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Potassium-Induced Melanosome Dispersion in Melanophores of *Oryzias latipes* Is Independent of Adrenergic Mechanisms

With 4 Text-figures

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ABSTRACT Using the denervated melanophores in an isolated scale of a freshwater teleost, *Oryzias latipes*, mechanisms of K-induced melanosome dispersion in the melanophores were examined. The melanophores in a state of pigment aggregation responded to isotonic KCl solution with rapid dispersion of the melanosomes. On subsequent return to physiological saline, the melanosomes became aggregated again. Isotonic NaCl solution was ineffective in producing such a dispersing response. The strength-response relations for adrenaline stimulation in the presence of various concentrations of K ions were obtained. Adrenaline-induced melanosome aggregation was significantly inhibited in high K-solution. The dispersing effect of K ions was not antagonized by beta adrenergic blocking agents. Therefore, K-induced melanosome dispersion response is not mediated through beta adrenoceptors. Isotonic $CaCl_2$ solution was slightly effective on pigment dispersion in the melanophores. Addition of Ca ions to the K-solution had no significant effect on the response produced by K ions.

INTRODUCTION

It is well known that K ions cause rapid melanosome aggregation in the melanophores in an isolated scale or an excised strip of fin of teleost fishes. On this pigment aggregation, it is now accepted generally that K ions do not act directly on the melanophores but act on the terminals of the pigment-aggregating nerve (Fujii, 1959; Iwata *et al.*, 1959) and that the released transmitter, which is supposed as noradrenaline, induces melanosome aggregation in the cells. Hence, this aggregation depends on the response aroused through an adrenergic mechanism on the cells.

Quite recently, on the other hand, it was indicated that K ions exerted an action opposite to the pigment aggregation, a melanosome-dispersing action, on the melanophores *in vitro*: When the denervated melanophores of *Oryzias latipes* in a state of pigment aggregation were immersed in isotonic KCl solution, prompt dispersion of the melanosomes was produced. The dispersed melanosomes became aggregated again on returning the preparation to physiological saline. The K-induced pigment-

dispersing response was reversible (Iga, 1976 a). Similar melanosome dispersion to KCl solution was observed on X-ray-induced melanophores of the goldfish, which did not yet receive the supplies of nerves by which the melanosome displacement within the melanophores was regulated (Iga, 1976 b). Based on these observations, it was concluded that melanosome dispersion produced by K ions was due to a direct action of K ions on the cells (Iga, 1976 a, b).

Meanwhile, Miyashita and Fujii (1975) reported that sympathomimetic monoamines in low concentrations induced melanosome dispersion in the guppy melanophores *in vitro* and that these dispersion responses were antagonized by beta adrenergic blocking agents. Thus, they assumed the presence of beta adrenoceptors in the melanophores, which mediate pigment dispersion. They also noted that the endogenous beta stimulating amine responsible for the darkening reaction of living fish may be epinephrine, and that the chromaffin cells, which may liberate a catecholamine, exist in the dermis of that fish. Considering these recent studies, the present study was designed to ascertain quantitatively the inhibitory effect of K ions on adrenaline-induced melanosome aggregation and to determine if the K-induced melanosome dispersion in the fish melanophores was mediated through adrenergic mechanism.

MATERIALS AND METHODS

The experiments were performed with isolated scale preparations of a freshwater teleost, *Oryzias latipes*. The denervated melanophores were employed. The denervated preparations were obtained according to the method of Iga (1968, 1975 a). An isolated scale was held epidermal side down under a cover glass, which was mounted on a perfusion chamber filled with physiological saline solution, which had the following composition: 128 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$, 5.0 mM Tris-HCl buffer (pH 7.2). On the perfusion chamber, the preparation was bathed in various experimental solutions by means of an inlet and outlet pipettes, the latter being operated by a stream pump.

