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Adenylate Cyclase Activity in Homogenates of Human Melanoma Cells. Effect of α-MSH and Isoprenaline

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The effects of the alpha-melanocyte-stimulating hormone (α-MSH) (10^{-7}–10^{-5} M) and the β-adrenoceptor agonist isoprenaline (10^{-9}–10^{-4} M) on adenylate cyclase (AC) activity were investigated in homogenates of the human IGR 1 melanoma cells with or without additional GTP. Basal AC activity was increased by the administration of 10 μM GTP. Alpha-MSH had no effect on cyclic AMP (cAMP) accumulation, while isoprenaline stimulated AC activity in a dose-dependent manner. ***Key words: MSH-receptor, β-adrenoceptor.***

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The melanocyte-stimulating hormone (MSH) is a peptide hormone which has numerous biological effects in mammals but is probably best known for its stimulation of melanogenesis. The hormone acts through specific, high-affinity membrane receptors and the formation of the hormone-receptor complex is followed by stimulation of the adenylate cyclase (AC) system and a net increase in the intracellular levels of cyclic AMP (cAMP) (1–4).

Melanocytes of all species of vertebrates so far studied appear to possess melanotropin receptors, but the largest part of our present knowledge is drawn from experiments on mouse melanoma cells (Cloudman S 91 or B 16) (1, 5–7). However, injection of MSH or modified melanotropins has also been shown to increase melanin pigmentation of the human skin (8, 9) and high α-MSH levels in plasma have been described in 50% of pregnant women at the term of gestation (10). Recent results have shown 3-fold detectable serum α-MSH levels in melanoma patients compared with controls. Furthermore, it has been demonstrated that the change in hormone level corresponds with the progression or remission of the disease (11).

MSH receptors have been identified on both normal and malignant human melanocytes by binding experiments with labelled MSH (12–14). However, the level of binding is much lower than that observed on murine melanoma cells (15) and concerning the hormone-cell interactions the data are controversial. Stimulation of tyrosinase activity and inhibition of cell growth following treatment with α-MSH have been demonstrated (12), while other findings indicate that both normal and malignant melanocytes are refractory to the hormone (15, 16).

Since the study of human MSH receptors might be important both with regard to melanogenesis and tumour therapy, we investigated the effect of α-MSH on the adenylate cyclase activity of the human melanoma cell line IGR 1.

Besides MSH, melanogenesis can be stimulated by a variety of known agents, e.g. ultraviolet light (17), prostaglandins (18), β-adrenergic agonists (19). Since the cellular response to the latter are also associated with stimulation of AC, in addition to α-MSH, isoprenaline was chosen to investigate the alterations of cAMP level in our system.

MATERIALS AND METHODS

Materials

Adenosine 5′-(α^{32}P)-triphosphate, triethylammonium salt (specific activity 30 Ci/mmol) (Amersham International plc, Amersham, UK), α-MSH, research grade (Serva, Feinbiochemica, Heidelberg/New York), (−)-isoprenaline HCl (Sigma Chemical Co., St. Louis, MO. USA). All other chemicals were of the purest grade commercially available. At every experimental occasion fresh solutions of α-MSH and (−)-isoprenaline were prepared.

Table I. *Adenylate cyclase activity of human melanoma cells after α-MSH and isoprenaline treatment*

The data are expressed as pmol cAMP/mg protein×min (mean ± SEM from 3 experiments)

