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Selective properties of C- and N-terminals and core residues of the melanocyte-stimulating hormone on binding to the human melanocortin receptor subtypes

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Abstract

We synthesised nine analogues of [Nle^4,D-Phe^7]α-MSH (melanocyte-stimulating hormone) (NDP) where (1) the N- or C-terminals were deleted or exchanged by those of β- or γ-MSH and (2) the core residues His^6, Phe^7, Arg^8 and Trp^9 were individually substituted by Glu^6, β-(2-naphthyl)-D-alanine (D-Nal^7), Lys^8 and His^9, respectively. We tested these analogues in ligand binding assays with cells transiently expressing the human melanocortin MC_1, MC_3, MC_4 and MC_5 receptors. The results show that the N-terminal segment (Ser^1-Tyr^2-Ser^3) of NDP was not important for binding to melanocortin MC_1 and MC_4 receptors whereas it affects binding to melanocortin MC_3 and MC_5 receptors. The C-terminal segment (Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}) of NDP was clearly important for binding to all the four melanocortin receptor subtypes. The data indicate that the low affinity of γ-MSH for the melanocortin MC_4 receptor is due to its C-terminal (Asp^{10}-Arg^{11}-Phe^{12}). Substitution of D-Phe^7 by D-Nal^7 increased the affinity for the melanocortin MC_4 receptor but not for the other melanocortin receptor subtypes. The other core residue substitutions lowered the affinity in a differentiated manner for each of the melanocortin receptors. These results are valuable for the molecular modelling and design of selective drugs for the melanocortin receptors.

Keywords: Melanocortin receptor subtype; MSH (melanocyte-stimulating hormone); Ligand binding; C-terminal; N-terminal; Core residue

1. Introduction

Molecular cloning has led to the identification of a family of five receptors for the melanocortic peptides (Chhajlani et al., 1993; Chhajlani and Wikberg, 1992; Gantz et al., 1993a,b; Mountjoy et al., 1992). The first member of this family was the well-characterised melanocortin MC_1 receptor which is expressed in melanocytes and melanoma cells and binds α-MSH (melanocyte-stimulating hormone) with high affinity. The melanocortin MC_1 receptor plays an important role in skin and fur pigmentation in a variety of vertebrates (Cone et al., 1996). The melanocortin MC_2 receptor (i.e., the ACTH (adrenocorticotropin) receptor) has a well-defined function in the regulation of steroid production in the adrenal gland. Much less is known about the physiological roles of the three newly discovered melanocortin (MC) receptors (melanocortin MC_3, MC_4, and MC_5. The melanocortin MC_3 receptor is found in the brain and in the placenta, gut tissues and the heart (Gantz et al., 1993a; Chhajlani, 1996). The melanocortin MC_4 receptor is found only in the brain, where it is distributed in several areas (Mountjoy et al., 1994). The melanocortin MC_4 receptor has recently been found to affect feeding in rodents and it might be important for weight homeostasis (Huszar et al., 1997; Fan et al., 1997). The melanocortin MC_5 receptor, which is less well characterised, is primarily located in various peripheral tissues but has also been found in the brain (Labbé et al., 1994; Fathi et al., 1995).

The melanocortin MC_2 receptor binds ACTH with high affinity but not the MSH peptides (Schiöth et al., 1996b). The natural melanocortins (α-, β-, γ-MSH, ACTH) are bound to the other melanocortin receptors with a specific

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and individual affinity profile each, but still they all are bound with highest affinity by the melanocortin MC_1 receptor, with intermediate affinity by the melanocortin MC_3 receptor and lower affinity by the melanocortin MC_4 and MC_5 receptors. The natural MSH-peptides are thus selective only for the melanocortin MC_1 receptor, whereas ACTH is selective for the melanocortin MC_2 receptor. The newly described MSH analogue SHU9119-(cyclic[Nle^4,Asp^5,β-(2-naphthyl)-D-alanine ($D\text{-}Nal^7$),$Lys\text{-}NH_2{}^{10}$]α-MSH-(4–10)) (Hruby et al., 1995) has approximately 2-fold higher affinity for the melanocortin MC_4 receptor than for other melanocortin receptors (Schiöth et al., 1997b), and some ACTH-(4–10) analogues have been found to display certain selectivity for the melanocortin MC_4 receptor (Adan et al., 1994b).

