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POSSIBLE INVOLVEMENT OF α- AND β-RECEPTORS IN THE NATURAL COLOUR CHANGE AND THE MSH-INDUCED DISPERSION IN *XENOPUS LAEVIS* IN VIVO

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Participation of adrenergic receptors in the darkening reaction has been demonstrated in *Xenopus laevis* in vivo. Blockade of the β-receptors inhibited adaptation to a black background as well as the artificially MSH-induced dispersion. α-Receptors could not be proved to be involved in the dispersion or aggregation of the melanophores. A possible mechanism of the melanophore dispersion by MSH and catecholamines is discussed

Adrenergic receptors Pigment migration MSH *Xenopus laevis*

1. INTRODUCTION

Amphibians adapt their skin colour to different backgrounds. The adaptation is caused by pigment migration in chromatophores.

The process of pigment migration has generally been studied in melanophores. These are melanin-containing pigmentcells, which play a role in black background adaptation. The movement of melanin granules in the melanophores of the skin is influenced by several hormonal and pharmacological agents. The most important hormonal agent involved in regulating the dispersion reaction is the melanophore stimulating hormone (MSH).

A number of authors have also described the effects of catecholamines on pigment migration (Burgers et al., 1953; Whright and Lerner, 1960; Abe et al., 1969; Van de Veerdonk and Konijn, 1970; Brouwer, 1970).

There are two types of adrenergic receptor at which catecholamines can act, alpha and beta (Alhquist, 1948).

In skin melanophores of several amphibian species both types of adrenergic receptor have been demonstrated (Lerner et al., 1954; Graham, 1961; Gupta and Bhide, 1967; Goldman and Hadley, 1969; Van de Veerdonk and Konijn, 1970).

Van de Veerdonk (1969) and Van de Veerdonk and Konijn (1970) assumed that adrenergic receptors were involved in the MSH-induced dispersion of the melanophores. In experiments with isolated tailfins of larval *Xenopus laevis*, dispersion of the melanophores induced by MSH was prevented by a β-blocking agent. Using α- and β-blocking agents, Graham (1961) in *Xenopus laevis* and Goldman and Hadley (1969) in *Scaphiopus couchi* found that the reaction of the melanophores to catecholamines was mediated by β-adrenergic receptors only. They did not find any influence of β-receptor blocking agents on the melanophore dispersion induced by MSH. In their opinion, therefore, β-receptors were not involved in the operative mechanism of the natural colour change.

The discrepancy between the results of Graham (1961) and Goldman and Hadley (1969) on the one hand and Van de Veerdonk and Konijn (1970) on the other hand have lead to a study of the influence of α- and β-receptor blocking agents on the pigment migration reaction of adult *Xenopus laevis* in vivo.

2. MATERIALS AND METHODS

In adult *Xenopus laevis* of 15–30 g, reared in our laboratory, the degree of melanin dispersion was estimated by applying the melanophore index (MI) of Hogben and Slome (1931). Before using toads in an experiment, a selection was made, based on the rate of aggregation and dispersion of their melanophores.

The toads were adapted to white or black backgrounds by placing them in illuminated white- or black-painted jars. In order to avoid complications due to central actions of the applied drugs, a study was made of the action of α and β-receptor blocking agents on natural colour change as well as on MSH-induced dispersion. The same toads served as control and test animals. This means that normal adaptation to white or black backgrounds, and also the MSH-induced dispersion of the melanophores, was examined with intervals of one day in the same animals as used in the experiments with α- and β-blocking agents.

The drugs and hormones were dissolved in amphibian Ringer according to Burgers and Van Oordt (1953) and injected into the dorsal lymph spaces.

α-MSH was generously supplied by Dr. W. Rittel of CIBA, Ltd., Basle, Switzerland. MSH was administered in doses of 0.25–0.5 μg per animal.

The α-blocking agents dibenamine and phentolamine were administered in doses of 5 mg/kg and 30 mg/kg respectively. Propranolol and DCI (1–(3′, 4′-dichlorophenyl)-2-isopropylamino ethanol hydrochloride) were used as β-blockers in doses of 30 mg/kg each.

In order to compare our results with those of Graham (1961), DCI was administered once in a dose of 5 mg/kg.

Phentolamine was a gift of CIBA, Basle. Dibenamine was obtained from K&K Lab., Inc. Plainview N.Y., U.S.A.

Propranolol was a gift of ICI, Ltd., London, England. DCI was obtained from Aldrich Chem. Co. Inc., Milwaukee, Wis., U.S.A.

