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Melanosome Aggregating Action of Dopamine on Fish Melanophores

Tetsuro IGA

Department of Biology, Faculty of Science, Shimane University, Matsue 690, Japan

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Abstract

The mechanism of melanosome-aggregating action of dopamine on fish melanophores was investigated, using isolated scales of *Oryzias latipes*. Dibenamine blocked the melanosome aggregation response of the melanophores to norepinephrine and to dopamine. Haloperidol inhibited the response to dopamine in both the innervated and denervated melanophores. The inhibition was pronounced in the denervated melanophores. Haloperidol accelerated the melanosome aggregating action of norepinephrine in the innervated melanophores, whereas in the denervated ones the drug inhibited the norepinephrine action. From these results, the following conclusions were suggested: The melanosome aggregation response induced by dopamine is not mediated through dopamine-specific receptors, but through alpha adrenergic receptors on the melanophores. Dopamine may act on the presynaptic endings to induce the release of the transmitter. Haloperidol also interacts with alpha adrenergic receptors of the melanophores.

Introduction

Melanophores in many teleost fishes are mainly under the control of the autonomic nervous system. The peripheral nerve fibers acting to aggregate melanosomes are adrenergic and the transmitter concerned may be norepinephrine (Fujii, 1961; Scheline, 1963; Scott, 1965; Fujii and Novales, 1972; Reed and Finnin, 1972). Recent pharmacological research has shown that adrenergic receptors mediating melanosome aggregation in teleost melanophores are of the alpha type (Iga, 1968; Fujii, 1969; Grove, 1969; Reed and Finnin, 1972; Fernando and Grove, 1974a, b; Fujii and Miyashita, 1975).

Catecholamines, such as norepinephrine, epinephrine, dopamine and isoproterenol, induce melanosome aggregation in the innervated and denervated melanophores of *Oryzias latipes*. Denervation causes supersensitivity of the melanophores to these amines. On the basis of their action mode to the melanophores, these catecholamines were classified as amines with a direct action (Iga, 1968). When comparing concentration-response curves for these amines, however, in the innervated melanophores only the curve of dopamine showed a steep slope, whereas in the denervated ones the curves for these amines showed almost the same slope. These results suggested that

dopamine might act on presynaptic elements to induce release of the transmitter in addition to its direct action on the melanophores (Iga, 1968).

In order to obtain more precise information about the mechanism of action of dopamine on melanosome aggregation in fish melanophores, the effect of haloperidol, which is considered a specific antagonist of dopamine (Rossum, 1966; Yeh *et al.* 1969; Goldberg and Yeh, 1971), on the action of dopamine on fish melanophores was investigated.

Materials and Methods

A freshwater teleost, *Oryzias latipes*, was used as experimental material. A scale isolated from the anterior dorsal region of the fish was attached, epidermis side down, to the under surface of a cover glass mounted on a perfusion chamber filled with a physiological salt solution with the following composition: 128 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$, 5 mM Tris-HCl buffer (pH 7.2).

The motile response of a single melanophore in the scale was measured photoelectrically as changes in the transmitted light due to melanophore responses (Iga, 1975a, 1977). Both innervated and denervated melanophores were employed. The denervated preparations were obtained by the same method as described previously (Iga, 1968, 1975a, 1978).

The drugs used were norepinephrine hydrochloride (Sigma Chemical, St. Louis), dopamine hydrochloride (Nakarai Chemicals, Kyoto), dibenamine hydrochloride (Kanto Chemicals, Tokyo) and haloperidol (G. D. Searle & Co, Chicago).

The drugs except haloperidol were directly dissolved in the physiological solution. Haloperidol dissolved in a drop of acetic acid was diluted with the physiological solution at 10^{-4} M. The pH of the solution was adjusted neutrally with 1 N NaOH.

The experiments were performed at a room temperature between 19–24°C.

Results and Discussion

Dibenamine, an alpha adrenergic receptor antagonist, blocked the melanosome aggregation response to norepinephrine or to dopamine. Dibenamine also blocked the response of melanophores to KCl (133 mM) (Fig. 1). The response is produced through the action of transmitter norepinephrine released from the nerve terminals which were stimulated by K ion, thus being the adrenergic one.

These results indicate that the response induced by dopamine is not mediated through dopamine-specific receptors, but through alpha adrenergic receptors of the melanophores. This conclusion, however, does not contradict any possibility that dopamine might act on a presynaptic mechanism. Quite recently, Miyashita and Fujii (1977) and Finnin *et al* (1979) have reported that receptors for dopamine are involved in the control of fish melanophores.

