Regulatory Peptides, 24 (1989) 27–35
Elsevier

RPT 00768

Melanocyte stimulating hormone and melanin concentrating hormone may be structurally and evolutionarily related

Ana Maria de L. Castrucci[1], Mac E. Hadley[2], Michal Lebl[3,*], Christian Zechel[3] and Victor J. Hruby[3]

*[1] *Departamento de Fisiologia Geral, Instituto de Biociências, Universidade de São Paulo, São Paulo, (Brazil),* [2] *Department of Anatomy, University of Arizona, Tucson, AZ 85724 (U.S.A.) and* [3] *Department of Chemistry, University of Arizona, Tucson, AZ 85721 (U.S.A.)*

(Received 18 July 1988; accepted 29 August 1988)

Summary

Two melanotropic peptides, melanin concentrating hormone (MCH) and α-melanocyte stimulating hormone (α-MSH), exert opposing actions on melanosome (melanin granule) movements within teleost pigment cells, melanocytes (melanophores). MCH stimulates melanosome aggregation to the cell center whereas α-MSH stimulates pigment organelle dispersion out into the dendritic processes of the melanocytes. The actions of α-MSH are dependent upon extracellular calcium (Ca^{2+}), whereas those of MCH are actually enhanced in the absence of the cation. At high concentrations (10^{-5}–10^{-8} M) MCH also exhibits MSH-like activity (autoantagonism), an effect which is abolished in the absence of Ca^{2+}. Therefore, MCH exhibits MCH-like as well as MSH-like activity depending on the presence or absence of extracellular Ca^{2+}. An analogue of MCH, [Ala^5, Cys^{10}]MCH, has been synthesized which is totally devoid of MCH activity but still exhibits MSH-like activity. These results suggest that the two melanotropic peptides share some component of structural similarity and may be evolutionarily related.

Melanotropin; Melanosome; Melanotropic peptide

* On leave from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 16610, Czechoslovakia.
Correspondence: M.E. Hadley, Department of Anatomy, University of Arizona, Tucson, AZ 85724, U.S.A.

Introduction

Many years ago evidence was provided that the pituitary gland of fishes contained a hormone that stimulated melanin granule (melanosome) aggregation within teleost melanocytes [1]. More recently Baker and Rance [2] provided additional evidence supporting the existence of this putative melanin concentrating hormone (MCH) in fishes. Very recently, Kawauchi et al. isolated and determined the primary structure of a peptide that proved to possess MCH-like activity [3]. The peptide was then synthesized and its melanotropic activities characterized [4]. A most interesting observation was that MCH also stimulated melanosome dispersion in tetrapod (frog and lizard) melanocytes [4,5]. In other words, the peptide possessed both MCH-like as well as MSH (melanocyte stimulating hormone)-like activities depending upon the species of melanocytes studied.

Studies using synthetic fragment analogues of MCH have revealed that the amino terminus of the melanotropin is associated with MSH-like activity whereas the carboxy terminus is particularly relevant to MCH-like activity [6]. The two contrasting actions of MCH, as might be expected, are apparently mediated through separate receptors. In all vertebrates studied to date (amphibians [7,8], reptiles [9], fish [10]), Ca^{2+} is required for α-MSH stimulation of melanosome dispersion within melanocytes. Melanosome aggregation, on the other hand, proceeds in the absence of this divalent cation. We have already demonstrated that MCH and MSH caused lightening or darkening of fish skins by stimulating melanosome aggregation or dispersion, respectively, within melanocytes [4,10]. A remaining question was whether only the actions of one of these peptides, α-MSH, required Ca^{2+} to mediate its actions on melanocytes. In the present communication we provide information on the role of this divalent cation on the actions of these two melanotropic peptides on teleost melanocytes. The results obtained suggest an evolutionary relationship between the two hormones.

