Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2647–2650

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Sub-Nanomolar hMC1R Agonists by End-Capping of the Melanocortin Tetrapeptide His-D-Phe-Arg-Trp-NH_2

L. N. Koikov,[a,*] F. H. Ebetino,[b] M. G. Solinsky,[b] D. Cross-Doersen[b] and J. J. Knittel[a]

[a]*College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA*
[b]*Procter and Gamble Pharmaceuticals, Mason, OH 45040, USA*

Received 6 November 2002; revised 2 June 2003; accepted 2 June 2003

Abstract—Twenty three derivatives of the core fragment His^6-D-Phe^7-Arg^8-Trp^9-NH_2 end-capped with carboxylic and sulfonic acids were synthesized and evaluated at human melanocortin receptors (hMC1, hMC3, and hMC4Rs). The SAR within this series allowed us to map the hMCRs near the His^6 binding site and design a superpotent MC1R agonist, LK-184, $Ph(CH_2)_3CO$-His-D-Phe-Arg-Trp-NH_2 (**19**) with EC_{50} 0.01 nM (5 nM at MC3 and MC4Rs).

Melanocortin receptors (MCRs) belong to the superfamily of seven transmembrane G-protein coupled receptors and stimulate the cAMP signal transduction pathway. Five MCRs have been identified (MC1R - MC5R) and are found both peripherally and in the CNS.[1] These receptors are involved in pigmentation and animal coat coloration (e.g., MC1R),[2–4] feeding behavior, obesity, diabetes, metabolism, and energy homeostasis[5–7] (e.g., MC3R and MC4R) as well as exocrine gland function (e.g., MC5R)[1]. The endogenous agonists for the MCRs, α-, β- and γ-melanocyte stimulating hormones (MSH) and adrenocorticotropin (ACTH), are derived from posttranslational processing of the pro-opiomelanocortin (POMC) gene transcript. All of these melanocortin peptide agonists contain the central message sequence His-Phe-Arg-Trp responsible for stimulation of MC1R.[8–10] The investigation of analogues of the natural α-melanocyte stimulating hormone (α-MSH) Ac-Ser-Tyr-Ser-Met-Glu-His^6-Phe^7-Arg^8-Trp^9-Gly-Lys-Pro-Val-NH_2 showed that the inversion of Phe^7 configuration gave compounds with increased potency and in vivo stability such as NDP-MSH (MT-I) Ac-Ser-Tyr-Ser-Nle-Glu-His^6-D-Phe^7-Arg^8-Trp^9-Gly-Lys-Pro-Val-NH_2.[11]Further work resulted in an even more potent and stable MC1-5R non-selective agonist MT-II Ac-Nle-c[Asp-His^6-D-Phe^7-Arg^8-Trp^9-Lys]-NH_2[12] and melanocortins synthesis became primarily focused on cyclic peptides containing the core fragment His-D-Phe-Arg-Trp (HfRW).[1,13–15]

Recently it has been shown that HfRW-NH_2 itself possesses full agonism albeit lower potency at human MC4R.[16] Ac-HfRW-NH_2 is a full agonist at all subtypes of murine melanocortin receptors with an 850-fold drop in potency relative to NDP-MSH for mMC1R, 2000-fold for mMC3R, 32-fold for mMC4R, and 40-fold for mMC5R.[17] This shows that Ac-HfRW-NH_2 can be used as a prototype for development of selective MCRs agonists.

The goals of this work were to explore the potential of end-capping of HfRW-NH_2 with a small diverse set of acids in order to 'map' the region of MCRs adjacent to His^6 and to design novel low molecular weight agonists of MCRs. We wish to report here how this simple approach allowed us to build a generalized model of the MCRs active site and to use this knowledge for creation of a linear superpotent MC1R agonist.[18] The validity of this assumption was confirmed by the fact, that during the preparation of this manuscript, the end-capping of HfRW-NH_2 with 29 aliphatic and aromatic acids had been reported.[19,20] Out of these, the most potent *n*-octanoic derivative had EC_{50} 0.36 nM at mMC1 and 0.38 nM at mMC4Rs. The derivatives of *p*-tolylacetic, and 2-naphthylacetic acids had EC_{50} ca. 5 nM at mMC1 and ca. 0.9 nM at mMC4Rs.[20]

