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Asp^{10} in Lys-γ_2-MSH determines selective activation of the melanocortin MC_3 receptor

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Abstract

The melanocortin MC_3 and MC_4 receptors are the main melanocortin receptors expressed in brain. Of the endogenous melanocortins, γ_2-melanocortin stimulating hormone (MSH) selectively activates the melanocortin MC_3 receptor, whereas α- and β-MSH activate all melanocortin receptors. The aim was to gain an insight into the contribution of amino acids in positions 5 and 10 of melanocortins to the selectivity of [Nle^4]Lys-γ_2-MSH for the melanocortin MC_3 receptor versus the melanocortin MC_4 receptor. Introduction of Asp^{10} into [Nle^4]α-MSH as in [Nle^4,Gly^5,Asp^{10}]α-MSH selectively increased the EC_{50} value for the melanocortin MC_4 receptor. Conversely, removal of Asp^{10}, as in [Nle^4,Gly^{10}]Lys-γ_2-MSH, selectively decreased the EC_{50} value for the melanocortin MC_4 receptor. Thus, Asp^{10} in Lys-γ_2-MSH determined selectivity for the melanocortin MC_3 receptor versus the melanocortin MC_4 receptor.

Keywords: Melanocortin receptor; ACTH; MSH

The mechanisms underlying the diversity of effects of melanocortins on the brain are poorly understood. The melanocortin MC_3 and MC_4 receptors are the main melanocortin receptors expressed in brain (Low et al., 1994). Therefore, it is important to identify and design ligands that can discriminate between activation of the melanocortin MC_3 receptor versus that of the melanocortin MC_4 receptor. Of the endogenous melanocortins, γ_2-melanocortin-stimulating hormone (MSH) selectively activates the melanocortin MC_3 receptor, whereas α- and β-MSH activate all melanocortin receptors except the melanocortin MC_2 (adrenocorticotropic hormone: ACTH) receptor (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992, 1994; Roselli-Rehfuss et al., 1993). α-MSH and Lys-γ_2-MSH contain an identical core sequence, His–Phe–Arg–Trp, a Tyr residue on position 2 and a Met residue on position 4. It has been demonstrated that position 12 in α-MSH is critical for melanocortin MC_3/MC_4 receptor selectivity (Miwa et al., 1995). These latter authors demonstrated that substitution of Phe^{12} for Pro^{12} into γ_2-MSH, as in α-MSH resulted in a 6-fold increase in melanocortin MC_4 receptor activity. However, the chemical nature of the amino acid residues at position 5 and 10 is very different, a negatively charged amino acid side-chain versus an uncharged side-chain, i.e., Glu^5 and Gly^{10} in α-MSH versus Gly^5 and Asp^{10} in Lys-γ_2-MSH. To find to what extent these positions determine selective receptor activation, we tested α-MSH and γ_2-MSH derivatives with exchanged amino acid residues on position 5 and 10 on in vitro activation of the melanocortin MC_3 receptor and the melanocortin MC_4 receptor. To exclude an influence of peptide length, N- and C-terminal modifications and oxidation of the Met^4 residue, we used synthetic [Nle^4]α-MSH (Ac-Ser-Tyr-Ser-Nle-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) and [Nle^4]Ac-Lys-γ_2-MSH-NH_2 (Ac-Lys-Tyr-Val-Nle-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-NH_2) as reference peptides (the latter will be referred to as [Nle^4]Lys-γ_2-MSH).

[Nle^4]α-MSH, [Nle^4,Gly^5]α-MSH, [Nle^4,Gly^5,Asp^{10}]α-MSH, [Nle^4]Lys-γ_2-MSH and [Nle^4,Gly^{10}]Lys-γ_2-MSH were synthesized by solid phase Fmoc chemistry and purified as described by Schaaper et al. (1998). The products were analyzed using liquid chromatography-mass spectrometry (LC-MS). Ion spray mass spectrometry (MS) performed on a Micromass Quattro sq

