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Review

# Melanocortin receptors: perspectives for novel drugs

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**Abstract**

The cloning of five different subtypes of melanocortin receptor subtypes have recently opened up new possibilities for the development of drugs. The physiological roles of the five melanocortin receptors have started to become understood, and compounds with selective actions on some of the five subtypes have become available. Presently, most clinically promising application for drugs active on melanocortin receptors are for control of feeding homeostasis and body weight and for treatment of inflammatory diseases. I review here the cloning, localisation, function and structure of the melanocortin receptors, in relation to the possibilities to develop selective drugs for these receptors. 

*Keywords:* Melanocortin receptor; Localization; Function; Structure; Selective drug

## 1. Introduction

The cloning of five different melanocortin receptors ($MC_{1-5}$) during the years 1992–1994 started a new era in the research on the melanocortin receptors. Before this time, the receptors for melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) were known mainly from the physiological effects that these hormones elicited; MSH causing skin pigmentation, and ACTH inducing the secretion of corticosteroids. However, from early literature, MSH/ACTH peptides were also known to induce an additional wide array of effects, including both central effects such as alterations in motor and sexual behaviour, analgesia, improvement of memory, antipyretic effects, and peripheral effects such as powerful anti-inflammatory and lipolytic actions.

With the cloning of the melanocortin receptors, the molecular mechanisms underlying all these effects have been started to become elucidated. It is now clear that most, if not all, of the effects of the MSH/ACTH peptides are mediated via specific subtypes of melanocortin receptors. Still there are many questions that remain to be answered before a full understanding of the actions of the MSH/ACTH peptides is achieved.

The melanocortin receptors show distinct distributions in the body. The melanocortin $MC_1$ receptor was first recognised as the peripheral MSH receptor which is present in the melanocytes, where it regulates the pigmentation of the skin, while the melanocortin $MC_2$ receptor was recognised as the ACTH receptor. The melanocortin $MC_3$ and $MC_4$ receptors were subsequently found at distinct loci in the central nervous system, while the melanocortin $MC_5$ receptor was found to have a wide distribution in the body. More recently, it has become known that the melanocortin $MC_1$ receptor is present in many cell types, both in the periphery and in the central nervous system. The melanocortin $MC_3$ receptor is also localised peripherally.

The melanocortin receptors belong to the G-protein coupled receptor family, and all of them couple in a stimulatory fashion to cAMP. Understanding of the physiological functions of the different melanocortin receptor subtypes have now started to emerge, and some of the new findings indicate that melanocortin receptors may serve as targets for novel drugs. Below I will review certain areas pertaining to the melanocortin receptor localisation, physi-

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ology, structure, novel compounds with selective actions, and the potential to use selective drugs for these receptors for treatment of diseases.

## 2. The cloning of the melanocortin receptors

The human melanocortin $MC_1$ receptor was cloned in 1992 independently by my group (Chhajlani and Wikberg, 1992) and the group of Dr. Roger Cone (Mountjoy et al., 1992). We identified the receptor from a novel DNA having 372 base pairs, obtained from the PCR (Polymerase Chain Reaction) amplification of human genomic DNA, by using degenerate primers deduced from the G-protein coupled receptors known at that time (Chhajlani and Wikberg, 1992). The tissue expression of the novel DNA was screened by using Northern blot analysis, and out of 16 different human tissues only the RNA from a human WM 266-4 melanoma cell line gave a signal. The RNA from these melanoma cells were then used for construction of a cDNA library, which was screened using the novel DNA fragment, resulting in the isolation of one clone encoding a protein with 317 amino acids. The clone was expressed and identified as an MSH receptor by its ability to bind MSH-peptides using an [$^{125}$I]NDP-MSH ([$^{125}$I] [Nle$^4$, D-Phe$^7$] α-MSH) radioligand binding assay. A similar approach was taken by Cone et al. By PCR-amplification of cDNA from a human melanoma cell line a gene encoding almost the same sequence as our melanocortin $MC_1$ receptor was isolated (Mountjoy et al., 1992). Following these initial discoveries four additional melanocortin receptors were then, within a short period of time, discovered by the use of homology screening. The melanocortin $MC_2$ receptor gene was first isolated from human genomic DNA and tentatively identified as the ACTH-receptor based on the ability of ACTH to stimulate cAMP in cells transfected with the DNA (Mountjoy et al., 1992). Moreover, the gene appeared to be quite uniquely expressed in the adrenal gland (Mountjoy et al., 1992; Xia and Wikberg, 1996). The human melanocortin $MC_3$ and $MC_4$ receptor genes were then isolated by Gantz et al. (1993, 1994), while the human melanocortin $MC_5$ receptor gene was isolated by us (Chhajlani et al., 1993) (please observe that we originally named the melanocortin $MC_5$ receptor 'MC-2', a terminology that we have now abandoned). Subsequently autologs of the human melanocortin receptors were cloned from several different species such as rodents, cows and birds (Barret et al., 1994; Labbé et al., 1994; Vanetti et al., 1994; Fathi et al., 1995; Takeuchi and Takahashi, 1998; and others).

The five melanocortin receptors show sequence homologies ranging between 40–60% and many of the characteristics that are common to other G-protein coupled receptors. All the melanocortin receptors have several potential *N*-glycosylidation sites in their N-terminal domains. They have also consensus recognition sites for protein kinase C, and in some cases also for protein kinase A, indicating that they may be subject to regulation by phosphorylation. The melanocortin receptors also have conserved cysteins in their C-termini, which may serve as sites for fatty acid acylation anchoring the C-terminus to the plasma membrane.

## 3. Expression and distribution of melanocortin receptors

### *3.1. Melanocortin $MC_1$ receptors*

As already stated above the melanocortin $MC_1$ receptor was first identified in cell lines derived from melanoma tumours (Chhajlani and Wikberg, 1992). Using antibodies directed against the N-terminus of the melanocortin $MC_1$ receptor we also demonstrated the presence of the receptor in a solid primary melanoma tumour (Xia et al., 1995a). However, it then becomes apparent that the melanocortin $MC_1$ receptor is also present in other tissues. By using both in situ hybridisation and immune histochemistry we could detect the melanocortin $MC_1$ receptor in a few scattered neurones of the periaqueductal grey in both rats and human brains (Xia et al., 1995b). However, despite extensive studies we did not find the melanocortin $MC_1$ receptor in other areas of the CNS (Central Nervous System). Chhajlani (1996) found evidence for the expression of melanocortin $MC_1$ receptors in the pituitary and testis. In our laboratory we also developed a monoclonal antibody, which was used to detect melanocortin $MC_1$ receptors in the leydig cells of the testis, as well as in the lutein cells of the corpus luteum (Thörnvall et al., 1997). Nuclei of trophoblastic cells of the placenta stained positively for melanocortin $MC_1$ receptors with the use of these monoclonal antibodies (Thörnvall et al., 1997). This is an interesting observation since we, by use of our polyclonal anti melanocortin $MC_1$ receptors sera, found prominent intracellular and perinuclear melanocortin $MC_1$ receptor staining also in melanoma cells (Xia et al., 1995c), as well as in the keratinocytes located adjacent to a solid melanoma tumour (Xia et al., 1995a). Still the melanocortin $MC_1$ receptor is clearly also located on the cell-membranes of melanoma cells as prominent melanocortin $MC_1$ receptors staining is seen on the cell-membranes of non-permeabilized melanoma cells (Xia et al., 1995c). There is also evidence that melanocortin $MC_1$ receptors on melanoma cells may become internalised upon exposure to MSH in some melanoma cell lines (Tatro, 1996). The function (if any) of intracellular/nuclear melanocortin $MC_1$ receptors remains to be established.

It has recently become evident that melanocortin $MC_1$ receptors are also expressed on macrophages and monocytes (Star et al., 1995; Hartmeyer et al., 1997), neutrophils (Catania et al., 1996), endothelial cells (Hartmeyer et al., 1997), glioma cells and astrocytes (Wong et al., 1997), fibroblasts (Boston and Cone, 1996), and keratino-

cytes (for reference see Luger et al., 1997). The localisation of melanocortin $MC_1$ receptors to these cell types have recently been much discussed in relation to anti-inflammatory actions of MSH-peptides (see further below).

### 3.2. Melanocortin $MC_2$ receptors

In situ hybridisation reveals that the melanocortin $MC_2$ receptor is highly expressed in the cortex of the adrenal gland; the densest expression occurring in the *zona reticularis* and *fasiculata*, with the expression in the *zona reticularis* being less pronounced. These findings are consistent with a role for the melanocortin $MC_2$ receptor in mediating the effect of ACTH on steroid secretion (Xia and Wikberg, 1996). Interestingly a few scattered cells of the adrenal medulla also expressed the melanocortin $MC_2$ receptor (Xia and Wikberg, 1996). The function of these cells is unknown.