3. RESULTS

3.1. *Effect of α- and β-blockers on the adaptation to a black background*

A possible co-operating effect of α- and β-adrenergic receptors in the dispersion reaction was investigated by administering α- and β-blocking agents into animals which were transferred from a white to a black background. The results of the action of α- and β-blockers upon the dispersion of the melanophores were compared with those obtained by normal adaptation to a black background of the same animals examined previously.

Propranolol and DCI injected into 5 animals each,

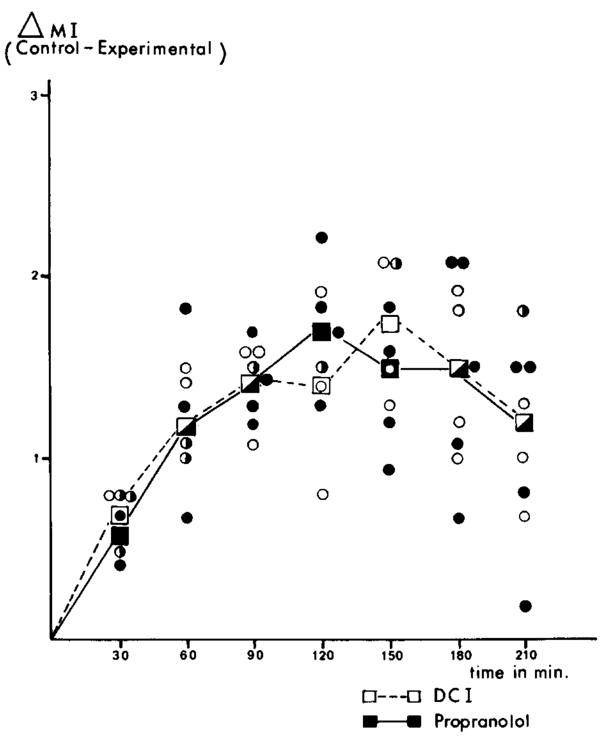

Fig. 1. The effect of propranolol, 30 mg/kg, and DCI, 30 mg/kg, on the dispersion of the melanophores after transferring the animals from a white to a black background. ΔMI is the value obtained by subtracting the MI values of animals treated with β-blocking agents from those of the same animals injected with solvent only. The MI values have been calculated with regard to the MI values at zero time. ○, values of control minus DCI treatment per animal. □, represents the mean value. ●, values of control minus propranolol treatment. ■ represents the mean value.

inhibited the dispersion of the melanophores. The MI values of the tested animals varied considerably, but the degree of dispersion in a particular treated toad was less than in the untreated control. In other words, the differences of the MI values between control and experimental situation of each toad were always positive (fig. 1); (the same applies to the results summarized in figs. 2 and 3, which will be presented hereafter).

The experiments were repeated with another 10 animals, with similar results.

Graham (1961) did not find any inhibitory activity of the β-blocking agent DCI in doses of 5 mg/kg per animal. In view of this, the experiments were repeated with doses of 5 mg/kg DCI. As may be seen in fig. 2, the smaller doses also had an inhibitory effect.

Black background adaptation was not influenced by the α-blocking agent dibenamine and only slightly by phentolamine (table 1).

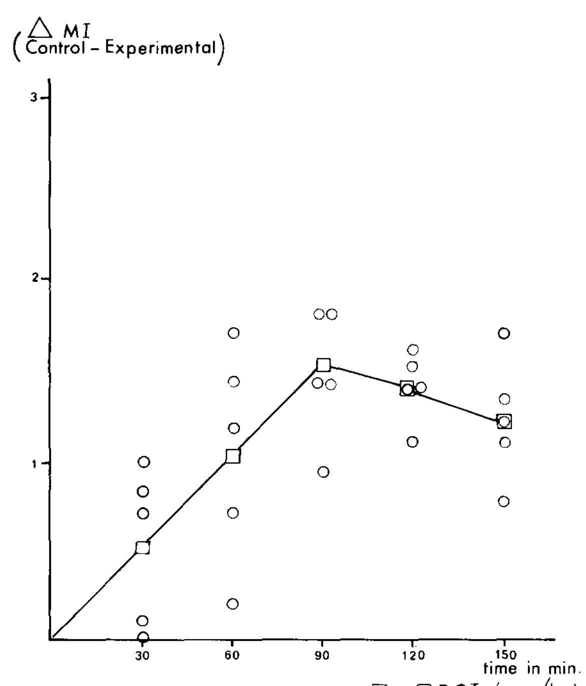

Fig. 2. The effect of a small dose of DCI (5 mg/kg) on the darkening reaction compared to the normal dispersion of the melanophores of the same animals, after transferring them from a white to a black background. For explanation of the symbols see fig. 1.

Table 1
The effect of dibenamine and phentolamine on black background adaptation.

