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The Nature of the Response of Scale Melanophores of the Teleost Fish, *Pseudorasbora parva*, to Light

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ABSTRACT

The primary color response of innervated and denervated scale melanophores of the adult motsugo, *Pseudorasbora parva*, was investigated. The intensity of the light used for illumination was about 2,000 ~ 10,000 lux. Innervated melanophores remained in a state of melanosome dispersion in physiological saline and in a state of melanosome aggregation in M/7.5 KCl solution, regardless of exposure to light or dark conditions. Intermittent exposure to light and darkness did not induce further dispersion or aggregation of melanosomes in innervated melanophores in which the melanosomes were in states of incomplete aggregation (or dispersion) due to incubation in 1.5K ~ 2K solutions. Denervated melanophores did not aggregate in the M/7.5 KCl solution, but did when treated with physiological saline containing adrenaline. Light did not induce the aggregation of melanosomes in denervated melanophores dispersed fully in physiological saline. In addition, light did not accelerate or inhibit the melanosome dispersion process in melanophores in physiological saline after their melanosomes had been induced to aggregate by the action of adrenaline. It is concluded that scale melanophores of the motsugo are not sensitive to light.

Keywords fish, primary color response, melanophore, light response, scale

A sea urchin, a species of Echinodermata, is known to change its body color in response to light and dark conditions, in spite of having no eyes (Millott, 1950). Light acts directly on chromatophores in the test of the sea urchin, *Diadema setosum* (Yoshida, 1956). This is a direct effect of light on the chromatophores themselves, and is called "a primary color response" to light.

In contrast, a color change induced by the action of light on the eyes is called "a secondary color response" to light. In most animals having eyes, the eyes function as a photoreceptive organ. In fishes having eyes, the secondary color response is generally stronger than the primary color response. Therefore, the ability of chromatophores on the body surface to respond directly to light (viz., the primary color response) is often obscured. It is necessary to use isolated scales and/or cultured chromatophores from scales to clarify light sensitivity. The light sensitivity of chromatophores on isolated scales, fins and skin pieces has been investigated in the following cases: melanophores of *Fundulus heteroclitus* (Spaeth, 1913), the blind fish, *Anoptichthys jordani* (Burgers et al., 1963) and the dark chum, *Zacco temmincki* (Iga and Takabatake, 1983); leucophores of the medaka, *Oryzias latipes* (Ohta and Sugimoto, 1980); xanthophores of the medaka, *O. latipes* (Kawai, 1989; Oshima et al., 1998); erythrophores of the tilapia, *Oreochromis niloticus and O. mossambicus* (Oshima and Yokozeki, 1999) and iridophores of the neon tetra, *Paracheirodon innesi* (Clothier and Lythgoe, 1987; Nagaishi and Oshima, 1989). So far, there are fewer reports that chromatophores of fishes show no response to light.

Since there are few reports, it is possible that the results obtained were negative, viz., there was no response to light. However, it is necessary to use and to compare chromatophores both with and without the ability to show a light response in order to clarify the details of chromatophore responses to light. Therefore, abundant information on the nature of chromatophore responses to light should be offered in many species of

fishes, even if results are negative. The present report describes a study to determine whether or not scale melanophores of the motsugo fish have the ability to undergo a light response.

MATERIALS AND METHODS

Adult motsugos, *Pseudorasbora parva*, were purchased from a fish farmer of Amagun, Yatomi-cho and kept in aerated aquaria. Scales on the dorso-caudal areas were removed by employing forceps with sharp tips. The scales were attached, epidermis side down, to a coverslip mounted on a glass trough (width 19 mm, length 55 mm, height 10 mm) filled with a physiological saline (Yamamoto, 1941). Following immersion in this saline for 10 min after having verified full aggregation in M/7.5 KCl, responses of the melanophores to light were investigated with the melanophores immersed in the following solutions: physiological saline, 10K (M/7.5 KCl, pH adjusted to 7.2 by N/10 KHCO3), 1.5K (M/7.5 KCl: physiological saline=1.5 : 8.5), 2K solution (M/7.5 KCl: physiological saline=2 : 8) or adrenaline in physiological saline. A stock solution (10^{-4} M) of adrenaline (Bosmin inj., Daiichi-seiyaku Co., Tokyo) in physiological saline was stored at about 4℃ and then diluted with physiological saline just before use.

