Vol. 210, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 5, 1995 Pages 205-210

A THREE DIMENSIONAL MODEL FOR THE INTERACTION OF MSH WITH THE MELANOCORTIN -1 RECEPTOR

Peteris Prusis*, Per-Anders Frändberg*, Ruta Muceniece[¤], Ivars Kalvinsh[#] and Jarl E.S. Wikberg*+ §

*Department of Pharmaceutical Pharmacology Uppsala University, Uppsala, Sweden

[¤]Laboratory of Pharmacology and [#]Department of Medicinal Chemistry, Institute of Organic Synthesis, Riga, Latvia

+Wa Pharm, Umeå, Sweden

Received March 31, 1995

SUMMARY: A model for the interaction of the melanocortin-1 (MC1) receptor with MSH peptide has been constructed. The model was built by homology modelling using bacteriorhodopsin as template. A cyclic analogue of MSH could be docked into a binding pocket located between transmembrane (TM) domains 2, 3 and 6 of the receptor. The most significant receptor to ligand interactions occur between D117 in TM3 of receptor with histidine in cyclic MSH-peptide, H260 in TM6 with glutamic in peptide and D121 in TM3 with arginine in peptide. The model finds support from earlier mutagenesis data.

The 3D modelling of several G-protein coupled receptors (GPCRs) has led to better understanding of the interactions of the receptors with their ligands. However, such modelling was in the past mainly done for the cationic GPRCs (1) and yet only two partial models for peptide hormone GPRCs were reported (2,3).

We and others recently cloned a new family of GPCRs, the melanocortin receptors, that contains 5 known members termed MC1 - MC5 (4-8). All of the melanocortin receptors interact with pro-opiomelanocortin derived peptides such as α-MSH, β-MSH, γ-MSH and ACTH, albeit with differing binding affinities (9).

§Address all correspondence to Jarl Wikberg, Pharmaceutical Pharmacology, Box 571, BMC, S-751 24, Uppsala, Sweden.

Abbreviations:
GPCR, G-protein coupled receptor; MCR, melanocortin receptor; MSH, melanocyte stimulating hormone; TM, transmembrane.

0006-291X/95 $5.00

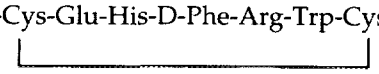
The melanocortin receptors show high sequence similarity within their group, but the sequence similarity to other GPCRs is comparatively low. Nevertheless the most conserved aminoacids among the GPCRs are also present in the melanocortin receptors and their all over features clearly identify them as belonging to the GPCR class. In a recent paper we reported specific alterations in the ligand binding properties in the MC1 receptor subtype by site directed mutagenesis of specific aminoacids in the third and sixth transmembrane segments, indicating that these aminoacids were part of the ligand binding pocket of the receptor (10). In the present paper we report for the first time a 3D model for a melanocortin receptor.

MATERIALS AND METHODS

Software and hardware. Molecular modelling was done by using the Sybyl (Tripos, St. Louis, Missouri, USA) package and a Sun IPX workstation. Sequence alignments were performed using the MacMolly package on a Macintosh computer (Soft Gene, Germany). Kyte-Doolitle plots were done using the GCG package (11) on a VAX computer.

Sequence analysis. Sequences were obtained from the Brookhaven Protein Data Bank or experimentally by DNA sequence analysis of the cloned receptor as earlier reported (4,5). The sequences of all five MC receptors were aligned using the MacMolly program. The location of transmembrane helices were determined from Kyte-Doolittle hydrophobicity plots. The alignment of MC receptor amino acid sequences to other GPCRs and to bacteriorhodopsin were taken from the literature (12) except for transmembrane segment 5 (TM5) which alignment will be detailed in the Results and Discussion section.

Peptide hormone modelling. For modelling the peptide hormone the cyclic peptide:



was used. Its structure was built using the standard random conformation protein building routine of Sybyl. Molecular dynamics simulations was applied at a temperature of 300K using 25 ps step time to yield 2000 conformations from which the 36 with lowest energy were chosen and energy minimised using the Tripos force field. The molecule with the lowest energy was used in our studies.

Receptor modelling. Modelling was performed using Sybyl with default parameters unless otherwise specified. The seven transmembrane helices of the MC1 receptor were first constructed separately using α-helical conformation. Helices 6 and 7 contains proline residues for which the geometry were fixed resulting in kinked helices. The helices were then superimposed on the helices of bacteriorhodopsin (13).

