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Melanin Concentrating Hormone (MCH): Structure-Function Aspects of Its Melanocyte Stimulating Hormone-Like (MSH-Like) Activity

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MATSUNAGA, T. O., V. J. HRUBY, M. LEBL, A. M. DE LAURO CASTRUCCI AND M. E. HADLEY. *Melanin concentrating hormone (MCH): Structure-function aspects of its melanocyte stimulating hormone-like (MSH-like) activity.* PEPTIDES 10(4) 773–778, 1989.—Melanin concentrating hormone (MCH) is a heptadecapeptide, Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val, synthesized in the brain and secreted from the pars nervosa of teleost fish. This hormone stimulates melanosome (melanin granule) aggregation within integumental melanocytes of fishes but, in contrast, stimulates melansome dispersion within tetrapod (frog and lizard) melanocytes. We determined the message sequence of the primary structure of MCH which is responsible for its MSH-like component of activity. Removal of the N-terminal amino acid results in an almost total loss of MSH-like activity. The C-terminal amino acid is also essential for full MSH-like activity since the analogue, MCH(1–16), is about 100 times less active than MCH. Therefore, the entire heptadecapeptide sequence of MCH appears to contribute to the MSH-like activity of MCH. Ring-contracted analogues (e.g., [Ala^5,Cys^{10}]MCH) of MCH are almost devoid of any melanosome aggregating (MCH-like) activity but generally possess considerable or as great an MSH-like activity as MCH. Racemization of MCH by heat-alkali treatment drastically reduces the MCH-like activity of MCH, but does not enhance the MSH-like activity of the hormone.

Melanin concentrating hormone (MCH) Melanocyte stimulating hormone-like activity Structure-function aspects

MANY species of frogs, lizards, and fish are dependent upon hormonally-induced chromatic responses for their existence and survival. Two hormones, melanocyte concentrating hormone (MCH) and α-melanocyte stimulating hormone (α-MSH), are primarily responsible for inducing skin lightening and skin darkening, respectively. MCH stimulates melanosome aggregation within melanocytes of teleost fish, and α-MSH stimulates melanosome dispersion within melanocytes of most vertebrate species (1, 5, 8, 10, 13, 14). MCH is a heptadecapeptide with a centrally located disulfide bridge between residues 5 and 14 (Fig. 1).

It has been demonstrated that MCH can also promote the dispersion (MSH-like activity) of melansomes in both the frog and lizard skin bioassays (21,22). The MSH-like activity of MCH has been confirmed by other laboratories as well (7,12). Recently, ring-contracted MCH analogues were found to exhibit drastically reduced or no MCH activity (16). Interestingly, however, some analogues still retained MSH-like activity (16). Because of their dual activities, Castrucci and co-workers (6) have suggested a possible evolutionary link between the two hormones. Thus, from a common sequence, mutations could have resulted in two different peptides with different biological functions.

We have synthesized an entire series of MCH fragment analogues consisting of sequential deletions of either or both the N- and C-termini. All of these truncated analogues still possessed the intact central cyclic disulfide bridge structure of MCH. In a previous publication we reported on the MCH-like activities of these fragment analogues as determined in fish skin bioassays (18). We concluded that the fragment analogue, MCH(5–15), is the minimal sequence for equipotency to MCH in the fish skin assay (eel, *Synbranchus marmoratus*). In the present report we

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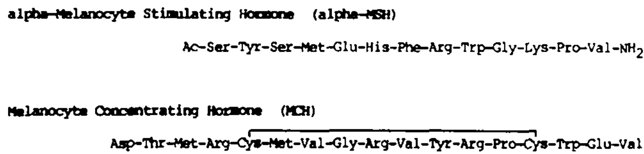

FIG. 1. Primary structures of melanin concentrating hormone (MCH) and melanocyte stimulating hormone (α-MSH).

determined the MSH-like activities of the analogues as determined in the frog and lizard skin bioassays. The results reveal some of the structural aspects of MCH related to its MSH component of melanotropic activity.

METHODS

All peptides were synthesized using a modification of the method of Lebl and co-workers (16), as described by Matsunaga *et al.* (18). It should be noted that strict attention was paid to avoiding any contact with α-MSH or any potent α-MSH analogues. This necessitated thorough washing of all glassware with base followed by an acid wash, and then rinsing with distilled H_2O. All columns utilized were free of any exposure to α-MSH or related peptides. As an added precaution, selected peptides were synthesized in a laboratory totally devoid of any synthesized melanotropins.