Besides the adrenal gland, the melanocortin $MC_2$ receptor is expressed in the white adipose tissue of the mice (Boston and Cone, 1996), a localisation which is well in line with the presence of ACTH binding site and lipolytic action of ACTH on murine adipocytes (White and Engel, 1958; Oelofsen and Ramachandran, 1983; Grunfeld et al., 1985). However, data from our laboratory indicate that the human adipocyte does not express the melanocortin $MC_2$ receptor (Chhajlani, 1996). Moreover, the primate adipocytic tissues, including that of the human, is reported to be non-responsive to any lipolytic action by ACTH (Bousquet-Melou et al., 1995). It seems thus that there are species differences in the expression of melanocortin $MC_2$ receptors in primates and rodents, an observation that may be of interest in relation to using melanocortin receptors as target for anti-obesity treatment (see also further below under localisation of the melanocortin $MC_5$ receptors).

Prompted by the known short loop negative feed back regulation of ACTH on corticotrophin release factor from the hypothalamus, we searched carefully for the expression of melanocortin $MC_2$ receptors in the hypothalamus and pituitary using in situ hybridisation, but we failed to detect such expression (Xia and Wikberg, 1996). It is thus likely that the negative regulation of corticotrophin release factor is mediated via some other melanocortin receptor (e.g., the melanocortin $MC_3$ or $MC_4$ receptor).

The expression of the melanocortin $MC_2$ receptor was recently identified in the skin (Slominski et al., 1996; Ermak and Slominski, 1997). This finding is of high interest as ACTH-(1–39) peptides and fragments thereof were recently demonstrated to be produced in the epidermis and cultured keratinocytes, in amounts exceeding those of the α-MSH-peptide, the latter being well known also to be expressed in the skin (Wakamatsu et al., 1997). It was recently produced that corticotropin [i.e., ACTH-(1–39) and ACTH-(1–24)] induces DNA synthesis and cell proliferation of keratinocytes (Kapas et al., 1998). Moreover, the mRNAs of the three cytochromes involved in steroid synthesis were demonstrated to be present in the human skin (Slominski et al., 1996). Thus, these observations prompts the search for novel roles for melanocortin $MC_2$ receptors and corticotropins in the physiology of the skin.

### 3.3. Melanocortin $MC_3$ receptors

The melanocortin $MC_3$ receptor was first reported to be expressed in the brain, placenta and gut (Gantz et al., 1993). Data from our laboratory indicate that the melanocortin $MC_3$ receptor is also expressed in the heart (Chhajlani, 1996).

A detailed mapping of the expression of melanocortin $MC_3$ receptors in the central nervous system was carried out by use of in situ hybridisation (Roselli-Rehfuss et al., 1993). The melanocortin $MC_3$ receptor mRNA shows a quite restricted distribution with the highest densities being found in regions of the hypothalamus and limbic system. High densities are also present in the dorsomedial part of the ventromedial nucleus of the hypothalamus and the posterior hypothalamus, including the arcuate nucleus. The melanocortin $MC_3$ receptor expression is also found in the septum, hippocampus, thalamus and midbrain including the ventral tegmental area. The ontogeny of the melanocortin $MC_3$ receptor expression was studied in the rat brain; the melanocortin $MC_3$ receptor expression being low at birth and then gradually rising until postnatal day 21, when steady adult levels are reached (Xia and Wikberg, 1997; Kistler-Heer et al., 1998).

Recently, we developed an autoradiographic approach to delineate melanocortin $MC_3$ and $MC_4$ receptor localisations (Lindblom et al., 1998). In these studies we incubated brain slices with the essentially non-selective [$^{125}$I]NDP-MSH radioligand in the presence of various concentrations of γ1-MSH (40-fold selective for rat melanocortin $MC_3$ vs. $MC_4$ receptor) and HS014 (300-fold selective for rat melanocortin $MC_4$ vs. $MC_3$ receptor), and then subjected the resulting autoradiograms to image analysis. The data suggest that in virtually all of the studied regions of the medial brain (nucleus accumbens, lateral septum, olfactory tubercle, optic nerve layer of the superior colliculus, central grey, medial preoptic area, and ventromedial nucleus of the hypothalamus) more than 60% of the [$^{125}$I]NDP-MSH binding sites are melanocortin $MC_3$ receptors. Very high relative densities of the melanocortin $MC_3$ receptors seem to be present in the ventromedial nucleus of the hypothalamus and the nucleus accumbens, followed by the medial preoptic area and the central grey. High relative levels of the melanocortin $MC_3$ receptors seem also to be present in the lateral septum and olfactory tubercle, whereas more equal levels of melanocortin $MC_3$ and $MC_4$ receptors are present in the optic nerve layer of the superior colliculus. With respect to the absolute densities, the ventromedial nucleus of the hypothalamus contained the highest levels of receptors (about 5 fmol/mg wet tissue), which was followed by the medial preoptic area (about 3.5 fmol/mg

wet tissue). The other studied areas contained between 0.7–1.5 fmol/mg wet tissue of melanocortin receptors (Lindblom et al., 1998).

These data are very exciting in view of the reported expression of melanocortin $MC_3$ (see above) and $MC_4$ receptors (see below) in the brain. Our autoradiographic data for the ventromedial nucleus of the hypothalamus align well with the reported mRNA expression of the melanocortin $MC_3$ receptors. However, for the nucleus accumbens only high levels of melanocortin $MC_4$ receptors expression were reported (Mountjoy et al., 1994), data which contrast strongly to the autoradiographic data. These data combined may suggest that the melanocortin $MC_3$ receptors are, in many cases, present on the nerve terminals projecting from the posterior hypothalamus, where the melanocortin $MC_3$ receptor expression is prominent. As will be detailed below, the majority of the MSH containing neurons of the central nervous system project from the arcuate nucleus of the hypothalamus to many parts of the brain, including the nucleus accumbens. The dominance of melanocortin $MC_3$ receptors in the nucleus accumbens is of interest in relation to a reported connection of the melanocortic system in morphine addiction (Mucha and van Ree, 1989; Alvaro et al., 1996, 1997), other forms of drug addiction, as well as possible roles for the melanocortin $MC_3$ receptors in psychiatric diseases.

With respect to the ventral tegmental area we found that affinities of HS014 and γ1-MSH for the [$^{125}$I]NDP-MSH binding site(s) were substantially lower than found in all the other studied areas of the medial brain (Lindblom et al., 1998). These low affinities seem to indicate that [$^{125}$I]NDP-MSH binds predominantly neither to melanocortin $MC_3$ nor to melanocortin $MC_4$ receptors. It is presently not known what these [$^{125}$I]NDP-MSH binding sites represent. A possible candidate is the melanocortin $MC_5$ receptor, but further studies are warranted to settle the issue. In this context, it should be mentioned that it has been speculated that the effect of MSH-peptides on cardiovascular function is elicited by some type of melanocortin receptor which is different from the presently cloned ones (Versteeg et al., 1998).

### 3.4. Melanocortin $MC_4$ receptors

The melanocortin $MC_4$ receptor is expressed in multiple sites in virtually every brain region including the cortex, thalamus, hypothalamus, brain stem and spinal cord (Mountjoy et al., 1994). The distribution of the melanocortin $MC_4$ receptor expression in the CNS is thus distinctly different, and much wider, than the expression of the melanocortin $MC_3$ receptor. However, as pointed out in the preceding paragraph, the melanocortin $MC_3$ receptor protein may still have a dominating role compared to the melanocortin $MC_4$ receptor in the medial part of the brain (Lindblom et al., 1998). In detailed studies covering 20 human organs the melanocortin $MC_4$ receptor expression was not detectable in the periphery (Chhajlani, 1996). However, the melanocortin $MC_4$ receptor was expressed in many peripheral tissues of the chicken (Takeuchi and Takahashi, 1998).

The ontogeny of the expression of the mammalian melanocortin $MC_4$ receptor expression was studied by Kistler-Heer et al. (1998). The melanocortin $MC_4$ receptor expression is predominant during the whole foetal period (Kistler-Heer et al., 1998), in contrast to the melanocortin $MC_3$ receptor expression which develops predominantly postnatally (Xia and Wikberg, 1996; Kistler-Heer et al., 1998). Mountjoy and Wild (1998) also reported that the melanocortin $MC_4$ receptor was highly expressed in the autonomic nervous system of the developing foetus.