To preserve the general structure of the receptor its backbone was first constrained and molecular dynamics simulation (100K temperature, 1 fs time step) was applied to yield 1000 conformations, from which 4 were chosen and energy minimised using the Kollman force field (14) The constraint of the backbone was then removed and the models were subjected further energy minimisation. The model with the lowest energy was selected for use in the docking studies.

Ligand docking. The cyclic ligand, obtained as described above, was manually docked to the receptor model and the complex subjected to energy minimisation using the Kollman force field (14).

RESULTS AND DISCUSSION

Modelling of ligand. Modelling of the natural MSH peptide hormones poses big problems because of their flexibility and the lack of any previous 3D structural data. The cyclic peptide used for the present study is related to α-MSH 5-9, which is the central core in α-MSH that earlier studies suggest is binding to the MSH-receptors (15). Earlier studies have also shown that the cyclisation at the 4-10 positions in MSH gives a compound with high affinity for the MSH-receptors (16). Moreover higher activity of both cyclic and linear MSH-peptides may be afforded by substitution of the L-Phe with D-Phe (17,18). While the chosen cyclic MSH-analogue has some restriction due its SS bridge, there still remains a possibility for many conformers. Its structure was therefore built by performing long molecular dynamics simulation and selecting the ones with lowest energy for further energy minimisation. The structure with the lowest energy thus obtained was used in the docking studies as is shown in fig 3.

Sequence alignments. The alignment of all the MC receptor sequences is shown in fig. 1. Most of the conserved amino acids are located within the presumed TM helices. The carboxyterminal, first extracellural and third intracellular loops differs significantly whereas the other extra and intracellular loops as well as the amino-terminal show regions of substantial similarity.

It was previously reported that the MCR transmembrane sequences can be aligned with the sequences of the whole group of GPCRs (12). Although most conserved amino acids among the GPCRs are also present in the MC receptors there are some exceptions; one of the most interesting being the lack of a proline in TM5 for the MC receptors. This fact might suggest a specific role for TM5 in the MC receptors as compared to other GPCRs. Moreover the extreme short extra-cellular loop between TM4 and TM5 in the MC receptors compared to other GPCRs (12) is an interesting observation.

None of the TM regions of the MCRs showed any apparent homology to bacteriorhodopsin, except TM5 which contains a nine amino acid long alignable motif in which up to five amino acids are equal and some others similar. Moreover, four aminoacids on each side of the motif are also similar (fig. 2).

Modelling of receptor and docking of ligand. The final MC1R model selected after the dynamics simulation and energy minimisations showed an all over preserved 7 transmembrane α-helical structure with substantial helice to helice interactions.

Our earlier mutagenesis studies (10) suggested that D117 in TM3 and H260 in TM6 is part of the ligand binding pocket. Moreover, it is plausible that TM2 is also close to the binding region. This is because a natural point mutation has been found in TM2 of the MC1R giving rise to a constitutively activated receptor (19).

