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Regulation of Motile Iridophores of the Floating Goby, *Chaenogobius* sp. 2

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Iridophores in the dermis of the floating goby, *Chaenogobius* sp. 2, are motile. Their movements involved centrifugal or centripetal migration of the light-reflecting platelets within the cells. High K^+ solution induced dispersion of the platelets in the cells. This response was lost in iridophores in denervated scale preparations. These results suggested that K^+ acted on the nerve controlling the iridophores to release the transmitter. Norepinephrine, which was assumed to be the transmitter in the nerve, caused the platelet dispersion. The action was inhibited by an alpha adrenergic antagonist, phenoxybenzamine, but not by a beta adrenergic one, propranolol. This indicated that norepinephrine acted on the alpha adrenoceptors on the cell membrane to induce the platelet dispersion. Forskolin, a unique activator of adenylate cyclase, was effective in inducing aggregation of the platelets. Melatonin enhanced aggregation of the platelets. Alpha MSH had no observable effect on the iridophores. Based on these results, it was concluded that *Chaenogobius* iridophores are under the dual control of the sympathetic adrenergic nerve and melatonin, the hormone from the pineal gland and that an increase in the intracellular cAMP induces the platelet aggregation within the cells.

Introduction

Iridophores are light-reflecting chromatophores and more frequently found in the skin of many poikilothermal vertebrates. They are responsible for the metallic sheen or whitish tone of the integument. Studies with electron microscope have revealed that iridophores in fishes contain a large number of light-reflecting platelets, which are usually arranged in highly oriented stacks (Kawaguti and Kamishima, 1966; Harris and Hunt, 1973; Hawkes, 1974).

Iridophores have been thought to be physiologically inactive, i.e., being without motility. Recently, however, iridophores with motility have been reported to exist in some fish species. The materials were the neon tetra, *Paracheirodon innesi* (Lythgoe and Shand, 1982), the blue damselfish, *Chrysiptera cyanea* (Oshima *et al.*, 1985), the blue-green damselfish, *Chromis viridis* (Fujii *et al.*, 1989) and a freshwater goby, *Odontobutis obscura* (Iga and Matsuno, 1986). In the former three species, the iridophores were of the round type, and contained the piles of thin light-reflecting platelets. Simultaneous changes in the distance between adjacent reflecting platelets result in the changes in the skin color of these fishes (Kasukawa *et al.*, 1987; Fujii *et al.*,

1989; Nagaishi *et al.*, 1989).

On the other hand, *Odontobutis* iridophores were of some dendritic and of motile iridophores of a different type, in which light-reflecting platelets aggregated toward the center of the cell and dispersed throughout the cytoplasm (Iga and Matsuno, 1986; Matsuno and Iga, 1989). The rate of migration of the platelets, in both dispersion and aggregation, in the cells was extraordinarily slow and the regulation of movements appeared to be peculiar. Thus, motile iridophores seems to provide an interesting subject on comparative studies of chromatophores.

Recently, we found that iridophores of the floating goby, *Chaenogobius* sp. 2 were motile. The present study was to examine the motility and the regulation mechanism of the iridophores.

Materials and Methods

Preparations

The floating goby, *Chaenogobius* sp. 2, of body length of 8–10 cm, was used.

Scales isolated from the dorso-lateral region of the trunk of the fish were used. In some experiments, scales from chemically sympathectomized fish were used as denervated ones. The denervation was made by intraperitoneal injection of 6-hydroxydopamine, as described elsewhere (Iga and Takabatake, 1982).

An isolated scale, for physiological and pharmacological studies, was fixed epidermal side down, under a coverslip, which was mounted on a glass trough filled with physiological saline of the following composition (mM): NaCl 128, KCl 2.6, $CaCl_2$ 1.8, Tris-HCl buffer 5.0 (pH 7.2). The trough was set on the stage of an epi-illuminated microscope.

Recording of iridophore responses

Degrees of responses of the iridophores were photographed at a given interval of time. From these photographs, responses of a single iridophore were shown as the change in the area covered with platelets measured by a planimeter (Iga *et al.*, 1987).