Materials and Methods

The teleost fish, *Synbranchus marmoratus,* an eel obtained from the Pantanal (Big Swamp) of Brazil was used. Skins were removed and prepared as originally described for the frog [11] and lizard [12] skin bioassays, and as detailed elsewhere [13]. In this bioassay skins become light in response to MCH due to melanosome aggregation within melanocytes [6]. The skins can then be redarkened by the addition of MSH which causes dispersion of melanosomes within melanocytes [14]. Movement of melanosomes within melanocytes results in color changes which can be monitored by a Photovolt reflectometer. Changes in skin color (reflectance) are recorded as percent changes from the initial base (zero) value.

The two melanotropic peptides, α-melanocyte stimulating hormone (MSH) and melanin concentrating hormone (MCH), were synthesized as previously reported [4]. Synthesis of the fragment analogue, MCH-(5–17), and related peptides has also been described [6,15]. The structures of the 4 melanotropic peptides used in these studies are shown in Table I.

TABLE I

Structures of 4 melanotropic peptides

alpha-MSH

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

MCH

Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val

MCH_{5-17}

Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val

[Ala^5, Cys^{10}]MCH

Asp-Thr-Met-Arg-Ala-Met-Val-Gly-Arg-Cys-Tyr-Arg-Pro-Cys-Trp-Glu-Val

Results

To determine the Ca^{2+} requirements of MCH and α-MSH to mediate their actions on melanosome movements within melanocytes, the melanotropins were added to fish skins residing in fish physiological solution (Ringer) in the presence and absence of the divalent cation. In the absence of extracellular Ca^{2+}, MCH still produced a maximal lightening of the skins (Fig. 1). When α-MSH was then applied to skins the potential darkening action of this melanotropin was totally abolished in the absence of Ca^{2+}. In fact, the skins continued to lighten in color, indicating a further stimulation of melanosome aggregation within the melanocytes by MCH even in the presence of MSH (Fig. 1). These results demonstrate that the actions of α-MSH, but not those of MCH, are dependent upon extracellular Ca^{2+}.

We had previously shown that MCH was a potent lightening agonist even when used at very low concentrations, but that the peptide exhibited auto-antagonism (self-antagonism) when used at higher concentrations, an action we suggested was due to its intrinsic MSH component of activity [14]. We predicted, therefore, that if this assumption were correct we should then be able to abolish this auto-antagonism in the absence of extracellular Ca^{2+}. This we were able to do (Fig. 2). If, indeed, MCH possesses a primary sequence that can exhibit either MCH-like or MSH-like activity depending upon the presence or absence of extracellular Ca^{2+}, then it might be expected that the potency of the peptide could actually be enhanced in the absence of the cation. Indeed, the dose–response curve was shifted to the left (2-fold) indicating increasing potency under these experimental conditions (Fig. 2). We had also shown that the NH_2-terminal 1–4 sequence of MCH was required for autoantagonism since the MCH_{5-17} fragment analogue lacking this sequence was devoid of antagonistic activity at any concentration employed. As expected, therefore, the action of this latter peptide was unaffected by the absence of Ca^{2+} from the medium in which the skins resided (Fig. 3).