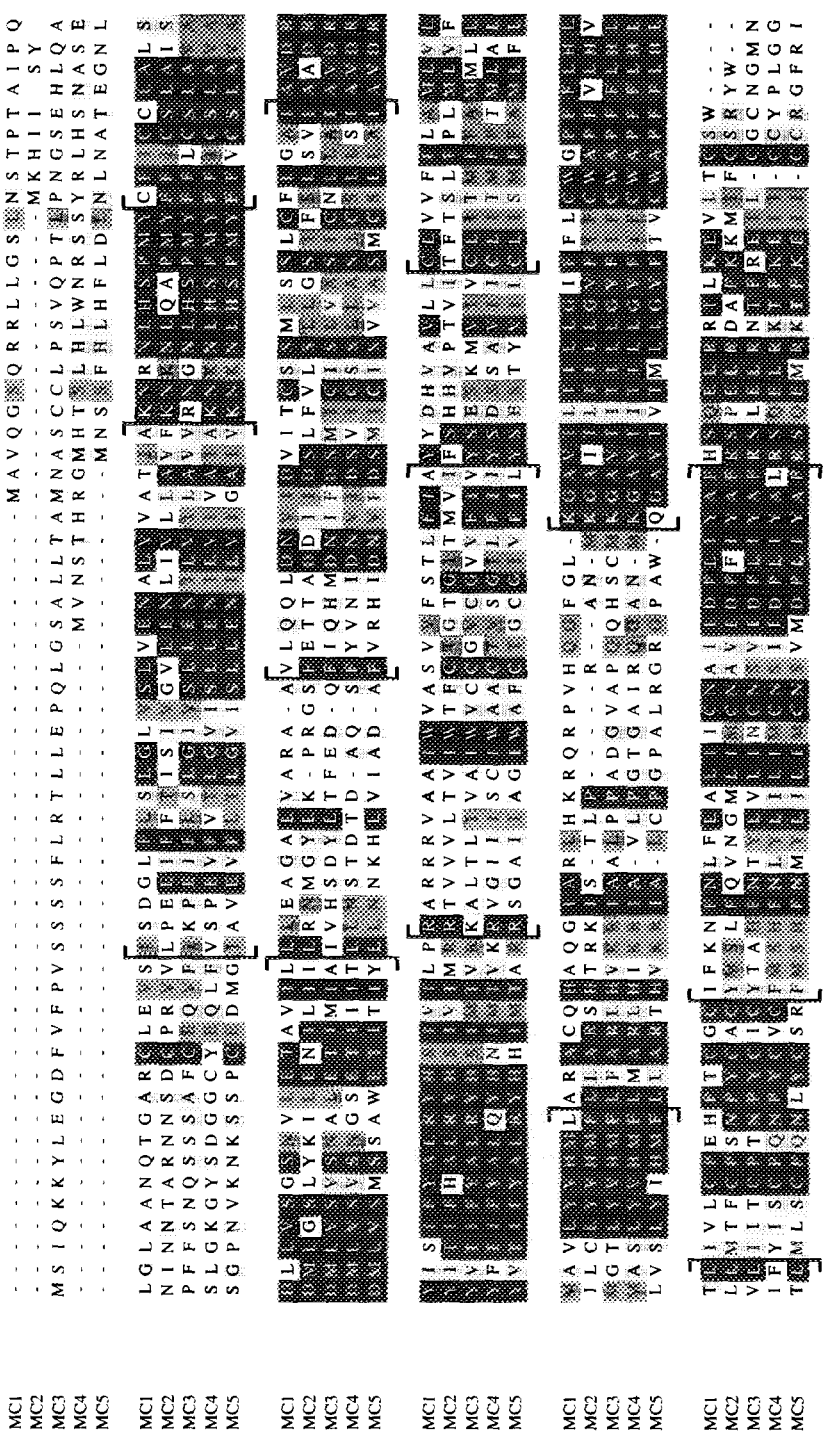


Fig.1. Alignment of MC receptor aminoacid sequences. TM regions used in modelling shown within brackets.

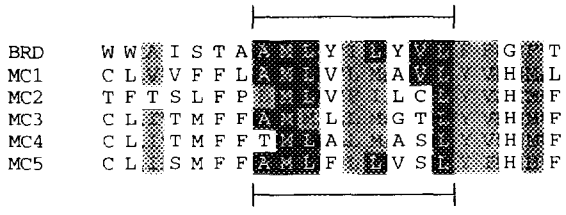


Fig.2. Alignment of TM5 of MC receptors and bacteriorhodopsin (BRD). The nine amino acid long alignable motif (see text) marked by horizontal bars.

The ligand could be docked into a pocket located between TM2, TM3 and TM6 of the model. Energy minimisation yielded a receptor-ligand complex with several points of interaction between receptor and ligand. The most interesting interactions are the formations of salt bridges between D117 in TM3 with the histidine of the cyclic peptide, between H260 in TM6 of the receptor with the glutamic residue of the ligand, and the D121 in TM3 with the arginine residue of the ligand. There is also an interaction between E55 of TM1 for the receptor and the tryptophan residue of the ligand (fig. 3). In our previous study (10) we found that the D117-->Ala, as well as the H260-->Ala mutation in the MC1R leads to a drop in affinity for the linear α-MSH but not for the analog [^{125}I]-Nle^{4}, D-Phe^{7}-MSH, a fact that indicates differences in their points of attachment to the MC1R. However, a cyclic peptide analog having the identical core as the peptide used in the present study was also found to loose affinity for the above mentioned mutated receptors in a similar fashion as α-MSH. (In fact the same pattern was also found when the D-Phe of the cyclic analogue was substituted with L-Phe, although the allover affinities became lower; Frändberg et al., unpublished