*Corresponding authors. Fax: +1-513-558-0978; e-mail: lkoikov@hotmail.com (L. N. Koikov); e-mail: james.knittel@uc.edu (J. J. Knittel)

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00552-3

Table 1. Binding and functional activity of melanocortins at human MC1, MC3 and MC4Rs. [a]

Peptide	R′-HfRWNH$_2$	hMC1R		hMC3R		hMC4R	
	R′	K_i (nM)	EC$_{50}$ (nM)	K_i (nM)	EC$_{50}$ (nM)	K_i (nM)	EC$_{50}$ (nM)
MT-I		0.50±0.10	0.07±0.01	0.68±0.20	0.36±0.14	0.95±0.23	0.51±0.07
1	H	51.0±3.0	31.8±14.1	890±100	350±110	180±18	29.7±12.5
2	MeCO	37.0±14.5	13.0±1.7	770±300	170±64	280±170	59.5±15.5
3	EtCO	47.5±0.5	7.5±1.5	540±250	450±260	160±35	38.5±1.5
4	PrCO	37.5±11.5	4.5±1.5	580±230	93.3±10.5	150±27	38.5±1.5
5	*i*-PrCO	40.0±6.0	7.0±1.0	280±28	130±75	97.5±8.5	24.0±12.0
6	BuCO	20.5±1.5	2.0±0.0	170±42	45.0±5.0	120±26	26.5±0.5
7	*t*-BuCO	43.0±21.0	5.0±0.0	130±8	13.0±7.0	160±72	14.5±6.5
8	*c*-PrCO	43.0±23.0	3.5±0.5	210±18	71.0±26.0	87.0±2.0	20.5±7.7
9	CF_3CH_2CO	63.5±27.5	6.5±3.5	540±100	120±19	170±28	22.5±6.5
10	$HOCH_2CO$	125±18	7.0±3.0	630±190	300±46	440±82	53.0±26.0
11	$NCCH_2CO$	86.5±21.5	8.0±2.0	350±8	160±4	110±47	18.0±6.0
12	NH_2COCO	62.0±31.0	7.0±3.0	160±13	58.0±5.0	140±78	16.5±5.5
13	$MeOCH_2CO$	91.5±23.5	9.5±0.5	380±130	570±290	350±27	42.5±17.5
14	$MeSCH_2CO$	57.5±17.5	9.0±1.0	230±40	230±70	210±74	41.0±21.3
15	$HOCH_2CH_2CH_2CO$	34.0±1.0	13.7±6.3	440±17	640±85	100±8.0	21.3±14.4
16	PhCO	30.0±1.0	6.8±2.1	260±47	98.3±23.3	160±53	28.0±5.0
17	$PhCH_2CO$	17.5±2.5	2.0±1.0	66.5±8.5	28.0±13.7	100±6	13.0±6.0
18	$Ph(CH_2)_2CO$	6.0±2.0	0.5±0.1	65.0±31.0	17.0±2.0	49.0±7.0	20.0±9.0
19	$Ph(CH_2)_3CO$ (LK-184)	**0.006±0.004**	**0.009±0.004**	13.5±1.5	4.7±1.2	7.3±1.8	4.6±2.8
20	*p*-$ClC_6H_4CH_2CO$	3.5±1.5	9.5±5.1	20.0±6.0	9.3±0.7	5.0±0.0	7.5±0.5
21	*p*-$ClC_6H_4OCH_2CO$	6.0±2.0	2.0±0.6	110±47	6.0±4.0	68.0±20.0	11.5±1.5
22	(D)-$C_6H_4CH_2CH(OH)CO$	11.5±1.5	8.0±2.0	140±15	40.5±26.5	15.5±6.5	6.3±2.7
23	CH_3SO_2	260±66	88.0±35.0	1900±260	840±43	170±29	21.5±9.5
24	*p*-$ClC_6H_4SO_2$	170±33	33.3±18.1	440±180	390±15	66.5±5.5	11.0±1.0

[a]Mean value±SEM.

