Synthesis and Biological Evaluation in Vitro of a Selective, High Potency Peptide Agonist of Human Melanin-concentrating Hormone Action at Human Melanin-concentrating Hormone Receptor 1*

Maria A. Bednarek‡§, Carina Tan‡, Donna L. Hreniuk‡, Oksana C. Palyha¶, Douglas J. MacNeil§, Lex H. Y. Van der Ploeg¶, Andrew D. Howard¶, and Scott D. Feighner∥

From the Departments of ‡Medicinal Chemistry and §Obesity and Metabolic Disorders, Merck Research Laboratories, Rahway, New Jersey 07065

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Human melanin-concentrating hormone (hMCH) is a nonselective natural ligand for the human melanin-concentrating hormone receptors: hMCH-1R and hMCH-2R. Similarly, the smaller peptide encompassing the disulfide ring and Arg6 of hMCH, Ac-Arg6-cyclo(S-S)(Cys7-Met8-Leu9-Gly10-Arg11-Val12-Tyr13-Arg14-Pro15-Cys16)-NH2, Ac-hMCH(6–16)-NH2, binds to and activates equally well both human MCH receptors present in the brain. To separate the physiological functions of hMCH-1R from those of hMCH-2R, new potent and hMCH-1R selective agonists are necessary. In the present study, analogs of Ac-hMCH(6–16)-NH2 were prepared and tested in binding and functional assays on cells expressing the MCH receptors. In these peptides, Arg in position 6 was replaced with various d-amino acids and/or Gly in position 10 was substituted with various l-amino acids. Several of the new compounds turned out to be potent agonists at hMCH-1R with improved selectivity over hMCH-2R. For example, peptide 26 with d-Arg in place of l-Arg in position 6 and Asn in place of Gly in position 10, Ac-d-Arg6-cyclo(S-S)(Cys7-Met8-Leu9-Asn10-Arg11-Val12-Tyr13-Arg14-Pro15-Cys16)-NH2, was a potent hMCH-1R agonist (IC50 = 0.5 nM, EC50 = 47 nM) with more than 200-fold selectivity with respect to hMCH-2R. Apparently, these structural changes in positions 6 and 10 result in peptide conformations that allow for efficient interactions with hMCH-1R but are unfavorable for molecular recognition at hMCH-2R.

In the last couple of years, melanin-concentrating hormone (MCH) has emerged as an important regulator of feeding behavior in rodents (1–7). Similarly to neuropeptide Y, this hormone stimulates appetite in rats when injected intracerebroventricularly, and this orexigenic effect is inhibited by anorectic peptides such as α-melanocyte stimulating hormone, glucagon-like peptide 1, and neuropeptide F. In hypothalamic nuclei, in addition to orexigenic effects, MCH also has an anorectic effect and stimulates the release of corticotropin releasing factor and adrenocorticotropin (8). Moreover, MCH has been retained for all cyclic analogs of this neuropeptide.

In humans, this 19-amino acid cyclic peptide is found in the brain, in the lateral hypothalamus and the zona incerta, and acts through specific receptors (8–12). At present, two receptors are known with which MCH interacts: hMCH-1R and hMCH-2R (13–23). These receptors are members of the family of G-protein-coupled receptors and their activation leads to mobilization of intracellular calcium. Binding of hMCH to hMCH-1R results in reduction of forskolin-elevated cyclic AMP levels, but binding to hMCH-2R does not cause this effect. The physiological role of hMCH-2R is less well understood than the physiological role of hMCH-1R, but the presence of the MCH-2R messenger RNA in the brain regions implicated in the regulation of body weight suggests that this receptor might also be involved in the regulation of feeding behavior (19–23).

To understand and separate the physiological functions of the MCH receptors, selective agonists are required, because MCH is a nonselective natural ligand for both hMCH-1R and hMCH-2R. Similarly, the synthetic ligands reported in the literature do not distinguish between the receptors (13–23).