### 3.5. Melanocortin $MC_5$ receptors

The melanocortin $MC_5$ receptor expression was initially recognised in the brain (Chhajlani et al., 1993). In subsequent studies from our laboratory the melanocortin $MC_5$ receptor expression was found to be ubiquitous, its mRNA being detected in many human tissues, namely adrenal glands, fat cells, kidneys, liver, lung lymphnodes, mammary glands, ovary, pituitary testis and uterus. Very weak expression seems also be present in vascular smooth muscle, stomach and spleen (Chhajlani, 1996). Clear melanocortin $MC_5$ receptor expression is also reported for several glands (lachrymal, prostate, seminal, pancreatic, preputial, Harderin and thyroid), as well as skeletal muscle (Chen et al., 1997; van der Kraan et al., 1998). Deletion of the melanocortin $MC_5$ receptor gene in mice resulted in essential total loss of [$^{125}$I]NDP-MSH bindings sites in skeletal muscle, Harderian, lachrymal and preputial glands, as well as the essentially complete loss of the ability of α-MSH to stimulate formation of cAMP in the Harderian and preputial glands (Chen et al., 1997), results which indicate that melanocortin $MC_5$ receptors represent the majority of melanocortin receptors in these tissues.

In view of what was reviewed above under the melanocortin $MC_2$ receptor together with the expression of the melanocortin $MC_5$ receptor in human fat cells, it seems possible that the lipolytic actions reported to be induced by MSH peptides, could predominantly be mediated in humans by an action on the melanocortin $MC_5$ receptor. The function(s) of the melanocortin $MC_5$ receptors expressed in the various tissues remains to be elucidated.

## 4. Genetic variation related to melanocortin receptors and agouti

A quite large number of mutations in the coding portion of melanocortin $MC_1$ receptor gene has been found, mutations which correlate with variations in the colour of skin and fur of mammals and man. The gene products for these mutations seem to fall into three categories, namely, recep-

tors that are activated by MSH peptides, receptors that are non-functional, and receptors that are constitutively active. Both single point and frame mutated melanocortin $MC_1$ receptors have been found (Robbins et al., 1993; Klungland et al., 1995; Valverde et al., 1995; Marklund et al., 1996; Koppula et al., 1997; Våge et al., 1997). These mutations affect distinctly the pigment formation in the melanocytes. However, the genetic variation in the melanocortin $MC_1$ receptors have not been reported to be associated with any other biological variation, besides that causing differences in the colour of skin and fur. This is of interest in the relation of the reported presence of melanocortin $MC_1$ receptors in other cells than pigment forming melanocytes. Careful population studies could perhaps reveal differences in predisposition to specific diseases that correlate with genetic variation in the melanocortin $MC_1$ receptor.

Fur colour pattern in mammals is also regulated by the *Agouti* locus, which encodes the agouti peptide. In mice more than 34 alleles are known in *Agouti*, which relate to different colour and banding patterns. A very close homologue to the mouse agouti is identified in humans (see Kucera et al., 1996), which has been termed agouti signalling peptide (ASP; or ASIP) (Wilson et al., 1995). ASP is expressed more widely in human tissues compared to mice, and ASP expression is seen in human skin, fat and heart (see references in (Fong et al., 1997)). The agouti peptide behaves like a competitive antagonist at the melanocortin $MC_1$ and $MC_4$ receptors, but it does not bind to the melanocortin $MC_3$ and $MC_5$ receptors (Lu et al., 1994; Blanchard et al., 1995). The over-expression of agouti in the skin of mice was shown to yield yellow pigmentation, while its over-expression in other areas of the body was related to obesity, diabetes and increased tumour susceptibility (Perry et al., 1995; Kucera et al., 1996). These observations has prompted a role for the melanocortin $MC_4$ receptor in control of feeding behaviour and body weight (see below). Recently, a novel protein was cloned, the agouti related protein (ART), which shows 25% identity with the human agouti (Shutter et al., 1997). ART was found to bind to and antagonise the action of MSH-peptides on melanocortin $MC_3$ and $MC_4$ (but not on the melanocortin $MC_5$) receptors (Fong et al., 1997). Interestingly the hypothalamic expression of ART was elevated approximately 10-fold in genetically obese ob/ob (leptin deficient) and db/db (leptin receptor deficient) mice (Shutter et al., 1997), indicating a possible role for ART as a signalling factor in the control of body weight.

By use of restriction fragment-length polymorphism (RFLP) mapping, Chagnon et al. (1997) found that both the human melanocortin $MC_4$ and $MC_5$ receptor genes are subject to polymorphisms within siblings of a Québec population (the melanocortin $MC_5$ receptor showing four alleles and the melanocortin $MC_4$ receptor two). Moreover, Chagnon et al. showed that these polymorphisms were linked with obesity phenotype; the melanocortin $MC_5$ mutations being more strongly linked with obesity, when compared with the melanocortin $MC_4$ mutations.

## 5. POMC derived peptides

The natural melanocortic peptides are derived from the precursor peptide pro-opiomelanocortin (POMC) by proteolytic cleavage in three regions of the POMC generating ACTH and α-MSH, β-MSH and γ-MSH peptides. POMC also generates a number of other peptides, among which β-endorphin should be mentioned.

Mammalian α-MSH is N-terminally acetylated and C-terminally amidated. Diacetyl and des-acetyl α-MSHs are also present (Eberle, 1988). POMC is expressed in the pituitary from which the ACTH and α-MSH are released systemically. α-MSH is also detectable in many peripheral tissues, such as stomach, kidney, intestine, testis, ovaries, placenta, adrenal medulla, pancreas and skin, where it may have paracrine roles. γ-MSH immunoreactive peptides have been detected in some tissues, such as the adrenal medulla, and in neurones of the intestine (Eberle, 1988). ACTH peptides were also recently identified in the skin (Wakamatsu et al., 1997). In the central nervous system, α-MSH immunoreactive perikarya are found in three regions: the nucleus arcuatus of the hypothalamus, the dorsolateral region of the hypothalamus (zona incerta) and the nucleus tractus solitarius (Eberle, 1988). From these regions nerve fibres project throughout virtually the whole brain, including the hypothalamus, thalamus, midbrain, amygdala, medulla, spinal cord, hippocamus and cerebral cortex. β-MSH have been detected in the human hypothalamus, and γ-MSH immunoreactive neurones are present at discrete locations in the CNS (Eberle, 1988).

The natural MSH peptides all bind the MC-receptors with an order of potency $MC_1 > MC_3 > MC_4 > MC_5$ (Table 1). MSH peptides do not bind to the melanocortin $MC_2$ receptor (Schiöth et al., 1996b). On the contrary, the sequence of ACTH beyond that of α-MSH influences negatively the binding of ACTH to the melanocortin $MC_1$, $MC_3$, $MC_4$ and $MC_5$ receptors (Schiöth et al., 1997a). In fact, part of the binding activity of ACTH in in vitro assays for the MSH binding melanocortin receptors may be due to the cleavage of ACTH into shorter MSH-fragments, unless measures are taken to prevent such cleavage (Schiöth et al., 1996b). None of the natural MSH peptides show selective actions for the melanocortin $MC_3$, $MC_4$ or $MC_5$ receptors, although γ-MSH is relatively much more selective for the melanocortin $MC_3$ vs. the melanocortin $MC_4$ and $MC_5$ receptors. Among the natural MSH-peptides, it is the β-MSH that shows the highest affinity for the melanocortin $MC_4$ receptor (Schiöth et al., 1996a). Whether or not these differences in melanocortin receptor subtype preferences by the natural MSH-peptides have any physiological relevance is at present not clear, but it is