Our choice of end-capping acids consisted of linear and branched aliphatic acids (**1**–**8**), their isosteres bearing polar and polarizable groups or heteroatoms (**9**–**15**), and a series of aromatic/alkylaromatic acids with a flexible C_0–C_3 spacer (**16**–**22**). These were used to explore spatial requirements of the binding site and to probe the site for possible polar or dipole interactions, H-bonding, and π–π interactions. Sulfonamides **23**–**24** were made to modify the N-terminal amide region (Table 1).

Compounds **1**–**24** were obtained on Rink resin using standard Fmoc methodology. Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-D-Phe-OH, Fmoc-His(Boc)-OH and end-capping acids were purchased from commercial sources. The peptides were purified by HPLC on a C_{18} column (purity >95%, most 97–98%, LK-184>98%) and characterized by ESI MS.

In vitro agonist assays were done in HEK293 cells transfected with human MC1R, MC3R, or MC4R. Binding was determined by measuring the displacement of a constant concentration of Eu-labeled NDP-MSH using time resolved fluorometry. Luciferase activity in the above MCR expressing HEK293 cells stably transfected with a cAMP responsive element (CRE) coupled to a luciferase reporter gene was used to determine the functional activity of MCR ligands. Responses were expressed as a % of maximum activity of MT-I (NDP-MSH) which is considered to be a full agonist at each of these MCRs. The K_i and EC$_{50}$ values for compounds **1**–**24** (all of them are full agonists) reported in Table 1 are the average of at least two separate experiments in duplicate.

The binding (K_i) and agonist potencies (EC$_{50}$) in Table 1 show good correlation. Tetrapeptide **1** is 450-fold less potent compared to MT-I at hMC1R and 80- and 60-fold less potent at hMC3R and hMC4Rs, correspondingly. This is close to the latest literature data for mMCRs.[19] Acetylation of the N-terminus does not noticeably change the potency of **2** relative to **1**. The acylation of **1** with longer or branched aliphatic acids (**3**–**8**) has practically no effect at MC4R and increases potency at MC1R and MC3R relative to **2**. The 'flat' response of MC1R (**3**–**8**) and MC4R (**1**–**8**) to the bulkiness and length of R′ (Table 1) suggests that the end-capping group does not have to fit into a confined pocket but stays in a large hydrophobic cavity or, rather, an open extracellular part of the receptors. The potencies of **2**–**8** at MC3R are fluctuating which indicates a more restricted fit. Despite the fluctuations, an increase in hydrophobicity appears to increase potency at MC3R and the bulky hydrophobic *t*-Bu (**7**) is best in this subseries.[†]

The results for the next subseries **9**–**15** confirm the above hypothesis. The introduction of various polar or polarizable groups or a heteroatom into the backbone of the parent aliphatic acid does not affect the potency at MC1 and MC4R. This agrees with the binding of R′ at an open site since it avoids unfavorable forced placement of a polar or H-bonding fragment in R′ next to a hydrophobic part of the receptor. The latter is the case with MC3R- all polar derivatives are less potent than **7**.

[†]Though data for end-capping of **HfRW**G-NH_2 with a series of lower aliphatic acids are not available, Bu-HfRWG-NH_2 is reported as a potent hMC1R and hMC4R agonist.[21]

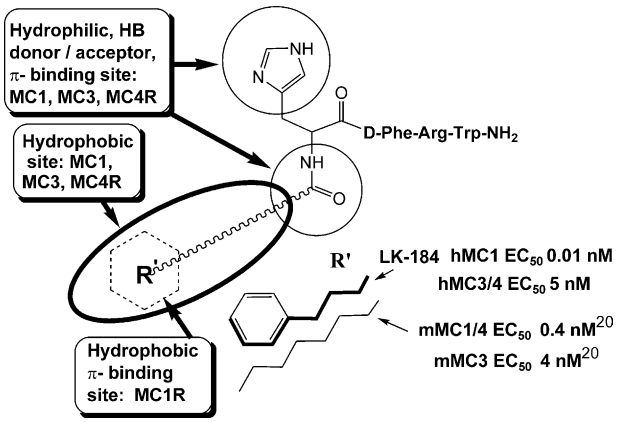

Figure 1. Model of the N-terminus- His[6] region of hMCRs.

