ROLE OF CALCIUM AND PROSTAGLANDIN (PGE_1) IN THE MSH-INDUCED ACTIVATION OF ADENYLATE CYCLASE IN XENOPUS LAEVIS

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SUMMARY

The effect of varying Ca^{2+} concentrations on the action of a number of pigment dispersing substances has been studied. The absolute Ca^{2+} requirement for MSH activity, contrary to the noradrenaline and cyclic AMP activities, suggests a role of Ca^{2+} in the formation or the primary action of the MSH-receptor-complex.

Prostaglandin (PGE_1) is also able to disperse the melanophores of Xenopus laevis. Its activity proves to be independent of the presence of Ca^{2+}.

A hypothesis has been put forward concerning the mechanism of MSH activity: formation of the MSH-receptor-complex may be responsible for prostaglandin synthesis in the membrane. This prostaglandin may produce displacement of Ca^{2+}, followed by a Na^+ influx, which is responsible for the activation of the catalytic unit of membranous adenylate cyclase.

INTRODUCTION

Dispersion of the pigment granules in the melanophores of Amphibia, resulting from adaptation to a black background, is a hormone regulated process. The melanophore stimulating hormone (MSH) initiates the migration of the melanin granules by activation of the enzyme adenylate cyclase (1,2,3), which is situated in the plasma membrane of the melanophore. Activation of adenylate cyclase results in a higher intracellular level of cyclic AMP (cAMP), which in turn is responsible for the migration of the melanosomes. The mechanism of this intracellular movement is still unknown. A possible involvement of microtubules and microfilaments has been suggested (4,5).

Investigation of hormone activated adenylate cyclase systems in other fields reveals the presence of distinct molecular components: at the outer side of the membrane one or more hormone specific receptors are located. A hormone-receptor-interaction induces a reaction process which is conducted through part of the membrane (named transducer) to the catalytic unit, situated at the inner part of the membrane. Activation of the catalytic unit results in the formation of cAMP (6,7,8).

Apart from activation by peptide hormones, many adenylate cyclase systems are sensitive to catecholamines as well. The corresponding adrenergic receptor appears to be of the beta type (9). The mechanism underlying activation of the catalytic unit is not uniform. In adipose tissue separate activation by each of the different receptors present is described (7,10). In the melanophores of Xenopus laevis, however, activation by MSH is only possible when the adrenergic receptor is operating concomitantly (3,11).

The function of Ca^{2+} in a number of stimulus-response reactions is generally acknowledged. Especially the influence of Ca^{2+} in hormonal stimulation has been studied by Rasmussen and Tenenhouse (12). However, their interesting hypothesis encounters much opposition (13). In many systems Ca^{2+} should be present extracellularly in order to facilitate hormone-receptor-interaction. In the case of melanophore dispersion Ca^{2+} indispensibility has been demonstrated earlier (14,15). In the present study an attempt will be made to localize this Ca^{2+} requirement in the series of reactions necessary for adenylate cyclase activation.

Apart from Ca^{2+}, prostaglandins are assumed to be involved in a number of cAMP mediated hormonal effects (16,17,18). This prostaglandin influence may result in either an increase or a decrease of the intracellular cAMP level. Ramwell and Shaw (18) presented evidence indicating an influence of prostaglandin on intracellular calcium content. The present paper describes the effect of exogenous prostaglandin on the pigment migration process.

MATERIAL AND METHODS

The melanophore dispersing activity was determined in vitro, using excised webs of adult Xenopus laevis. The animals (weighing about 30 grams) were kept in white illuminated jars for 48 hours. Then the webs of the hindlegs were excised, cut into pieces of 2 x 3 mm and soaked in Ringer solution of the following composition: NaCl (110.10^{-3}M); KCl, $CaCl_2$ and $NaHCO_3$ (each 2.10^{-3}M) and ascorbic acid (10^{-4}M) to prevent oxidation. The compounds to be tested for dispersing or inhibiting activities were dissolved in the Ringer solution and the melanophore index (MI) according to Hogben and Slome (19) was recorded at various time intervals.