Time (min)	Ringer	Dibenamine, 5 mg/kg	Ringer	Phentolamine, 30 mg/kg
−30	1.7	1.8	1.6	1.8
0	1.8	1.8	1.7	1.7
30	3.0	3.0	2.6	2.4
60	3.5	3.4	3.2	2.5
90	3.8	3.7	3.5	2.8
150	4.3	4.2	4.4	3.4

The average MI-values of eight toads are represented. The α-blocking agents and amphibian Ringer were administered 30 min before ($t = -30$) transferring the toads from a white to a black background ($t = 0$).

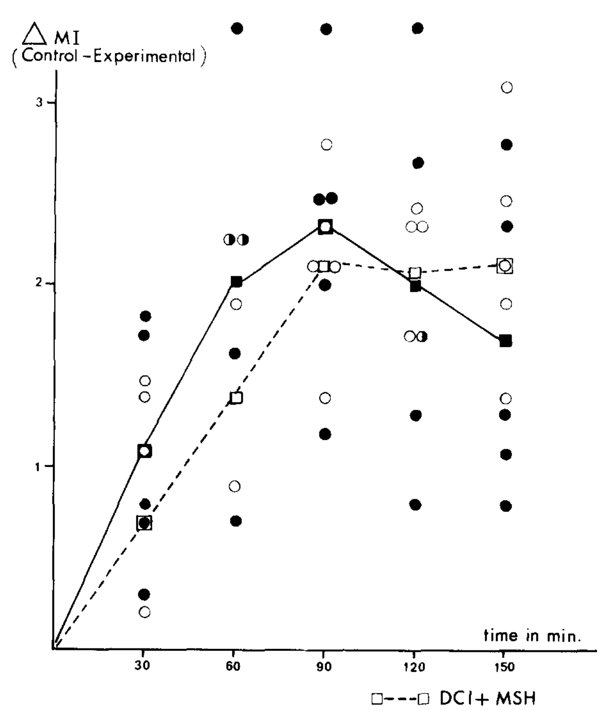

Fig. 3. The effect of propranolol, 30 mg/kg, and DCI, 30 mg/kg, on the dispersion reaction by MSH (0.25 μg per animal). Δ MI is the value obtained by substracting the MI values of animals treated with β-blocking agents in combination with α-MSH, from those of the same animals treated with αMSH only. The MI values have been calculated with regard to the MI values at zero time. ○, values of α-MSH-minus (α-MSH+DCI)-treatment per animal. □ represents the mean value. •, values of α-MSH-minus (α-MSH+propranolol)-treatment per animal. ■ represents the mean value.

3.2. *Effect of β-receptor blocking on MSH-induced dispersion*

β-Receptors were shown to be directly involved in the MSH-induced dispersion of the melanophores.

White background adapted animals were injected with α-MSH (0.25 μg per toad) and replaced on an illuminated white background. The melanophore dispersion was recorded. The same animals were used the next day to verify the influence of propranolol and DCI on the darkening reaction after an identical dose of α-MSH. The β-blocking agents were injected 30 min prior to administering α-MSH. The MSH-induced dispersion was inhibited to a considerable extent (fig. 3).

3.3. *Effect of α-receptor blocking on the adaptation to a white background*

The effect of α-receptor blockade on the dispersion reaction was not convincing. It might be possible that α-receptors, contrary to β-receptors, are involved in the aggregation reaction. To test this assumption, toads were injected with dibenamine, 5 mg/kg, or phentolamine, 30 mg/kg, 30 min before transferring them from a black to a white background. The rate of aggregation in these animals was compared with that in the same animals which the day before were adapted to a white background without administering any drug.

Dibenamine and phentolamine did not have an inhibiting effect on the aggregation of the melanin granules (table 2).

Table 2
The effect of dibenamine and phentolamine on the white background adaptation.

Time (min)	Ringer	Dibenamine, 5 mg/kg	Ringer	Phentolamine, 30 mg/kg
−30	4.9	4.9	5.0	4.9
0	4.0	4.9	5.0	4.5
30	3.6	3.7	3.7	2.8
60	3.1	3.1	3.1	2.2
90	2.7	2.8	2.7	2.0
150	2.4	2.5	2.3	1.8

The average MI-values of eight toads treated with dibenamine and five toads with phentolamine are represented. Ringer and drugs were administered half an hour before (t = 30) transferring the toads from a black to a white background (t = 0).

4. DISCUSSION

The literature indicates an effect of catecholamines in the pigment migration in amphibians. The catecholamines have a dispersing or an aggregating activity depending on the species under investigation. A darkening action of adrenaline in adult *Xenopus* skin in vitro and in vivo has been described by Burgers (1956). Goldman and Hadley (1969) also demonstrated a dispersing activity of noradrenaline in *Scaphiopus couchi*. In species of *Rana* an aggregating activity of catecholamines has been observed (Lerner and Case, 1959; Gupta and Bhide, 1967; Hagmüller and Umrath, 1969).