		48 h		72 h	
	Conc. (M)	−GTP	+GTP	−GTP	+GTP
Basal	0	7.3±1.1	13.7±3.0	6.1±1.9	13.1±2.9
α-MSH	10^{-7}	7.0±1.4	13.2±2.8	5.8±1.7	13.1±2.6
	3×10^{-7}	6.7±1.1	12.6±2.5	5.0±1.2	13.0±2.9
	10^{-6}	6.5±1.7	12.6±2.9	5.6±1.5	12.3±2.4
	3×10^{-6}	7.0±1.2	12.8±3.3	5.3±1.4	13.4±3.1
	10^{-5}	6.4±1.1	12.0±3.1	5.1±1.4	13.1±2.2
Isoprenaline	10^{-9}	6.2±1.1	12.6±2.5	5.5±1.4	13.2±2.6
	10^{-8}	6.5±0.8	14.0±3.2	6.5±1.6	15.9±3.7
	10^{-7}	11.4±1.4	20.5±3.6	14.9±3.5	25.1±5.5
	10^{-6}	27.5±5.5	34.8±6.8	31.5±3.0	42.7±6.4
	10^{-5}	40.0±9.8	51.5±13.9	43.9±4.4	52.5±3.8
	10^{-4}	39.0±9.8	52.7±15.7	45.5±5.3	59.1±6.1

Culture

Cultures of a pigment producing human melanoma cell line (IGR 1) were obtained from Dr Christian Aubert, Marseille, and have been kept since March, 1982 in culture at the Tornblad Institute, University of Lund using methods previously described (20). The medium used was Eagle's minimal essential medium (MEM) with 15% fetal calf serum.

Cells were allowed to attach and grown for 48 or 72 h, respectively. (The doubling time was approximately 24 h.) Before harvesting, cultures were washed twice with 20 ml phosphate buffered saline (pH 7.2) and then removed in the same buffer using a rubber policeman. The contents of the flasks were centrifuged, 180×g, for 10 min, the pellet was resuspended in water and homogenized (5 stroke) using a B. Braun Melsungen tissue homogenizer at 1 500 rpm. Homogenates were then used without further purification.

Adenylate cyclase assay

The adenylate cyclase activity was assayed according to the method of Salomon et al. (21), modified by Minneman et al. (22). The enzyme reaction was carried out in a final volume of 200 µl, containing cell homogenate (25–228 µg protein) and 50 mM Tris HCl (pH 7.5), 0.25 mM ATP, 1.0 mM $MgCl_2$, 0.5 mM ethylene glycol-(bis-β-aminoethyl ether)*N,N,N'N'*-tetraacetic acid, 5 mM cAMP, 0.75 mM 3-isobutyl-1-methyl xanthine, 0.1 mg/ml of creatine phosphokinase, 10 mM creatine phosphate and 1 to 2×10^6 cpm (α-^{32}P)ATP in the absence or presence of 10 µM GTP. The effects of α-MSH (10^{-7}–10^{-5} M) and (−)-isoprenaline (10^{-9}–10^{-4} M) were studied at increasing concentrations. The enzyme reaction, which was carried out at 37°C for 10 min, was terminated by the addition of 100 µl of 10% sodium dodecyl sulphate containing 5 mM ATP and by boiling in a water-bath for 15 min. The adenylate cyclase activity was expressed in pmol cAMP/mg protein×min and the pK_{act}-value ($-\log K_{act}$) for a drug was defined as the −log concentration of drug that induces 50% of maximum adenylate cyclase activity of that drug.

Protein determination

Protein was determined by the method of Lowry et al. (23) using bovine serum albumin as standard.

RESULTS

Basal adenylate cyclase activity was 7.3±1.1 and 6.1±1.9 pmol cAMP/mg protein×min in the cell homogenates prepared 48 or 72 h after replating (Table I). Addition of 10 µM GTP markedly increased the enzyme activity (13.7±3.0 and 13.1±2.9 pmol cAMP/mg protein×min). However, the increase was of about the same extent regardless of the age of the cultures.

Adenylate cyclase activity in the presence of α-MSH (10^{-5}–10^{-7} M) remained at the basal level, no stimulation could be demonstrated either with or without additional GTP.

Dose-dependent and considerable increase of AC activity was observed after isoprenaline (10^{-7}–10^{-4} M) treatment, with higher values in the presence of additional GTP. However, the activation constant for half-maximal stimulation of cAMP accumulation did not depend either on the age of the cultures or on the addition of exogenous GTP (Table II).