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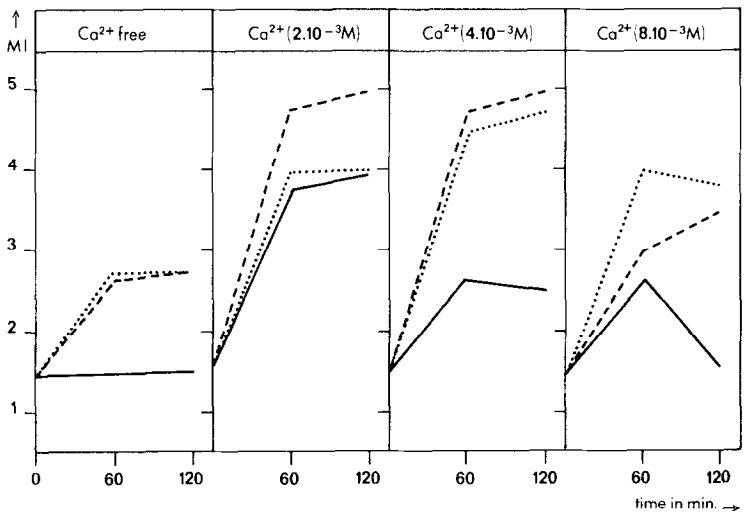

Fig 1. Melanophore dispersion, initiated by αMSH (.1 µg/ml, ———), noradrenaline (10^{-4}M, ·····) and cAMP (10^{-2}M, ------) in media containing various Ca^{2+} concentrations.

RESULTS

The dispersing capacity of MSH, noradrenaline and cAMP was determined in various calcium concentrations (0-½-1-2-4-8-16-32 mM). In each of the various solutions isotonicity was maintained by varying the NaCl amount of the Ringer. The Ca^{2+} free Ringer solution contained in addition EGTA (10^{-3}M).

The results of some Ca^{2+} concentrations are presented in Fig 1. As expected, MSH induced the best dispersion reaction in the normal Ringer solution, containing 2.10^{-3}M Ca^{2+}. But all other Ca^{2+} concentrations, except the Ca-free solution, did show any dispersion. The dispersion caused by noradrenaline, cAMP or dibutyryl-cAMP is influenced by fluctuations in the external Ca^{2+} concentration to a certain degree, but not entirely inhibited.

In order to test a possible role of prostaglandin in the MSH-induced dispersion reaction, PGE_1 was used in various concentrations. As shown in Fig 2a, PGE_1 (5.10^{-5}M) is capable of inducing a marked dispersion in Xenopus' melanophores. In order to test whether synthesis of prostaglandin is underlying the MSH activity, the inhibitor indomethacin (20) was injected in a black background adapted toad (24 mg/kg). After three hours maximal aggregation was reached (Fig 3).

To check whether prostaglandin may be involved in the role

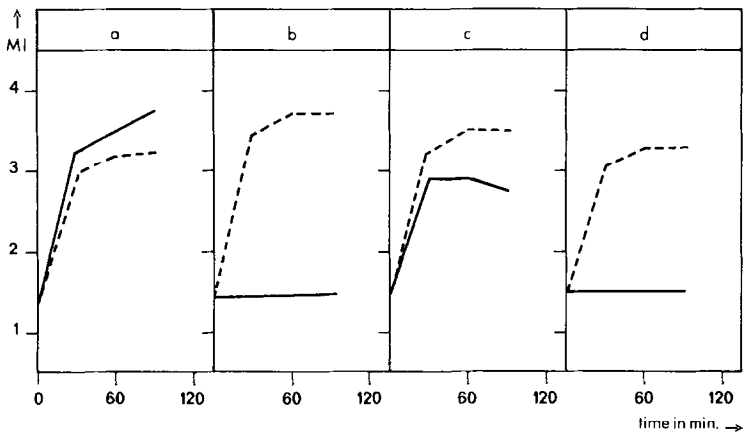

Fig 2. Melanophore dispersion, initiated by ∝MSH (.03 µg/ml, ———), and PGE_1 (5.10^{-5}M, ------) in (a) Ringer solution, (b) propranolol (5.10^{-4}M), (c) phentolamine (10^{-3}M) and (d) Ca^{2+} free Ringer solution containing EGTA (10^{-3}M).

played by catecholamines (11,21,22), the adrenergic blockers phentolamine and propranolol were tested (Fig 2b,c).

In order to determine whether prostaglandin activity depends on the presence of Ca^{2+}, an experiment was made in Ca^{2+} free Ringer solution (Fig 2d).

D I S C U S S I O N

The dispersing activity of noradrenaline and cAMP in excised webs of Xenopus laevis is only moderately influenced by the external Ca^{2+} concentration. MSH activity, however, behaves different : whereas an increase of Ca^{2+} concentration results in a (slightly) decreased activity, absence of Ca^{2+} in the medium completely blocks MSH activity (Fig 1). This points to an involvement of Ca^{2+} in one of the early steps between the formation of the hormone-receptor-complex and activation of the catalytic unit of the enzyme adenylate cyclase.

