Peptides, Vol. 2, pp. 121–123, 1981. Printed in the U.S.A.

Melanotrophin-Potentiating Factor (MPF) Potentiates MSH-Induced Melanogenesis in Hair Follicle Melanocytes

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Received 26 February 1981

LOGAN, A., R. J. CARTER, S. SHUSTER, A. J. THODY AND B. WEATHERHEAD. *Melanotrophin-potentiating factor (MPF) potentiates MSH-induced melanogenesis in hair follicle melanocytes.* PEPTIDES 2(2) 121–123, 1981.—Melanotrophin-potentiating factor (MPF) is a fragment of human β-lipotrophin (LPH 88–91) which potentiates the action of α-MSH on *Anolis* skin. In the present study, we investigated the effect of MPF on MSH-induced melanogenesis. Pooled hair follicle scrapings from Siberian hamsters *(Phodopus sungorus)* were incubated for 48 hours with or without α-MSH and/or MPF. Melanogenesis was monitored by measuring tyrosinase activity and melanin accumulation. 10^{-8} M MPF had no effect on melanogenesis, but 10^{-9} to 10^{-7} M α-MSH caused a dose-related increase. 10^{-8} M MPF potentiated the effect of each dose of α-MSH. Thus MPF potentiated MSH action on mammalian melanogenesis as well as on reptilian melanosome dispersion. Although each of these processes involve different intracellular responses the receptor mechanisms involved in each may therefore be the same.

α-MSH Melanotrophin-potentiating factor Mammalian melanogenesis *Phodopus sungorus*

MELANOTROPHIN-potentiating factor (MPF) is a fragment of human β-lipotrophin (β-LPH) which potentiates melanosome dispersing activity of melanocyte-stimulating hormone (MSH) peptides on the skin of the lizard *Anolis* [2] and which has been identified as β-LPH 88–91 (Lys-Lys-Gly-Glu), the C terminal tetrapeptide sequence [3]. As well as causing melanosome dispersion in the melanophores of amphibian and reptilian skin [1] the MSH peptides also induce melanogenesis in the skin [17,18] and hair [8,22] of some mammals, through a stimulation of the enzyme tyrosinase [12]. We therefore set out to examine whether MPF potentiates MSH-induced melanogenesis in the mammal and now report that it does.

METHOD

The experiments utilised short-term (48 hr) cultures of hair follicles from the Siberian hamster *(Phodopus sungorus)*. Hair follicle scrapings were taken from several animals during their spring moult, and were pooled and incubated in culture medium (RPMI 1640) containing 10% foetal calf serum at 37°C. Two aspects of melanogenesis were monitored: tyrosinase activity and melanin accumulation. Tyrosinase, thus far the only fully characterised enzyme involved in melanin biosynthesis, was measured by a radiometric assay based upon the method described by Pomerantz [13]. Approximately 10 μCi of L-(3,5-^{3}H)-tyrosine (Radiochemical Centre) was added to each hair follicle incubation and tyrosinase activity assessed by determining the release of tritiated water. Melanin content of the incubated follicles was determined using a spectrofluorimetric assay [14] which has been described in detail elsewhere [9]. Synthetic melanin (Koch Light Laboratories) was used as the standard. In control cultures, melanin content increased approximately four-fold during the incubation. Incubations were set up in quadruplicate and the synthetic peptides, α-MSH (Ciba-Geigy) and MPF (ICI Ltd.), were added at the commencement of the incubation and not subsequently replenished. The dose-related increased melanogenesis with α-MSH was compared with that including added MPF by analysis of variance.

RESULTS

Figure 1 demonstrates that in hair follicle cultures, both tyrosinase activity and melanin production are stimulated by α-MSH compared to control cultures although the doses were larger than those which stimulate melanosome dispersion on *Anolis* skin. Furthermore, while MPF (10^{-8} mol/l) has no inherent melanotrophic activity, it does potentiate the effect of each dose of MSH examined ($p<0.01$) and although there was a falloff in response to MSH at high concentrations the potentiating effect of MPF was still maintained.

DISCUSSION

These findings show that MPF potentiates the action of α-MSH on the mammalian melanocyte and this is in keeping with our earlier findings on the reptilian melanophore [2,3].