Our previous structure-function studies on hMCH yielded a cyclic peptide consisting of the disulfide ring and Arg6 of hMCH: the so-called “active core” of hMCH with the four residues, Arg6, Met8, Arg11, and Tyr13, critical for molecular recognition at hMCH-1R and hMCH-2R (24–27). This compound, Ac-hMCH(6–16)-NH2 (Structure 2), was equipotent to the full-length hMCH at both receptors.2


STRUCTURE 2

Moreover, at hMCH-1R, an analog of Ac-hMCH(6–16)-NH2 with d-Arg in position 6 was as potent as the parent compound, but, at hMCH-2R, this analog was a noticeably weaker agonist. The d-Arg6 compound was the first described peptide agonist with enhanced hMCH-1R selectivity with respect to hMCH-2R. The study presented here was designed to expand on the...
above observation and prepare potent agonists of high hMCH-1R selectivity. First, analogs of Ac-hMCH(6–16)-NH2 were synthesized in which Arg in position 6 was replaced with various α-amino acids, with anticipation that the hMCH-1R selectivity of these peptides will be improved. In the binding and calcium release assays at the human MCH receptors, most of the new compounds displayed lower affinity and potency at hMCH-2R than hMCH-1R. Further, structure-function studies on hMCH(6–16)-NH2 revealed that incorporation of various α-amino acids in place of Gly10 affects interactions of the new analogs with hMCH-2R but not with hMCH-1R.

These observations led us to design analogs of Ac-hMCH(6–16)-NH2 modified at both positions 6 and 10. Syntheses and biological evaluation in vitro at hMCH-1R and hMCH-2R of several cyclic peptides that are potent and selective hMCH-1R agonists is reported here.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis, Purification, and Characterization (24)—Elongation of peptide chains on 4-(2′,4′-dimethoxybenzyl)-Fmoc (N-(9-fluorenylmethoxycarbonyl)-aminomethyl)-phenoxy resin, deprotection and cleavage of peptides from a resin, and formation of the disulfide ring in solution were performed as previously described in detail (24).** The lyophilized crude peptides were analyzed by analytical reverse-phase high pressure liquid chromatography (HPLC) on a C18 Vydac column attached to a Waters 600E system with automatic Wisp 712 injector and 991 Photodiode Array detector. A standard gradient system of 0–100% buffer B in 30 min was used for analysis; buffer A was 0.1% trifluoroacetic acid in water, and buffer B was 0.1% trifluoroacetic acid in acetonitrile. HPLC profiles were recorded at 210 and 280 nm. Preparative separations were performed on a Waters Delta Prep 4000 system with a semipreparative C18 RP Waters column. The above-described solvent system of water and acetonitrile, in a gradient of 0–70% buffer B in 60 min, was used for separation. The chromatographically homogeneous products (purity >97%) were analyzed by electrospray mass spectrometry.

**hMCH-1R and MCH-2R Radioligand Filter Binding Assays—Membrane binding assays were performed on transiently transfected COS-7 cells expressing human MCH-2R from the plasmid vector pCI-neo (Promega, Madison, WI), and a CHO cell line stably expressing human MCH-1R from pcDNA3.1. For transient expression, COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% heat-inactivated fetal calf serum. A suspension of 7 × 10^6 COS-7 cells were transplanted with 20 µg of pCI-neo/MCH-2R plasmid by electroporation (26), and cells were harvested after 60–72 h. Membranes were prepared from transient and stable transfectants by hypotonic lysis, frozen in liquid nitrogen, and stored at −80 °C. A filter binding assay was developed to measure the specific binding of [125I]Phe13,Tyr19]hMCH displaced at 10 µM peptide concentration.