It is generally accepted that Ca^{2+} regulates membrane permeability. Ramwell and Shaw (18) studied the regulation of the permeability of frog skin and described the activity of prostaglandin in this respect. They put forward the hypothesis that PGE_1 displaces membrane bound Ca^{2+}. The result is an increased Na^+ influx which may be responsible for subsequent intracellular events, such as activation of adenylate cyclase. Their suggestion is in agreement with earlier statements (12) that an initial event of hormone action may be a displacement of Ca^{2+} from the membrane. In agreement with these

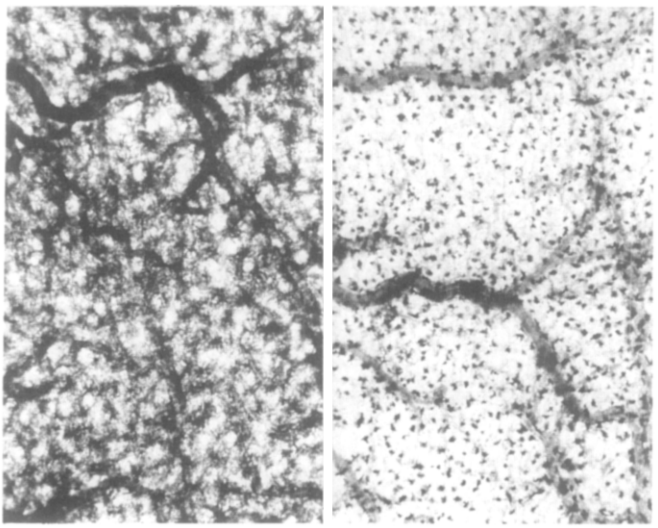

Fig 3. Melanophores in the web of a hindleg of Xenopus laevis, adapted for 3 hours and 45 minutes to a black background; (a) control animal, (b) animal after injection of indomethacin (three hourly injections of each 24 mg/kg).

results we also noticed a dispersing effect of PGE_1 administration (Fig 2a). The results obtained with indomethacin, which has been described as a blocker of prostaglandin synthesis (20), point to a de novo synthesis of prostaglandin as a result of MSH action (Fig 3). In the field of pigment migration prostaglandin also displays its dispersing activity in a Ca^{2+} free medium (Fig 2d). Consequently, the absolute Ca^{2+} requirement of MSH cannot be explained by the Ca^{2+} displacing activity of prostaglandin.

Since the limiting factor in endogenous prostaglandin biosynthesis is the concentration of free essential fatty acids (23,24), MSH activity may fundamentally consist of an activation of membrane bound phospholipase, in order to release polyunsaturated fatty acids (PUFA) from the membranous phospholipid stores. The liberated fatty acids are rapidly transformed into prostaglandin. A similar suggestion has been made for the hormone induced permeability change of frog skin (25) and rat fundus (26).

The exact nature of the phospholipase involved is still unknown. The absolute Ca^{2+} requirement of the enzyme from porcine pancreas (27) and from several snake venoms (28,29), may be extended to the enzyme present in melanophores. This might explain the specific Ca^{2+} requirement of MSH.

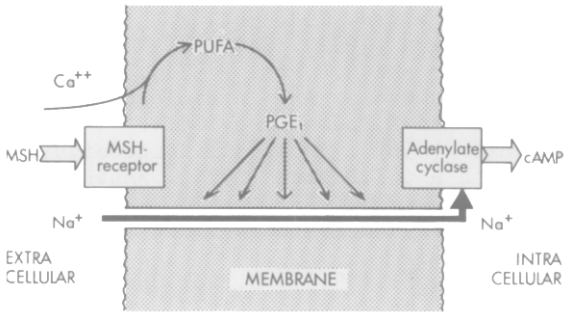

Fig 4. Suggestion of the role played by PGE_1 in the darkening reaction induced by MSH.

Liberation of catecholamines from certain reservoirs in the skin of Xenopus laevis, as a result of MSH activity (11,22) might well be explained by an identical action of MSH, since binding of catecholamines to phospholipids is one of the possible ways in which these amines are stored (30,31,32).

Involvement of catecholamines in the MSH induced darkening process is firmly established for Xenopus laevis (21), but does not likely play a role in Rana species, where these amines induce a lightening of the skin, due to the presence of alpha adrenergic receptors (33). Sih et al.(34) suggest a coenzymatic role of catecholamines in the mechanism of prostaglandin biosynthesis.