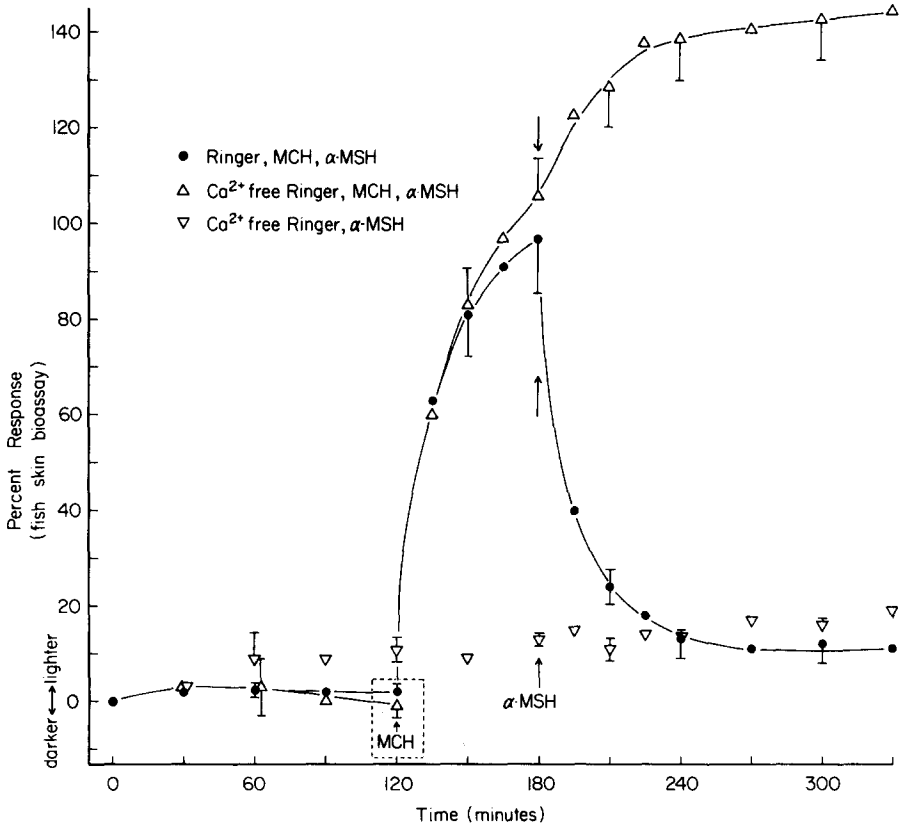

Fig. 1. In vitro demonstration that the melanosome-aggregating activity (lightening of fish skins) of MCH (10^{-10} M) does not require Ca^{2+} whereas the melanosome dispersing action (darkening of skins) by α-MSH (10^{-8} M) does require Ca^{2+}. Each value is the mean ± S.E.M. darkening response of the skins (n = 6) to the peptides.

The initial experiment (Fig. 1) actually suggested that α-MSH might also exhibit contrasting activities which were dependent upon the presence or absence of extracellular Ca^{2+}. In other words, in the absence of extracellular Ca^{2+} the normal actions of α-MSH might be shifted from its own receptor to the MCH receptor. The actions of α-MSH were repeatedly abolished in every experiment in the absence of Ca^{2+}. Nevertheless, α-MSH added to skins residing in Ca^{2+}-free Ringer did not cause them to darken or to lighten further. MSH did not, therefore, exhibit MCH-like activity in the absence of Ca^{2+}.

We have found that certain structural modifications of MCH lead to analogues devoid of MCH activity but still possessing MSH-like activity. For example, the analogue, [Ala^5, Cys^{10}]MCH, is totally devoid of MCH-like activity in the fish skin bioassay but is equipotent to MCH in exhibiting MSH-like activity in the frog skin bioassay (Fig. 4). These results suggest that the integrity of the 5–14 disulfide bridge is important for the MCH-like activity of MCH. Contraction of the ring does not,

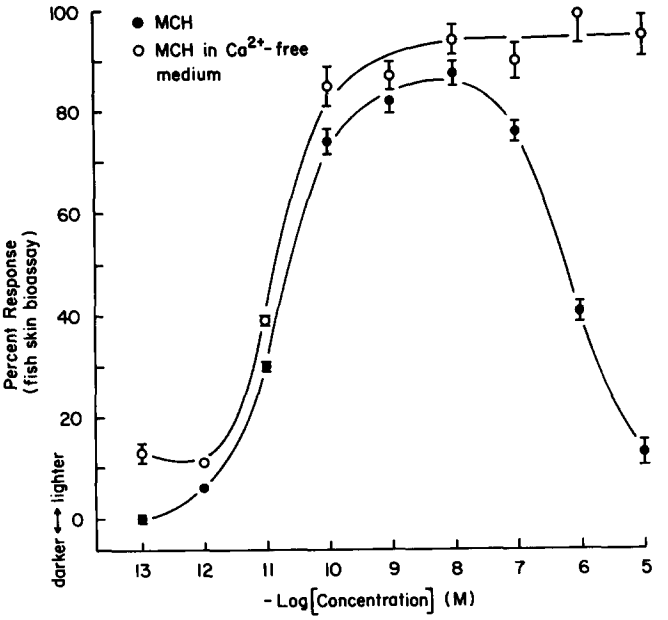

Fig.2. In vitro demonstration that the autoantagonism of MCH on fish melanocytes can be reversed in the absence of Ca^{2+}. The potency of MCH is enhanced (shift in dose–response curve to the left) in the absence of Ca^{2+}. Each value is the mean ± S.E.M. lightening response of the skins (n = 10) to MCH at the various concentrations employed.

