles and provide examples. We will utilize examples primarily from our approach to develop receptor selective, potent agonist and antagonist analogues and derivatives that are active at the melanocortin receptors (MC1R, MC3R, MC4R, or MC5R). These analogues will be primarily based on the endogenous linear peptide hormone and neurotransmitter α -MSH (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂), which is a product of the precursor protein proopiomelanocortin (POMC). Other products of this gene including ACTH, β -endorphin, etc. will not be discussed here though they also have very important biological functions critical to our survival.

General Approaches to Ligand Design

A basic hypothesis of our approach (1) is that the peptide hormone or neurotransmitter when it binds to its receptor will generally assume a conformation that is a low energy conformation for the peptide or protein (e.g. α -helix, β -turn, β -sheet, etc.) at its backbone conformation (Ramachandran space). An additional critical consideration is the disposition of the side chain group in chi space, gauche (–), gauche (+), and trans confirmations (4). Generally, the design process involves converting a linear peptide into a cyclic peptide, use of novel chi constrained amino acids, use of D-amino acids or novel amino acids, and/or amide bond replacements. These and other structural modifications (Table 1) are utilized toward the following major goals: 1) obtain the bioactive conformation (template) for agonist, antagonist and inverse agonist analogues and derivatives; 2) improve receptor affinity, selectivity and efficacy; 3) stabilize peptide against proteolytic degradation; 4) improve bioavailability; and 5) minimize the structure. As shown in Table 1, there are several strategies that can be applied, all of which generally require enhanced synthetic organic chemistry, as applied to amino acid asymmetric synthesis, peptide conjugation methods and careful design of structures in three dimensional space. We will illustrate these approaches with a few examples from our research on receptor selective agonist and antagonist analogues for the melanocortin receptors MC1R, MC3R, MC4R and MC5R.

Design of Receptor Selective Peptides & Peptidomimetic Ligands for the Melanocortin Receptors

Proopiomelanocortin (POMC) is a primordial gene found in virtually all terrestrial animal life, both on the land and in the sea. It is found throughout the body and produces a variety of peptide hormones and neurotransmitters that are essential for life and survival, including in humans. Depending upon the cell type, it is processed to a variety of peptides, including α -MSH, β -MSH, γ -MSH, ACTH, β -endorphin and several others. The major targets for these peptides (5,6) are the five melanocortin receptors (MC1R, MC2R, MC3R, MC4R and MC5R) and the three opioid receptors (μ , δ and κ). All of these receptors have been cloned and are found throughout the body. They are involved in most of the major biological functions critical to our survival and well-being including: 1) pigmentation, 2) response to stress, fear-flight, etc., 3) feeding behavior, 4) sexual function and behavior, 5) energy homeostasis, 6) cardiovascular function, 7) pain, 8) kidney function, 9) temperature control, 10) sebaceous gland secretion and many others still being discovered (e.g. 5-7).

In this short paper, I will concentrate most of our discussion on the design of ligands related to α - melanocyte stimulating hormone (α -MSH) which has the structure Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂. This natural product from processing POMC binds to four of the melanocortin receptors (MC1R, MC3R, MC4R and MC5R) in the nanomolar range and acts as an agonist at all four. Previous studies in our group demonstrated that the key pharmacophore for all four receptors was the sequence -His-Phe-Arg-Trp- (8) which is preserved throughout 700 million years of evolution.

The major goals of this research effort, as is the case for many peptide hormone and neurotransmitter studies are: 1) enhance in vivo stability; 2) enhance receptor selectivity and

potency; 3) design agonists and antagonists, both orthosteric and allosteric; 4) enhance blood brain barrier penetration (or not); and 5) evaluate and understand peptide conformations in relation to biological function and receptor interactions.

In Vivo Stability

α -MSH is quite susceptible to breakdown by proteases. Its half-life in human serum is only a few minutes. Thus, an early goal of structure activity studies was the development of α -MSH analogues that would be stable for biological activity studies and for possible application as drugs. There are many ways to make peptides more stable to proteolytic breakdown, including use of D-amino acids and novel amino acids. An early major success was the design of [Nle⁴, D-Phe⁷] α -MSH(Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂) (MT-1) (9) in which Nle⁴ was a novel pseudoisosteric amino acid replacement of Met⁴ to prevent sulfur oxidation which readily occurs in α -MSH and reduces its potency. The replacement of Phe⁷ by D-Phe⁷ was introduced to stabilize a β -turn conformation. The two “simple” changes led to an α -MSH analogue that was more potent and considerably more stable than α -MSH (half-life in serum was now 2-3 hours). The analogue is now a commercial drug used to treat pigmentary disorders. It also has been used by hundreds of laboratories for a wide variety of biological and pharmacological investigations of MSH functions in animals, fish, etc.

