Conformationally Constrained Ac-His-D-Nal(2')-Nle-Trp-NH₂ Analogues Leads to Selective Melanotropins

Saghar Mowlazadeh Haghighi, Minying Cai*, Yang Zhou, Jonathon Sawyer, Bailey Lahtinen, Charley Chen, and Victor Hruby

Department of Chemistry, University of Arizona, Tucson, AZ, 85721, USA

Introduction

Skin pigmentation shows substantial variation within human populations and within regional groupings or races. High levels of UVR can lead to skin damage such as sunburn or skin cancer, as well as the breakdown of folic acid. Melanin provides some protection from UVR induced damage [1]. MC1-R has a well-defined role in the regulation of skin pigmentation, provides protection against damaging and mutagenic effects of UV radiation (skin cancer) and has been recently implicated in the control of inflammation [2,3].

Results and Discussion

Introduction of stereochemical modifications (D-amino acids), functional group modification, conformational constraints, and topographical constraints to the “core” peptide sequence provide an effective means to investigate these preferences [4]. Aromatic amino acids (Phe, Tyr, Trp, and His) are often a key element in bioactive peptides [5]. The procedure allows the introduction of a variety of substituents (halogens) at the phenylalanine derivatives. Phenylalanine and histidine are also conserved among all five melanocortin receptors. Mutation of His to proline resulted in significant decrease in endogenous agonist binding and receptor signaling, suggesting that these residues are also involved in melanocortin orthosteric binding. Therefore, in our effort to produce selective melanocortin ligands, we devoted a great deal of time to obtaining ligands with selective activity for the hMC1R and to developing the pharmacological properties that are suitable for medical applications of these compounds. Among our synthesized peptides, Ac-His-4-Br-D-Phe-Nle-Trp-NH₂ (1), Ac-His-4-Cl-D-Phe-Nle-Trp-NH₂ (2), show enhanced drug efficacy and MC1R activity compared to our previously synthesized tetrapeptides.

Fig. 1. Novel selective peptides.

Peptide Design and Synthesis. We have used standard solid-phase methods. The peptides were synthesized on Rink Amide resin using Fmoc chemistry. Initially, the resin was deprotected with 20% (v/v) piperidine in DMF solution. The first amino acid was coupled to the resin. The following amino acids were coupled sequentially to the growing peptide chain. Each coupling reaction was achieved using amino acid, HCTU in the presence of DIEA. The peptide was cleaved from the resin, and the other side chain protecting groups were removed in a cleavage cocktail with 95% TFA, 2.5% TIS, 2.5% water.

In conclusions, one of the major goals of medicinal chemistry has been the design and synthesis of novel peptides with conformationally constrained side chains in order to obtain highly selective and potent peptide analogues. Our biological data suggests that these compounds are selective MC1R agonists and have the EC₅₀ within nanomolar range. Substitution of Nle5 produced a more balanced activity in these peptides.

Table 1. Binding and cAMP Assays of Novel Peptides toward Melanocortin Receptors.

	MC1R				MC3R				MC4R				MC5R			
	Binding		cAMP		Binding		cAMP		Binding		cAMP		Binding		cAMP	
	IC ₅₀	%BE	EC ₅₀	Act %	IC ₅₀	%BE	EC ₅₀	Act %	IC ₅₀	%BE	EC ₅₀	Act %	IC ₅₀	%BE	EC ₅₀	Act %
(1)	1621	61	9.6	100	NB	0	111	93	189	46	40	79	NB	0	NA	0
(2)	1053	81	8.5	100	>10000	45	102	87	NB	0	19	55	NB	0	NA	0
MT-II	1 ± 0.1	100	1.02 ± 0.4	100	2.0 ± 0.1	100	5.1 ± 0.3	100	2.3 ± 0.85	100	2.1 ± 0.6	100	4.2 ± 1.3	100	5.7 ± 2.2	100

^aMT-II = Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂. ^bIC₅₀ = concentration of peptide at 50% specific binding (N = 4). NB = 0% of 125I-NDP- α -MSH displacement observed at 10 μ M. %BE (binding efficiency) = maximal % of 125I-NDP- α -MSH displacement observed at 10 μ M. EC₅₀ = Effective concentration of peptide that was able to generate 50% maximal intracellular cAMP accumulation (N = 4). Act% = % of cAMP produced at 10 μ M ligand concentration, in relation to MT-II. NA = 0% cAMP accumulation observed at 10 μ M. The peptides were tested at a range of concentration from 10⁻¹⁰ to 10⁻⁵ M.

Fig. 2. The comparison between Melanocortin activity of synthesized Peptides and MT-II.

Acknowledgments

Supported in part by a grant from the U.S. Public Health Service, National Institutes of Health, DK017420, GM 108040 and DA06284.

References

- Norton, H.L., Friedlaender, J.S., Merriwether, D.A., Koki, G., Mgone, C.S., Shriver, M.D. *Am. J. Physical Anthropology* **130**, 254-268 (2006), <http://dx.doi.org/10.1002/ajpa.20343>
- Rinne, P., Ahola-Olli, A., Nuutinen, S., Koskinen, E., Kaipio, K., Eerola, K., Juonala, M., Kähönen, M., Lehtimäki, T., Raitakari, O.T., Savontaus, E. *Arterioscler. Thromb. Vasc. Biol.* **35**, 1678-1686 (2015), <http://dx.doi.org/10.1161/ATVBAHA.114.305064>
- Ying, J., Gu, X., Cai, M., Dedek, M., Vagner, J., Trivedi, D.B., Hraby, V.J. *J. Med. Chem.* **49**, 6888-6896 (2006), <http://dx.doi.org/10.1021/jm060768f>
- Morgan, A.A., Rubenstein, E. *PLoS One* **8**, 53785 (2013), <http://dx.doi.org/10.1371/journal.pone.0053785>
- Pulka, K., et al. *Tetrahedron* **63**, 1459-1466 (2007), <http://dx.doi.org/10.1016/j.tet.2006.11.069>