**Aequorin Bioluminescence Functional Assays (24, 26, 27)—** Functional receptor activation assays, stable cell lines expressing either the MCH-1R or the MCH-2R and the aequorin reporter protein were written for a PC-compatible computer. 293AEQ17/MCH-1R (or MCH-2R) cells were measured in three different experiments using wheat germ agglutinin-polyvinyltoluene beads (Amersham Biosciences), in 96-well OptiPlates (Packard, Meriden, CT). Each well contained 0.5–10 µg of membrane protein and 200 µl of binding buffer (50 mM Tris, pH 7.4, 10 mM MgCl2, 2 mM EDTA, 12% glycerol, 0.1% bovine serum albumin). Binding buffer contained 50 mM Tris, pH 7.4, 10 mM MgCl2, 2 mM EDTA, and protease inhibitors; 200 µM μg/mL bacitracin (Sigma), 1 µM phosphoramidon (Peninsula Laboratories), Assays were optimized with respect to membrane preparations: for CHO/ MCH-1R membranes, 1 µg of membranes/well yielded a 10× specific binding window and for COS MCH-2R membranes, 8 µg of membrane protein yielded a window of 8×. Specific binding is defined as the difference between total binding and nonspecific binding conducted in the presence of 500 nM unlabeled hMCH. In 96-well dishes, the membranes were combined with peptide at various dilutions and the radioligand [125I]Phe13,Tyr19]hMCH at 0.3 nM final concentration and incubated at room temperature for 1 h. The membrane-bound counts were collected by filter harvesting through a Filtermate harvester (Packard Instruments) and washing with binding buffer as described above with added 0.04% Tween detergent, dried, scintillant added, and the plates were read in a TopCount (Packard). IC50 calculations were performed using Prism 3.0 (GraphPad Software, San Diego, CA). The IC50 values were measured in three different experiments.

**Table 1**

<table>
<thead>
<tr>
<th>Ac</th>
<th>No.</th>
<th>Binding assay</th>
<th>Aequorin functional assay</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MCH-1R IC50</td>
<td>MCH-2R IC50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nM</td>
<td>nM</td>
</tr>
<tr>
<td>hMCH</td>
<td>0.17 ± 0.01</td>
<td>2.9 ± 2.7</td>
<td>17</td>
</tr>
<tr>
<td>Ac-hMCH(6–16)-NH2</td>
<td>0.16 ± 0.03</td>
<td>2.7 ± 1.1</td>
<td>17</td>
</tr>
<tr>
<td>Ac-6-Arg</td>
<td>0.25 ± 0.22</td>
<td>650 ± 110</td>
<td>2600</td>
</tr>
<tr>
<td>1</td>
<td>Gva</td>
<td>0.37 ± 0.16</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>d-Arg</td>
<td>0.35 ± 0.20</td>
<td>47 ± 17</td>
</tr>
<tr>
<td>3</td>
<td>Ac-6-Ala</td>
<td>0.77 ± 0.13</td>
<td>2700 ± 410</td>
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<tr>
<td>4</td>
<td>Ac-6-Nle</td>
<td>4 ± 3</td>
<td>1460 ± 270</td>
</tr>
<tr>
<td>5</td>
<td>Ac-6-Pro</td>
<td>1.6 ± 1.1</td>
<td>1260 ± 180</td>
</tr>
<tr>
<td>6</td>
<td>Ac-6-Phe</td>
<td>0.9 ± 1.1</td>
<td>1250 ± 220</td>
</tr>
<tr>
<td>7</td>
<td>Ac-6-Asn</td>
<td>5.8 ± 0.8</td>
<td>4040 ± 900</td>
</tr>
<tr>
<td>8</td>
<td>Ac-6-Ser</td>
<td>0.64 ± 0.05</td>
<td>3000 ± 510</td>
</tr>
<tr>
<td>9</td>
<td>Ac-6-Glu</td>
<td>16 ± 4</td>
<td>6900 ± 1900</td>
</tr>
<tr>
<td>10</td>
<td>Ac-6-Lys</td>
<td>0.27 ± 0.21</td>
<td>1030 ± 500</td>
</tr>
<tr>
<td>11</td>
<td>Ac-6-Cit</td>
<td>3.1 ± 1.9</td>
<td>114 ± 72</td>
</tr>
<tr>
<td>12</td>
<td>Ac-6,D-Pro,Glu</td>
<td>7 ± 2</td>
<td>3500 ± 1600</td>
</tr>
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</table>

**IC50 is a concentration of peptide at 50% specific binding. When peptide is not able to reach 50% specific binding, the percentage of IC50 is a concentration of peptide at 50% maximum calcium accumulation. 100% activation is the bioluminescence value obtained with 10 µM hMCH.**

**RESULTS**

Analogs of hMCH listed in Tables I–III were prepared by solid-phase syntheses as described under “Experimental Procedures.” They were evaluated for their respective binding affinities for cloned human MCH receptor 1 and 2 in the competition binding assays with [125I]-[Phe13,Tyr19]hMCH as the radiolabeled ligand (28) and, also, for their ability to stimulate inositol trisphosphate-coupled mobilization of intracellular calcium in human HEK-293 cells expressing hMCH-1R and hMCH-2R (24, 29, 30).