Drugs used

The following drugs were used: alpha-MSH, forskolin, 6-hydroxydopamine hydrobromide, melatonin, norepinephrine hydrochloride, propranolol hydrochloride, which were obtained from Sigma Chemical, St. Louis, and phenoxybenzamine hydrochloride (Tokyo Chemical Ind., Tokyo). These drugs were diluted appropriately with physiological saline immediately before use.

A high K^+ solution, which was a mixture of the saline and isotonic KCl solution in equal volumes, was used as an ionic stimulation.

All experiments were performed at room temperature which varied between 20–26°C.

Results

Motile iridophores

Microscopic examination revealed three kinds of chromatophores in the skin of the floating goby, i.e., melanophores, xanthophores and iridophores. These chromatophores were present in the dermis. The melanophores, with many slender branches, were located in the uppermost part of the dermis and the xanthophores, if existed, appeared to be distributed in a little lower part. The iridophores were generally found in small groups lower than the part, where the melanophores were located.

In scales isolated from the dark-adapted fish, the iridophores appeared in punctate in shape, with the platelets aggregated in the perikarya. While, the cells in the scales from the white-adapted one assumed in somewhat dendritic, with their platelets dispersed throughout the cytoplasm.

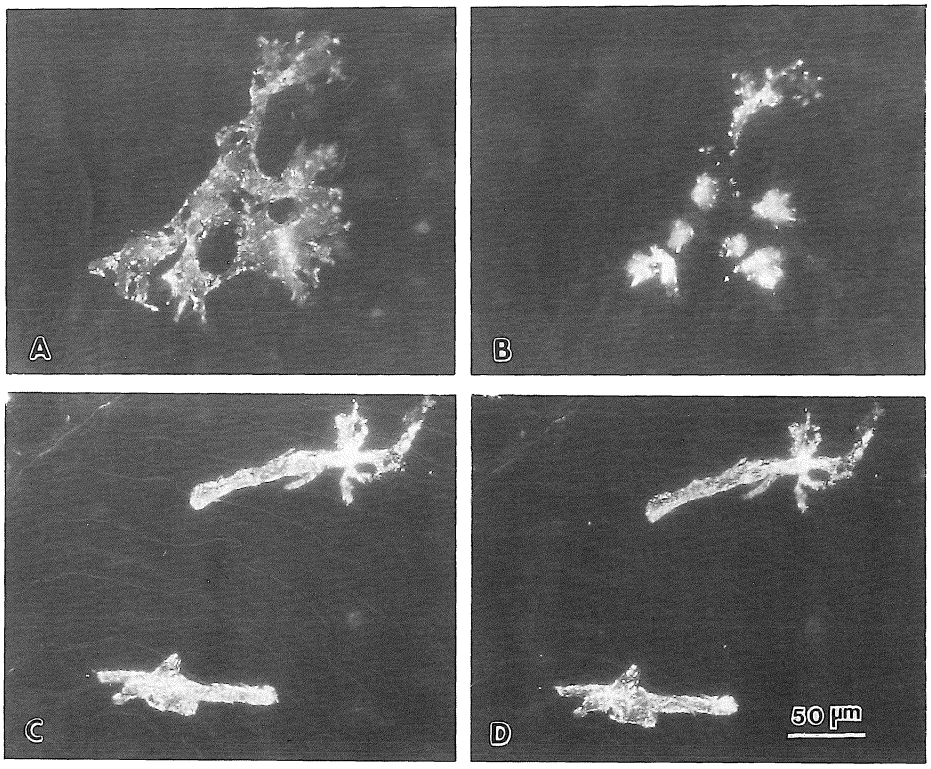


Fig. 1. Photomicrographs showing two types of iridophores (upper row, motile type; lower row, non-motile type) of the floating goby, *Chaenogobius* sp. 2, taken with dark-field epi-illuminated optics. A and C; in the saline. B and D: 60 min after application of 10^{-6} M melatonin.

Now, if the iridophores in the platelet dispersion were immersed in an appropriate concentration of melatonin solution, they slowly become punctate with the platelet aggregation (Fig. 1A and B). The response was reversible. Thus, the iridophores of the floating goby are motile. There seemed to be some iridophores which failed to respond (Fig. 1C and D).