Table 1
$K_i$ values (in nM) of natural and synthetic peptides for human melanocortin $MC_1$, $MC_3$, $MC_4$ and $MC_5$ receptors expressed in COS cells obtained in competition with [$^{125}$I]NDP-MSH using the same experimental conditions

| | $MC_1$ | $MC_3$ | $MC_4$ | $MC_5$ |
|---|---|---|---|---|
| ACTH-(1–39)[a] | 2.5 | 87 | 690 | 17,000 |
| α-MSH[a,b,c,d] | 0.12 | 31 | 660 | 5700 |
| Desacetyl α-MSH[b] | 0.28 | 14 | 250 | 1900 |
| Diacetyl α-MSH[b] | 2.0 | 72 | 2800 | 5900 |
| β-MSH[a] | 1.2 | 13 | 380 | 14,000 |
| γ1-MSH[a] | 2.7 | 7.1 | 29,000 | 43,000 |
| γ2-MSH[a] | 11 | 18 | > 100,000 | > 100,000 |
| γ3-MSH[a] | 1.4 | 11 | 34,000 | > 100,000 |
| [Nle$^4$, D-Phe$^7$] α-MSH[a] | 0.085 | 0.40 | 3.8 | 5.1 |
| ACTH-(4–10)[c] | 300 | 7700 | 18,000 | 100,000 |
| [Phe-I$^7$]ACTH-(4–10)[c] | 2700 | 4800 | 13,000 | 29,000 |
| [Ala$^6$]ACTH-(4–10)[c] | > 3,000,000 | 100,000 | 67,000 | 130,000 |
| SHU9119[d] | 0.71 | 1.2 | 0.36 | 1.1 |
| HS014[f] | 110 | 54 | 3.2 | 690 |
| HS024[g] | 19 | 5.5 | 0.29 | 3.3 |
| MS04[h] | 7.6 | 21,000 | ≫ 50,000 | ≫ 50,000 |
| MS05[i] | 0.76 | > 50,000 | > 100,000 | > 100,000 |
| HP-228[e] | 1.6 | 74 | 74 | 53 |
| Melanotan II[d] | 0.67 | 34 | 6.6 | 46 |

[a]Schiöth et al. (1996a); [b]Schiöth et al. (1997a); [c]Schiöth et al. (1997b); [d]Schiöth et al. (1997c); [e]Schiöth et al. (1997d); [f]Schiöth et al. (1998a); [g]Kask et al. (1998b); [h]Szardenings et al. (1997); [i]Szardenings et al., to be published in detail elsewhere.
For α-MSH, the value given is the geometric means of the values from the indicated studies.

notable in this context that α-, β- and γ-MSH are present in the CNS with differing distributions (Eberle, 1988).

## 6. Synthetic compounds showing activity on melanocortin receptors

Based on assays on melanophores or melanocytes, a large number of linear and cyclic peptide agonists for MSH receptors have been developed (De Wied and Wolterink, 1988; Eberle, 1988; Al-Obeidi et al., 1989). A very useful linear peptide is [Nle$^4$, D-Phe$^7$]α-MSH (Melanotan-I; NDP-MSH) as it shows high affinity for the melanocortin receptors, and as it can be labelled with iodine and used as radioligand (Table 1). However, most (if not all) of these linear or cyclic peptides (at least as far as they have been tested) seem not to deviate from the potency order $MC_1 > MC_3 > MC_4 > MC_5$.

Adan et al. (1994) reported that some linear ACTH-(4–10) analogues show selective antagonistic actions on melanocortin receptors. The data of Adan et al. are difficult to interpret as comparisons were done on melanocortin receptors from three different species. It is well known that large differences in receptor binding specificities may exist for species variants of the same receptor. In our hands the order of affinities of one of these analogues, namely [Ala$^6$]ACTH-(4–10), did indeed differ from the pattern of peptides mentioned in the preceding paragraph, but the affinity of the compound was very low, as were also the affinities of ACTH-(4–10) and the[Phe-I$^7$] ACTH-(4–10) (Table 1).

Hruby et al. (1995) synthesized a cyclic lactam peptide, SHU9119, which was claimed to show selectivity and antagonistic activity at the melanocortin $MC_4$ receptor. However, SHU9119 is a partial agonist at the melanocortin $MC_3$ receptor, and a full agonist at the melanocortin $MC_5$ receptor (Hruby et al., 1995). In radioligand binding assays we actually found the compound to be essentially non-selective for the melanocortin receptor subtypes (Table 1). Thus the usefulness of SHU9119 as an experimental tool for melanocortin receptor subtype classification seems to be limited.

Among a series of cyclic MSH analogues with large ring sizes (26 atoms) we recently discovered the melanocortin $MC_4$ receptor selective antagonist HS014 (Table 1). The selectivity of HS014 is about 20-fold over the melanocortin $MC_3$ receptor, 30-fold over the melanocortin $MC_1$ receptor and 200-fold over the melanocortin $MC_5$ receptor. Besides the melanocortin $MC_4$ receptor, HS014 was found to be an antagonist at the melanocortin $MC_3$ receptor, but a partial agonist at the melanocortin $MC_1$ and $MC_5$ receptor (Schiöth et al., 1998a). Among a more recent series of cyclic MSH peptide analogues, where the ring size was extended to 29 atoms, we discovered another very potent melanocortin $MC_4$ receptor selective antagonist, HS024 (Table 1). The affinity of HS024 for the melanocortin $MC_4$ receptor is in the sub-nanomolar range, and the compound is about 70-, 20- and 10-fold melanocortin $MC_4$ receptor selective when compared to the melanocortin $MC_1$, $MC_3$, and $MC_5$ receptor, respectively. Moreover, HS024 did not show agonistic activity at any of the melanocortin $MC_1$, $MC_3$, $MC_4$ or $MC_5$ recep-

tors (Kask et al., 1998b). Thus, HS024 appears to be a true antagonist for all the MSH-peptide binding melanocortin receptors.

In a recent study we developed a phage display library screening method and used it to sort for peptides capable of binding to melanocortin $MC_1$ receptors (Szardenings et al., 1997). From a number of clones we found one that encoded the MS04 peptide. When we characterised the properties of MS04 it showed a remarkable selectivity for the melanocortin $MC_1$ receptor (Table 1), although its affinity was not as high as that of α-MSH. MS04 was also found only to be a partial agonist at the melanocortin $MC_1$ receptor (Szardenings et al., 1997). We therefore applied extensive chemical modifications to the MS04, and among a series of new compounds we obtained MS05 (Szardenings et al., to be published). MS05 shows sub-nanomolar affinity for the melanocortin $MC_1$ receptor, and virtually no binding affinity at all for the melanocortin $MC_3$, $MC_4$ and $MC_5$ receptors (Table 1). Moreover, MS05 appears to share virtually the same capacity as α-MSH to stimulate cAMP. This is illustrated in the experiment of Fig. 1A, which compares the ability of MS05 and α-MSH to induce melanocortin $MC_1$ receptor mediated accumulation of cAMP in B16-melanoma cells. Thus, our data indicate that MS05 is a full agonist at melanocortin $MC_1$ receptors. Our success in generating the MS05 show the capacity of the phage display library screening method to sort out lead compounds, followed by chemical development to achieve high affinity, high-selectivity compounds.

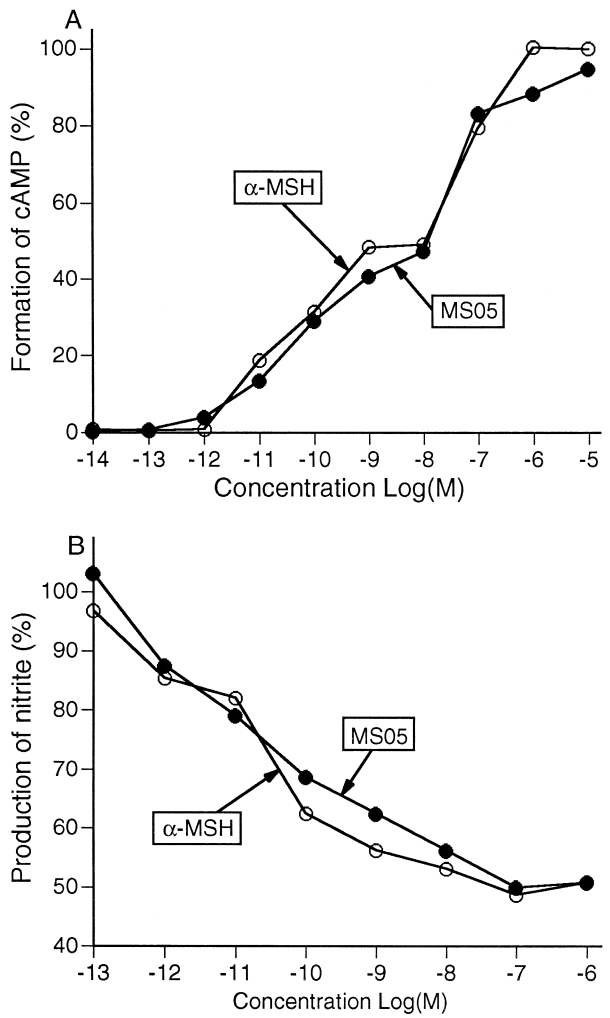


Fig. 1. (A) Effects of α-MSH and MS05 on cAMP stimulation in B16 mouse melanoma cells. Effect expressed in percent of stimulation afforded by α-MSH. (B) Effects of α-MSH and MS05 on production of nitrite in mouse RAW-264 cells. NO production was stimulated with bacterial lipopolysacharide and interferon and quantified by measurement of the formation of nitrite in the presence of different concentrations of α-MSH and MS05 (data taken from Muceniece et al., intended to be published in full detail elsewhere).