The photoelectrical system for recording melanophore responses was fundamentally the same as that described by Iga (1975 a). Minor modifications included the use of two sheets of a blue (Olympus, C) and a yellow (Y–48) filter, which were placed under the condenser lens. The yellow filter could eliminate the activity of xanthophores found in the dermis of the preparation. The response of a single melanophore was measured. For this purpose, a circular diaphragm was put on the level of the real image inside the eyepiece, the area of the scale through which light was transmitted was restricted to about 130 μm in diameter. The changes in photoelectric current were recorded on a paper chart recorder (Yokogawa, type 3049), where the upward deflection of the trace was set to indicate the increase in transmittance, that is, the melanosome aggregation, while the downward deflection represented melanosome dispersal. The magnitude of the melanophore response was ex-

pressed by the percent aggregation: The recording at maximal aggregation was taken as 100 and that at full dispersion as zero.

Isotonic (133 mM) KCl solution containing 5.0 mM Tris-HCl buffer (pH 7.2) was used as K-solution. In some experiments, mixtures of the K-solution and NaCl (133 mM, containing 5.0 mM Tris-HCl buffer) solution in various volume ratios were also used, where the concentration of K ions was expressed as volume parts of KCl solution in the total 10 volumes of the mixture. For instance, 2K represented a mixture composed of 2 volumes of the KCl solution and 8 volumes of the NaCl solution. The following drugs were used: adrenaline hydrochloride (Adrenaline-injection fluid, Sankyo, Tokyo), propranolol hydrochloride (Sigma Chemical, St. Louis), dichloroisoproterenol hydrochloride (Aldrich Chemical, Milwaukee). Adrenaline solutions in various concentrations were prepared by diluting the adrenaline-injection fluid with the physiological saline immediately before use, except for the experiment 2, where the injection fluid was diluted with the KCl solution or the Na-K mixture. The other drugs were made up with the physiological saline.

The experiments were carried out at room temperature (18–25°C).

Results and Discussion

1. *K-induced melanosome dispersion*

Denervated melanophores in an isolated scale maintain their pigment dispersal in the physiological saline. Even when the perfusion solution was changed from the physiological saline to isotonic KCl solution, their dispersed state remained unchanged, because the nervous elements on which K ions act had completely lost their function. However, the melanophores themselves gain rather high sensitivity to catecholamines. The perfusion of a preparation with the physiological saline was followed by that with 10^{-6} M adrenaline-contained saline. The 10^{-6} M adrenaline was sufficient to induce full aggregation of the melanosomes in denervated melanophores. After being returned to the physiological saline, the melanophores maintained their state of aggregation for fairly long time. If KCl solution was applied to the melanophores then, the melanosomes within the melanophores responded with rapid dispersion and their dispersal was maintained throughout the period of the KCl application. On subsequent replacement of the KCl solution with the physiological saline, the melanosomes became aggregated again. Isotonic NaCl solution could not induce such a dispersion of the melanosomes. A typical recording on this experiment was shown in Fig. 1. These results were fundamentally the same as those in a previous paper (Iga, 1976 a).

2. *Inhibitory effect of K ions on adrenaline-induced pigment aggregation in melanophores*

There was a marked decrease in the magnitude of melanosome aggregation to adrenaline diluted with isotonic KCl solution (adrenaline-K solution) as compared with that to the adrenaline-physiological saline solution containing the same