The iridophores in a punctate state appeared bluish-white in color, while the same cells appeared yellowish with a greenish tint when the platelets were dispersed.

Responses of the iridophores to K^+

Iridophores in a punctate state maintained its state in the saline. When immersed in a high K^+ solution, the iridophores responded with the platelet dispersion. The rate of dispersion was quite slow and more than 30 min were required for maximal dispersion. Upon cessation of the stimulation, the iridophores kept the state of dispersion at least for 1 hr (Fig. 2, solid circle).

For examining a possible site of action of K^+, scales isolated from 6-OH dopaminized fish were used for experiments. Iridophores in the scales did not respond to the high K^+ solution, continuing their punctate state. The iridophores, however, responded to norepinephrine with the platelet dispersion (Fig. 2, empty circle), as will be mentioned in the next experiment.

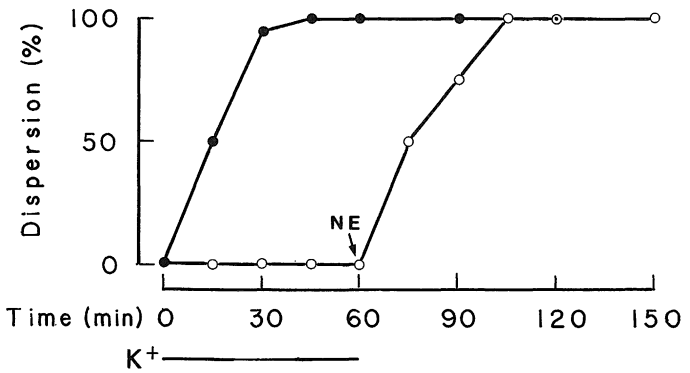
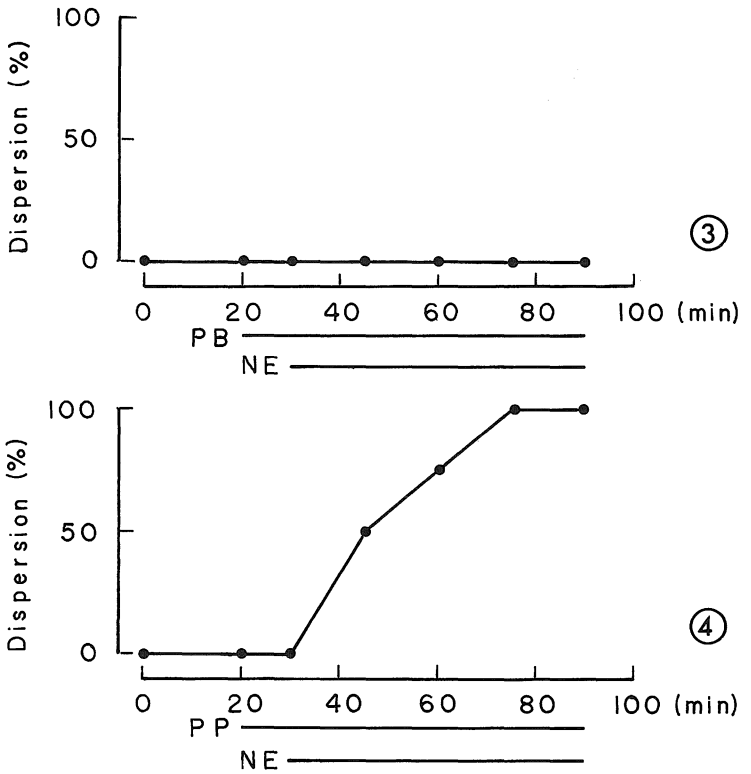


Fig. 2. Responses of iridophores of the floating goby to a high K^+ solution (the saline : isotonic KCl = 1 : 1 vol). ●, innervated iridophores. ○, denervated iridophores. The scale was treated with 10^{-6} M norepinephrine (NE) following the K^+ solution.

Responses of the iridophores to norepinephrine

Norepinephrine (10^{-6} M) induced a dispersion of the platelets in the iridophores. The process of the response was almost the same as that of the response to K^+. The dispersion response was blocked by an alpha adrenergic antagonist, phenoxybenzamine, but not by a beta adrenergic one, propranolol (Figs. 3 and 4).