There is little information available about how the MSH peptides bind to the different melanocortin MC receptor subtypes. This knowledge is essential for the design and synthesis of highly selective and potent ligands for the melanocortin MC_3, MC_4, and MC_5 receptors. The aim of the present study was to synthesise MSH peptides with different residues in the core and with different C- and N-terminal segments, using the high affinities ligand [Nle^4,D-Phe^7]α-MSH (NDP) as a model, and to investigate the subtype specific binding of these substances to the human melanocortin MC_1, MC_3, MC_4, and MC_5 receptors in order to provide information for molecular modelling and drug design.

2. Materials and methods

2.1. Chemicals

NDP (Sawyer et al., 1980) was purchased from Bachem, Switzerland. NDP was radio-iodinated by the chloramine T method and purified by HPLC (high performance liquid chromatography). D-Nal was purchased from Bachem, Switzerland. All other amino acid derivatives were purchased from PerSeptive, USA.

2.2. Peptide synthesis

The peptides tested in this study (except NDP) were synthesised in our laboratory by using the solid phase approach and then purified by HPLC. The correct molecular weights of the peptides were confirmed by mass spectrometry. The peptide sequences were assembled by using the Pioneer (PerSeptive) peptide synthesis system. Fmoc(9-fluorenylmethoxycarbonyl)–amino acid derivatives were used in coupling steps. When OPfp (pentafluorophenyl) esters were used, the synthesis cycle was as follows: (a) the Fmoc group was removed by 20% piperidine in DMF (*N*,*N*-dimethylformamide) (5 min); (b) to form a new peptide bound side chain-protected Fmoc–amino acid OPfp ester (4 eq.) and HOAt (1-hydroxy-7-azabenzotriazole) (4 eq.) were dissolved in DMF and circulated through the column for 30–60 min; (c) to cap residual amino groups the support was treated with 0.3 M Ac_2O (acetic anhydride) in DMF for 5 min. If free acids were used, then in step (b) side chain-protected Fmoc–amino acid (4 eq.), HATU (*O*-[7-azabenzotriazol-1-yl] 1,1,3,3-tetramethyluronium hexafluorophosphate) (4 eq.) and DIEA (*N*,*N*-diisopropylethylamine) (4 eq.) were applied. For deprotection a reagent mixture (trifluoroacetic acid–phenol–anisole–1,2-ethanedithiol–water, 82:2:2:2:2, 2.5 h) was used. The raw peptides formed were purified by HPLC (10 mm × 250 mm column, Vydac RP C18, 90A, 201HS1010, eluent—20–35% MeCN (acetonitrile) in water + 0.1% trifluoroacetic acid, detection at 240 nm).

2.3. Expression of receptor clones

The human melanocortin MC_1 and human melanocortin MC_5 receptors (Chhajlani and Wikberg, 1992; Chhajlani et al., 1993) were cloned into the expression vector

Peptide \ position nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
γ1-MSH		Tyr	Val	Met	Gly	His	Phe	Arg	Trp	Asp	Arg	Phe			
β-MSH	Pro	Tyr	Arg	Met	Glu	His	Phe	Arg	Trp	Gly	Ser	Pro	Pro	Lys	Asp
α-MSH	Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Trp	Gly	Lys	Pro	Val		
NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	Trp	Gly	Lys	Pro	Val		
NDP(1-9)	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	Trp						
NDP(4-13)				Nle	Glu	His	D-Phe	Arg	Trp	Gly	Lys	Pro	Val		
C-β-NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	Trp	Gly	Ser	Pro	Pro	Lys	Asp
C-γ-NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	Trp	Asp	Arg	Phe			
N-γ-NDP		Tyr	Val	Met	Gly	His	D-Phe	Arg	Trp	Gly	Lys	Pro	Val		
[Glu^6]-NDP	Ser	Tyr	Ser	Nle	Glu	Glu	D-Phe	Arg	Trp	Gly	Lys	Pro	Val		
[D-Nal^7]-NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Nal	Arg	Trp	Gly	Lys	Pro	Val		
[Lys^8]-NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Lys	Trp	Gly	Lys	Pro	Val		
[His^9]-NDP	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	His	Gly	Lys	Pro	Val		