Frog skin assays were based upon previous work described in the literature (3, 11, 19). *Rana pipiens,* a species of frog native to North American regions, were obtained from Kohn's Scientific Supply Company, Germantown, WI. Animals were sacrificed by decapitation and the leg and thigh skins were removed and kept viable in a bath of physiological saline (Ringer) solution. Skins were stretched over PVC rings and then baseline light reflectance was measured via a Photovolt (model 670) reflectometer. When stimulated by melanotropins, melansomes are induced to migrate from the perinuclear space throughout the dendritic processes of melanocytes. This leads to a generalized darkening of the entire skin. Since skin darkening results in an increase in light absorbance, reflectance measurements yield a decrease in reflectance. Upon removal of the melanotropin (i.e., by rinsing with Ringer solution) a reaggregation of melanosomes to the perinuclear space occurs, and the skins generally relighten to their original baseline values.

Racemization studies were conducted according to the procedures of Bool *et al.* (4) and Eberle *et al.* (7). Skin darkening was determined via photoreflectance as above. All data were compiled and analyzed by plotting the percent skin darkening *vs.* −log concentration.

RESULTS

Melanin concentrating hormone (MCH) stimulates melanosome aggregation within teleost melanocytes but not within pigment cells of tetrapods (frogs and lizards). Rather, MCH exhibits MSH-like activity when used at high concentrations in tetrapods (Fig. 2). We previously demonstrated that MCH, as well as the fragment analogue, MCH(1–14), possessed MSH-like activity whereas MCH(5–17) and the central cyclic sequence, MCH(5–14), did not. This suggested that the N-terminal 1–4 sequence, or some component thereof, was responsible for the MSH-like activity of MCH. Synthesis of MCH analogues with N-terminal deletions revealed that removal of the terminal amino acid resulted in a near total loss of MSH-like activity relative to MCH. MCH(2–17), MCH(3–17) and MCH(4–17) exhibited greatly

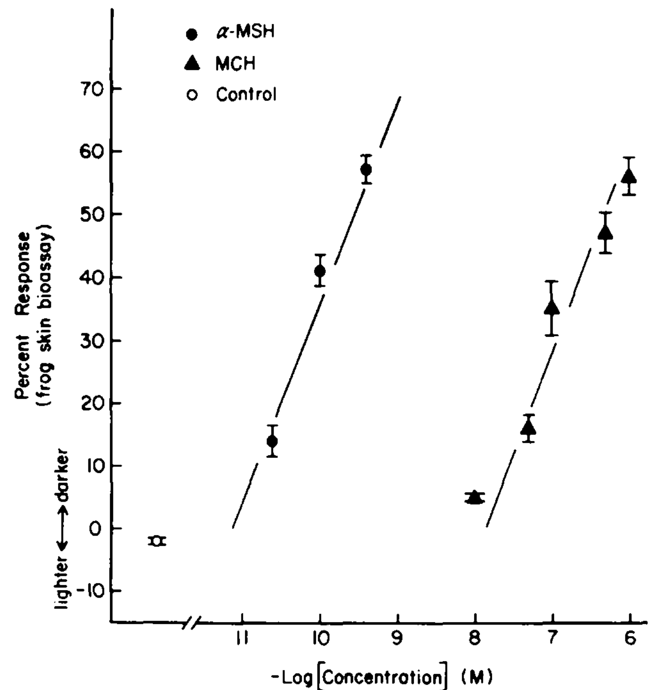

FIG. 2. In vitro demonstration of the MSH-like activity of MCH as seen in the frog (*R. pipiens*) skin bioassays. Each value is the mean, ±S.E., darkening response of the skins to α-MSH (N = 10) or MCH (N = 5) at the concentrations indicated.

diminished MSH-like activity in both the frog and the lizard (data not shown) skin bioassays (Fig. 3). At very high concentrations, these MCH analogues were full MSH-like agonists although they were more than 100-fold less active than MCH (Fig. 3). Although the melanotropic activity of MSH is rapidly reversed following