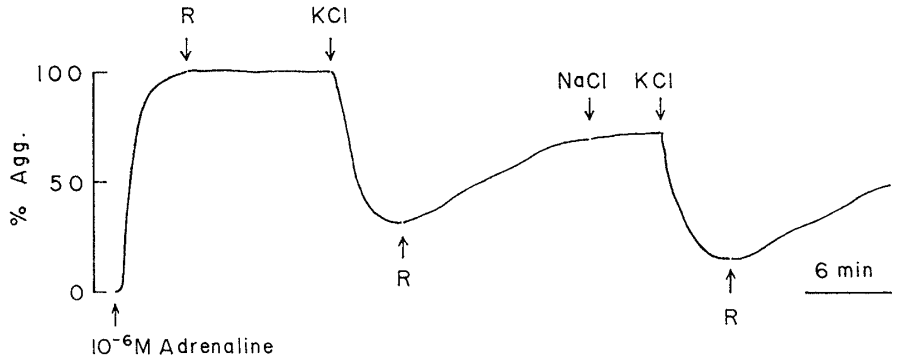

Fig. 1. Typical recording showing the melanosome-dispersing effect of K ions (133 mM) on denervated melanophores in a state of melanosome aggregation. NaCl, 133 mM NaCl. R, Ringer (physiological saline) solution. Ordinate, magnitude of response as a percentage of the maximal level of aggregation.

concentration of adrenaline. Furthermore, the magnitude of melanosome dispersion produced by K ions depended on the concentrations of K ions in the Na-K mixtures: Their concentration to arouse a discernible melanosome dispersion lay between 0.4 and 0.6. With an increase in K-concentration, the magnitude of the response increased and at 3.0 K, the maximal level as well as the maximal rate of response was attained (Iga, 1976 a).

Figure 2 illustrated the strength-response relations for adrenaline stimulation in the presence of various concentrations of K ions on the denervated melanophores. In a control experiment, where adrenaline was diluted with the physiological saline, the minimal concentration of adrenaline to arouse a barely visible melanosome aggregation was about 10^{-9} M, and at 5×10^{-7} M, the full aggregation was attained. The strength-response curve showed a sigmoid pattern, the same as that obtained previously (Iga, 1968). In the experiment where adrenaline was diluted with 1 K solution, the threshold concentration of adrenaline was increased to be about 10^{-8} M. At 10^{-6} M, almost full aggregation response was obtained. As a consequence, the strength-response curve was shifted to the right from the curve of the control experiment, and the slope of the curve slightly increased. With an increase of K ions in the dilution media of adrenaline, the curves for adrenaline were shifted downwards. However, between 5 K solution and 10 K (isotonic KCl) solution no significant difference was found in the strength-response relations for adrenaline stimulation.

Fujii and Taguchi (1969) reported recently that even in K-Ringer, where all Na ions were replaced with K ions, adrenaline could induce melanosome aggregation within denervated melanophores of the goby, *Chasmichthys gulosus*. However, they did not point out the inhibitory effect of K ions on adrenaline-induced aggregation. As shown in Fig. 2, adrenaline-induced aggregation response was significantly inhibited in high K-solution. Thus, the K-induced melanosome dispersion may be explained as inhibition of the pigment-aggregating action of adrenaline by K ions.