Fig. 1. Alignment of peptides used in the present study with natural MSH peptide. All peptides used in the present study had an acetyl-group at the N-terminal, except N-γ-NDP (which is similar to that of natural γ-MSH). All peptides used in the present study also had an amide group on the C-terminal, except for C-β-NDP which was free acid. The N-terminal H-Ala-Glu-Lys-Lys-Asp-Glu-Gly– sequence of β-MSH has been omitted from the figure.

Table 1
K_i values (mean ± S.E.M), obtained from competition curves, for MSH analogues at human melanocortin MC_1, MC_3, MC_4, and MC_5 receptors transfected in COS-1 cells together with relative affinity ratios

Ligand	Receptor						
	MC_1	MC_3	MC_4	MC_5	MC_3/MC_1	MC_4/MC_1	MC_5/MC_1
	K_i (nmol/l)	K_i (nmol/l)	K_i (nmol/l)	K_i (nmol/l)			
NDP	0.078 ± 0.020	0.653 ± 0.082	4.03 ± 0.22	3.43 ± 0.57	8.4	52	44
NDP(1–9)	0.345 ± 0.123	62.8 ± 33.4	51.2 ± 21.5	40.0 ± 6.7	180	150	120
NDP(4–13)	0.099 ± 0.037	4.85 ± 1.19	2.30 ± 0.44	15.3 ± 2.1	49	23	150
C-β-NDP	0.448 ± 0.092	3.05 ± 1.23	2.37 ± 0.95	8.81 ± 4.52	6.8	5.3	20
C-γ-NDP	0.850 ± 0.172	89.7 ± 6.8	513 ± 279	109 ± 23	110	600	130
N-γ-NDP	0.052 ± 0.013	0.722 ± 0.320	3.17 ± 1.28	3.31 ± 1.06	14	6163	
[Glu^6]NDP	7.66 ± 2.64	122 ± 17	111 ± 49	134 ± 115	16	16	17
[D-Nal^7]NDP	0.110 ± 0.080	0.740 ± 0.036	0.877 ± 0.278	2.51 ± 0.82	6.7	9.1	23
[Lys^8]NDP	0.786 ± 0.136	24.5 ± 10.3	70.3 ± 5.2	57.7. ± 12.3	31	89	73
[His^9]NDP	6.66 ± 2.64	2830 ± 830	4820 ± 1850	38000 ± 12000	64	720	5700
NDP(1–9)/NDP	4.4	96	13	12			
NDP(4–13)/NDP	1.3	7.4	0.57	4.5			
C-β-NDP/NDP	5.7	4.7	0.59	2.6			
C-γ-NDP/NDP	11	137	127	32			
N-γ-NDP/NDP	0.67	1.1	0.79	0.97			
[Glu^6]NDP/NDP	98	187	28	39			
[D-Nal^7]NDP/NDP	1.4	1.1	0.22	0.73			
[Lys^8]NDP/NDP	10	38	17	17			
[His^9]NDP/NDP	85	4300	1200	11000			