The only exception is that an extremely hydrophilic oxamic derivative **12** is almost equipotent with its hydrophobic cyclopropyl isostere **8**, that is, MC3R has a small amphiphilic site within the hydrophobic area. Thus, notwithstanding the different shape of N-terminal binding sites of hMC1/4 and hMC3Rs, they share a common feature—a rather large hydrophobic area adjacent to the polar His[6] binding site (Fig. 1).

The above model of an 'open' MC1R strongly suggests that it can accommodate not only flexible hydrophobic chains but flat aromatic rings as well, thus adding a favorable π–π interaction to a purely hydrophobic one. The SAR found with compounds **16**–**19** confirms this idea. The parent benzoyl derivative **16** has no advantage over the aliphatic series while the increase in chain length in $Ph(CH_2)_n$ from 0 to 3 (**16**–**19**) leads to a steady increase in binding and potency due to a hydrophobic term contributed by each next CH_2 group. This trend is observed for all MCRs but with MC1R there is a dramatic 20-fold jump between compounds **18** and **19**. The latter cannot be attributed to hydrophobic interaction only which with high probability implies π–π interaction when Ph finally reaches the putative aromatic binding site of the MC1R hydrophobic area. The resultant compound LK-184 (**19**) has EC_{50} 0.01 nM at MC1 (ca. 10-fold better than the commonly used non-selective melanocortin agonist MT-I) and is ca. 500-fold more selective to MC1R compared to MC3 and MC4Rs (Table 1).

p-Cl-Phenyl derivatives **20**–**21** with extended conjugated π-systems have no advantages compared to their prototypes **17** and **18**. The introduction of an α-hydroxy group in **18** (compound **22**) decreases the potency at MC1 and MC3R but slightly increases it at MC4R. Sulfonyl derivatives **23**–**24** show essentially the same biological profile as their carbonyl analogues **2** and **16**.

In summary, using the series of end-capped tetrapeptides **2**–**24** we have demonstrated that human MC1, MC3, and MC4Rs share a common feature- a rather large hydrophobic area adjacent to the polar His[6] and N-terminal amide binding sites for the core sequence His[6]-D-Phe[7]-Arg[8]-Trp[9]-NH_2 (Fig. 1). This hydrophobic area seems to be more confined for hMC3R as compared to the 'open' region in hMC1 and hMC4Rs. The hydrophobic area in hMC1R has a putative aromatic π-binding zone about 3 carbons apart from the N-terminal binding site (Fig. 1). This knowledge allowed us to differentiate hMC1 and hMC3/4Rs and design LK-184 (**19**) (Table 1), a sub-nanomolar MC1R agonist with high MC1R versus MC3/MC4Rs selectivity.

Acknowledgements

This work was funded by a grant from Procter and Gamble Pharmaceuticals to J.J.K.