A microscope stage was covered with dark cloths to produce darkness. Scale melanophores were illuminated through a microscope condenser using an illumination apparatus (Halogen lamp, Techno light, KLS 2100, Kenko Co., Tokyo). The intensity of the illumination was about 2,000, 4,000 and 10,000 lux at the position of the scales in the trough. The intensity of the light was measured using a photodiode (Hamamatsu photonics K. K., 2386-18K, Hamamatsu) and a lux meter (Tokyo photo-electric Co., ANA-313, Tokyo). The light intensity in the expeimental room was below about 10 lux. The temperature during the experiments was 24 - 28℃.

Denervated melanophores were obtained as described in a previous paper (Ohta, 1972). Scales were removed alternately from the dorso-caudal area with forceps, and then immediately returned to their original positions. The fish were kept for greater than 24 hr at about 25℃ in a black container. Immediately before the experiments, the scales were removed again and it was ascertained that the melanophores on the scales did not respond by melanosome aggregation to M/7.5 KCl.

The melanophore response was expressed as a percentage of the length of a branch at full extension by measuring the length of a branch with a micrometer set in the ocular lens of a microscope.

RESULTS

Innervated melanophores in a state of melanosome dispersion in physiological saline

Melanophores with fully dispersed melanosomes in physiological saline were continuously illuminated for 15 min at two light intensities (2,000 and 4,000 lux). In the control, melanophores were kept in darkness for the same period with momentary illumination only to allow observations. The numbers of samples observed were 10 at 2,000 lux, 14 at 4,000 lux and 16 controls.

Under conditions of both illumination and darkness, the melanophores maintained almost full dispersion of melanosomes throughout the time of observation. Although some melanophores showed a fluctuation in

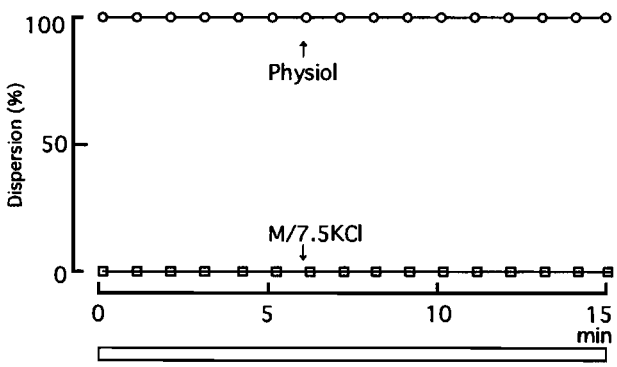

Fig. 1 Typical responses of melanophores exposed to light (4,000 lux, white bar) in physiological saline (white circles) and in M/7.5 KCl solution (white squares).

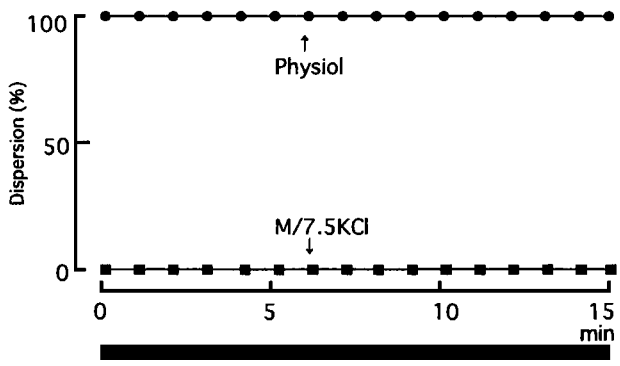

Fig. 2 Typical responses of melanophores exposed to darkness (black bar) in physiological saline (black circles) and in M/7.5 KCl solution (black squares).

branch length under both conditions, the fluctuation range was below 10 %. The melanophore response did not differ depending on the difference in light intensity, or between illumination and darkness.

Figures 1 and 2 show examples of the responses under the respective conditions (white circles in figure 1 and black circles in figure 2).