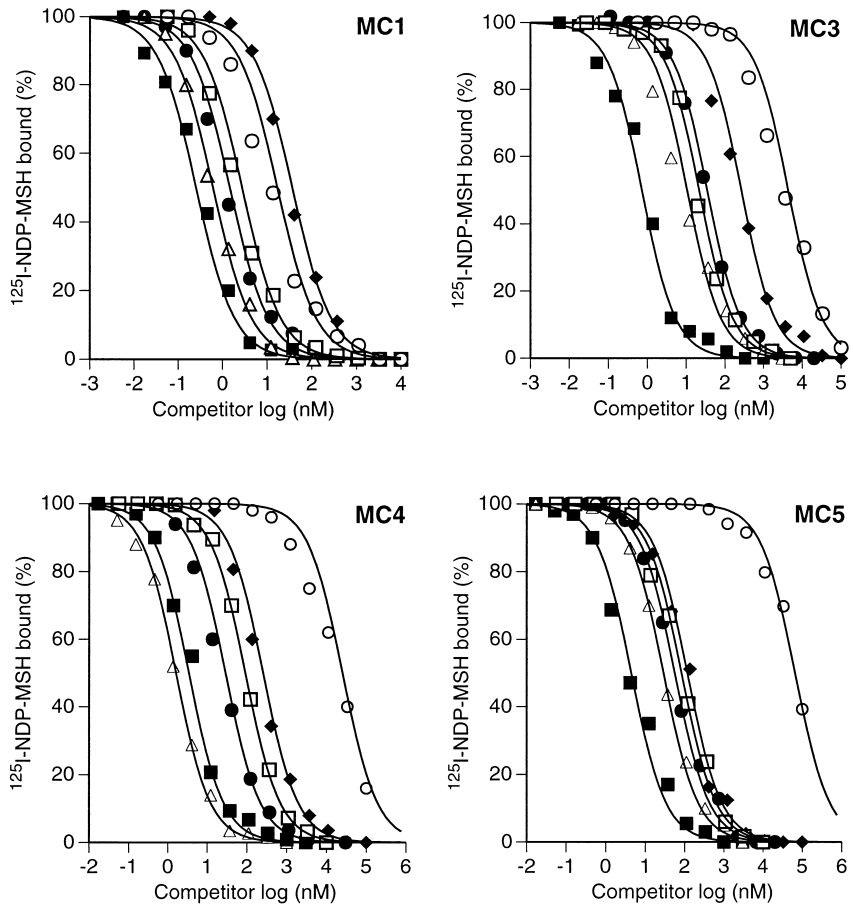

Fig. 2. Competition curves for NDP (■), NDP(1–9) (●), NDP(4–13) (△), [Glu^6]NDP (◆), [Lys^8]NDP (□), [His^9]NDP (○) binding to COS-1 cells transfected with the different melanocortin receptor subtypes. The curves were obtained by using a fixed concentration of [^{125}I]NDP and varying concentrations of the non-labelled competing peptide.

pRc/CMV (In Vitrogen). The human melanocortin MC_3 and human melanocortin MC_4 receptors, cloned into the expression vector pCMV/neo, were a gift from Dr. Ira Gantz (Gantz et al., 1993a,b). For receptor expression COS-1 (*C*V-1 *O*rigin, *S*V40) cells were grown in Dulbecco's modified Eagle's medium with 10% foetal calf serum. Eighty-percent confluent cultures were transfected with the DNA mixed with liposomes in serum-free medium (for details see Schiöth et al., 1996b). After transfection, the serum-free medium was replaced by serum-containing medium and the cells were cultivated for about 48 h. Cells were then scraped off, centrifuged, and used for radioligand binding.

2.4. Binding studies

The transfected cells were washed with binding buffer (see Schiöth et al., 1995) and distributed into 96-well non-culture-coated plates, which were centrifuged and the binding buffer was removed. The cells were then immediately incubated in the well plates for 2 h at 37°C with 0.05 ml binding buffer in each well containing a constant concentration of [^{125}I]NDP and appropriate concentrations of the competing unlabelled ligand. After incubation the cells were washed with 0.2 ml of ice-cold binding buffer and detached from the plates with 0.2 ml of 0.1 M NaOH. Radioactivity was counted (Wallac, Wizard automatic gamma counter) and data were analysed with a software package suitable for radioligand binding data analysis (Wan System, Umeå, Sweden). Data were analysed by fitting to formulas derived from the law of mass action by the method generally referred to as computer modelling. The K_d values for [^{125}I]NDP for the melanocortin receptors were taken from Schiöth et al. (1995, 1996a). The binding assays were performed in duplicate wells and repeated three times. Untransfected COS-1 cells did not show any specific binding for [^{125}I]NDP.