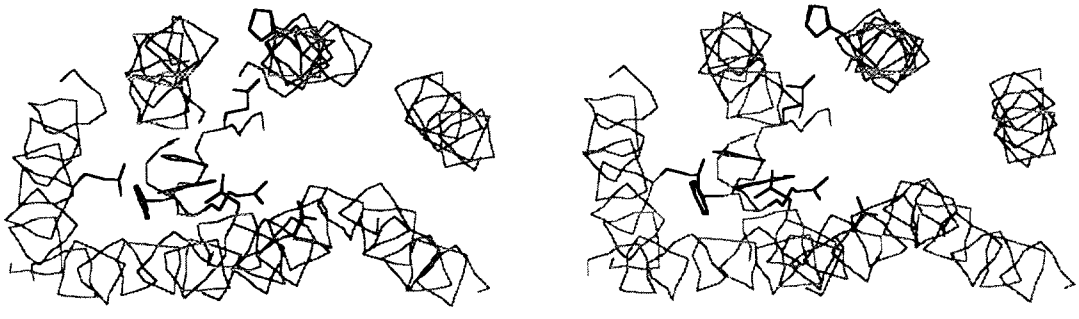


Fig.3. The model of the interaction of the cyclic MSH analogue with the MC1 receptor. Shown are the backbones of the receptor and ligand (gray) with the side chains of some of the aminoacids (black) having ligand to receptor interactions; from left to right E55-R, F4-L, W6-L, H3-L, D121-R, E2-L, R5-L, H260-R and D117-R. (Code used denotes aminoacid number followed by R for aminoacid of receptor or L for aminoacid of ligand.)

data). Thus these data seem to indicate that the cyclic peptide may have a similar conformation as the natural MSH. The future modelling studies must take into account that different MSH-analogues may bound with slightly different points of attachment to the MC1R.

Acknowledgments: Supported by the Swedish MRC (04X-05957), CEC (ERBCHRXCT 940505), CFN, the Royal Swedish Academy of Sciences and the Howard Hughes Medical Institute.

REFERENCES

1. Hibert, M.F., Trumpp-Kallmeyer, S., Bruinvels, A. and Hoflack, J. (1991) Mol. Pharmacol. 40, 8-15.
2. Hakala, J.M.L. (1994) 202, 1569-1573.
3. Krystek Jr, S.R., Patel, S., Rose, P.M., Fisher, S.M., Kienzle, B.K., Lach, D.A., Liu, E.C.-K., Novotnu, J . and Webb, M.L. (1994) J. Biol. Chem. 1994, 269, 12383-12386.
4. Chhajlani, V., and Wikberg, J.E.S. (1992) FEBS Lett. ,309, 417-420.
5. Chhajlani, V., Muceniece, E. and Wikberg, J.E.S. (1993) Biochem. Biophys. Res. Commun. 1195, 866-873.
6. Mountjoy, K.G., Robbins, L.S., Mortrud, M.T. and Cone, R.D. (1992) Science. 257, 1248-1251.
7. Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S.J., Del Valle, J., and Yamada, T. (1993) J. Biol. Chem. 268, 8246-8250.
8. Gantz, I., Miwa, H., Konda, ., Shimoto, Y., Tashiro, T., Watson, SJ., DelValle, J. and Yamada, T.: (1993) J. Biol. Chem. 268, 15174-15179.
9. Schiöth, H.B., Muceniece, R., Wikberg, J.E.S. and Chhajlani, V. (1995) Eur. J. Pharmacol, Mol. Pharm Sect. 288, 311-317.
10. Frändberg, P.-A., Muceniece, R. Prusis, P., Wikberg, J.E.S. and Chhajlani, V. (1994) Biochem. Biophys. Res. Commun. 202, 1266-1271.
11. Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucl. Acids Res. 12, 387-395.
12. Rippman, F. (1994) In TM7 (G. Vriend, Ed.) EMBL, Heidelberg 4.
13. Henderson, R., Baldwin, J.M., Ceska, T.A., Zemlin, F., Beckmann, E. and Downing, K.H. (1990) J. Mol. Biol. 213, 899-929.
14. Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.A., Ghio, C., Alagona, G., Profeta Jr, S. and Weiner, P. (1984) J. Am. Chem. Soc. 106, 765-784.
15. Sahm, U.G., Qarawi, M.A., Olivier, G.W.J., Ahmed, A.R.H., Branch, S.K., Moss, S.H. and Pouton, C.W. (1994) FEBS Lett. 350, 29-32.
16. Hirsch, M., O'Donohue, T.L., Wilson, R., Sawyer, T.K., Hruby, V.J., Hadley, M.E., Cody, W., Knittel, J.J. and Crawley, J.N. (1984) Peptides. 5, 1197-1201.
17. Kobobun, K., O'Donohue, T.L., Handlemann, G.E., Sawyer, T.K., Hruby, V.J. and Hadley, ME (1983) Peptides. 4, 721-724.
18. Beckwith, B.E., Tinius, T.P., Hruby, V.J., al-Obeidi, F., Sawyer, T.K. and Affholter, J.A. (1989) Peptides. 10, 361-368.
19. Robbins, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K.G. and Cone, R.D. (1993) Cell. 72, 827-834.