Linear to Cyclic Analogues

Conversion of linear to cyclic peptides has been developed over many years into a very powerful approach to peptide design, biological stability in vivo, agonist and antagonist development, blood brain barrier penetration and numerous other applications (e.g. 1,10,11). There are many modes for converting linear to cyclic peptides with maintenance and enhancement of biologically active (side chain to side chain; N-terminal to C-terminal, “side chain to backbone”, backbone to backbone, etc.) and many different modes of cyclization (disulfides, sulfides, lactones, lactams, ethers, carbon-carbon, etc.).

In the case of α -MSH, based on the β -turn hypothesis, Sawyer et al. synthesized the first cyclic α -MSH analogue [Cys⁴Cys¹⁰] α -MSH (12), which was a highly potent and more stable analogue. Later, based on computational considerations, Al-Obeidi et al. designed a series of cyclic lactam analogues of α -MSH including Ac-Nle⁴[Asp⁵, D-Phe⁷, Lys¹⁰]- α -MSH-NH₂(Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂) (MT-II) (13), which is a highly potent ligand for all four melanocortin receptors as an agonist. It was found to be very stable to proteolytic degradation (half-life in serum of 2-3 days) and to cross the blood brain barrier. Extensive NMR studies in conjunction with computational studies showed that this compound has a β -turn structure around the D-Phe⁷ residue (14). This compound has been used very extensively worldwide to study melanotropin biological activity, and also has been in clinical trials for erectile dysfunction (15).

Cyclization of peptides via side chain to side chain, carbon-carbon, carbon-sulfur, terminal to side-chain, etc. has a long and storied history in peptide design for biological activity. Historically, peptides with carbon-carbon or carbon-sulfur bonds have been referred to as

carba analogues, but more recently they have been referred to as stapled peptides. The chemistry of forming such compounds will not be discussed, except to say that the application of Grubbs' catalysts to peptide chemistry has greatly expanded the potential of these compounds for a wide variety of biological applications, which are being explored in many academic and industrial laboratories. A recent application in the melanocortin area is the design of a novel thioester analogue of α -MSH c[(CH₂)₂CO-Gly-His-D-Phe Arg-D-Trp-Cys(S)]-Asp-Arg-Phe-Gly-NH₂ (16) which is a highly selective MC1R receptor antagonist. It has been used to unequivocally demonstrate sex specific female mediation of opioid-induced hyperalgesia by the hMC1R (17).

Development of Antagonists

Though many efforts were made to obtain antagonists for the MC1R, MC3R, MC4R and MC5R, only weak partial antagonists were found until quite recently. In this regard, a major breakthrough in this area was the finding of Hruby, et al. (18), that using novel topographically constrained amino acids (19) in the cyclic α -MSH structure could be a useful approach to developing antagonists. In this case, the D-Phe⁷ residue was substituted by a 2'-D-Naphthylalanine [D-Nal(2')] residue in MT-II to give the analogue Ac-Nle-Ic[Asp-His-D-Nal(2')-Arg-Trp-Lys]-NH₂ (SHU-9119) which was found to be a potent antagonist at the MC3R and MC4R and an agonist at the MC1R and MC5R (18). Interestingly, the D-Nal (1') analog was a potent agonist at all four melanocortin receptors (MC1R, MC3R, MC4R and MC5R). This demonstrated the exquisite sensitivity of G-protein coupled receptors to how minor changes in topographical structure can modulate their biological functional efficacy. In this regard, it is interesting to note that the structure of SHU-9119 has no obvious structural relation to the natural endogenous antagonist protein for the melanocortin receptor AGRP. The discovery of a small peptide antagonist of the MC3R and MC4R has had a large impact on the biology of several important human behaviors, including the discovery of the critically central importance of these receptor in appetite and feeding behavior (20) and sexual function (21) and many others (e.g. 5,6).