Binding and functional data for analogs of Ac-hMCH(6–16)-NH₃ modified in position 6 are compiled in Table I. Omission of Ac and the amino group of Arg⁶, through incorporation of 5-guanidinovaleric acid (des-aminocaragine) in position 6, resulted in compound 1, which was not a fully effective agonist at hMCH-1R (29% activation at 10 μM concentration), but was a full agonist at hMCH-2R of potency similar to that of the parent compound. In contrast, at hMCH-1R, analog 2 without the Ac group but with D-Arg in position 6 was equipotent to Ac-hMCH(6–16)-NH₃ but, at hMCH-2R, this des-acetyl-peptide was an ~10-fold weaker agonist.

In compounds 3–10, D-enantiomers of hydrophobic amino acids, Ala, Nle, Pro, and Phe, and hydrophilic amino acids, Asn, Ser, Glu and Lys, were incorporated in position 6 of hMCH(6–16)-NH₃. The new peptides were efficient binders to hMCH-1R, but their signal transduction efficacies at this receptor were more than 4-fold lower than those of Ac-hMCH(6–16)-NH₃. These compounds poorly activated the second hMCH receptor, thus showing the enhanced selectivity for hMCH-1R. Replacement of Arg⁶ with D-enantiomer of citrulline, yielded analog 11 of binding affinity for hMCH-1R diminished ~20-fold and of agonist potency at both MCH receptors significantly reduced (>50-fold).

In analog 12, the Ac-Arg⁶ segment of Ac-hMCH(6–16)-NH₃ was omitted and 3-mercaptopropanic acid (des-aminocysteine) was used instead of Cys in position 7 to form the disulfide ring. This peptide showed ~40-fold lower binding affinity and activity at hMCH-1R than Ac-hMCH(6–16)-NH₃. Similarly to the other peptides modified at position 6, compound 12 was a very weak ligand for hMCH-2R (IC₅₀ and EC₅₀ > 3000 nM).

The disulfide cycle of Ac-hMCH(6–16)-NH₃ encompasses Gly in position 10. This residue is known to facilitate formation of reversed turns in peptide chains. To test the effect of conformational changes in position 10 on biological activity, analogs of Ac-hMCH(6–16)-NH₃ were synthesized and tested in which various l-amino acids were incorporated in place of Gly¹⁰; their binding and functional data are compiled in Table II.

Replacement of Gly¹⁰ with hydrophobic l-amino acids which possess long, branched, or aromatic side chains yielded compounds 13–18 (Ala¹⁰, Leu¹⁰, Nle¹⁰, Cha¹⁰, Phe¹⁰, and (2′)Nal¹⁰ analogs). These peptides bound to hMCH-1R almost as efficiently as the parent compound but, at hMCH-2R, these analogs were 2–10 times less potent. The Cha¹⁰ analog with the bulky branched side chain in position 10, compound 16, activated poorly both receptors. Additionally, incorporation of the conformationally constraining Pro in place of Gly¹⁰ was deleterious to agonism at both hMCH receptors; the Pro¹⁰ analog was virtually inactive at micromolar concentrations (peptide 19).

In compounds 20–25, ionogenic residues were incorporated in position 10. Peptides with Lys, Asn, Ser, and Cit in this position were high affinity binders to hMCH-1R, but their affinities for hMCH-2R were 20–700 times lower than that of Ac-hMCH(6–16)-NH₃. At both hMCH receptors, the Asn¹⁰, Ser¹⁰, and Cit¹⁰ analogs were 2–50-fold weaker agonists than the parent compound, but the Lys¹⁰ analog was even less potent (more than 250 times). Peptide 25 with an acidic residue in position 10, the Glu¹⁰ analog, was virtually inactive at micromolar concentrations.