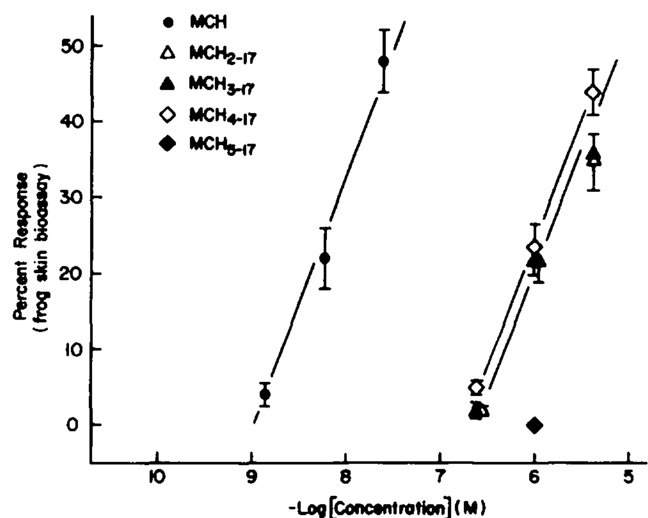

FIG. 3. MCH fragment analogues minus the first [MCH(2–17)], second [MCH(3–17)], third [MCH(4–17)], or fourth [MCH(5–17)] amino acid residues of the N-terminus lack of MSH-like activity. Each value is the mean, ±S.E., darkening response of frog skins (N = 10) to the melanotropins at the concentrations noted.

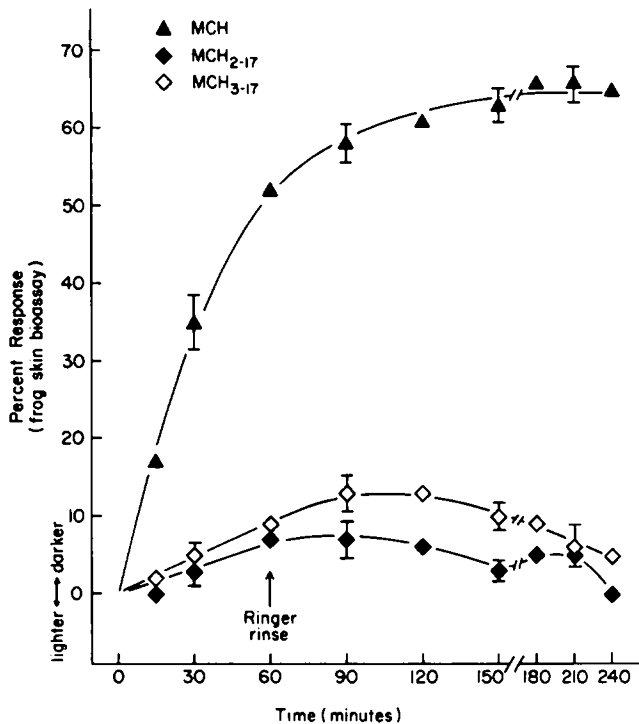

FIG. 4. In vitro demonstration that N-terminal deleted fragment analogues of MCH have greatly reduced MSH-like potency. Following transfer of the skins to fresh Ringer in the absence of the peptides (Ringer rinse), the skins continued to remain darkened in response to MCH but not to the fragment analogues even over a long period of time. Each value is the mean, ± S.E., darkening response of frog skins (N = 6) to the melanotropins (10^{-6} M).

transfer of frog or lizard skins to medium which lacks the hormone, the MSH-like activity of MCH is often prolonged in either species when used at high concentrations (Fig. 4). However,