3. Results

We tested NDP and the nine new NDP analogues on intact COS-1 cells that express the human melanocortin MC_1, MC_3, MC_4, and MC_5 receptors by competitive

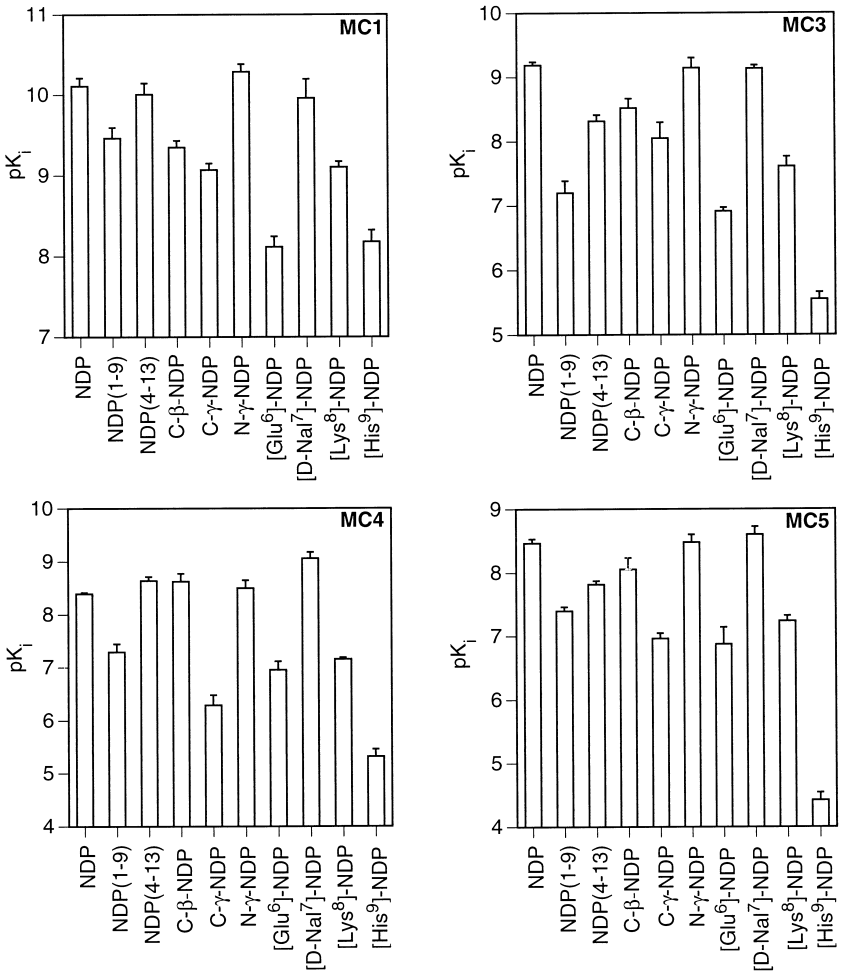

Fig. 3. Graphical presentation of the pK_i values for the different peptides for the melanocortin receptor subtypes.

receptor binding assays using [^{125}I]NDP as radioligand. The structure of the substances are aligned with the structures of α-MSH, β-MSH, γ-MSH and NDP in Fig. 1. The resulting K_i values calculated from competition tests of these compounds are summarised in Table 1. The competition curves for NDP, NDP(1–9), NDP(4–13), [Glu^6]NDP, [Lys^8]NDP, [His^9]NDP at the different melanocortin receptor subtypes are shown in Fig. 2. Schematic presentation of the pK_i values for each of the receptors are presented in Fig. 3.