Catecholamines act at adrenergic receptors (Ahlquist, 1948). According to Graham (1961), only β-receptors are responsible for the dispersing effects of catecholamines in *Xenopus*. Similar results were obtained by Goldman and Hadley (1969) in *Scaphiopus couchi*. Gupta and Bhide (1967), among others, demonstrated that in *Rana tigrina* α-receptors are mainly responsible for aggregation of the melanophores after administration of catecholamines in vivo.

In the present study it was confirmed that the melanophore reaction in *Xenopus laevis* is controlled by adrenergic receptors. Blockade of the α-receptors with dibenamine was not effective in inhibiting dispersion or aggregation of the melanophores. Phentolamine did have a slight inhibiting effect on black background adaptation. Using tailfinds of *Xenopus* tadpoles, Van de Veerdonk and Konijn (1970) likewise did not find more than a minor effect of phentolamine in the MSH-induced dispersion. When using α- and β-receptor blocking agents, one has to consider the possibility that a drug which effects adrenergic mechanisms may well have other actions, as was demonstrated by Iversen (1965). Iversen found that several α- and β-blocking agents, for example imipramine, cocaine a.o., were also inhibitors of noradrenaline and adrenaline uptake into sympathetically innervated tissue. Imipramine was found to be a blocking agent of adaptation to a black background and MSH-induced dispersion (Brouwer, 1970). In view of this date, it might be possible that the action of phentolamine is comparable to that of imipramine, as regards its slight inhibitory effect on the dispersion reaction.

The present study shows that the β-blocking agents

DCI and propranolol inhibit adaptation to a black background as well as dispersion of the melanophores induced by administering MSH.

It may be concluded that β-receptors are present on the melanophores of *Xenopus laevis*, as was stated by Graham (1961). In addition, the results of the present study point to β-receptors being involved in the natural colour change and the MSH-induced dispersion of the melanophores. Since β-blocking agents may have other actions (see comments made above), a restriction has to be made on the interpretation of the results.

The differences between the activities of identical doses of DCI on the darkening of the skin after transferring *Xenopus* from a white to a black background as described by Graham (1961) and in our experiments cannot be explained. Considerable individual differences in the rate of pigment migration in toads have been noticed. The selection of toads with comparable velocities in colour change, as well as the use of the same animals for control and experiment, may be responsible for the discrepancy between Graham's results and ours.

Experiments of Van de Veerdonk and Konijn (1970) are in support of our results. They observed that the dispersion of the melanin granules caused by MSH in tailfins of *Xenopus laevis* tadpoles in vitro was likewise inhibited by propranolol.

In 1969 Brouwer and Van de Veerdonk proposed the hypothesis that catecholamines may be involved in the physiological melanophore reaction induced by MSH, at least in *Xenopus laevis.* The data obtained on the action of β-blocking agents upon the dispersion reaction confirm in an indirect way that catecholamines are involved in the natural darkening reaction in *Xenopus laevis*. These results are in agreement with the previously described relation between skin catecholamines and pigment dispersion (Brouwer and Van de Veerdonk, 1969; Brouwer, 1970) as well as with the relation between catecholamines and the intracellular cyclic AMP level (Van de Veerdonk and Konijn, 1970).

At the moment it is not clear how catecholamines are involved in the dispersion reaction induced by MSH. Investigations by Bär and Hechter (1969) on the lipolytic effects of ACTH, glucagon and adrenaline on fat cells of rat adipose tissue indicated that in the membrane of the fat cell a single catalytic unit of adenyl cyclase is coupled to distinctive selectivity sites for the three lipolytic hormones. A similar interpretation may be applied to the dispersing effects of MSH and catecholamines in the darkening process; however, the variation is that MSH and a catecholamine, freed from the skin reservoir, are co-operating in producing an activation of adenyl cyclase.

Another possibility concerning the relation of MSH and catecholamines is that MSH releases dopamine from the reservoirs in the skin of *Xenopus laevis* and this substance or one of its metabolites, noradrenaline or adrenaline, causes, by activating adenyl cyclase, the dispersion of the melanophores.

The results so far obtained point to the first possibility.

This conclucion is based on the following observations. In *Xenopus laevis*, blocking of the synthesis of catecholamines or blockade of the β-adrenergic receptors results in an inhibition of the darkening reaction. A dispersing activity of catecholamines comparable with that of MSH has not been demonstrated in vivo.

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