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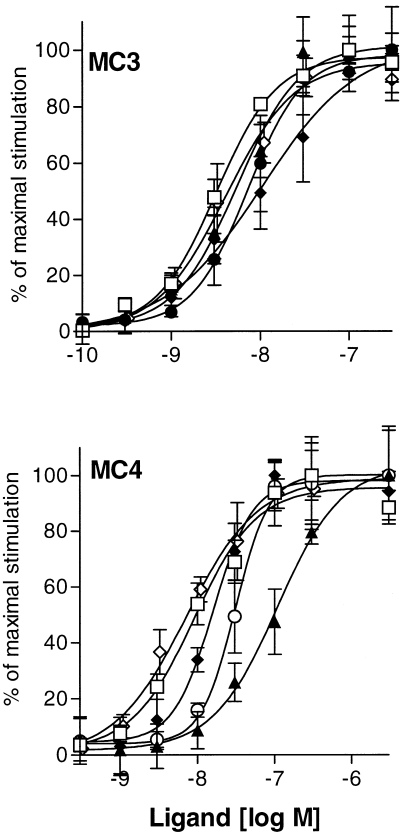
confirmed the expected molecular weights. The receptor activation assay makes use of the β-galactosidase (*lacZ*) gene fused to five copies of the cyclic AMP response element (CRE) to detect the activation of CRE-binding protein (CREB) resulting from increased intracellular cAMP and Ca^{2+} (Chen et al., 1995). Two hundred ninety-three human embryonal kidney (HEK) cells, expressing either the rat melanocortin MC_3 receptor (Roselli-Rehfuss et al., 1993) or the human melanocortin MC_4 receptor (Mountjoy et al., 1994), were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal calf serum. Approximately 7×10^6 cells were transfected with 7 μg of the pCRELacZ construct using the calcium phosphate precipitation method (Chen et al., 1995). Twenty hours after transfection 293 HEK cells were distributed into 96-well plates (Primaria). The next day agonist activity was measured by stimulation of the cells for 6 h with varying concentrations of peptides in DMEM supplemented with 0.5% bovine serum albumin, 25 mM HEPES (pH 7.4) and 50 μg/ml (150 kIU/ml) aprotinin (Sigma). After treatment, the cells were lysed in phosphate-buffered saline with 0.1% Triton X-100, frozen, thawed and assayed for β-galactosidase activity. Twelve data points for each peptide were measured in quadruplicate. EC_{50} values were then calculated with a 95% confidence interval, using GraphPad Prism software (sigmoidal dose–response curve fitting, variable slope). The data are expressed as percentages of the maximal receptor activation under these assay conditions. The measurements were repeated at least three times with the same results. The aim of this study was to gain insight into the contribution of amino acids in positions 5 and 10 of melanocortins to the selectivity of [Nle^4]Lys-γ_2-MSH for the melanocortin MC_3 receptor versus the melanocortin MC_4 receptor.

When Glu^5 of α-MSH was replaced by Gly^5, as in Lys-γ_2-MSH, the selectivity for the melanocortin MC_3 and melanocortin MC_4 receptors did not change significantly (Fig. 1). However, when an Asp^{10} as in Lys-γ_2-MSH was also introduced in this peptide, as in [Nle^4,Gly^5,Asp^{10}]α-MSH, affinity for the melanocortin MC_4 receptor decreased, whereas the affinity for the melanocortin MC_3 receptor remained unaffected. Conversely, when Asp^{10} in [Nle^4]Lys-γ_2-MSH was replaced by Gly^{10} as in α-MSH, the affinity for the melanocortin MC_3 receptor was unaltered whereas that for the melanocortin MC_4 receptor increased 7-fold. Thus, we showed Asp^{10} in Lys-γ_2-MSH to determine selectivity for the melanocortin MC_3 receptor versus the melanocortin MC_4 receptor. However, Asp^{10} probably does not interact directly with the melanocortin MC_3 receptor, since [Nle^4,Gly^{10}]Lys-γ_2-MSH has a potency similar to that of [Nle^4]Lys-γ_2-MSH for this receptor.

The identification of Asp^{10} as selectivity-driving residue will allow the design of further peptides with enhanced melanocortin MC_3 receptor selectivity. These peptides could serve as essential tools to delineate the physiological and pharmacological significance of the melanocortin MC_3 receptor. This study did not explain how Asp^{10} in Lys-γ_2-MSH is able to recognize critically the melanocortin MC_3 receptor. This will require further analysis of the structural properties of the melanocortin MC_3 receptor in conjunction with Lys-γ_2-MSH derivatives.



Ligand	MC_3 receptor	MC_4 receptor
[Nle^4]α-MSH	3.2×10^{-9}	9.1×10^{-9}
[Nle^4, Gly^5]α-MSH	4.0×10^{-9}	7.2×10^{-9}
[Nle^4, Gly^5, Asp^{10}]α-MSH	7.1×10^{-9}	3.1×10^{-8}
[Nle^4]Lys-γ_2-MSH	5.4×10^{-9}	1.1×10^{-7}
[Nle^4, Gly^{10}]Lys-γ_2-MSH	1.0×10^{-8}	1.6×10^{-8}

EC^{50} values [M] of melanocortin peptides on rat melanocortin MC_3 and melanocortin MC_4 receptors

Fig. 1. (a) Melanocortin MC_3 receptor activation by melanocortin peptides 293 HEK cells expressing the melanocortin MC_3 receptor were stimulated with various concentrations of [Nle^4]α-MSH (□), [Nle^4,Gly^5]α-MSH (◇), [Nle^4,Gly^5,Asp^{10}]α-MSH (○), [Nle^4]Lys-γ_2-MSH (▲) or [Nle^4,Gly^{10}]Lys-γ_2-MSH (◆). Data are expressed as percentages of maximal receptor activation (mean ± S.D.). (b) Melanocortin MC_4 receptor activation by melanocortin peptides 293 HEK cells expressing the melanocortin MC_4 receptor were stimulated with various concentrations of [Nle^4]α-MSH (□), [Nle^4,Gly^5]α-MSH (◇), [Nle^4,Gly^5,Asp^{10}]α-MSH (○), [Nle^4]Lys-γ_2-MSH (▲) or[Nle^4,Gly^{10}]Lys-γ_2-MSH (◆). Data are expressed as percentages of maximal receptor activation (mean ± S.D.).

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