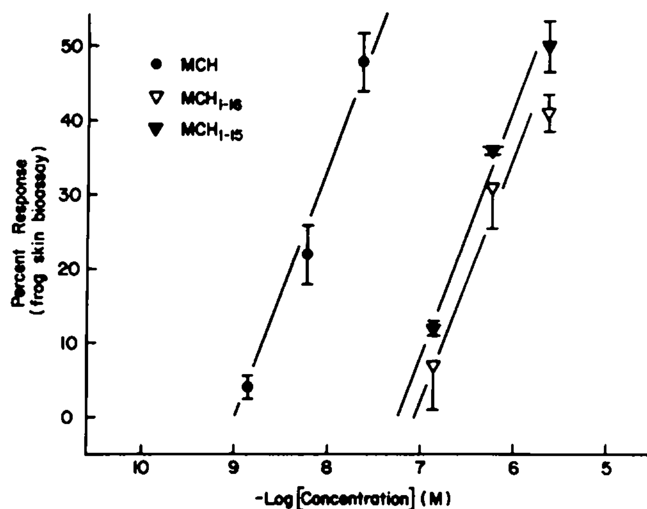

FIG. 5. In vitro demonstration that C-terminal deleted fragment analogues lacking C-terminal amino acids also lose considerable (compared to MCH) MSH-like potency. Each value is the mean, ± S.E., darkening response of lizard skins (N = 6) to the melanotropins (10^{-6} M).

TABLE 1

RELATIVE POTENCIES OF MCH FRAGMENT ANALOGUES AS DETERMINED BY THE IN VITRO FISH (*SYNBRANCHUS MARMORATUS*) AND FROG (*RANA PIPIENS*) SKIN BIOASSAYS

PEPTIDE	Fish Skin Bioassay Potency Relative to MCH		Frog Skin Bioassay Potency Relative to MSH
MCH(1–17)	1.0		0.001
MCH(2–17)	1.0		0.000005
MCH(3–17)	1.0		0.000005
MCH(4–17)	1.0		0.000005
MCH(5–17)	1.0	(17)	<0.000001
MCH(1–16)	1.0		0.00003
MCH(2–16)	1.0		0.000005
MCH(3–16)	1.0		0.00004
MCH(4–16)	1.0		0.00001
MCH(5–16)	1.0		0.000007
MCH(1–15)	1.0		0.00003
MCH(2–15)	1.0		0.00001
MCH(3–15)	1.0		0.00001
MCH(4–15)	1.0		0.000004
MCH(5–15)	1.0		0.000004
MCH(1–14)	0.1	[0.07 (17)]	0.000006
MCH(2–14)	0.07		0.000005
MCH(3–14)	0.05		<0.000001
MCH(4–14)	0.023		<0.000001
MCH(5–14)	0.014	[0.003(17)]	<0.000001

even over a long period of incubation these analogues failed to exhibit prolonged activity on frog melanocytes (Fig. 4). MCH, as it had done previously, maintained its prolonged activity (Fig. 4).

Most fragment analogues of MCH, like the parent hormone, possessed some MSH-like activity when used at high concentrations (e.g., 10^{-5} M). Removal of the C-terminal amino acid, Val, from MCH resulted in a peptide, MCH(1–16), which exhibited about 1/100 the MSH-like activity of MCH (Fig. 5). The shorter C-terminal deleted fragment analogues, MCH(1–15) and MCH(1–14), also exhibited a similar diminished MSH-like activity (Fig. 5; Table 1); MCH(1–14) showing the more drastic decrease in activity (Table 1). Thus, removal of residues from either the amino or carboxy termini led to reductions of melanotropic activity of at least two orders of magnitude relative to MCH. One may note that in Table 1, the most active fragment analogue is but 1/20,000 the potency of α-MSH. Even using the most meticulous care, one cannot reliably assess the purity of a sample (from contamination) beyond this limit using current purification schemes. Hence, the very low in vitro potency may be an indication that these analogues could be even less potent or even completely inactive.

The melanotropic activity of α-MSH (and β-MSH) is enhanced by heat-alkali treatment (Fig. 6) (4). Heat-alkali treatment of α-MSH also leads to a peptide that exhibits prolonged (residual) activity (Fig. 6). A recent report has suggested a similar result upon heat-alkali treatment of MCH (7). We have repeated the experiment and have found that heat-alkali treatment did not enhance the melanotropic activity of MCH. In fact, we observed in several experiments a modest reduction in potency (Fig. 7).