Figs. 3 and 4. Effects of adrenergic blockers, phenoxybenzamine (PB, 10^{-5} M) and propranolol (PP, 10^{-5} M) on the platelet-dispersing action of norepinephrine (NE, 10^{-6} M).

Effect of forskolin

Forskolin, an activator of adenylate cyclase, was used for examining the involvement of adenylate cyclase system and cAMP as a second messenger in the iridophore response of the floating goby.

Forskolin acted effectively to induce aggregation of the platelets on the iridophores in the dispersed state (Fig. 5). The drug did not evoke the platelet dispersion.

Responses of the iridophores to melatonin

Melatonin enhanced the aggregation of the platelets. The rate of the aggregation was also slow and 45 to 60 min were required for maximal aggregation (Fig. 6). These iridophores maintained the punctate state in the saline. Upon application of 10^{-6} M norepinephrine, the platelets dispersed gradually again.

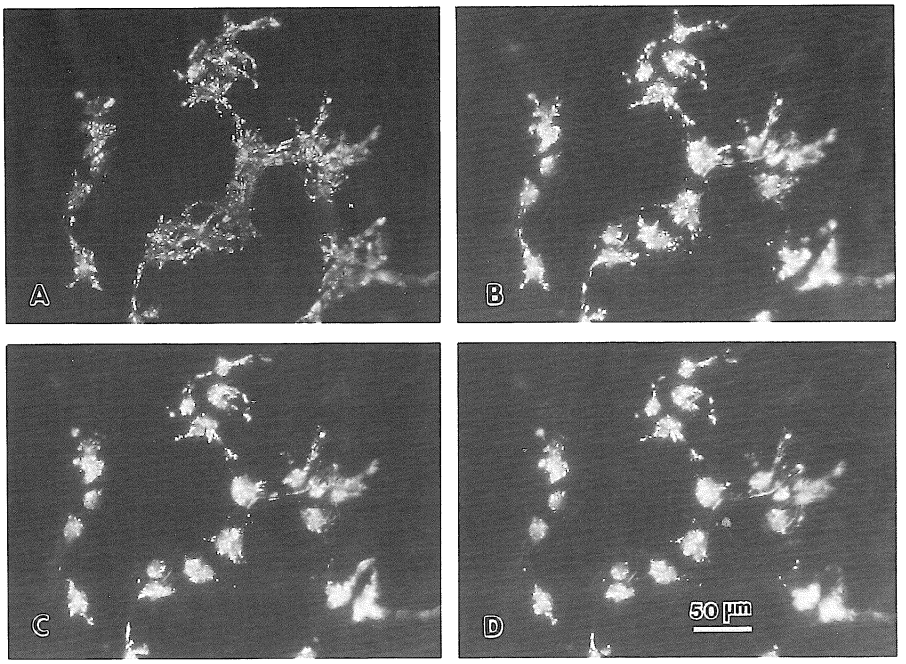


Fig. 5. Serial photomicrographs of iridophores of the floating goby, showing responses of the iridophores to 5×10^{-7} M forskolin. A: in the saline. B, C and D: 20, 40 and 60 min after application of forskolin.

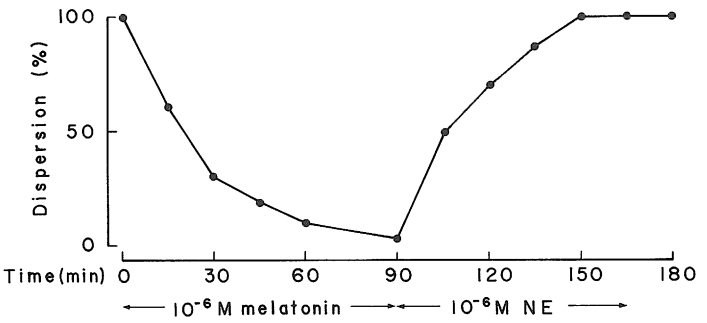


Fig. 6. Responses of iridophores of the floating goby to melatonin and norepinephrine (NE).