The cyclic peptides listed in Table III, compounds 26–31, were designed to incorporate changes in the structure of Ac-hMCH(6–16)-NH₃, which were reported above as favorable for hMCH-1R selectivity. Hence, in compounds 26–31, amino acid residues in both positions 6 and 10 were replaced; Arg⁶ with D-Arg or D-Cit, and Gly¹⁰ with Asn or Gln. The new peptides turned out to be poor ligands for hMCH-2R, IC₅₀ > 1000 nM, EC₅₀ > 1300 nM. However, the D-Arg⁶,Asn¹⁰ analog, peptide 26, activated hMCH-1R almost as efficiently as Ac-hMCH(6–16)-NH₃ and, thus, was more than 200-fold selective with respect to hMCH-2R. Fig. 1 depicts binding and functional agonism of compound 26 (hMCH-1R selective agonist) and the parent peptide, Ac-hMCH(6–16)-NH₃ (nonselective agonist at hMCH-1R and hMCH-2R). Rather conservative replacement of
Asn in position 10 with Gln turned out to be detrimental to potency at hMCH-1R. The D-Arg6,Gln10 analog was 50-fold weaker agonist at hMCH-1R (EC50 = 2300 nM) than the D-Arg6,Asn10 peptide.

In peptides 28 and 29, the critical for molecular recognition Met8 of peptide 26 was replaced with the isosteric Nle. The new peptides were more than 5-fold less potent at hMCH-1R than compound 26.

Omission of the guanidine group in position 14 in compound 26, through the replacement of Arg with Ala, yielded peptide 30, which was 5-fold weaker agonist at hMCH-1R than the parent compound. Also unfavorable to molecular recognition at

### Table III

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Binding assay&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Aequorin functional assay&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hMCH-1R IC50</td>
<td>hMCH-2R IC50</td>
</tr>
<tr>
<td>hMCH</td>
<td>0.17 ± 0.01</td>
<td>2.9 ± 2.7</td>
<td>17</td>
</tr>
<tr>
<td>Ac-hMCH(6–16)-NH2</td>
<td>0.16 ± 0.03</td>
<td>2.7 ± 1</td>
<td>17</td>
</tr>
<tr>
<td>26</td>
<td>D-Arg6,Asn10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>6.1 ± 2.5</td>
<td>1400 ± 260</td>
</tr>
<tr>
<td>27</td>
<td>D-Arg6,Gln10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.28 ± 0.17</td>
<td>1600 ± 210</td>
</tr>
<tr>
<td>29</td>
<td>ΔAc,D-Arg6,Nle6,Asn10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>3.2 ± 1</td>
<td>48 ± 9%@10</td>
</tr>
<tr>
<td>30</td>
<td>D-Arg6,Asn10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>8.3 ± 6.7</td>
<td>55 ± 7%@10</td>
</tr>
<tr>
<td>31</td>
<td>ΔCit6,Asn10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>8.3 ± 6.7</td>
<td>55 ± 7%@10</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC50 is a concentration of peptide at 50% specific binding. When peptide is not able to reach 50% specific binding, the percentage of 125I-[Phe13,Tyr19]hMCH displaced at 10 μM peptide concentration is reported.

<sup>b</sup>EC50 is the concentration of peptide at 50% maximum calcium accumulation. 100% activation is the bioluminescence value obtained with 10 μM hMCH.

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**Fig. 1.** Binding and functional activities of selected MCH peptides: Ac-hMCH(6–16)-NH2 and analog 26. Panels A and C represent binding and function of these peptides on the human MCH-1R, respectively, and panels B and D represent binding and function of the same peptides on the human MCH-2R, respectively.
hMCH-1R was replacement of D-Arg in position 6 of compound 26 with d-citrulline; the d-Cit\textsuperscript{6},Asn\textsuperscript{10} analog (compound 31) was a weak hMCH-1R agonist of EC\textsubscript{50} = 1500 nM.