PGE_1 activity is not influenced by either alpha or beta blockers (Fig 2b,c), indicating that the role of PGE_1 is localized in the MSH- rather than in the catecholamine route of adenylate cyclase activation. The special mechanism of this catecholamine involvement in Xenopus' darkening is still obscure. Whether or not the suggested MSH-PUFA-PGE_1 route (Fig 4) may be of general validity for all Amphibian species, has to be investigated.

A C K N O W L E D G E M E N T S

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R E F E R E N C E S

1. M.W.Bitensky and S.R.Burstein (1965). Nature, 208, 1282.
2. K.Abe, R.W.Butcher, W.E.Nicholson, C.E.Baird, R.A.Liddle and G.W.Liddle (1969). Endocrin., 84, 362.
3. F.C.G.van de Veerdonk and Th.M.Konijn (1970).Acta Endocrin., 64, 364

4. S.E.Malawista (1971). Nature, 234, 364.
5. J.McGuire and G.Moellmann (1972). Science, 175, 642.
6. M.Rodbell, L.Birnbaumer and S.L.Pohl (1971). In: Coll.Role of Adenyl Cyclase and Cyclic AMP in Biological Processes. Ed. P.Condliffe and M.Rodbell; p.103.
7. H.P.Bär and O.Hechte (1969). Proc.Natl.Acad.Sci.U.S., 63, 350.
8. R.J.Lefkowitz, J.Roth and I.Pastan (1971). Ann.N.Y.Acad.Sci., 185, 195.
9. G.A.Robison, R.W.Butcher and E.W.Sutherland (1967). Ann.N.Y. Acad.Sci., 139, 703.
10. L.Birnbaumer and M.Rodbell (1969). J.Biol.Chem., 244,2377.
11. E.Brouwer and F.C.G.van de Veerdonk (1972). Eur.J.Pharmac., 17,234.
12. H.Rasmussen and A.Tenenhouse (1968). Proc.Natl.Acad.Sci.U.S., 59, 1364.
13. A.G.Gilman and T.W.Rall (1971). In: The Actions of Hormones. Ed.P.P.Foa; p.87.
14. R.R.Novales and B.J.Novales (1965). Gen.Comp.Endocrin., 5, 568.
15. D.L.Vesely and M.E.Hadley (1971). Science, 173, 923.
16. D.Steinberg, M.Vaugham, P.J.Nestel and S.Bergström (1963). Biochem.Pharmac., 12, 764.
17. R.W.Butcher and E.W.Sutherland (1967). Ann.N.Y.Acad.Sci., 139,849.
18. P.W.Ramwell and J.E.Shaw (1970). Rec.Progr.Horm.Res., 26, 139.
19. L.T.Hogben and D.Slome (1931). Proc.Roy.Soc.(B), 108, 10.
20. J.R.Vane (1971). Nature New Biol., 231, 232.
21. E.Brouwer (1973). Diss.Utrecht, (in press).
22. E.Brouwer and F.C.G.van de Veerdonk (1969). Exper., 25, 391.
23. W.E.M.Lands and B.Samuelsson (1968). Biochim.Biophys.Acta,164, 426.
24. H.Vonkeman and D.A.van Dorp (1968). Biochim.Biophys.Acta,164, 430.
25. S.J.Jessup, W.J.McDonald-Gibson, P.W.Ramwell and J.E.Shaw (1970). Feder.Proc., 29, 387.
26. F.Coceani, J.J.Dreifuss, L.Puglisi and L.S.Wolfe (1969). In: Prostaglandins, Peptides and Amines. Ed. P.Mantegazza and E.W.Horton; p.73.
27. G.H.de Haas, N.M.Postema, W.Nieuwenhuizen and L.L.M.van Deenen (1968). Biochim.Biophys.Acta, 159, 103.
28. J.F.Uthe and W.L.Magee (1971). Can.J.Biochem., 49, 776.
29. B.Arnesjo and A.Grubb (1971). Acta Chem.Scand.,25,577.
30. U.S.von Euler (1958). Acta Physiol.Scand., 43, 155.
31. R.E.Coupland and T.D.Heath (1961). J.Endocrin., 22, 71.
32. J.P.Green (1962). Adv.Pharmac., 1, 349.
33. I.Gupta and N.K.Bhide (1967). J.Pharm.Pharmac., 19, 768.
34. C.J.Sih, C.Takeguchi and P.Foss (1970). J.Amer.Chem.Soc., 92, 6670.