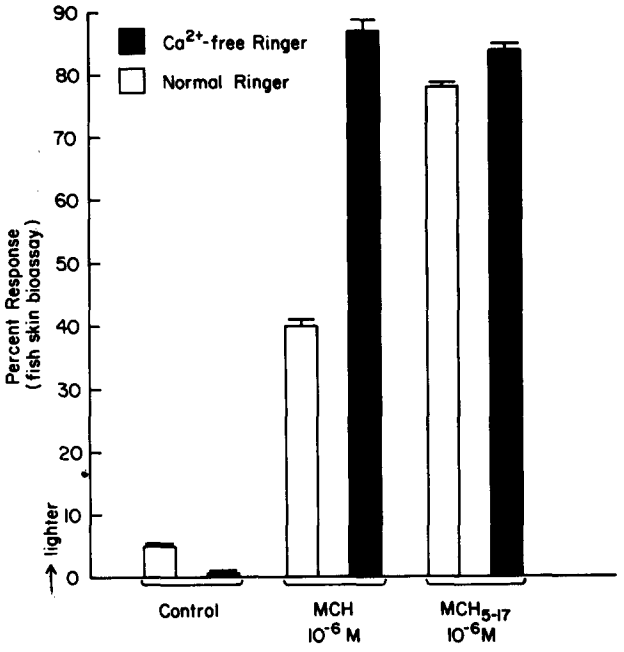

Fig. 3. As in Fig. 2, the actions of MCH (10^{-6} M) are enhanced in the absence of Ca^{2+} whereas the actions of MCH-(5–17) (10^{-6} M) are uncompromised in the absence of the divalent cation. Each value is the mean ± S.E.M. lightening response of the skins (n = 6) to MCH or MCH-(5–17) (10^{-6} M) in the presence or absence of Ca^{2+}.