Responses of the iridophores to alpha MSH

MSH (10^{-7} M) was applied to the iridophores in a punctate or an expanded state. The drug did not induce any observable response in the iridophores in either state.

Discussion

In the floating goby, *Chaenogobius* sp. 2, with which the present experiment has been performed, the iridophores in the dermis were motile, the motility involved aggregation and dispersion of the platelets within the cells. The pattern of the movement was quite similar to that of *Odontobutis* iridophores. In the present material, some iridophores failed to respond to various stimuli (Fig. 1C and D). No differences in morphological features between motile and non-motile iridophores were observed. Fujii *et al.* (1989) have reported that, in the blue-green damselfish, *Chromis viridis,* two types of motile and non-motile iridophores, which were similar in morphological features, were found in the dermis.

The platelet-dispersion in response to K^+ was lost in iridophores in the denervated scale preparations. These results suggested that K^+ acted on the nervous elements to release the neuro-transmitter inducing the platelet dispersion within the cells. The transmitter is supposed to be norepinephrine. This indicates that the iridophores of the floating goby are under the nervous control, as those of *Odontobutis* goby (Iga *et al.,* 1987).

Norepinephrine induced dispersion of the platelets. The response was effectively blocked by an alpha adrenergic antagonist, but not by a beta adrenergic one, indicating that adrenoceptors mediating platelet dispersion in the iridophores of the floating goby are of an alpha type in nature. The present results showed that the adrenoceptor mechanism was the same as that in *Odontobutis* iridophores. While, leucophores, another sort of the light-reflecting chromatophores, of *Oryzias latipes* also responded to norepinephrine with dispersion of the leucosomes. The response was mediated through beta adrenoceptors on the cell membrane (Iga *et al.,* 1977), and the stimulation of alpha adrenoceptors caused leucosome aggregation (Iga, 1979).

Forskolin has been found to be a potent and unique activator of adenylate cyclase and to increase intracellular cAMP levels in a variety of eukaryotic cells (Seamon and Daly, 1981; Sano *et al.,* 1983). Forskolin, thus, has provided an invaluable tool for the investigation of the role of cAMP in physiological responses to hormones and transmitters. In chromatophores in fishes, forskolin effectively blocked the noradrenaline-induced pigment aggregation in melanophores (Andersson *et al.,* 1984; Karlsson *et al.,* 1987) and erythrophores (Karlsson *et al.,* 1988), and, in leucophores of *Oryzias latipes,* forskolin elicited dispersion of the leucosomes (Namoto and Yamada, 1987). Increase in intracellular concentration of cAMP has been shown to cause dispersion of pigment within melanophores of the frog (Abe *et al.,* 1969) and the fish (Fujii and Miyashita, 1976; Negishi *et al.,* 1982). Thus, it has been generally considered that increases in levels of intracellular cAMP result in dispersion of pigment organelles in chromatophores.

In the iridophores of the floating goby, on the other hand, forskolin acted on

aggregation of the platelets. The same effect of forskolin has been obtained in iridophores of *Odontobutis obscura* (Maeno and Iga in preparation). These results suggested that, in the motile iridophores of the gobiid species, increases in levels of intracellular cAMP elicit "aggregation" of the platelets. This is an important characteristic found in the motile iridophores.

In the present material, melatonin induced aggregation of the platelets, while, in the iridophores of *Odontobutis* goby, the drug acted to disperse their platelets (Iga and Matsuno, 1986; Iga *et al.*, 1987). These results suggest that two types of melatonin receptors may exist: one which mediates the platelet dispersion and the other which is responsible for eliciting its aggregation. From studies on the day and night coloration of the pencil fish, *Nannostomus beckfordi anomalus,* Reed (1968) has pointed out that two types of melanophores are found in the pencil fish; melanophores that expand to melatonin and ones that contract to melatonin.

Alpha MSH acted on *Odontobutis* iridophores to accelerate aggregation of the platelets within the cells. While, in the present material, this hormone did not appear to affect the iridophore responses.

We were able to find some important differences on the receptor mechanisms between iridophores in two species of gobiid fish. These suggest a variety of regulation of movements in motile iridophores and therefore it is interesting to develop comparative researches on motile iridophores in various species of fish.

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