**DISCUSSION**

Previous structure-function studies (24) on a short analog of hMCH, the Ac-hMCH(6–16)-NH\textsubscript{2} peptide, indicated that chirality of Arg\textsuperscript{6} determines, to some extent, the receptor selectivity of peptide agonists; the D-Arg\textsuperscript{6} analog was ~4 times more potent at hMCH-1R than hMCH-2R. We concluded (24) that, for maximum agonist activity at hMCH-1R, the side chain of Arg\textsuperscript{6} does not need to be in a preferred orientation but, for hMCH-2R activation, the side chain orientation provided by the chiral center of D-Arg\textsuperscript{6} is desired. These conclusions were supported in the present study through testing in binding and functional assays at hMCH-1R and hMCH-2R of several Ac-hMCH(6–16)-NH\textsubscript{2} analogs with various D-amino acid residues in position 6. Similarly to the D-Arg\textsuperscript{6} peptide, these compounds displayed enhanced selectivity for hMCH-1R. At hMCH-2R, peptides with hydrophobic, hydrophilic, basic, or acidic side chains in position 6 were significantly weaker binders and agonists than Ac-hMCH(6–16)-NH\textsubscript{2}. In contrast, at hMCH-1R, their binding affinities were similar to those of Ac-hMCH(6–16)-NH\textsubscript{2} and its D-Arg\textsuperscript{6} analog, but their agonist potencies were noticeably lower. In fact, none of the new compounds was more potent at, and selective for, hMCH-1R than the D-Arg\textsuperscript{6} analog. This confirmed yet again that the guanidine group in the side chain of residue 6 is essential for maximum activity at hMCH-1R (24–27), and that its orientation allowed by the D-chiral center contributes to hMCH-1R selectivity.

The disulfide ring of the Ac-hMCH(6–16)-NH\textsubscript{2} peptide encompasses two residues, Gly and Pro, known to facilitate formation of bends in peptide chains. Although conformationally constrained pyrrolidine ring of the Pro residue imposes steric restrictions on the peptide backbone, the flexible chain of the Gly residue is able to adopt various conformations. To stabilize some of the low energy conformers of biological significance, Gly is frequently replaced with sterically constraining amino acids such as o-amino acids (L or D) or o,\alpha-di-alkylated amino acids. These replacements are usually done with anticipation that changes observed in peptide-receptor recognition pattern can indicate conformational requirements necessary for ligand-receptor interactions and aid to the design of new potent and selective agonist and antagonists.

In the present study, the effect on biological activity of Ac-hMCH(6–16)-NH\textsubscript{2} of conformational changes in position 10 was explored through analogs in which Gly\textsuperscript{10} was replaced with various L-amino acids. Peptides with hydrophobic, linear, or branched side chains in position 10 (the Ala\textsuperscript{10}, Leu\textsuperscript{10}, or Nle\textsuperscript{10} analogs), and with the hydrophobic aromatic side chain in the same position (the Phe\textsuperscript{10} analog), were slightly less potent at both receptors than the parent compound, suggesting that their biologically active conformations are rather similar. However, further decrease of conformational freedom in analogs with bulky Cha or (2′)Nal in position 10 affected significantly the interactions with hMCH-2R and hMCH-1R. Thus, the side chains of Cha\textsuperscript{10} and (2′)Nal\textsuperscript{10} unfavorably alter the conformation of the peptide backbone and/or hinder formation of ligand-receptor complexes.

The steric constrain introduced by Pro in position 10 was deleterious to binding and activity at both receptors. Inability of the Pro\textsuperscript{10} analog to activate hMCH-1R and hMCH-2R even at micromolar concentrations could also be attributed to the lack of free \(\alpha\)NH in position 10 available for H-bond formation. However, the only slightly lower agonist potency of a further analog lacking free \(\alpha\)NH in position 10, the N-Me-Gly\textsuperscript{10} peptide,\textsuperscript{3} did not support this assumption. This suggested that potential H-bonds involving \(\alpha\)NH of Gly\textsuperscript{10} do not contribute significantly to the stabilization of the biologically active conformers and/or peptide-receptor complexes.