Two other melanocortin receptor agonists have been devised that deserve to be mentioned. These are HP-228 (Abou-Mohamed et al., 1995) and Melanotan-II (Hadley et al., 1989). HP-228 is a linear MSH-hepta peptide analogue, which has been subject to some clinical trials. HP-228 shows the affinity order $MC_1 > MC_3 > MC_4 > MC_5$ for the melanocortin receptors (Table 1). In comparison to the selectivity of MS05 the selectivity of HP-228 is marginal. Melanotan-II is a cyclic MSH analogue synthesized quite long time ago (Hadley et al., 1989). Melanotan-II has recently been used in various functional studies. Melanotan-II is quite potent at melanocortin $MC_1$ receptors, but it is not particularly selective (Table 1).

## 7. Potential of the use of melanocortin receptor active drugs for control of eating and body weight

Eating is regulated by a complex network of physiological regulatory pathways that involves both the central nervous system and peripheral sites. Peripherally released leptin and insulin are important mediators that act on hypothalamic sites. Within the central nervous system various regulatory factors are well known to be involved, among which may be mentioned neuropeptide Y, orexins and CRF (Corticotropin-Releasing Factor). These systems control the amount of food intake both in short and long term, something which secondarily affects body weight, body fat mass and growth rate. Thus, e.g., neuropeptide Y administered intracerebroventricularly (i.c.v.) or directly into specific regions of the hypothalamus, is shown to dramatically increase food intake followed by body weight increase and gain of body fat (Stanley et al., 1986). The melanocortic system was known more than a decade ago to be involved in feeding homeostasis, as injections of the melanocortic peptides α-MSH and ACTH-(1–24), either i.c.v. or directly into the hypothalamus, were shown to markedly inhibit intake of food in experimental animals (Poggioli et al., 1986; Vergoni et al., 1986). The discovery that agouti was capable of inhibiting subtypes of melanocortin receptors and that its ectopic expression resulted in obesity further strengthened this view. Additional evidence were provided by Fan et al. (1997) who showed

that i.c.v. injections of the non-specific melanocortin receptor agonist Melanotan-II potently reduced food intake. Moreover, i.c.v. administration of SHU9119 blocked the effect of Melanotan-II. Given alone, SHU9119 increased food intake (Fan et al., 1997). Huszar et al. (1997) reported that targeted inactivation of the melanocortin $MC_4$ receptor gene in mice resulted, for homozygous litters, in about 50% increased food consumption and body weight at 15 weeks of age, as well as an about 10% increase in body length. Even furthermore, Krude et al. (1998) recently reported two different mutations in the human POMC gene, leading to deficiency in ACTH and MSH, that results in a syndrome consisting of severe early onset obesity, hyperphagia, adrenal insufficiency and red hair pigmentation; symptoms which are entirely consistent with a role for MSH in control of feeding homeostasis and regulation of skin pigmentation, as well as a role for ACTH for regulation of corticosteroid secretion. We showed recently that both the melanocortin $MC_4$ receptor selective antagonists HS014 and HS024 cause a dramatic (i.e., up to 160%) dose dependent increase in food intake in free feeding rats after their i.c.v. administration (Kask et al., 1998a,b). Thus, the combined data give strong support for a role of the melanocortic system in control of feeding, an effect likely to be mediated mainly (and perhaps even exclusively) by central melanocortin $MC_4$ receptors. The effect of HS014 and HS024 on food intake is illustrated in Fig. 2A.

The mechanism of action of the melanocortin receptor active drugs for control of feeding is currently under investigation in several laboratories. Leptin is suggested to act on POMC neurones of the arcuate nucleus of the hypothalamus causing increased expression of the MSH-precursor (Schwartz et al., 1997). The leptin signalling on the arcuate nucleus appears to be directed to the paraventricular nucleus of the hypothalamus as the administration of leptin leads to increase c-*fos* expression in the latter area, an effect that was blocked by the non-specific

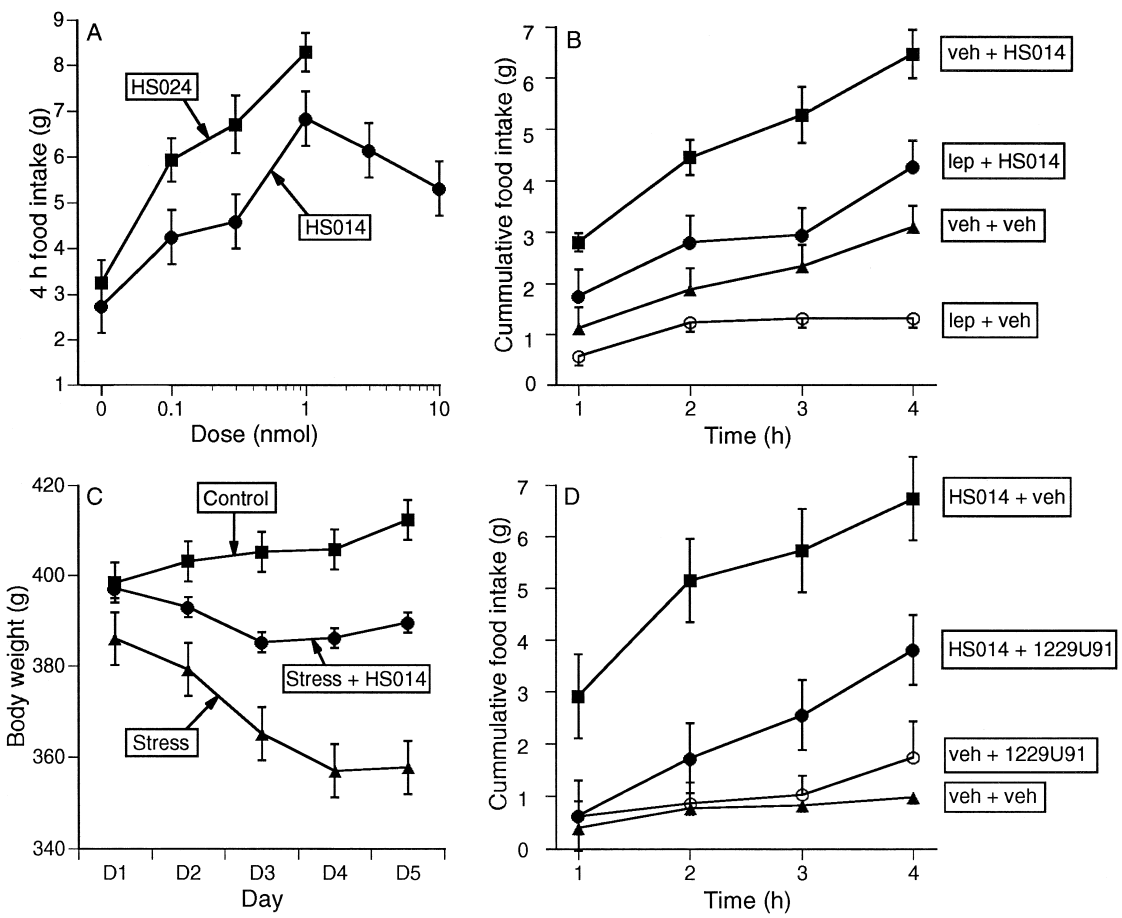


Fig. 2. (A) Cumulative food intake in free feeding rat during 4 h after i.c.v. injections of the MC4 receptor selective antagonists HS024 and HS014 (re-drawn from Kask et al., 1998b, courtesy of the publisher). (B) Effect of HS014 and leptin on food intake in rats. HS014 (1 nmol) and/or leptin (0.3 nmol) was administered into the lateral ventricle, the leptin being administered 30 min after the HS014, and the cumulated food intake assessed for up to 4 h. veh = i.c.v. administration of vehicle for HS014 and leptin, respectively (re-drawn from Kask et al., 1998d, courtesy of the publisher). (C) Body weight gain in normal (control) and rats subjected to immobilisation stress (Stressed). HS014 signifies i.c.v. treatment with 10 μg/rat of HS014 5 min before the stress (re-drawn from Vergoni et al., 1999, courtesy of the publisher). (D) Cumulative food intake in free feeding rat during 4 h after i.c.v. injections of 1 nmol HS014 in normal rats and rats treated with the neuropeptide Y Y1 receptor antagonist 1229U91 (12 nmol iv). veh = vehicle of HS014 and 1229U91, respectively. The 1229U91 was given 5 min before the injection of HS014 (re-drawn from Kask et al., 1998c, courtesy of the publisher).

