

NOTE

A Contribution to the Analysis of the Mechanism of MSH-Induced Dispersion in the Melanophores of *Xenopus laevis*

The way in which MSH exerts its dispersing influence on the melanin granules in the melanocytes of amphibians has been the subject of many investigations. Different aspects of the dispersion process have been discussed by a number of investigators. The facts point to a rather complicated series of reactions, starting with the (hypothalamically regulated) secretion of MSH from the intermediate lobe of the pituitary gland into the blood stream, and resulting in the migration of the melanin granules from the central part into the processes of the melanocyte. The direct stimulus for the movement of the granules is probably related to a change in the electrolyte composition of the cytoplasm.

It is not likely that MSH, being a peptide hormone, has to enter the melanocyte in order to effect these changes in the internal medium. In the concept of Sutherland *et al.* (1965), hormonal action is limited to the cellular membrane in a number of cases. This cellular membrane contains the enzyme adenylyl cyclase which converts ATP into cyclic adenosine 3',5'-monophosphate (3,5-AMP). The 3,5-AMP influences, as a second messenger, certain receptors in the internal medium. Demonstrating a dispersing effect of 3,5-AMP on the melanophores of *Rana pipiens*, Bitensky and Burstein (1965) suggested a similar mechanism for the dispersion reaction. The question remained whether or not MSH directly influenced the adenylyl cyclase. Recent results of Brouwer (1969) demonstrated that during the adaptation of adult *Xenopus laevis* to a black background, dopamine disappeared from certain

reservoirs in the skin. So, dopamine or its metabolites (norepinephrine and epinephrine) may be involved in the action of MSH on the enzyme adenylyl cyclase. This paper deals with some results obtained in an attempt to answer this question.

When dopamine or its metabolites are involved in the series of reactions induced by MSH, inhibition of the dispersion requires substances which are known to be adrenergic receptor blocking agents. MSH-dispersion was therefore studied in the presence of the blockers Inderal (or propranolol) and Regitine (or phentolamine). Excised webs of white-background-adapted adult *X. laevis* were incubated in Inderal (3.4×10^{-4} M) or Regitine (10^{-3} M). After 45 min of incubation, α -MSH (0.3 μ g/ml) was added to the bathing solution and the melanophore index (Hogben and Slome, 1931) was estimated every 15 min from this time (0 min) until 180 min. α -MSH in *Xenopus*' Ringer (Dikstein and Sulman, 1964) was used as control solution. The MSH-induced dispersion appeared to be greatly influenced by the presence of the blocking substances. In the presence of Regitine, MSH did not show any dispersion at all, and in the presence of Inderal a slight dispersion was noticed which gradually decreased after 60 min, resulting in a completely aggregated state again at 120 min. The upper part of Fig. 1 shows the results at 0 and 120 min. These results strongly suggest that catecholamines mediate, or at least cooperate, in the dispersion evoked by α -MSH.