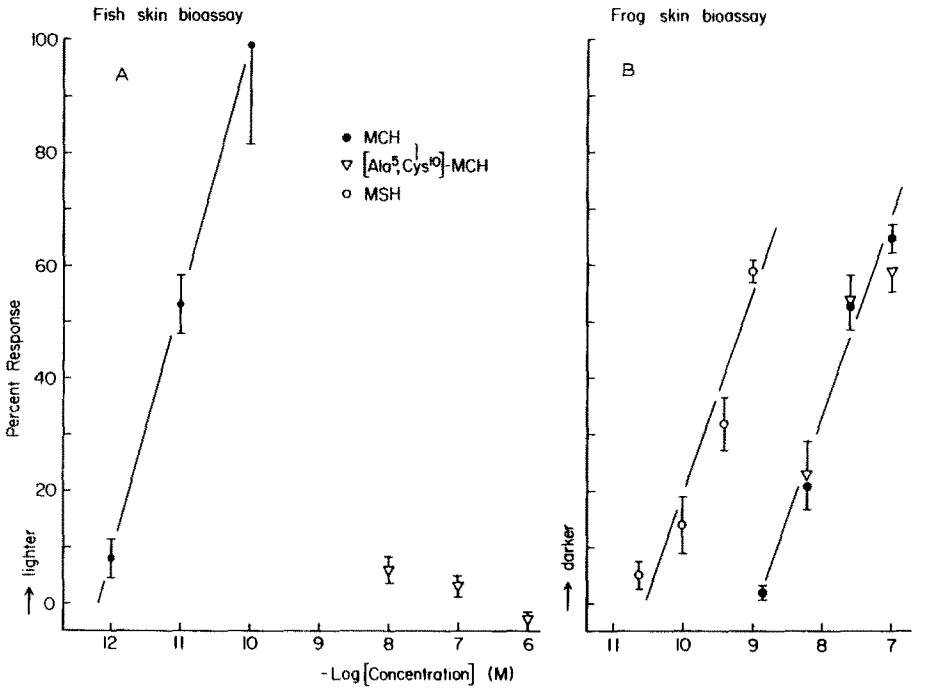

Fig. 4. A: in the fish skin bioassay MCH is a very potent stimulator of melanosome aggregation whereas the analogue, [Ala^5, Cys^{10}]MCH, is without MCH-like activity at any concentration employed. B: in contrast, in the frog skin bioassay [Ala^5, Cys^{10}]MCH is equipotent to MCH in stimulating melanosome dispersion within melanocytes. The analogue is devoid of MCH-like activity but still possesses MSH-like activity as does MCH. Each value is the mean ± S.E.M. response of the skins to the melanotropic peptides in the frog (*Rana pipiens*) or fish (*Synbranchus marmoratus*) skin bioassays (n = 7) at the concentrations noted.

however, affect the MSH-like component of MCH activity. Clearly MCH possesses structural features responsible for both the MCH-like and MSH-like activities of the hormone. In the absence of the normal disulfide bridge between the 5 and 14 residues, the analogue exhibits only the MSH-like component of activity.

Discussion

These results demonstrate that the two melanotropic peptides, α-MSH and MCH, can each exhibit MSH-like activity depending upon the presence or absence of extracellular Ca^{2+}. The MSH-like component of MCH activity is only revealed at high (unphysiological) concentrations and therefore is probably unimportant relative to the normal physiological actions of the peptide, although at physiological concentrations, the latent aggregating potential of MCH is, to some extent, masked by its intrinsic MSH-like activity (as shown by the leftward shift of the dose-response curve in Ca^{2+}-free saline, Fig. 2). Nevertheless, the cryptic actions that the two peptides can manifest on melanocytes are of considerable evolutionary interest.

The importance of the present studies is that, although α-MSH and MCH do not appear to share any similarity of primary structure, they must bear a closely related topographical structural component which allows both of them to bind to an MSH receptor and initiate MSH-like activity. How a linear amino acid sequence determines the 3-dimensional topography of a peptide or protein is still not understood [16]. Given the relatively small size of MSH and MCH, these peptides may provide useful models for the determination of the relationship between primary structure and three-dimensional structure using conformationally constrained analogues [17].

α-MSH as well as β-MSH, corticotropin (ACTH), and β-lipotropin (β-LPH) are all derived from a common precursor molecule, pro-opiomelanocortin, and all bear some primary structural relationships. It is likely that one or more gene duplications gave rise to the melanocorticotropic family of peptides. Since MCH possesses intrinsic MSH-like activity, it would be expected that one or more of the other melanocorticotropins may also possess some structural similarity to MCH. Computer as well as biophysical analyses of these structures compared to MCH may provide important insights into the evolution of peptide hormones. Such studies are in progress in our laboratory.

It has been pointed out that MCH bears some structural similarity to the carboxy-terminal cyclic portion of salmon prolactin (PRL) [3]. In fact, it has been reported [3] that a chymotryptic peptide of salmon prolactin, Arg-Cys-Ala-Thr-Lys-Met-Arg-Pro-Glu-Thr-Cys, exhibits very weak MCH-like activity (5×10^4 times less potent than MCH). It is interesting to note that purified preparations of salmon PRL are less effective in maintaining plasma sodium levels at concentrations above the maximally effective dose [18]. Therefore, both MCH and PRL, which may be structurally (and presumably evolutionarily) related, also both exhibit auto-inhibition at higher concentrations.