DISCUSSION

These results clearly demonstrate that MCH possesses two contrasting melanotropic activities. The hormone stimulates mel-

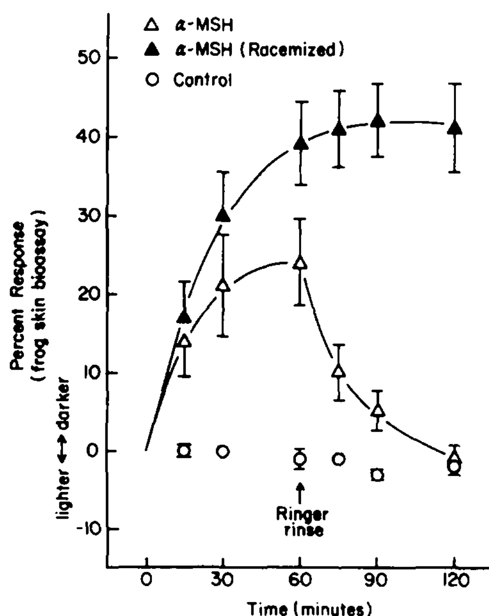

FIG. 6. In vitro demonstration that heat-alkali treatment (racemization) of α-MSH leads to enhanced and prolonged biological activity. Each value is the mean, ± S.E., darkening response of frog skins (N = 6) to α-MSH and to racemized α-MSH (10^{-10} M).

anosome aggregation at physiological concentrations in teleost fishes and at higher concentrations stimulates melanosome dispersion within melanocytes of fishes, frogs and lizards.

Our original results demonstrated that some component of the N-terminal tetrapeptide tail of MCH was necessary for MSH-like activity (9). That original observation was supported by our present study. Removal of the N-terminal amino acid, aspartic acid, resulted in an analogue with an almost total lack of MSH-like activity relative to MCH (at 10^{-6} M) in both the frog and lizard skin bioassays. Not surprising, therefore, was the observation that

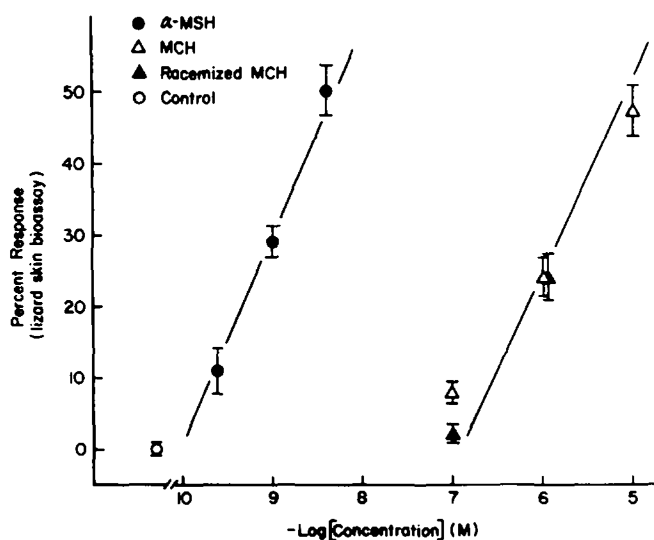

FIG. 7. In vitro demonstration that heat-alkali treatment of MCH does not enhance the MSH-like activity of the peptide. Each value is the mean, ± S.E., darkening of the skins (N = 6) at the concentrations noted.

the MCH(3–17), MCH(4–17) and MCH(5–17) analogues also showed decreased MSH-like activity (Fig. 2). Removal of the C-terminal amino acid residue also resulted in a dramatic (100-fold) loss of MSH-like activity compared to MCH (Table 1). The C-terminal truncated peptides, MCH(1–14) and MCH(1–15), were even less potent than MCH(1–16). These results, taken together, suggest that the entire heptadecapeptide sequence of MCH contributes in some way to the MSH-like potency of the hormone.

Although the N-terminal amino acid residue was essential for the full MSH-like activity of MCH, all analogues, whether truncated at the N- or C-termini, or both, exhibited some skin darkening (melanosome dispersion) when used at very high concentrations (i.e., 10^{-5} M). This was most dramatically demonstrated by Lebl and co-workers with a series of ring-contracted analogues (16). The most potent of these latter analogues was the analogue [$\overset{|}{\text{C}}$ys[7]]MCH(7–17). This peptide, particularly in the lizard skin bioassay, possessed the greatest MSH-like activity, being about 10 times more potent in that respect than MCH itself (17). Hence, manipulation of ring size has apparently resulted in conformational and/or topographical features that are less favorable for MCH-like activity but, rather, more conducive to stimulating MSH-like activity.