melanocortin $MC_3/MC_4$ receptor blocker SHU9119 (Seeley et al., 1997). Also our melanocortin $MC_4$ receptor selective blocker HS014 is capable of counteracting the anorexogenic effect induced by leptin (Fig. 2B). It is also an important observation that the HS014 was capable of completely attenuating the loss of body weight induced by leptin in animals (Kask et al., 1998d). These data thus indicate that POMC neurones originating in the arcuate nucleus contacts synaptically at melanocortin $MC_4$ receptor containing neurones (presumably in the paraventricular nucleus or the medial hypothalamic nucleus; see below) that participate in control of feeding motivation. This POMC related melanocortin $MC_4$ receptor signalling thus appears clearly to occur down-stream to the leptin signalling. This interpretation is also supported by the observation that the Melanotan-II induced anorectic effect was seen in leptin deficient C57BL/6J-$Lep^{ob}$ mice (Fan et al., 1997).

An interaction with the melanocortic system and neuropeptide Y is also present. Kesterson et al. (1997) observed that while normal mice lacked detectable mRNA expression of the neuropeptide Y gene in neurones of the medial hypothalamic nucleus, an intense expression occurred in the lethal yellow agouti mice as well as in melanocortin $MC_4$ receptor knockouts (Kesterson et al., 1997). It is also shown that deletion of the neuropeptide Y gene in leptin deficient ob/ob animals partially attenuates the obesity syndrome caused by the leptin deficiency (Erickson et al., 1996). We have also shown that the neuropeptide Y Y1 receptor blocker 1229U91 attenuates the orexigenic effect induced by HS014 (Fig. 2D). The combined data would thus suggest that the effect of the melanocortic peptides exerted on the melanocortin $MC_4$ receptors leads to increased activity of neuropeptide Y containing neurones of the medial hypothalamic nucleus, something which in turn exerts the effect on feeding motivation. It appears thus that neuropeptide Y signalling is a down-stream event to the POMC-signalling. However, the observation that a neuropeptide Y knock out in ob/ob animals only partially counteract the animals weight gain (Erickson et al., 1996) indicates that neuropeptide Y is not the only factor of importance for regulation of feeding; perhaps the MSH-peptides are involved in other signalling pathways controlling feeding, as well.

The robustness of the POMC system in control of feeding motivation prompts it as a target for novel drugs for treatment of obesity and anorexia. Thus, an MSH-peptidomimetic (i.e., melanocortin receptor agonist) is predicted to be useful to reduce motivation for food intake, whereas an agouti-mimetic (i.e., melanocortin receptor antagonist) is predicted to be useful for increasing it.

We have recently evaluated the ability of HS014 to prevent stress induced anorexia and the body weight loss caused by stress. Stress was induced by short daily immobilisations of rats which causes profound anorexia and weight loss (Fig. 2C). HS014 was capable of quite effectively counteracting this weight reduction (Fig. 2C). Moreover, HS014 reduced powerfully the anorexia (Vergoni et al., 1999). We have also assessed the long term effects of HS014 on feeding and body weight (Kask et al., 1999). During 6 days of twice daily i.c.v. administration of HS014, no sign of tachyphylaxia to its orexigenic effect was noted. The increase in food intake was associated with a marked increase in body weight, an effect that could be attributed to deposition of fat. After cessation of the injections a rebound in eating was seen and the body weight returned to normal (Kask et al., 1999). Continuos 2-week i.c.v. infusion of HS014 also increased feeding and body weight without sign of tachyphylaxia (Kask et al., 1999).

Anorexia nervosa is a disease of unknown aetiology, with a high morbidity and a significant lifetime mortality, where both psychological and biological mechanisms are involved, and for which there exist currently only ineffective and costly psychological and behavioural therapies (Gilchrist and Ben-Tovim, 1998). Disturbances in serotonin have been implicated (Kaye, 1997; Smith et al., 1999), and patients suffering from the disease have low plasma and cerebrospinal fluid contents of leptin (Mantzoros and Flier, 1997). However, the low levels of leptin are shown to normalise long before regain of body weight upon traditional therapy attempts. It is believed that the increase in leptin has a braking effect leading to resistance of weight gain, and the incomplete weight recoveries generally seen in clinical practice (Mantzoros and Flier, 1997). In view of what was reviewed above it is possible that a blockade of the melanocortic transmission at the level of the hypothalamus will give the patient the necessary motivation for eating and help ameliorating the condition. It is also possible that melanocortin receptor blocking agents may be useful in treatment of other anorectic conditions, such as trauma, cancer, AIDS (Acquired Immuno-Deficiency Syndrome) and the anorexia often seen in the elderly. Also for these cases, the loss of appetite leading to cachexia makes a major contribution to the severity and high mortality for these patients.

Obesity has multifactorial aetiology where both genetical and environmental factors contribute. Obesity is correlated with measurable changes in factors such as galanine, neuropeptide Y and leptin, as well as it correlates with a growing number of genetic markers. Only when melanocortin receptor agonists become available for clinical trials it will be possible to assess their usefulness in treatment of obesity.

## 8. Potential of the use of melanocortin receptor active drugs for treatment of inflammation

Another area that has recently become subject to wide interest is the possibility to use melanocortin receptor active drugs in treatment of inflammatory processes. A steadily growing number of publications are appearing

showing that MSH-peptides have a broad capacity to inhibit inflammatory processes. Thus, α-MSH is shown to powerfully inhibit the inflammation in experimental bowel disease, arthritis, brain inflammation/ischemia, kidney ischemia, contact hypersensitivity and dermatitis, as well as it may induce hapten tolerance (Ceriani et al., 1994; Chiao et al., 1997; Huh and Lipton, 1997; Luger et al., 1997; Rajora et al., 1997a,b; Lipton et al., 1998). These effects of MSH-peptides have in many cases been shown to be associated with the reduction of the production of pro-inflammatory cytokines such interleukin-1α, interleukin-1β, interleukin-6 and TNF-α (Tumour Necrosis Factor-α) (Luger et al., 1997; Lipton et al., 1998), while the production of the anti-inflammatory suppressor factor interleukin-10 and angiogenic factor interleukin-8 has been shown to increase (Luger et al., 1997). α-MSH was also reported to inhibit the inflammatory responses caused by bacterial lipopolysacharide, interferon and interleukin-1, as well as to inhibit the chemotactic migration of neutrophils and reduce the expression of Major histocompatibility class I antigens on monocytes and keratinocytes (Catania et al., 1996; Luger et al., 1997). α-MSH was also shown to reduce the ICAM-1 (Intercellular cell adhesion molecule-1) on melanoma cells and melanocytes (Morandini et al., 1998). Moreover, it was shown to inhibit formation of nitric oxide (NO) in cultured murine macrophages stimulated with bacterial lipopolysaccharide and γ-interferon, an effect presumed to be caused by the inhibition of the production of inducible NO synthase (Star et al., 1995). NO is believed to be a common mediator of all forms of inflammation and all the data summarised here indicate a very wide role for MSH-peptides in the regulation of inflammatory processes.

The melanocortin $MC_1$ receptor was recently found on cells that are involved in the inflammatory responses, namely macrophages, monocytes, neutrophils, endothelial cells, and astrocytes (for references see above) and it is tempting to assume that the anti-inflammatory effects of α-MSH are caused by the stimulation of melanocortin $MC_1$ receptors. Strong support for this conclusion comes from the observation that our highly melanocortin $MC_1$ receptor selective compound MS05 shows about equal capacity as α-MSH to inhibit the production of NO (measured as formation of nitrite) in a macrophage cell line (Fig. 1B). These RAW-264 cells also express melanocortin $MC_1$ receptors as can be assessed by [$^{125}$I]NDP-MSH radioligand binding (Muceniece et al., unpublished), in support of the hypothesis.

However, it is also possible that additional mechanisms may prevail. Lipton et al. (1998) have shown that the tripeptide fragment MSH-(11–13) is able to mimic many of the effects caused by α-MSH on the inflammatory processes, and it is possible that this fragment causes its effects by acting on a site different from the melanocortin $MC_{1-5}$ receptors. Both α-MSH and MSH-(11–13) inhibit the binding of radiolabelled interleukin-1β to the interleukin-1 receptor (Mugridge et al., 1991), an observation that should deserve further attention. Interestingly MSH-(11–13) was reported not to bind to MSH receptors on melanoma cells as could be assessed by radioligand binding (Lyson et al., 1994).