These observations raise the question of whether MSH might bear some structural relatedness to PRL [19]. It has been reported on several occasions that PRL exhibits MSH-like activity [20]. One documented biological response to PRL preparations was yellowing of fish scales due to carotenoid dispersion within yellow pigment cells, a response that is also elicited by MSH [21]. Unfortunately, it remains to be resolved whether these responses were due to the intrinsic melanotropic activity of PRL or due to the contamination of the preparations by a melanotropin. Sage has argued for a direct effect of PRL on xanthophores [22] and it should also be recalled that MSH-producing cells, like PRL cells, were reported to be affected by a sodium-deficient diet in mice [23]. Taken together these observations and conjectures suggest that MCH, PRL and the melanocorticotropins might be evolutionarily derived from a common ancestral cistron or else structurally related by way of a convergent evolutionary process.

It would be expected that the actions of two different peptide hormones that exhibit opposing actions should be mediated through separate cellular receptors. This view is supported by the observation that the actions of MSH were inhibited in Ca^{2+}-free medium whereas the actions of MCH under similar experimental conditions were uncompromised. The demonstration that MCH only exhibits melanosome aggregating activity on teleost melanocytes (not on tetrapod, frog and lizard, melanocytes) is

further proof that the two receptors are separate. Tetrapods apparently lack MCH or MCH-like melanocyte receptors. It is possible that MCH functions as a humoral hormone only in teleostean fishes.

Both MSH and MCH are present within the brain and/or the pituitary gland of several vertebrates [24–26]. Indeed, MCH has been reported to inhibit corticotropin secretion from the isolated rat pituitary gland [27]. This observation suggests that MCH may be a peptidergic neurohormone functioning as a hypophysiotropic factor. If both melanotropic peptides play a role as neuroregulatory hormones, then their actions are probably mediated through separate receptors. The present studies provide a useful beginning for an understanding of these putative peptidergic receptors.

Acknowledgements

These studies were partially supported by the Fundacao de Amparo a Pesquisa do Estado de São Paulo, Grants 84/1967 and 85/0718-7 (Brasil), U.S. Public Health Service grant AM-17420, and National Science Foundation grant DCB-8615706.

References

1 Enami, M., Melanophore-contracting hormone (MCH) of possible hypothalamic origin in the catfish, Science, 121 (1955) 36–37.
2 Baker, B.I. and Rance, T.A., Further observations on the distribution and properties of teleost melanin concentrating hormone, Gen. Comp. Endocrinol., 50 (1983) 423–431.
3 Kawauchi, H., Kawazoe, I., Tsubokawa, M., Kishida, M. and Baker, B.I., Characterization of melanin-concentrating hormone in chum salmon pituitaries, Nature (Lond.) 305 (1983) 321–323.
4 Wilkes, B.C., Hruby, V.J., Sherbrooke, W.C., Castrucci, A.M.L., and Hadley, M.E., Synthesis and biological actions of melanin concentrating hormone, Biochem. Biophys. Res. Commun., 122 (1984) 613–619.
5 Wilkes, B.C., Hruby, V.J., Castrucci, A.M.L., Sherbrooke, W.C. and Hadley, M.E., Synthesis of a cyclic melanotropic peptide exhibiting both melanin concentrating and dispersing activities, Science, 224 (1984) 1111–1113.
6 Hadley, M.E., Zechel, C., Wilkes, B.C., Castrucci, A.M.L., Visconti, M.A., Pozo-Alonso, M. and Hruby, V.J., Differential structural requirements for MSH and MCH activities of melanin concentrating hormone, Life Sci., 40 (1987) 1139–1145.
7 Vesely, D.L. and Hadley, M.E., Ionic requirements for melanophore stimulating hormone (MSH) action on melanophores, Comp. Biochem. Physiol., 62A (1979) 501–508.
8 Van de Veerdonk, F.C.G. and Brouwer, E., Role of calcium and prostaglandin (PGE_1) in the MSH-induced activation of adenylate cyclase in *Xenopus laevis*, Biochem. Biophys. Res. Commun., 52 (1973) 130–134.
9 Vesely, D.L. and Hadley, M.E., Calcium requirement for melanophore stimulating hormone action of melanophores, Science, 173 (1971) 923–925.
10 Oshima, N., Kasukawa, H., Fujii, R., Wilkes, B.C., Hruby, V.J., Castrucci, A.M.L. and Hadley, M.E., Melanin concentrating hormone (MCH) effects on teleost (*Chrysiptera cyanea*) melanophores, J. Exp. Zool., 235 (1985) 175–180.
11 Shizume, K., Lerner, A.B., and Fitzpatrick, T.B., In vitro bioassay for the melanocyte stimulating hormone, Endocrinology, 54 (1954) 555–560.
12 Castrucci, A.M.L., Hadley, M.E. and Hruby, V.J., Melanotropin bioassays: in vitro and in vivo comparisons, Gen. Comp. Endocrinol., 55 (1984) 104–111.