As can be seen from Table 1, the deletion of the N-terminal of NDP (NDP(4–13)) or replacement of it by that of γ-MSH (N-γ-NDP) did not appreciably affect binding to the melanocortin MC_1 and MC_4 receptors. For the melanocortin MC_3 receptor, however, the deletion of the N-terminal of NDP clearly lowered affinity, whereas replacement of the NDP N-terminal with the N-terminal of γ-MSH did not affect binding. Deletion of the N-terminal of NDP clearly decreased the affinity for the melanocortin MC_5 receptor, but the NDP affinity was only slightly reduced when the N-terminal was replaced by that of γ-MSH.

Deletion of the C-terminal of NDP (NDP(1–9)) and replacement of it by the C-terminal of β- or γ-MSH (C-β-NDP and C-γ-NDP, respectively) lowered the affinity for the melanocortin MC_1 receptor. For the melanocortin MC_3 receptor, deletion of the C-terminal and replacement of it with that of γ-MSH greatly lowered the affinity (around or more than 100-fold), whereas C-β-NDP displayed only a slightly lower affinity than NDP. The low affinity of C-γ-NDP was surprising to us, as γ-MSH has a slightly higher or similar affinity for the melanocortin MC_3 receptor as that of α-MSH (Schiöth et al., 1995). We repeated the synthesis of C-γ-NDP but the results were the same for all of the receptors. Replacement of the C-terminal of NDP by that of β-MSH slightly increased the affinity for the melanocortin MC_4 receptor, whereas deletion of the C-terminal resulted in 13-fold lower affinity. C-γ-NDP had 130-fold lower affinity for the melanocortin MC_4 receptor than did NDP. C-β-NDP showed only slightly lower affinity for the melanocortin MC_5 receptor than NDP whereas NDP(1–9) and C-γ-NDP displayed 12- and 32-fold lower affinity than NDP, respectively.

Replacement of the basic hydrophilic His^6 by the acidic hydrophilic Glu^6 reduced the affinity of NDP for all the melanocortin receptors, in particular for the melanocortin MC_3 (190-fold) and for the melanocortin MC_1 (98-fold) receptors, whereas the reductions for the melanocortin MC_4 (28-fold) and melanocortin MC_5 (39-fold) receptors was less. Replacement of the hydrophobic D-Phe^7 in NDP by the hydrophobic but more bulky D-Nal^7 resulted in similar affinity for the melanocortin MC_1, MC_3, and MC_5 receptors but an about 4-fold increased affinity for melanocortin MC_4 receptor. Replacement of the basic hydrophilic Arg^8 with the structurally similar Lys^8 lowered the affinity for the melanocortin MC_1 receptor 10-fold, for the melanocortin MC_3 receptor 38-fold and for the melanocortin MC_4 and MC_5 receptors 17-fold. Replacement of the non polar hydrophobic Trp^9 by the basic hydrophilic His^9 lowered the affinity for the melanocortin MC_1 receptor 85-fold and for the melanocortin MC_3, MC_4, and MC_5 receptors more than 1000-fold.