Innervated melanophores in a state of melanosome aggregation in M/7.5 KCl

Fully aggregated melanophores were continuously illuminated at two intensities (2,000 and 4,000 lux, 11 and 10 samples, respectively) of light for 15 min. Controls consisted of melanophores (15 samples) maintained under the same conditions except that they remained in darkness. Most melanophores maintained full aggregation of their melanosomes during the observation interval under both light and dark conditions (white squares of figure 1 and black squares of figure 2).

Denervated melanophores in a state of melanosome dispersion in physiological saline

Denervated melanophores did not aggregate at all when exposed for three min to M/7.5 KCl. Fully dispersed melanophores in physiological saline were illuminated at 4,000 lux for 15 min (10 samples). Controls (13 samples) were treated the same except for darkness only. All denervated melanophores dispersed in physiological saline remained dispersed regardless of illumination or darkness.

Responses to continuous illumination of innervated melanophores in a state of incomplete aggregation

Although innervated melanophores aggregated fully in M/7.5 KCl (10K), they showed incomplete aggregation of melanosomes in 1.5K and 2K solutions. The melanophore responses to illumination (4,000 lux) were observed for 15 min. In the 1.5K solution, the fifteen melanophores observed aggregated and maintained a state of incomplete melanosome aggregation with fluctuating branch length (Fig. 3).

No clear tendency for melanosome dispersion or aggregation was observed as a result of illumination (white circles in figure 3).

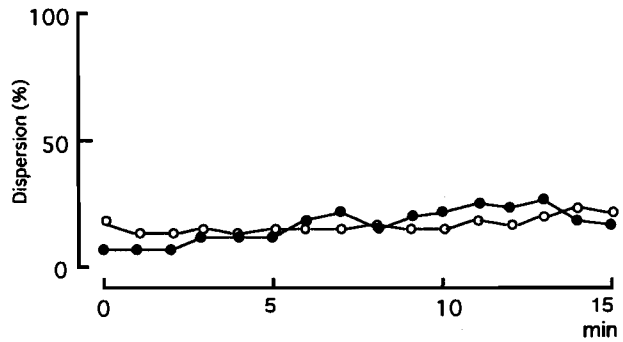

Fig. 3 Typical responses of melanophores in a state of aggregation in 1.5K solution. White circles show melanophore responses when exposed to light at 4,000 lux. Black circles show melanophore responses in the dark.

Furthermore, thirteen melanophores observed under dark conditions (control) also maintained the state of incomplete melanosome aggregation (black circles of figure 3). The response was not different from that of illuminated melanophores.

In the 2K solution, twelve observed melanophores showed a response similar to that of melanophores in the 1.5K solution, except for a slight increase in the aggregation rates of the melanosomes. However, there were no differences between the illuminated melanophores and those kept in darkness (twelve controls).

Responses to intermittent illumination and darkness of innervated melanophores in a state of incomplete aggregation

Melanophores immersed in the 1.5K solution for 10 min under dark conditions underwent incomplete aggregation of melanosomes of about $77 \pm 14\%$ (mean$\pm$S.D., $n=23$). These melanophores were then exposed to alternate 5-min intervals of illumination (4,000 and 10,000 lux) and darkness. The numbers of experiments were 12 at 4,000 lux and 10 at 10,000 lux. However, illumination at these intensities did not necessarily induce clear melanosome dispersion or aggregation responses in these melanophores (Fig. 4). There were no differences in melanophore responses depending on the light intensity.

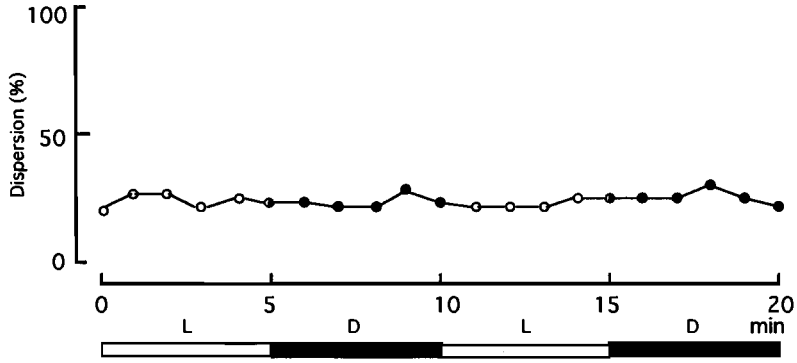

Fig. 4 A typical response of melanophores in 1.5K solution exposed to intermittent illumination (4,000 lux) and darkness. White (L) and black (D) bars show light and dark intervals, respectively.