References and Notes

1. *The Melanocortin Receptors*. Cone, R. D., Ed. Humana: Totowa, 2000.
2. Hruby, V. J.; Wilkes, B. C.; Cody, W. L.; Sawyer, T. K.; Hadley, M. E. *Peptide and Protein Rev*. **1984**, *3*, 1.
3. Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Cone, R. D. *Science* **1992**, *257*, 1248.
4. Lu, D.; Väge, D. I.; Cone, R. D. *Mol. Endocr*. **1998**, *12*, 592.
5. Chen, A. S.; Marsh, D. J.; Trumbauer, M. E.; Frazier, E. G.; Guan, X. M.; Yu, H.; Rosenblum, C. I.; Vongs, A.; Feng, Y.; Cao, L.; Metzeger, J. M.; Strack, A. M.; Camacho, R. E.; Mellin, T. N.; Nunes, C. N.; Min, W.; Fischer, J.; Gopal-Truter, S.; MacIntyre, D. E.; Chen, H. Y.; Van der Ploeg, L. H. *Nat. Genet*. **2000**, *26*, 97.
6. Butler, A. A.; Kesterson, R. A.; Khong, K.; Cullen, M. J.; Pelleymounter, M. A.; Dekoning, J.; Baetscher, M.; Cone, R. D. *Endocrinology* **2000**, *141*, 3518.
7. Huszar, D.; Lynch, C. A.; Fairchild-Huntress, V.; Dunmore, J. H.; Smith, F. J.; Kesterson, R. A.; Boston, B. A.; Fang, Q.; Berkemeir, L. R.; Gu, W.; Cone, R. D. *Cell* **1997**, *88*, 131.
8. Hruby, V. J.; Wilkes, B. C.; Hadley, M. E.; Al-Obeidi, F.; Sawyer, T. K.; Staples, D. J.; DeVaux, A.; Dym, O.; Castrucci, A. M.; Hintz, M. F.; Riehm, J. P.; Rao, K. R. *J. Med. Chem*. **1987**, *30*, 2126.
9. Castrucci, A. M.; Hadley, M. E.; Sawyer, T. K.; Wilkes, B. C.; Al-Obeidi, F.; Staples, D. J.; DeVaux, A.; Dym, O.; Hintz, M. F.; Riehm, J. P.; Rao, K. R. J.; Hruby, V. J. *Gen. Comp. Endocrinol*. **1989**, *73*, 157.
10. Haskell-Luevano, C.; Sawyer, T. K.; Hendrata, S.; North, C.; Panahinia, L.; Stum, M.; Staples, D. J.; Castrucci, A. M.; Hadley, M. E.; Hruby, V. J. *Peptides* **1996**, *17*, 995.
11. Sawyer, T. K.; Sunfilippo, P. J.; Hruby, V. J.; Engel, V. J.; Heward, C. B.; Burnett, J. B.; Hadley, M. E. *Proc. Natl. Acad. Sci. U.S.A*. **1980**, *77*, 5754.
12. Al-Obeidi, F.; de Castrucci, L.; Hadley, M. E.; Hruby, V. J. *J. Med. Chem*. **1989**, *32*, 2555.
13. Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Van der Ploeg, L. H. T.; Weinberg, D. H. *Biochem. Biophys. Res. Comms*. **1999**, *261*, 209.
14. Bednarek, M. A.; Silva, M. V.; Arison, B.; MacNeil, T.; Kalyani, R. N.; Huang, R.-R. C.; Weinberg, D. H. *Peptides* **1999**, *20*, 401.
15. Prusis, P.; Muceniece, R.; Mutule, I.; Mutulis, F.; Wikberg, J. E. S. *Eur. J. Med. Chem*. **2001**, *36*, 137.
16. Yang, Y. K.; Fong, T. M; Dickinson, C. J.; Mao, C.; Li, J.-Y.; Tota, M. R.; Mosley, R.; Van der Ploeg, L. H. T.; Gantz, I. *Biochem*. **2000**, *39*, 14900.
17. Haskell-Luevano, C.; Holder, J. R.; Monck, E. K.; Bauzo, R. M. *J. Med. Chem*. **2001**, *44*, 2247.

18. Koikov, L. N.; Knittel, J. J.; Solinsky, M. G.; Cross-Doersen D.; Ebetino F. H. *Abstracts of Papers*, 224 National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 2002; Abstract 337.
19. Marques, F. F.; Holder, J. R.; Bauzo, R.; Xiang, Z. and Haskell-Luevano, C. *Abstracts of Papers*, 223 National Meeting of the American Chemical Society, Orlando, FL; American Chemical Society: Washington, DC, 2002; Abstract 74.
20. Holder, J. R.; Marques, F. F.; Xiang, Z.; Bauzo, R.; Haskell-Luevano, C. *Eur. J. Pharm.* **2003**, *462*, 41.
21. Cheung, A. W.-H.; Danho, W.; Swistok, J.; Qi, L.; Kurylko, G.; Franco, L.; Yagaloff, K. A.; Chen, L *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2407.