4. Discussion

The melanocortin receptors are a family of 7TM (transmembrane) spanning receptors that bind melanocortic peptides. The melanocortin receptors display a number of common structural features and show considerable amino acid homology, especially within TM1, TM2, TM3 and TM7, which may contain key elements for the binding of MSH peptides. They are also characterised by short N- and C-terminal regions as well as a very small second extracellular loop. The core of the MSH peptide (i.e., His^6-Phe^7-Arg^8-Trp^9) is known to be crucial for MSH binding to melanocortin receptors (Eberle, 1988; Schiöth et al., 1995, 1996a, 1997a; Haskell-Luevano et al., 1996, 1997). All compounds that bind to the melanocortin receptors have important elements from this core (except the agouti peptide which has no homology to α-MSH). It is also evident that the C- and/or the N-terminal side chains of MSH peptides may play an important role in determining the subtype- specific binding, which is in particular displayed by γ-MSH, which has a comparatively much higher selectivity for the melanocortin MC_3 receptor than for the melanocortin MC_4 receptor (Adan et al., 1994a; Miwa et al., 1995; Schiöth et al., 1995, 1996a). NDP is an α-MSH analogue which has L-Phe^7 substituted by D-Phe^{7,} which led to a higher potency and prolonged biological activity, and Met^4 substituted by Nle^4, which further enhanced the affinity and stability of this peptide. NDP has earlier been shown by us and others to have a high affinity for all of the melanocortin receptors and is therefore suitable as a reference substance for characterisation of the importance of the C- and N-terminals and core residues of MSH peptides for the binding to the human melanocortin receptors. Mutagenesis data indicate that there might be an alternative point of attachment for NDP and α-MSH at the melanocortin MC_1 receptor (Frändberg et al., 1994). However, more thorough testing with several linear and cyclic Phe^7-substituted MSH analogues (Schiöth et al., 1997c) has shown that this hypothesis may not be correct.

Our results show that the N-terminal segment (Ser^1-Tyr^2-Ser^3) in the NDP peptide is not important for binding to the melanocortin MC_1 receptor whereas the C-terminal segment (Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}) is clearly important because deletion or changes in the C-terminal caused an about 5-fold decrease in affinity for the melanocortin MC_1 receptor. This is very much in line with early data on the melanotropic activity of MSH analogues on several melanophores (for review see Eberle, 1988), and with more recent data showing that individual replacement of

N-terminal residues by Ala in α-MSH resulted in negligible influence on receptor binding or tyrosinase activity in murine melanoma cells, whereas Ala substitutions in the C-terminal, in particular the replacement of Pro^{12}, resulted in loss in affinity (Sahm et al., 1994b). The lack of importance of the N-terminal segment and the significance of the C-terminal for the melanocortin MC_1 receptor has also been shown by use of cyclic [Cys^4-Cys^{10}]α-MSH analogues in frog and lizard skin bioassays (Cody et al., 1984). The residues Lys^{11} and Pro^{12} were in particular shown to be important for the activity of cyclic analogues. Our present and earlier (Schiöth et al., 1995) data for the melanocortin MC_1 receptor seems to indicate that the differential binding of α-, β- and γ-MSH is related to the C-terminal sequence, where the absence of Lys^{11} in β-MSH and γ-MSH and Pro^{12} in γ-MSH causes the lower affinity of β-MSH and γ-MSH compared to α-MSH.

Much less is known about the influence of the N- and C-terminal segments of MSH peptides for binding to the other melanocortin receptors. Our present results show that both the N- and the C-terminal segments are important for binding to the melanocortin MC_3 receptor. This is in line with alanine scanning data for the rat melanocortin MC_3 receptor (Sahm et al., 1994a) which indicated the importance of both Tyr^2 in the N-terminal and Lys^{11} in the C-terminal, whereas other Ala substitutions in both terminals did not significantly affect ligand binding. Also, truncation of both the N- and C-terminals of MSH peptides influence the cAMP response of the rat (Adan et al., 1994b) and human melanocortin MC_3 receptors (Miwa et al., 1995).

The melanocortin MC_4 receptor can be distinguished from the melanocortin MC_3 receptor because the former binds γ-MSH with much lower affinity than α-MSH, whereas the latter shows a similar affinities for both of these peptides. Recently, it was shown that both linear and cyclic core MSH analogues are bound by melanocortin MC_3 and MC_4 receptors with equal affinity (Schiöth et al., 1997a), which indicates that it is indeed the N- or/and C-terminals that determine the differences in affinity of these receptors for α- and γ-MSH. Our present data show that the C-terminal of NDP but not the N-terminal is important for binding to the melanocortin MC_4 receptor. Our data indicate that it may be the C-terminal end of γ-MSH (Asp^{10}-Arg^{11}-Phe^{12}) that perturbs binding to the melanocortin MC_4 receptors. However, it should be considered that chimeric or engineered peptides may not bind in the same manner as natural peptide hormones simply due to incompatibility of the different structural elements which are artificially brought together. The effects of deletions or replacements of terminal residues may be caused by a direct contribution of the amino acid residues involved or the changes may cause indirect effects due to conformational changes of core residues.