Many years ago it was demonstrated that heat-alkali treatment of α-MSH led to several phenomena relative to the melanotropic activity of the peptide: 1) retardation of response, 2) enhanced potency, and 3) prolonged activity (4,10). Since MCH exhibits MSH-like activity, the question arose as to whether similar changes could be induced following heat-alkali treatment of MCH. Although the MCH-like activity was drastically reduced (data not shown), no enhancement of the MSH-like activity of the hormone could be effected in MCH or several of its analogues. These observations were consistently observed. Therefore, we do not support the claim (7) that the MSH-like activity of MCH could be enhanced by heat-alkali treatment. In fact, with respect to our experiments, the MSH-like activity of MCH was modestly diminished.

In several other communications we have commented on the possible evolutionary relationships of α-MSH and MCH. It appears that the melanotropic activity (melanosome aggregation) of MCH is restricted to teleost fishes and their immediate forbearers (Holostean fishes). α-MSH, on the other hand, regulates melanosome dispersion and/or melanin synthesis in all species of vertebrates (including cartilaginous fishes and humans). Therefore, since the bony fishes were an early off-shoot from the main stem of vertebrate evolution, it might be assumed that MSH is ancestral to MCH.

Since an MCH-like peptide is claimed to be present within the mammalian (including human) brain (23), an MCH-like peptide must have been present before the bony fishes evolved as an off-shoot of the mainline vertebrate evolution. Since MCH lacks MCH-like activity in all members of the tetrapod line, then it remains to be determined whether MCH or a related peptide plays some other physiological role in vertebrates.

Because of its ability to cause melanosome dispersion/aggregation, it is conceivable that MCH may have evolved by gene duplication of a melanotropin (e.g., α- or β-MSH, or ACTH) followed by mutations in one of the redundant MSH codons. On this note, we have compared the mRNA sequences for both α-MSH and MCH (20) (Fig. 8). Although we could not readily find any significant amount of primary sequence homology either from sequence comparison, from changes due to possible frame-shift mutations, or from residue deletions, one possibly interesting component did emerge. In α-MSH, structure-activity relationships have suggested the importance of Phe^{7}-Arg^{8} (10) to its biological potency. In MCH, Matsunaga *et al.*, have suggested the importance of the Tyr^{11}-Arg^{12}-Pro^{13} residues of MCH. Interestingly, a

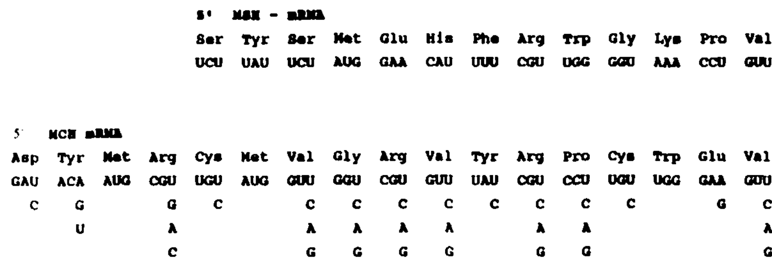

FIG. 8. Messenger RNA (mRNA) sequences of α-MSH and MCH.

point mutation of uracil to adenine for the Phe codon would convert the Phe to a Tyr (Fig. 8). If a point mutation had occurred precociously, this would imply that both MCH and α-MSH would have had a Phe-Arg part of the sequence in common. This would be very interesting because this part of the sequence (Phe^7-Arg^8) in α-MSH and (Tyr^{11}-Arg^{12}) in MCH is considered extremely important for their respective activities (2, 15, 18). Certainly, it would be interesting to see if conversion of Tyr^{11} to Phe^{11} in MCH would lead to an increase in MSH-like activity. In addition, this would be interesting from an evolutionary standpoint. We currently are testing this hypothesis.

The fact that MCH has the ability to exhibit MSH-like activity leads one to consider a functional homology between the two peptides. In other words, there may be a topographical similarity in structure despite a lack of any primary sequence homologies. One may propose that the MSH-like activity of MCH could be due to the transducing component of the MSH receptor having a similar topology, but a different (binding) potency requirement than the MCH receptor (or vice versa). Alternatively, one could propose a common receptor that may be activated in different ways by MCH and α-MSH. Although the second argument appears unlikely (9), neither proposal has been unequivocally ruled out.

ACKNOWLEDGEMENTS

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