It was also recently reported that α-MSH is capable of inhibiting the activation of NF-κB (Nuclear transcription factor-κB) brought about by a variety of inflammatory stimuli, namely TNF-α, bacterial lipopolysacharide, okadaic acid and ceramide (Manna and Aggarwal, 1998). NF-κB is a ubiquitous transcription factor which is broadly involved in activation of the immune system; its activation causing expression of the genes of inducible NO synthase, adhesion molecules ICAM-1 (Intercellular cell adhesion molecule-1), VCAM-1 (Vascular cell adhesion molecule-1), ELAM-1 (Endothelial-leucocyte adhesion molecule-1), MHCs (Major Histocompatibility complexes), acute phases proteins, and various grow factors and cytokines (Siebenlist et al., 1994). Manna and Aggarwal (1998) reported that inhibitory effect of α-MSH on NF-κB was mimicked by a cyclic AMP analogue (dibutyryl cAMP) and blocked by protein kinase A inhibitors. These findings seem to be quite compatible with an activation of an MC-receptor (possibly the melanocortin $MC_1$ receptor) leading to increased formation of cAMP (Manna and Aggarwal, 1998). However, there still exist many controversies to resolve and questions to answer before the role of cAMP and melanocortin receptors in the regulation of NF-κB activity can be completely understood (see discussion section of Manna and Aggarwal, 1998).

Recently, a role for the melanocortin $MC_5$ receptor in the activation of the tyrosine phosphorylation pathway JAK/STAT (Janus [tyrosine] kinase/Signal transducers and activators of transcription) was proposed (Buggy, 1998). In this study, which was conducted on mouse pro-B-lymphocyte cells, α-MSH was shown to promote the tyrosine phosphorylation of JAK2, a member of the intracellular JAK tyrosine kinase family. The effect was accompanied by tyrosine phosphorylation and translocation into the nucleus of the cytoplasmic transcription factor STAT1. The effect of α-MSH was linked to the melanocortin $MC_5$ receptor because only the melanocortin $MC_5$ receptor subtype was found to be expressed in the pro-B-lymphocytes. Moreover, the effect on JAK2 phosphorylation could be reproduced in a fibroblast like cell line stably expressing the melanocortin $MC_5$ receptor, whereas it could not be seen when these cells expressed the melanocortin $MC_1$ receptor (Buggy, 1998). Both α-MSH and [Nle$^4$, D-Phe$^7$] α MSH (Table 1) were shown to induce proliferation of the B-lymphocytes in this study (Buggy, 1998).

The JAK pathway is considered to be involved in cytokine and interferon-mediated signalling. Although, it is definitely at present too early to state that there exists a direct link between the G-protein coupled receptor melanocortin $MC_5$ receptor and a tyrosine phosphorylation

pathway, the results presented by Buggy are intriguing and open up a new dimension in melanocortin $MC_5$ receptor signalling. There exist yet another example for the direct coupling of a G-protein coupled receptor with tyrosine phosphorylation pathways, namely for the angiotensin II $AT_1$ receptor, an effect thought to be related to growth responses by vascular smooth muscle (Marrero et al., 1995).

The data reviewed above indicate broad roles of the MSH-peptides and melanocortin receptors in regulation of the immune system and inflammation and prompts the speculation that the systemic release and/or local formation of the α-MSH is part of the host-defence mechanism to stress, a function it would then exert together with the ACTH/corticosteroids and catecholamines. It is predicted that selective agents can be developed for MSH receptors involved in the regulation of inflammatory processes, and that these agents will be both effective remedies for inflammatory diseases and as immune suppressants, without causing the troublesome side effects of the corticosteroids and other anti-inflammatory drugs now in general use for the treatment of these conditions.

## 9. Structure of the melanocortin receptors and their peptide binding pockets

The melanocortin $MC_1$, $MC_3$, $MC_4$ and $MC_5$ receptors show 40–60% amino acid homology. The similarity with which the MSH-peptides bind to the melanocortin receptors indicate that the structure of the melanocortin receptors binding pocket is conserved. All the natural MSH-peptides show a conserved sequence: His–Phe–Arg–Trp, which is a sequence that is also incorporated in many of the synthetic MSH-peptide analogues. The His–Phe–Arg–Trp sequence seem to constitute a central core that is important of the MSH-peptide binding to the melanocortin receptors.

Insight into the structural organisation of the MSH-peptide binding to melanocortin receptors and their ligand binding pockets have now started to emerge. By using site directed mutagenesis we initially identified two aminoacid residues, $Asp^{117}$ and $His^{260}$, in the 3:d and 6:th transmembrane segment of the human melanocortin $MC_1$ receptor, that seemingly were involved in the binding of α-MSH (Frändberg et al., 1994). In a following study we used this information to develop a 3-dimensional model for the melanocortin $MC_1$ receptor based on homology modelling using bacteriorhodposin as template (Prusis et al., 1995). Docking of a cyclic pentapeptide placed the ligand binding pocket of the melanocortin $MC_1$ receptor between transmembrane segments 2, 3 and 6 (Prusis et al., 1995).

However, due to the lack of experimental data that could substantiate our model we felt it necessary to perform extensive analysis of the melanocortin $MC_1$, $MC_3$ and $MC_5$ receptors by use of directed mutagenesis. As the different melanocortin receptors show quite large differences in their binding affinities for various peptides, the construction of chimeras between receptor subtypes should give useful information of which parts of the receptor molecules that mediate the selective binding of the peptides. Thus, in one of these studies we exchanged the transmembrane segment 4 and 5, as well as the extracellular loop 2 of the melanocortin $MC_3$ receptor with the corresponding regions of the melanocortin $MC_1$ receptor ('casette mutations') and found it to not at all affect the binding of various MSH-peptides (Schiöth et al., 1996c). These data thus indicate that the transmembrane segments 4 and 5, and extracellular loop 2 are not part of the peptide binding pocket of the melanocortin receptors. Further analysis of a number of melanocortin $MC_1/MC_3$ receptor chimeras, that extended from the N-terminus to the C-terminus and systematically exchanged all the seven transmembrane segments of the two receptors, indicated that the peptide binding site was located somewhere between transmembrane segments 1, 2, 3, 6 and 7. Only the chimeras with exchanges in these receptor segments resulted in changes in the MSH-peptide binding (Schiöth et al., 1997e). Thus, these data are in complete accord with our study on the cassette mutated receptors, and indicate the transmembrane segments 4 and 5 are not part of the peptide binding pocket.

We also truncated the N-terminal region of the melanocortin receptors. Thus, 27, 25, 28 and 20 aminoacids could be deleted from the N-termini from the melanocortin $MC_1$, $MC_3$, $MC_4$ and $MC_5$ receptors, respectively, (leaving only 11, 14, 17 and 17 aminoacids in the N-termini) without affecting the receptors ligand binding properties. For the melanocortin $MC_1$ and $MC_4$ receptors these truncations deleted all potential *N*-glycosylidation sites. These results indicate that the N-termini of the melanocortin receptors are not likely to be involved in the binding of MSH peptides, as well as is probably not *N*-glycosylidation for the all over integrity of the receptor structure. However, further deletions of the N-termini resulted in total loss of MSH-peptide binding, indicating that problems in receptor folding probably ensued (Schiöth et al., 1997g). We also exchanged the extracellular loops 1 and 3 in the mouse melanocortin $MC_5$ receptor with the corresponding portions of the human melanocortin $MC_1$ receptor, without the peptide binding properties being changed (Schiöth et al., 1998b). However, specific amino acids of the extracellular loops and N-termini may still be important for maintaining the overall receptor structure. All the human MSH binding receptor contain one conserved cystein in the N-terminus, and three conserved cysteins in the extracellular loop 3. However, for the human melanocortin $MC_5$ receptor one cystein of the extracellular loop is exchanged to arginine at position 272, while for the mouse melanocortin $MC_5$ receptor this cystein is conserved. As the human melanocortin $MC_5$ receptor show comparatively low affinity for the natural MSH peptides of the

other melanocortin receptors, including the mouse melanocortin $MC_5$ receptor (Schiöth et al., 1998b), this observation prompted the hypothesis that cysteins of the extracellular loop and N-terminus might form an SS bridge that maintains the structure of the receptor in a conformation yielding high affinity for the MSH-peptides. The hypothesis was verified by mutating $Arg^{272}$ in the human MC5 receptor to Cys which resulted in a 700-fold increase in the affinity of α-MSH (Frändberg et al., 1997).