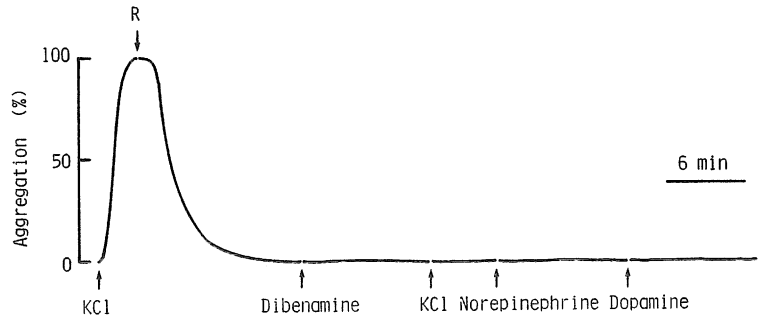

Fig. 1. Effect of dibenamine (10^{-4} M) on melanosome aggregation response to KCl (133 mM), norepinephrine (10^{-5} M) and dopamine (10^{-5} M) in the innervated melanophores of *Oryzias latipes*.

In both the innervated and denervated melanophores, the melanosome aggregation response to dopamine in various concentrations was examined in the absence and presence of 10^{-4} M haloperidol. The treatment time was fixed for 10 minutes, which was enough to cause a maximal response in a given solution. In haloperidol experiments the melanophores were pretreated with 10^{-4} M haloperidol for 10 minutes. Haloperidol itself, in concentrations used, did not induce any pigment aggregation in the melanophores. The maximal aggregation in the melanophores was attained by the KCl solution in the innervated melanophores and by 10^{-6} M norepinephrine in the denervated ones.

Figure 2 shows concentration-response curves for dopamine in the absence (control) and presence of haloperidol, where the percent response of melanophores is plotted to the log molar concentrations of dopamine. As is indicated in this figure, the minimal concentration to arouse a discernible melanosome aggregation was 10^{-6} M and 10^{-7} M in the innervated and denervated melanophores, respectively. The maximal aggregation of melanosomes was obtained at 10^{-5} M in both the innervated and denervated melanophores. Thus, the curve in the innervated melanophores showed a steep slope. The increase ratio in sensitivity following denervation was 3.1 at the concentration required to produce 50% of the maximal aggregation (EC_{50}). These results were almost the same as those obtained previously (Iga, 1968). As can be seen by the triangular symbols in this figure, haloperidol significantly inhibited the melanosome aggregating action of dopamine in both the innervated and denervated melanophores. The curve in the innervated melanophores in the presence of haloperidol was parallel to the control curve in the denervated melanophores. The inhibition by haloperidol was more pronounced in the denervated melanophores than in the innervated ones. In the presence of haloperidol, the curve in the innervated melanophores sat rather leftside than that in the denervated ones (at 5×10^{-6} M and 10^{-5} M, $0.025 > P > 0.01$). These results can be explained as in the following:

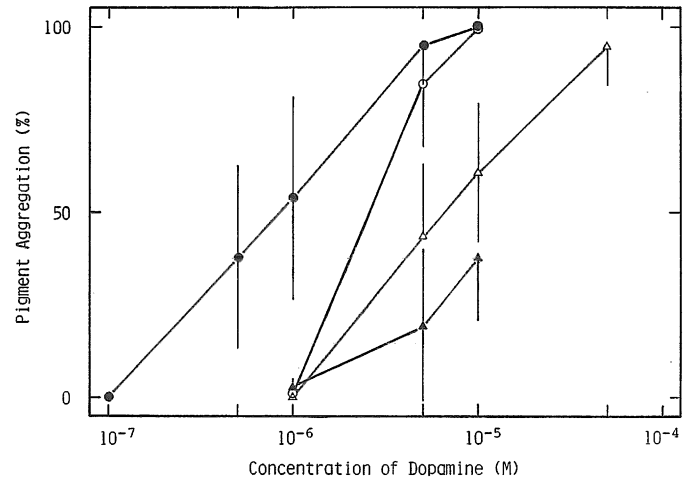

Fig. 2. Inhibitory effect of haloperidol (10^{-4} M) on melanosome aggregating action of dopamine in various concentrations in the innervated (empty symbol) and denervated (solid symbol) melanophores. circle, in the absence of haloperidol (control). triangle, in the presence of haloperidol. Vertical bars indicate standard deviations.