Melanophores in the 2K solution aggregated incompletely and their degrees of aggregation (about 84 ±14%, n=30) were higher than those of melanophores in the 1.5K solution. Illumination did not induce clear melanosome dispersion or aggregation responses, the same as in the 1.5K solution. The responses were independent of the intensity of illumination.

Responses of denervated melanophores to intermittent illumination and darkness

a) Melanophores aggregated by adrenaline treatment

Denervated melanophores were treated with 10^{-7} or 10^{-6}M adrenaline in physiological saline. Denervated melanophores showed rates of aggregation above 30% in 10^{-7}M and above 80% in 10^{-6}M adrenaline. The denervated melanophores in various states of melanosome aggregation were illuminated at 4,000 lux for 5 min followed by darkness for 5 min (Fig. 5).

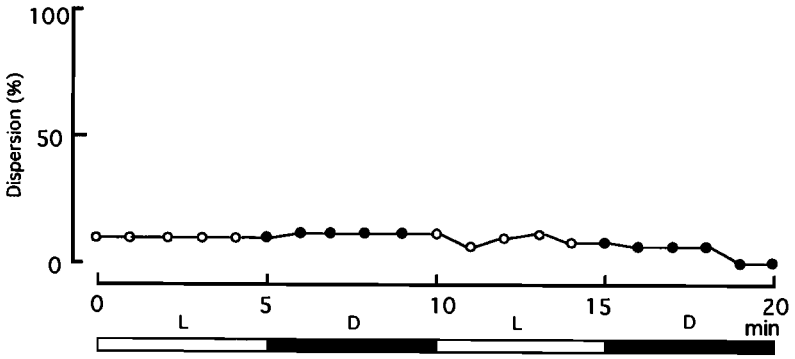

Fig. 5 A typical response of denervated melanophores, first treated with 10^{-7}M adrenaline in physiological saline to aggregate their melanosomes, and then exposed to intermittent illumination (white bar, L) and darkness (black bar, D).

The melanophores did not respond to the illumination by clear dispersion or aggregation of their melanosomes. The order of light and darkness did not influence the melanophore response.

b) Melanophores in the process of recovery from a state of melanosome aggregation

Melanophores aggregated when exposed to 5×10^{-7}M adrenaline in physiological saline. Ten minutes after application of the adrenaline, the solution was changed to physiological saline without the adrenaline. The melanophores underwent gradual dispersion. During the dispersion process, illumination and darkness were repeated intermittently (n=22). The illumination did not accelerate or inhibit melanosome dispersion (Fig. 6). The order of light and darkness did not influence the melanophore response.

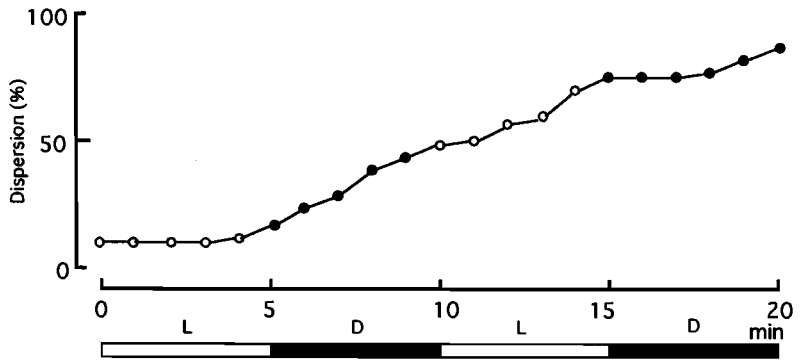

Fig. 6 Denervated melanophores were treated for 5 min with 10^{-7}M adrenaline in physiological saline to aggregate their melanosomes. They were then immersed in physiological saline. The figure depicts a typical response of the denervated melanophores during the process of melanosome dispersion in saline as the melanophores were exposed to intermittent light (white bars, L) and darkness (black bars, D).