Recent studies on the membrane potential of *Fundulus* melanophores indicated

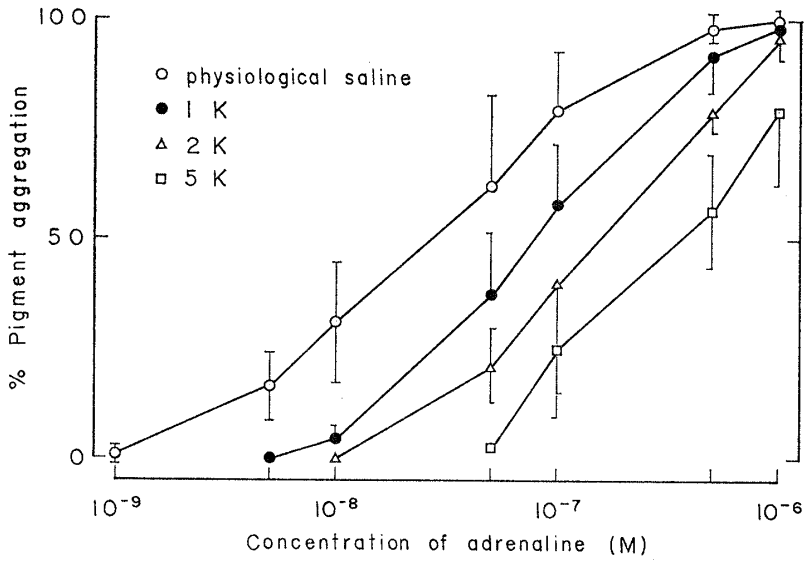

Fig. 2. Strength-response relationships for adrenaline stimulation in the presence of various concentrations of K ions on denervated melanophores. The solution with which adrenaline was diluted in various concentrations was a mixture of isotonic KCl solution and isotonic NaCl solution. The concentration of K ions in the mixture was expressed as volume parts of the KCl solution in the total 10 volumes of the mixture; 1 K solution indicating the mixture of the KCl solution and the NaCl solution in volume ratio of 1: 9. Test solutions were applied for 10 min. Each point is the mean of 10 measurements on different scales. Vertical lines indicate standard deviations of the means.

that the value was very low in K-rich medium, and that no significant differences were found between the means in K-rich solutions containing some pigment-motor substances and that in control K-rich saline (Fujii and Novales, 1969). K-solution containing adrenaline, depending on its concentrations, could produce the migration of melanosomes within denervated melanophores in two opposite directions, i. e., the aggregation and the dispersion (Iga, 1976 a). The present results also supported the previous findings. It seems to be difficult to explain these observations from the standpoint of the electrophoretic theory of Kinosita (1953, 1963) that the melanosomal movements are directly dependent on the changes in the membrane potential.

3. *Effects of beta adrenergic blocking agents*

Recent pharmacological studies have indicated that the melanosome aggregation in fish melanophores by catecholamines is induced through an interaction with alpha adrenoceptors on the cells (Iga, 1968; Grove, 1969; Reed and Finnin, 1972; Fernando and Grove, 1974 a, b; Fujii and Miyashita, 1975). Meanwhile, Reed and Finnin (1972), in the angel fish (*Pterophyllum eimekei*), and Miyashita and Fujii (1975), in the guppy (*Lebistes reticulatus*), suggested a possible involvement of beta adrenoceptors in melanosome dispersion in the melanophores.

Considering these recent studies, in order to determine if the melanosome dispersion by K ions is brought about through interaction with beta adrenoceptors, the