The effect of conformational changes in position 10 on biological activity of Ac-hMCH(6–16)-NH\textsubscript{2} was further studied in conjunction with potential ionic interactions between the residues in position 10 and the MCH receptors. Evaluation of the Arg\textsuperscript{10}, Lys\textsuperscript{10}, and Glu\textsuperscript{10} analogs of Ac-hMCH(6–16)-NH\textsubscript{2} in binding and functional assays revealed that both basic and acidic side chains in position 10 are deleterious to agonism at the MCH receptors. These side chains might be repelled by identical charges in the side chains of the receptors or might form salt bridges and new H-bonds unfavorable to stabilization of biologically active conformations or ligand-receptor complexes. At hMCH-1R, binding and agonist activities of compounds with the hydrophilic side chains of Asn and Ser in position 10 were similar to, or slightly lower than, those of Ac-hMCH(6–16)-NH\textsubscript{2}. This seems to suggest that, in the ligand-receptor complexes, these hydrophilic side chains are not in direct contact with the receptors but are facing the outside environment. In contrast, the side chain of Asn\textsuperscript{10} appears to affect significantly interactions with hMCH-2R; this analog was ~40 times less active at hMCH-2R than the parent compound. As a consequence of these differences in molecular recognition pattern of compounds with \(\alpha\)-amino acids in place of Gly\textsuperscript{10}, the Asn\textsuperscript{10} analog emerged as the most selective high affinity ligand for hMCH-1R.

Subsequently, the cyclic peptide designed to accommodate structural changes at both positions 6 and 10 of Ac-hMCH(6–16)-NH\textsubscript{2}, described above as the most favorable for high potency and selectivity at hMCH-1R, was evaluated. The new compound with D-Arg\textsuperscript{6} and Asn\textsuperscript{10} analog 26 (Structure 3), was a potent high affinity agonist at hMCH-1R (IC\textsubscript{50} = 0.5 nM, EC\textsubscript{50} = 47 nM) but a poor ligand for hMCH-2R (see also Fig. 1).

\[ \text{Ac-D-Arg}^6-\text{Cys}^7-\text{Met}^8-\text{Leu}^9-\text{Asn}^{10}-\text{Arg}^{11}-\text{Val}^{12}-\text{Tyr}^{13}-\text{Arg}^{14}-\text{Pro}^{15}-\text{Cys}^{16}-\text{NH}_2 \]

**Structure 3**

It displayed ~6000 times higher binding affinity and more than 200-fold higher agonist potency at hMCH-1R than hMCH-2R. Apparently, the combined structural changes evoked by D-Arg in position 6 and Asn in position 10 resulted in peptide conformations that were unfavorable to molecular recognition at hMCH-2R but allowed efficient interactions with hMCH-1R.

To gain further insight into the conformational properties of our new hMCH-1R selective agonist, several analogs of compound 26 were synthesized and evaluated in binding and functional assays at both hMCH-1R and hMCH-2R. Similarly to the parent peptide, these compounds were virtually inactive at hMCH-2R and, rather unexpectedly, their binding affinities and agonist potencies were noticeably lower at hMCH-1R than those of Ac-hMCH(6–16)-NH\textsubscript{2}. Replacement of Asn\textsuperscript{10} in position 10 of compound 26 with Gln showed that the side chain of Gln in position 10, merely one carbon atom longer than the side chain of Asn, significantly disturbs molecular recognition at hMCH-1R. Additionally, potency and selectivity of compound 26 at hMCH-1R were affected by incorporation of Nle in place of Met\textsuperscript{8} (compound 28), or by the simultaneous omission of the N-terminal acetyl group (compound 29). The observed drop of potency was larger than previously reported (24) for other analogs of Ac-hMCH(6–16)-NH\textsubscript{2} with Nle\textsuperscript{8}. Not unexpectedly, substitution of Arg\textsuperscript{14} with Ala in compound 26 yielded peptide 28, that was only 4-fold less potent at hMCH-1R than the parent compound, thus confirming that the Arg\textsuperscript{14} side chain is not essential for ligand-receptor interactions (24–27).

Our present study yielded a cyclic peptide that is a potent and selective agonist at hMCH-1R. The new compound is an
We are continuing our investigation in this direction. The insight gained in this study should also be helpful in the design of new agonists; we are continuing our investigation in this direction.

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Synthesis and Biological Evaluation in Vitro of a Selective, High Potency Peptide Agonist of Human Melanin-concentrating Hormone Action at Human Melanin-concentrating Hormone Receptor 1

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