INDUCTION OF MELANOCYTOGENESIS IN EXPLANTS OF CURASSIUS AURATUS L THE XANTHIC GOLDFISH BY DIBUTYRYL-cAMP[a]

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Summary Di-butyryl-cAMP has been shown to induce the differentiation of melanoblasts into melanocytes in cultured explants of the caudal fin of the goldfish.

We have reported previously that MSH (melanocyte stimulating hormone) and structurally related polypeptides (such as ACTH, adrenocorticotrophic hormone)can induce the differentiation of melanoblasts into melanocytes in organ cultures of xanthic goldfish tailfins, a process which we have termed melanocytogenesis. Evidence was also presented which strongly indicates that an obligatory mitosis of a stem cell (the melanoblast) is involved and that the hormone acts near the time of mitosis (see references 1 and 2). We wish to report here that this effect of MSH, like the classical melanin granule dispersion effect of MSH (3, 4), can be duplicated by dibutyryl-cyclic-AMP.

The incubation of tailfin explants (approximately 1 mm. by 2 mm.) is carried out essentially as previously described (2). Each experiment (from one fish) contains two groups of explants used as controls (basal tissue culture medium only and maximal stimulation by hormone) and several groups of explants treated with different concentrations of dibutyryl-cAMP. The results of several experiments are combined and summarized in Table I.

The results shown here are the averages obtained from several fish. With individual fish, the dose-response curve is somewhat variable, with

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TABLE I

Number of Melanocytes Formed per Explant

Addition	Concentration	Number of Explants	Average Number of Melanocytes per Explant
NONE		28	0.4
ACTH	0.2 I. U./ml.	24	26
dibutyryl - cAMP	1×10^{-8} M	9	0.1
	1×10^{-7} M	10	0.7
	1×10^{-6} M	29	3.2
	3×10^{-6} M	19	2.2
	1×10^{-5} M	23	1.3
	3×10^{-5} M	13	16
	1×10^{-4} M	24	27
	3×10^{-4} M	21	27
	1×10^{-3} M	27	21

Legend to Table I: Explants were incubated essentially as described previously except that the incubation period was four instead of three days. The results are the averages obtained with five fish. ACTH (Acthar, Armour Pharmaceutical Company) was employed instead of MSH at a level that gives maximum response (1).

Similar experiments with cAMP gave variable results. This is probably due to permeability barriers. It is not known whether this is due to cellular permeability barrier or tissue permeability barrier or both. In previous studies, we have noted high permeability barriers, presumably of tissue origin, towards various analogues of amino acids, purines and pyrimidines (5). It is possible

that the outgrowth of cells from the explants during incubation may lead to disintegration of the normal epidermal structure and the tissue permeability barrier. If this event were to be variable from explant to explant, it could lead to the variable responses obtained with cAMP. Further experiments are in progress to clarify this uncertainty.

maximum stimulation occurring at either 10^{-4} or 10^{-3} M dibutyryl-cAMP. Averaging the maximum response obtained with dibutyryl-cAMP obtained with several fish, the response compares favorably with stimulation by ACTH.

These results add another effect of cAMP to the long list of effects of cAMP to both eucaryotic and procaryotic cells. Although this list of effects of cAMP is too long to be discussed here, it should be mentioned that the classical granule dispersion effect of the MSH has been demonstrated to be mediated via cAMP (3,4). The present study indicates that the melanocytogenic activity of this hormone is probably also mediated via cAMP. To our knowledge, this is the first example where cAMP is implicated in the differential mitosis of a stem cell.

References

1. T. T. Tchen, R. N. Ammeraal, K. Kim, C. M. Wilson, W. Chavin and F. Hu, Natl. Cancer Inst. Monograph, 13, 67 (1964).
2. C. D. Foote and T. T. Tchen, Exptl. Cell Research, 47, 596 (1967).
3. M. W. Bitensky and S. R. Burstein, Nature, 208, 1281, (1965).
4. R. R. Novales and W. J. Davis, Endocrinology, 81, 283, (1967).
5. R. N. Ammeraal, Dissertation, Wayne State Univ., Detroit, Mich. (1963).