Moreover, a detailed analysis of the ligand binding properties of our earlier point mutations $Asp^{117}$ and $His^{260}$ by using a doubly mutated $Asp^{117}/His^{260}$ melanocortin $MC_1$ receptor led us to believe that actually, these two amino acids were not directly involved in the interactions with the peptide hormone (Schiöth et al., 1997f). It rather appeared that these amino-acids participated in the maintenance of the overall structure of the receptor (Schiöth et al., 1997f). Alterations in these amino acids probably gave rise to a slight perturbation in the receptor 3D structure that gave subtle changes in the affinity patterns for MSH-peptides (see Frändberg et al., 1994). These conclusions prompted us to develop improved models for the melanocortin receptors. In these attempts we developed a new unbiased objective approach for the homology modelling of G-protein coupled receptors. In our approach, projection maps of rhodopsin was used together with orientational restraints derived from the presence of conserved and non-conserved amino acids in all the melanocortin $MC_1$, $MC_3$, $MC_4$ and $MC_5$ receptors cloned in different species. The data was used with Monte Carlo simulated annealing procedure to yield initial receptor templates, followed by molecular dynamics simulated annealing to full atoms representation and ligand docking (Prusis et al., 1997). A major problem in developing the model was that linear MSH-peptide are too flexible to allow accurate ligand docking. We therefore developed a novel series of small rigid cyclic MSH core peptides, the smallest one from this series showing melanocortin receptor binding affinity being the cHFRWG peptide (Schiöth et al., 1997h). Our final model indicates that the binding of the MSH-core peptide occurs to a pocket located mainly between transmembrane segments 1, 2 and 7, with some extension of the core tryptophane of the MSH-peptide in between transmembrane segments 3 and 6 (Fig. 3). Interactions of the residues of the core ligand with the human melanocortin $MC_1$ receptor is as follows: for the arginine, $Ser^{52}$, $Glu^{55}$, $Asn^{290}$ and $Asp^{294}$; for the tryptophane. $Asp^{121}$, $Thr^{124}$, $Phe^{257}$, $Phe^{238}$ and $Leu^{286}$; for phenylalanine $Leu^{48}$, $Thr^{95}$ and $Ile^{98}$; and for histidine, $Glu^{94}$, $Ile^{98}$ and $Asn^{91}$. The model is well supported by the

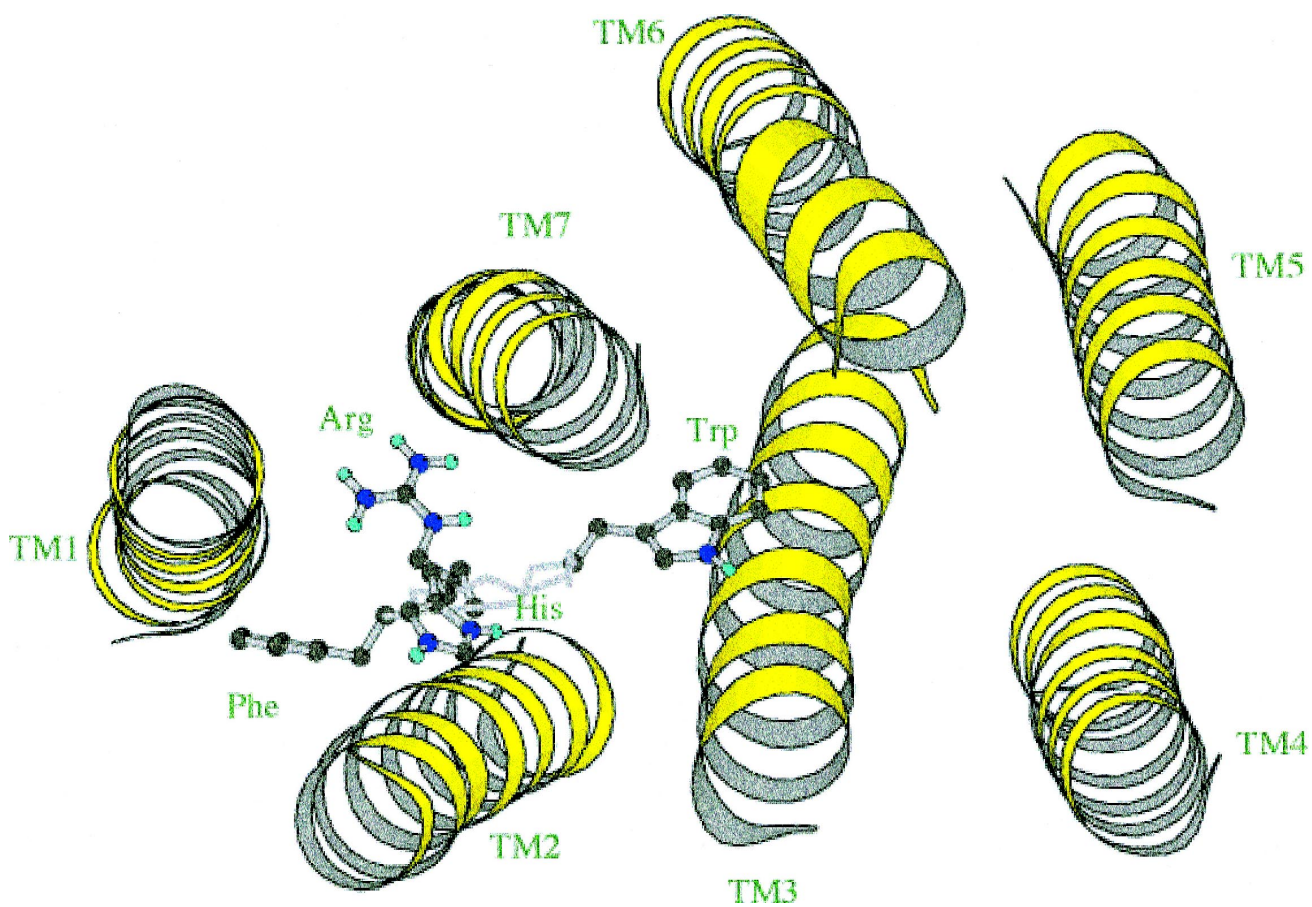


Fig. 3. Overview of the three dimensional model of the human melanocortin $MC_1$ receptor, with the cyclic core peptide cHFWRG docked into the model.

mutagenesis data summarised above with the localisation of the peptide binding pocket between transmembrane segments 1, 2, 3, 6 and 7 (Prusis et al., 1997).

In our model most of the mutated amino acid residues found in natural melanocortin $MC_1$ receptor mutations (see above) face the ligand binding pocket. The locations of the natural mutations to this region of the receptor also validate the model and give good explanation for the loss of MSH-binding for some mutations, as well as the constitutive receptor activation for some other mutations (for further discussion on this topic see Prusis et al., 1997). The model is also fully compatible with an SS-bridge being formed between the N-terminus and extracellular loop 3.

It should be mentioned that another 3-dimensional model of the melanocortin $MC_1$ receptor was proposed (Haskell-Luevano et al., 1996). This model places the peptide binding pocket between transmembrane segments 4, 5, 6 and 7. However, the collective experimental evidence reviewed above gives no support for this placement of the peptide binding pocket of the melanocortin receptors.

## 10. Concluding remarks

I have reviewed here some aspects of the dynamically evolving melanocortin receptor field. The accumulated data indicate that drugs with selectivity for melanocortin $MC_1$ receptors might find use for treatment of inflammatory conditions, whereas the melanocortin $MC_4$ receptor may serve as a target for drugs useful for control of eating behaviour and body weight. The melanocortin $MC_5$ and in particular the melanocortin $MC_3$ receptors are still awaiting to be ascribed clear functional roles. It seems quite likely that additional opportunities for drug development related to the melanocortin receptors will open up in the future. Areas of clear interest to pursue are pain control (see Follenfant et al., 1989), drug addiction (see Alvaro et al., 1996) and nerve regeneration (see van de Ment et al., 1997). The most important forthcoming issues will be to develop small organic compounds showing selective agonistic and antagonist actions on the five melanocortin receptor subtypes, and evaluate the pharmacology, pharmacokinetics and toxicology of these, as well as the already existing melanocortin receptor selective compounds. It is hoped that some of melanocortin receptor selective compounds will prove to be useful in clinical practices.

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