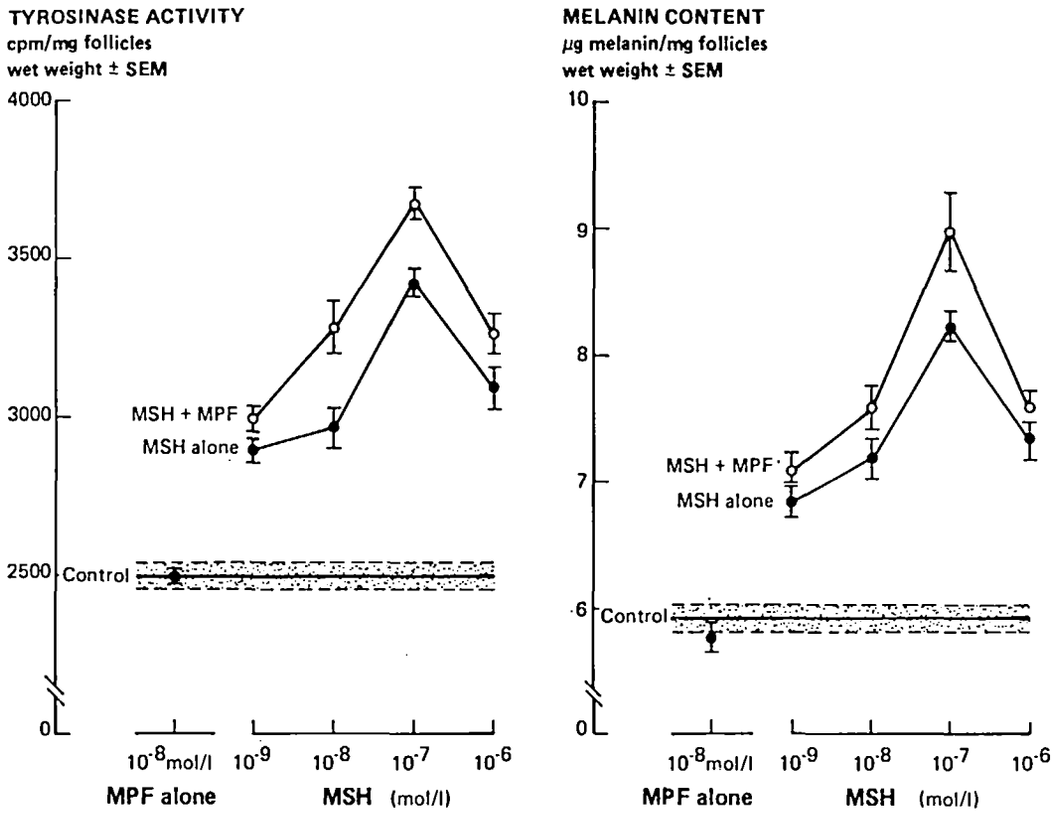

FIG. 1. Effects of α-MSH and MPF on melanogenesis in hair follicle cultures. Each point represents the mean ± SEM of the cultures set up in quadruplicate. The horizontal line and stippling show the mean values found in control cultures.

Thus, although these two different pigmentary models ultimately depend on different intracellular responses, viz melanosome dispersion and melanin synthesis the receptor mechanisms involved may be the same. The correlation between the pigmentary responses in *Anolis* and the mammalian melanocyte contrasts with the lack of correlation in *Rana* which responds poorly to β-LPH [16] and does not respond to MPF (Eberle, A. and R. Schwyzer, personal communication, 1978). How MPF potentiates the action of α-MSH is not yet clear. Although MPF has no intrinsic melanotrophic activity it enhanced the effect of each dose of α-MSH. It is interesting that this potentiating effect was maintained at high concentrations of α-MSH where there was a decline in the melanogenic response. It is not known whether this fall off in the melanogenic response to α-MSH at high concentrations is due to changes in receptor mechanism or to intracellular events.

It has been reported that MPF does not augment the effect of α-MSH in cAMP levels in B16 mouse melanoma cells [6], suggesting that either the MPF potentiation of MSH action does not involve adenylate cyclase or else the MSH receptor in the B16 mouse melanoma is different from that in the normal hair follicle.

Pigmentation has only been associated with changes in MSH like peptides when they achieve high levels in the blood as in Addison's disease, Cushing's syndrome, and chronic renal failure and these peptides were therefore not believed to have a physiological role in pigmentation in man. The present findings that MPF, either on its own or as part of β-endorphin (unpublished data), effectively increases the pigmentary potency of the MSH peptides reopens the question of the role of these peptides in mammalian pigmentation. It also raises the more general question of interaction between MSH and other peptides, particularly those which, like MPF, are derived from the ACTH-β-LPH precursor. The possibility should also be considered that MPF can modulate other effects of MSH such as its action on the sebaceous glands [21]. MSH peptides are produced by the brain [5, 10, 11, 15, 20] and have numerous behavioural effects [4, 7, 19]. Whether any of these actions are influenced by MPF must now be investigated.

ACKNOWLEDGMENTS

This work was supported by grants from the MRC to B. W. and to S. S. and A. J. T. Synthetic MPF was generously provided by ICI Ltd., and synthetic α-MSH by Ciba-Geigy. We thank Gillian Richardson for her invaluable technical assistance.

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