DISCUSSION

Previous studies of cultured murine melanoma cells have demonstrated that MSH mediates melanin synthesis via the enzyme tyrosinase in direct correlation

Table II. *The pK_{act} values of isoprenaline (mean ± SEM of 3 experiments)*

48 h		72 h	
−GTP	+GTP	−GTP	+GTP
6.29±0.12	6.18±0.20	6.41±0.26	6.35±0.30

with binding to high affinity cell surface receptors (1, 2) and subsequent stimulation of AC activity (24). A few minutes after exposure of cells to MSH, the intracellular levels of cAMP rise to several times those of the controls, reaching a peak between 10 and 30 min (4, 25). The increased cAMP level results not only in increased tyrosinase activity but also in changes of cell morphology and proliferation (3).

Concerning the human studies, there are results indicating specific binding of radiolabelled α- and β-MSH also by normal and malignant human melanocytes (12–15). However, the level of binding of (^{125}I)-β-MSH by these human cells is much lower than the binding observed in murine melanoma cells (15). Furthermore, Legros et al. (12) reported an increase of intracellular cAMP followed by stimulation of tyrosinase activity, significant inhibition of DNA synthesis and cell growth after 30 min treatment with 10^{-9} to 10^{-7} M α-MSH in HM6A human melanoma cell line. Ranson et al. (26) demonstrated a 7-fold increase in intracellular cAMP level after treatment of melanocyte cultures of human foreskin origin with 5×10^{-7} M α-MSH for 12 min. However, this was followed only by a small (<20%) increase in tyrosinase activity.

Using the human melanoma cell line IGR 1 we did not observe any increase in AC activity after a 10 min incubation with 10^{-7} to 10^{-5} M MSH. Negative results have been reported also by other authors. Halaban et al. (15) found tyrosinase activity and rate of cellular proliferation of normal and malignant human melanocytes to be insensitive to MSH. Hadley & Dawson (16) reported that a potent MSH analog, the (Nle^4,D-Phe^7)-α-MSH was ineffective in stimulating cultured human melanocytes as determined by tyrosinase bioassay.

Thus, data concerning the in vitro effects of MSH on human melanocytes are conflicting. A discrepancy also seems to exist between the in vitro and in vivo observations, since human skin is darkened by systematically administered MSH (8, 9). The reason for these and the difference between the responses of murine and human cells is not yet clear. Recently it has been reported by Warren (27), that melanogenesis within primary cultures of human skin explants can be stimulated by (Nle^4,D-Phe^7)-α-MSH. This suggests that the structural integrity of the skin might play an important role in the effect of MSH on human melanocytes.

Adenylate cyclase in partially purified plasma membrane fraction of M2R murine melanoma cell line responds to β-MSH only in the presence of GTP (28). However, preparation of plasma membrane fraction eliminates endogenous soluble GTP, which is necessary for the activation of AC. In our experiments cell homogenates were used without further purification, but the basal AC activity could be further increased by addition of exogenous GTP. The reason for this is either the dilution of endogenous GTP during the preparation of cell homogenates or the intracellular GTP level of the IGR 1 melanoma cells is originally too low for maximal AC activity.

The β-adrenergic agent, isoprenaline, has been reported to stimulate tyrosinase activity in hair follicular melanocytes of the mouse (19). Pigment cells in the lower vertebrates are known to be controlled by both β- and α-agonists (29, 30). We have demonstrated a 4 to 7-fold increase of AC activity after isoprenaline treatment, indicating that the IGR 1 human melanoma cell line possesses β-adrenoceptors. We used this substance since it is well recognized that β-adrenoceptors, like receptors of MSH, operate through cAMP dependent mechanisms. Thus it could serve as a control for our model system. The present result may suggest that catecholamines could have a role in the regulation of human melanogenesis.

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