N-Methylation in Backbone

N-Methylation of the peptide backbone of peptides has been utilized by nature, and more recently by the peptide community, to modify peptide conformations and to stabilize peptides against degradation by peptidases. More recently, Kessler and co-workers have developed robust chemistry for the application to solution phase and solid phase peptide synthesis which has general applicability to peptide and peptidomimetic synthesis. We have collaborated with Kessler's group for the systematic synthesis of N-Methylated peptide analogues of MT-II (22) and SHU-9119 (23) as an approach to obtaining orally bioavailable, receptor selective agonist and antagonists for the MC1R, MC3R, MC4R and MC5R. We also performed extensive conformational analysis using NMR, and computational chemistry methods, including receptor docking experiments. A few examples of outcomes will be presented here. The papers can be examined for extensive details. The basic strategy was to make all possible combinations of N-Methylated analogues of the amino acid residues in the ring portion of MT-II and SHU-9119 (22,23), to evaluate their binding and second messenger efficacies for the melanocortin receptors, and then to selectively examine in detail

the conformational properties of the most interesting ligands in solution and on their binding interactions with the relevant melanocortin receptors, using computer assisted modeling and computational studies. For example, we found that the tetra methylated analogue of MT-II, Ac-Nle-c[Asp-N-Me-HisD-Phe-N-MeArg-N-MeTrp-N-MeLys]-NH₂ was a potent highly selective agonist ligand for the MC1R with no efficacy at the MC3R, MC4R, and MC5R. Conformational and computational studies provided new insights into the selectivity of this compound for the MC1R, and when it was examined for biological activity, it could cause death to melanoma cancer cells.

When similar studies were made on SHU-9119, the MC3R and MC4R antagonist and MC5R and MC1R agonist, a much different pattern of biological potency and efficacy emerged. Keep in mind that except for the replacement of D-Phe in MT-II with a D-Nal(2') in SHU-9119 in position 7, the structures are identical. For example, the tri-substituted analogue of SHU-9119 Ac-Nle-c[Asp-N-MeHis-D-Nal(2')-Arg-N-MeTrp-N-MeLys]-NH₂ is a universal antagonist for all four melanocortin receptors (MC1R, MC3R, MC4R and MC5R), the first such reported analogue in the literature.

Conclusions

The potential of melanotropin ligands to be drugs for a very wide variety of diseases is enormous. These include for example, such diverse diseases as pigmentary disorders and melanoma cancer, pain modulation, obesity, anorexia and other feeding disorders, sexual function, response to stress, immune response, inflammatory response, cardiovascular function, kidney function and many others. This requires the development of ligands with novel biological activity profiles at melanocortin receptors. In this brief over view, I have discussed an overall approach to peptide and peptide mimetic design that will be needed to develop peptide-based drugs using design principles, synthetic chemistry, biophysics, pharmacology and physiology considerations to obtain useful drugs with minimal or no toxicities. Figure 1 illustrates a few of the key structural/conformational modifications that have been made to provide melanotropin analogues that may be useful ligands for biological studies and/or as drugs.

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Figure 1.
 Selected Modifications of Melanocyte Stimulating Hormone Leading to Greater Receptor
 Selectivity, Stability, Efficacious and Bioavailability

Table 1**Peptide Structures Needed & Used in Peptide Ligand Design**

I. CYCLIC STRUCTURES
Disulfides, Sulfides, Lactams, Lactones, Carba, Backbone to Side Chain, Backbone to Backbone, Others
II. BACKBONE MODIFICATIONS
A. Amide Bond Replacements
B. N-Methylation of Backbone
III. CHI SPACE MODIFICATIONS
A. β -Substitutions
B. Intermolecular Cyclizations
C. Aromatic Substitutions/Cyclizations
IV. PEPTIDE CONJUGATES
A. Glycopeptides
B. Lipopeptides
C. With Spacers
D. Fluorescent, etc. Probes
E. Dendrimers
F. With Polymers
G. Others
V. MULTIVALENT PEPTIDES – NEW SCAFFOLDS
1. Homo-multivalent Ligands
2. Hetero-multivalent Ligands
3. Overlapping Pharmacophores
4. Separated Pharmacophores
5. Others