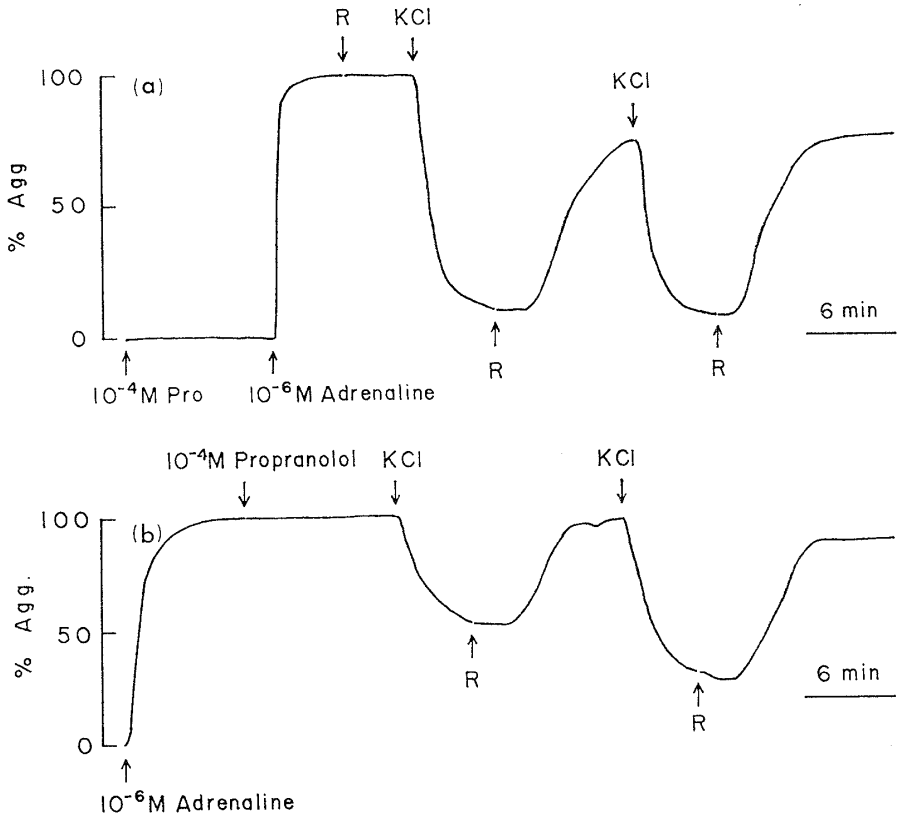

Fig. 3. Typical recording showing the effect of propranolol (Pro) on the K-induced melanosome dispersion response of denervated melanophores.

effect of specific antagonists was examined, though, up to now, the existence of beta adrenoceptors on the melanophores of this species remains unexamined. As shown in Figs. 3 a and 3 b, treatment with propranolol (10^{-4} M), a beta adrenergic blocking agent, for 10–15 min did not affect the dispersion response produced by K ions; the melanosomes which had been aggregated under the influence of adrenaline showed rapid dispersion by replacement of physiological saline with KCl solution. More prolonged application of the blocker was also ineffective to block the K-induced response. Dichloroisoproterenol could not block the K-induced dispersion, either.

The pigment dispersion in *Oryzias* leucophores induced by catecholamines was mediated through beta adrenoceptors, since the response was antagonized by pretreatment with beta adrenergic blocking agents (Iga, 1975 c). In this experiment also the pigment-dispersing response of the leucophores to adrenaline was completely blocked with the pretreatment of these blockers during the period of the experiment. These may imply that these blockers are sufficient to block the beta adrenergic mechanism at their concentrations and for the treatment time, if the mechanism is involved in the melanophores of this species, though the difference in the pharmacological nature of beta adrenoceptors in different cell types should be considered. Thus,

the results obtained suggest that the melanosome dispersion produced by K ions is not mediated through beta adenoceptors, being independent of adrenergic mechanisms.

4. *Effects of Ca ions*

On denervated melanophores keeping a state of pigment aggregation, the influence of Ca ions was examined. On application of isotonic $CaCl_2$ solution, the melanophores showed slight dispersion of their pigment following a quiescent period for 2 or 3 min. After returning to the physiological saline, the melanophores retained their affected state during the subsequent perfusion with the physiological saline. Addition of Ca ions to the KCl solution did not affect the K-induced dispersion response (Fig. 4).

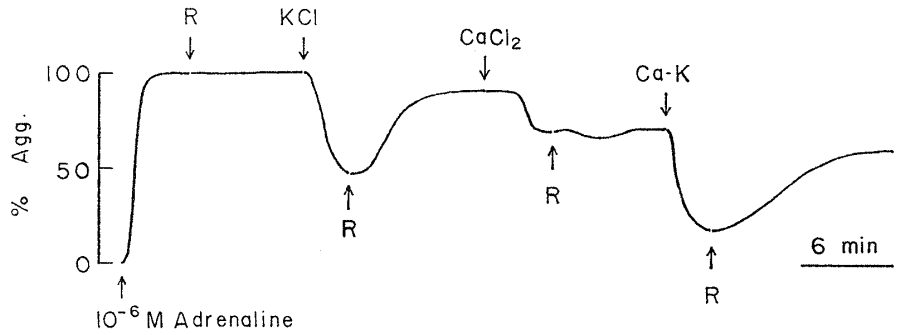

Fig. 4. Typical recording showing the effect of Ca ions on denervated melanophores in a state of melanosome aggregation. $CaCl_2$, isotonic (90.9 mM) $CaCl_2$, Ca-K, a mixture of the $CaCl_2$ solution and the KCl solution in volume ratio of 2 : 8.

At the present time, little information is available about the pigment dispersing action of K ions on the fish melanophores. To understand the action mechanism of K ions further intensive studies are needed at the cellular as well as the subcellular levels.

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