The melanocortin MC_5 receptor binds the different MSH peptides with the same order of preference as the melanocortin MC_1 receptor but in general with much lower all over affinity. Our present data show that both the N- and C-terminals are important for the binding of MSH peptides to the melanocortin MC_5 receptor. Our data indicate that it is in particular the C-terminal which determines the differential binding of the MSH peptides to this receptor.

Replacement of Phe^7 by D-Phe^7 is the only substitution that is known to enhance the affinity of the core of α-MSH. Now we demonstrate that replacement of D-Phe^7 by D-Nal^7 further enhances the affinity for the melanocortin MC_4 receptor but not for the other melanocortin receptor subtypes. Replacement of D-Phe^7 by D-Nal^7 in the cyclic lactam analogue MTII resulted in SHU9119, which has been shown to be a melanocortin MC_4 receptor antagonist (Hruby et al., 1995). However, SHU9119 showed higher affinity for the melanocortin MC_3 and MC_5 receptors compared to MTII (Schiöth et al., 1997b), which reveals that the D-Nal^7/D-Phe^7 substitution has a different effect in the lactam analogue than the linear NDP used in the present study. The other substitutions in the MSH core resulted in more or less pronounced loss in affinity for the different melanocortin receptors, as one might have predicted. The above-mentioned alanine scanning experiments by Sahm et al. (1994a,b), which were performed with murine melanoma cells and rat melanocortin MC_3 receptors, as well as earlier structure–activity studies (Eberle, 1988), have indicated that Arg^8 and Trp^9 are the most important residues in the MSH core for receptor binding. It is interesting to note that the relatively subtle change in the Arg side chain, converting it to a Lys, resulted in a more than 10-fold loss in affinity for all the melanocortin receptors. It is not unexpected that the substitution of His^6 by Glu^6 or of Trp^9 by His^9, which changes the polarity of the residue in respective position, causes a much more dramatic decrease of the affinities. Interestingly, these changes depend to a large extent on the receptor subtype. These differences might be useful to verify or reject hypotheses formulated on the basis of results from molecular modelling studies of the melanocortin receptors or from direct mutagenesis studies which pinpoint the specific interaction between residues in the ligand and the receptors.

It is also important for the design of compounds which bind selectively to a specific receptor subtype to determine residues or regions whose modification can increase the selectivity. Taken together, our present data show that both the melanocortin MC_1 and MC_4 receptors are not sensitive to changes in the N-terminal of the MSH peptide, whereas the melanocortin MC_3 and MC_5 receptors recognise important binding elements in this part of the MSH peptide. Moreover, binding to the melanocortin MC_4 and MC_3 receptors seems in particular to be sensitive to changes in the C-terminal of the MSH peptide, whereas the melanocortin MC_1 and MC_5 receptors are affected in a more subtle way by changes in this part of an MSH peptide. Furthermore, His^6 replacement by Glu^6 reduced

binding to the melanocortin MC_3 and MC_1 receptors to a much greater extent than it did binding to the melanocortin MC_4 and MC_5 receptors. The Trp^9 replacement by His^9 reduced binding to the melanocortin MC_4 and MC_3 receptors to a much greater extent than to the melanocortin MC_1 receptor. The D-Phe^7/D-Nal^7 or the Arg^8/Lys^8 substitutions did not lead to any marked changes in the selectivity of the MSH ligand for the melanocortin MC receptor subtypes. The substitution of NDP by D-Nal^7 (resulting in [D-Nal^7]NDP) gave the ligand with the highest affinity for the melanocortin MC_4 receptor, a property which might find potential use in studies of the pharmacology of the melanocortin MC_4 receptor.

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