13 Castrucci, A.M.L., Hadley, M.E. and Hruby, V.J., A teleost skin bioassay for melanotropic peptides, Gen. Comp. Endocrinol., 66 (1987) 374–380.
14 Castrucci, A.M.L., Hadley, M.E., Wilkes, B.C., Zechel, C. and Hruby, V.J., Melanin concentrating hormone exhibits both MSH and MCH activities on individual melanophores, Life Sci., 49 (1987) 1845–1851.
15 Lebl, M., Hruby, V.J., Castrucci, A.M.L., Visconti, M.A. and Hadley, M.E., Melanin concentrating hormone analogues: contraction of the cyclic structure. I. Agonist activity, J. Med. Chem., 31 (1988) 949–954.
16 Kolata, G., Trying to crack the second half of the genetic code, Science, 233 (1986) 1037–1039.
17 Hruby, V.J., Conformational restrictions of biologically active peptides via amino acid side chain groups, Life Sci., 31 (1982) 189-199.
18 Grau, E.G., Prunet, P., Gross, T., Nishioka, R.S. and Bern, H.A., Bioassays of salmon prolactin using hypophysectomized *Fundulus heteroclitus,* Gen. Comp. Endocrinol., 53 (1984) 78–85.
19 Kawauchi, H., Abe, K.-I., Takahashi, A., Hirano, T., Hasegawa, S., Naito, N. and Nakai, Y., Isolation and properties of chum salmon prolactin, Gen. Comp. Endocrinol., 49 (1985) 446–458.
20 Ball, J.N. and Hawkins, E.F., Adrenocortical (interrenal) hormones in the teleost *Poecilia latipinna,* Gen. Comp. Endocrinol., 28 (1976) 59–70.
21 Pickford, G.E. and Atz, J., The Physiology of the Pituitary Gland of Fishes, Zoological Society, New York, 1957.
22 Sage, M.J., Assay of prolactin in vertebrate pituitaries by its dispersion of xanthophore pigment in the teleost, J. Exp. Zool., 173 (1970) 121–128.
23 Kobayashi, Y., A morphometric study on pars intermedia of the hypophysis during impairment of the renin-angiotensin-aldosterone system in sodium depleted mice, Cell Tissue Res., 154 (1974) 321–327.
24 Zamir, N., Skofitsch, G., Bannon, M.J. and Jacobowitz, D.M., Melanin-concentrating hormone: unique peptide neuronal system in the rat brain and pituitary gland, Proc. Natl. Acad. Sci. U.S.A., 83 (1986) 1528–1531.
25 Naito, N., Nakai, Y., Kawauchi, H. and Hayashi, Y., Immunocytochemical identification of melanin-concentrating hormone in the brain and pituitary gland of teleost fishes *Oncorhynchus keta* and *Salmo gairdneri,* Cell Tissue Res., 252 (1985) 41–48.
26 Skofitsch, G., Jacobowitz, D.M. and Zamir, N., Immunohistochemical localization of a melanin concentrating hormone-like peptide in the rat brain, Brain Res. Bull., 15 (1985) 635–649.
27 Baker, B.I., Bird, D.J. and Buckingham, J.C., Salmonid melanin-concentrating hormone inhibits corticotropin release, J. Endocrinol., 106 (1985) R5–R8.