DISCUSSION

It is well known that scale melanophores of fish are controlled by sympathetic nerves (Fujii, 1961). Iwata et al. (1959) found that scale melanophores undergo three successive stages, i.e. initial dispersion, concentration and a final dispersion phase. For one or two hours after a scale isolated from the fish's body surface (initial dispersion), the severed nerves attached to the scale continue to function. However, the melanophore nerves cease functioning in the final dispersion phase after scale isolation (Iwata et al., 1959). Scale melanophores in the initial and final dispersion phases are called "innervated melanophores" and "denervated melanophores", respectively. In the present results, the responses to light by the scale melanophores hardly differed notwithstanding innervation or denervation. The evidence obtained did not suggest that the light used in the experiment acts on the melanophore nerves; that is, the scale melanophore response to light is considered to be unrelated to the presence of melanophore nerves.

Scale melanophores of the motsugo in a state of full dispersion or aggregation of melanosomes did not respond to light by undergoing melanosome aggregation or dispersion. In most cases in which the melanophores of fish are light sensitive, they respond to light by melanosome dispersion (Iga and Takabatake, 1983; Negishi, 1985; Ohta, 1983; Ohta et al., 1995). In M/7.5 KCl, in which K^+ acts on melanophore-aggregating nerve endings (Iwata et al., 1959), melanosomes in innervated melanophores are forced into the aggregated state. Therefore, light effect may be difficult to appear in melanophores of such state, if it were light-sensitive. Melanophores in this state may not be able to respond to light even if they are light sensitive. Therefore, the light response was also investigated in innervated melanophores that were in an incomplete aggregation state in K-poor and K-effective solutions (1.5～2K). In denervated melanophores the light response was investigated during the course of melanosome-dispersion in physiological saline after adrenaline-induced aggregation. The results showed that light did not induce further dispersion or aggregation of the incompletely aggregated melanosomes. Moreover, light did not accelerate or inhibit melanosome dispersion in physiological saline after adrenaline-induced aggregation. From these results, it is concluded that scale melanophores of the motsugo do not show a primary color response. From the view point of the primary color response, reports in which scale melanophores are not light-sensitive are few, in spite of the importance.

The effective intensity of light is important. In cases reported so far, the effective intensity of light was from hundreds to thousands of lux. The intensity of the light (2,000～10,000 lux) that the authors employed in this study was enough to induce responses of light-sensitive melanophores of fry (3,500 lux, Ohta, 1983) or of scale and incubated melanophores from scales of fish (100-1,000 lux, the rose bitterling, Ohta et al., 1995; 600 lux, *Z. temmincki*, Iga and Takabatake, 1983; 600 lux, medaka, Negishi, 1985). Scale melanophores of the motsugo failed to respond not only to light in almost the same intensity range, but also to stronger light (10, 000 lux).

Scale chromatophores that do respond to light are known in some species of fish: leucophores of *Oryzias latipes* (Ohta and Sugimoto, 1980), melanophores of *Fundulus heteroclitus* (Spaeth, 1913), of *Anoptichthys jordani* (Burger et al., 1963), of *Zacco temmincki* (Naora and Iga, 1989), xanthophores of *O. latipes* (Kawai, 1989; Oshima et al., 1998). Light sensitive scale melanophores known up to now are limited to the above four species of fishes. Scale melanophores of *Zacco temmincki* respond notably to light (Iga and Takabatake, 1983). However, melanophores of the present motsugo (*Pseudorasbora parva*) are insensitive to light at the intensities tested. At present, the reason for the light insensitivity is not clear, although we can speculate about some possibility, viz., fewer or no photopigments.

The following points on the primary color response of fish are clear: (1) there are light-sensitive and insensitive chromatophores. (2) the direction of pigment movement in chromatophores in response to visible light and ultraviolet light is not consistent in the same or different chromatophores. (3) There is not now a common hypothesis to explain the pigment movement in light-sensitive chromatophores. Therefore, it is necessary to obtain much more information on the primary color response in different species of fishes and species of chromatophores, as well as to compare scale and cultured chromatophores.

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