In order to establish the sequence of events, the same blocking substances were

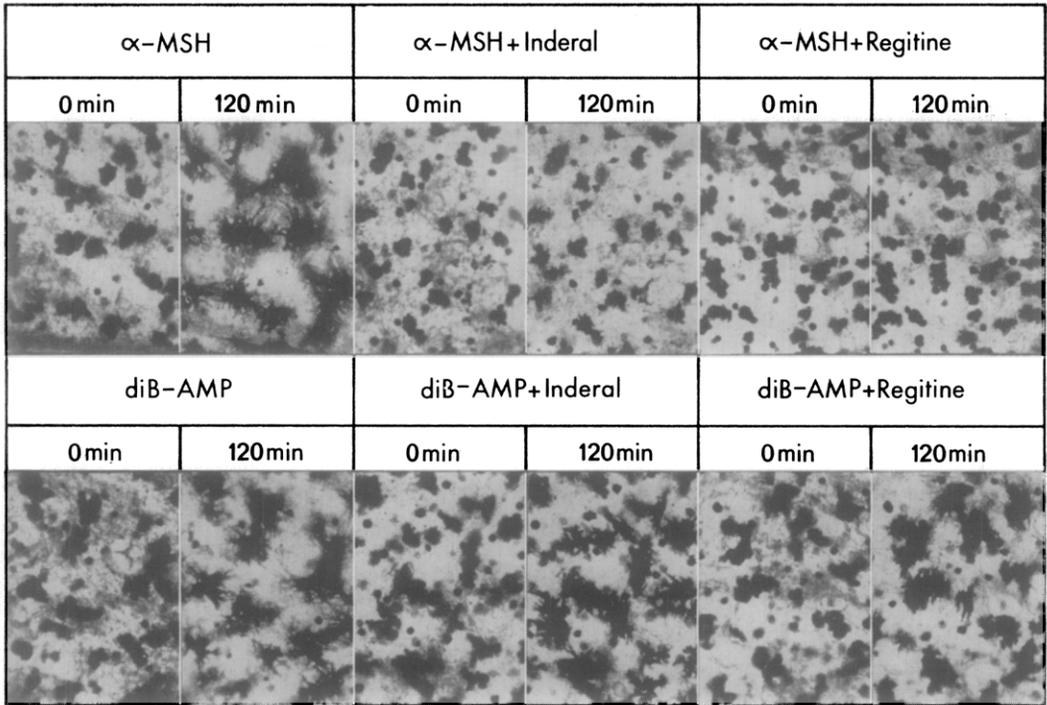


FIG. 1. The effect of Inderal and Regitine on the dispersion induced by α -MSH and dibutyryl 3,5-AMP, at 0 and 120 min.

applied to the dispersion brought about by 3,5-AMP. Since 3,5-AMP is known to penetrate cellular membranes rather poorly, its analogue dibutyryl cyclic adenosine 3',5'-monophosphate (diB-AMP) was also used. After a preincubation of webs during 45 min in Inderal or Regitine, 3,5-AMP (10^{-2} M) or diB-AMP (5×10^{-3} M) were added to the bathing solutions. Again, 3,5-AMP and diB-AMP in Ringer solution

were used as controls. The melanophore index recorded every 15 min revealed that 3,5-AMP as well as diB-AMP dispersed the pigment granules and that this dispersion was not influenced by Inderal or Regitine. The lower part of Fig. 1 shows the result at 0 and 120 min in the case of diB-AMP.

Summarizing these results (Table 1), it is suggested that the part played by MSH

TABLE 1
ESTIMATION OF DISPERSION AND AGGREGATION OF THE MELANOPHORES IN EXCISED WEBS OF *Xenopus laevis*

Estimation after 120-min incubation with α -MSH, 3,5-AMP, dibutyryl-3,5-AMP, and Ringer solution, in the presence of the adrenergic receptor blocking substances Inderal and Regitine.

	Ringer solution	Ringer + Inderal (3.4×10^{-4} M)	Ringer + Regitine (10^{-3} M)
α -MSH (0.3 μ g/ml)	++ ^a	—	—
3,5-AMP (10^{-2} M)	+	+	+
diB-AMP (5×10^{-3} M)	++	++	++
Ringer solution	—	—	—

^a According to the Hogben and Slome Index: (++) Represents a dispersion of about 3.5-4; (+) represents a dispersion of about 3-3.5; and (—) represents no dispersion (1-2).

in the dispersion reaction is mainly the liberation of a catecholamine, which in turn (or together with MSH) stimulates the membraneous enzyme adenylyl cyclase, resulting in the production of 3,5-AMP. The way in which 3,5-AMP changes the internal medium is still obscure, although a relation between 3,5-AMP and the electrolyte composition of the cytoplasm has been described in other fields (Friedman and Park, 1968; Zor *et al.*, 1968).

As compared to the lipolytic effects of 3,5-AMP (Butcher and Sutherland, 1967), the dispersing effects of this substance do not permit any definite conclusion regarding the α - or β -type of the adrenergic receptor. Prostaglandin E_1 (10^{-4} – 10^{-6} M) and insulin (10^{-6} – 10^{-9} M) did not effect the MSH-induced dispersion. Our results have to be confirmed by investigating the 3,5-AMP content of *Xenopus* skin in relation to MSH-secretion; i.e., to the dispersion state of the melanophores. Furthermore, we are carrying out, in collaboration with E. Brouwer, investigations in which the synthesis of catecholamines has been blocked.

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