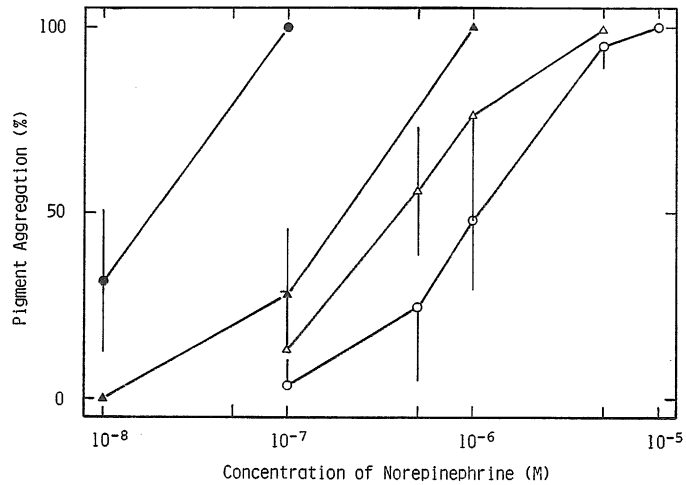

Fig. 3. Effect of haloperidol (10^{-4} M) on melanosome aggregating action of norepinephrine in various concentrations in the innervated (empty symbol) and denervated (solid symbol) melanophores. circle, in the absence of haloperidol (control). triangle, in the presence of haloperidol. Vertical bars indicate standard deviations.

Dopamine acts on the presynaptic elements to induce the release of the transmitter and haloperidol interfers with the presynaptic events.

By the same procedures as described in the dopamine experiments, the effects of haloperidol on norepinephrine action were examined in the innervated and denervated melanophores. These results are presented in Fig. 3 as concentration-response curves of norepinephrine in the absence and presence of 10^{-4} M haloperidol.

In the innervated melanophores, haloperidol rather accelerated the melanosome aggregating action of norepinephrine (at 5×10^{-7} M, $0.01 > P > 0.005$; 10^{-6} M, $0.5 > P > 0.25$). In the denervated melanophores, however, the drug inhibited the norepinephrine action, showing that haloperidol interacts with alpha adrenergic receptors. The antagonistic action of haloperidol on adrenergic receptors also has been shown in certain peripheral systems (Simon and Maanen, 1976; Finnin *et al.* 1979). Although, at present, we have no evidence to explain the accelerated effect of norepinephrine by haloperidol in the innervated melanophores, it appears most likely that uptake into the nerve terminals of norepinephrine is impaired by haloperidol and the concentration at the receptors increases, *i.e.* an enhanced effect ensures.

The inhibitory effect of haloperidol at various concentrations on melanosome aggregation induced by dopamine and norepinephrine was examined. In order to eliminate some possible participation of nervous elements, the denervated melanophores were used in this experiment. 10^{-5} M dopamine and 10^{-7} M norepinephrine were used, at concentrations which the melanophores showed almost full melanosome aggregation as shown in Figs. 2 and 3. Following a 10-minute pretreatment with haloperidol at various concentrations, the resultant degree of melanosome aggregation to the amine solution was measured during a 10-minute application in the presence of

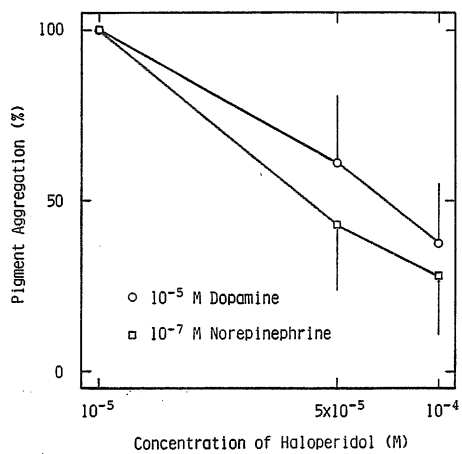

Fig. 4. Inhibitory effect of haloperidol in various concentrations on the melanosome aggregation response to dopamine and norepinephrine in the denervated melanophores of *Oryzias latipes*. Vertical bars indicate standard deviations.

haloperidol in the same concentration used as the pretreatment.

These results illustrated in Fig. 4 show the degree of melanosome aggregation occasioned by the two amines in the presence of haloperidol at various concentrations. 10^{-5} M haloperidol did not change the degree of the aggregation response to these amines. In higher concentrations (5×10^{-5} M, 10^{-4} M), haloperidol inhibited the response to dopamine and norepinephrine. It was observed that the inhibitory effect of haloperidol was somewhat marked with norepinephrine (5×10^{-5} M, $0.05>P>0.02$; 10^{-4} M, $0.4>P>0.3$). However, a precise comparison between the two amines is difficult, because the concentrations of these amines used are not always equivalent in their melanosome aggregation potency.

Acknowledgments

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