Involvement of Ca2 in the Direct Effect of K on Xanthophores of the Medaka, Oryzias latipes

Authors: Oshima, Noriko, Sekine, Hiroki, and Tanooka, Miki

Source: Zoological Science, 15(5) : 645-650

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.645

ZOOLOGICAL SCIENCE **15**: 645–650 (1998)

Involvement of Ca^{2+} in the Direct Effect of K^+ on Xanthophores of the Medaka, *Oryzias latipes*

Noriko Oshima*, Hiroki Sekine and Miki Tanooka

Department of Biomolecular Science, Faculty of Science, Toho University, Miyama, Funabashi, Chiba 274-8510, Japan

ABSTRACT—The direct effect of K^+ on xanthophores of the medaka, *Oryzias latipes*, was studied using denervated scale preparations and cultured cells. At concentrations of 30 mM or higher, K^+ ions caused almost complete aggregation of pigment in denervated xanthophores. The withdrawal of divalent cations from the perfusing solution, or the addition of Ca^{2+} channel blocker to the medium, made the cells unresponsive to K^+. When Fluo 3-loaded xanthophores were stimulated by K^+, a temporary increase in the fluorescence intensity was observed. Experimental elevation of cytosolic Ca^{2+} concentration induced aggregation of pigment in denervated xanthophores pretreated with A23187. Forskolin, an activator of adenylate cyclase, inhibited the K^+-induced aggregation of pigment in a dose-dependent manner, whereas W-7, a calmodulin antagonist, did not. From these results, it is likely that membrane depolarization caused by elevated K^+ concentration in the perfusing medium may be accompanied by Ca^{2+} entry into the cytoplasm through voltage-dependent Ca^{2+} channels present in the xanthophore membrane. This would result in inhibition of adenylate cyclase activity, since it is well established that decreases in intracellular cAMP level induce pigment aggregation in fish chromatophores.

INTRODUCTION

The rapid physiological color changes of teleost fishes are due to motile activities of integumentary colored cells, termed chromatophores. Their motility, i.e. aggregation or dispersion of pigment granules within them, is well known to be under hormonal and neural regulation (Fujii and Oshima, 1986, 1994). In light-absorbing chromatophores (melanophores, xanthophores and erythrophores) of many fish species, pigment aggregation is also caused by elevated K^+ concentration in the perfusing solution, although K^+ does not affect directly those cells (Fujii, 1959; Iwata *et al*., 1959). Rather, K^+ acts on neural elements surrounding chromatophores to liberate the neurotransmitter (norepinephrine), which in turn induces the centripetal migration of pigment granules mediated by α-adrenoceptors on the cell membrane. The dispersion of light-scattering organelle in leucophores of the medaka *via* β-adrenoceptors (Iga, 1978) and the coloring response of motile iridophores of the blue damselfish (Oshima *et al*., 1985) are also elicited by K^+ through a similar mechanism.

Using scales of the medaka, *Oryzias latipes* (wild type and red type), Iga (1969) first observed that denervated xanthophores aggregated their pigment as did innervated xanthophores, when the perfusing solution was changed from physiological saline to isotonic KCl, whereas denervated melanophores and leucophores never responded to the K^+-rich solution. Since xanthophores of the medaka also respond to norepinephrine, α-melanophore stimulating hormone (α-MSH), melanin-concentrating hormone, etc., the motile activities of those cells are surely controlled by nervous and hormonal systems (Fujii and Oshima, 1986).

In the present study, the pigment-aggregating action of K^+ on medaka xanthophores extraneous to the nervous system was confirmed by the use of cultured cells in addition to denervated xanthophores on scales, and the mechanism of the direct action of K^+ was further examined. It may facilitate understanding of delicate and subtle color changes of fish to clarify the difference in characteristics among chromatophore species.

MATERIALS AND METHODS

Medaka (*Oryzias latipes*, wild type and orange-red variety) of both sexes, 2.5-3.5 cm in length, were used. For denervation, reserpine (1 mg) obtained in commercial ampoules (Daiichi Pharmaceutical) was diluted with tap water (500 ml), and medakas were kept alive for 12 hr in the dark in that solution. Since melanophores (wild type) or amelanotic melanophores (orange-red variety) in the skin tissue of scales from these medakas were subsequently refractory to treatment with isotonic K^+ solution (128 mM), chromatophores were considered to be completely denervated (cf. Fujii, 1959).

Scales on the dorsal trunk were isolated in a physiological saline solution (PSS) of the following composition: NaCl 125.3 mM, KCl 2.7 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.8 mM, D-glucose 5.6 mM, Tris-HCl buffer 5.0 mM (pH 7.2). Scales were immersed for 5 min in Ca^{2+}- and Mg^{2+}-

* Corresponding author: Tel. +81-474-72-7512; FAX. +81-474-75-1855.

free saline (CMFS; NaCl 130.7 mM, KCl 2.7 mM, D-glucose 5.6 mM, EDTA 3 mM, Tris-HCl buffer 5.0 mM; pH 7.2) and the epidermal layer of skin tissue on each scale was removed with forceps. Scales were fixed on a holder with a fine glass fiber, both sides of which had been attached to a coverslip with epoxy adhesive; the holder was then turned over and placed on a perfusion chamber with a narrow trough filled with saline (Oshima and Fujii, 1984).

Treatment with K^+

K^+-rich solutions (10, 20, 30, 40, 50, 70, 90, 110, 128 mM) were prepared by substitution of equimolar KCl for NaCl in PSS. After high K^+ stimulation for 3 min, denervated scale preparations were treated with 5 μM norepinephrine (NE) for 3 min to obtain perfect (100%) responses of chromatophores. The NE solution was prepared by dilution of an injection fluid of norepinephrine hydrochloride (Sankyo). Responses of xanthophores were continuously photomicrographed and a video image analyzing system, Luzex F (Nireco), was employed for quantitative analysis. In this system, changes in the area occupied by pigment granules that are present inside a rectangular frame on a monitor screen are calibrated (cf. Oshima *et al.*, 1988).

In addition to scale preparations, cultured xanthophores were also used: xanthophores dissociated from scales were cultured for 48 hr at 25°C by methods described in detail previously (Oshima *et al.*, 1996). In case pigment granules in cultured xanthophores were aggregated in PSS, they were dispersed beforehand with 1 nM α-melanophore stimulating hormone (α-MSH, Sigma Chemical).

Effects of divalent cation-free medium and channel blockers

To prepare Ca^{2+}- and Mg^{2+}-free 50 mM K^+ solution containing 3 mM EDTA, equimolar NaCl in CMFS was replaced by KCl. After rinsing with CMFS for 7-8 min, denervated xanthophores were stimulated with the divalent cation-free K^+-rich solution for 4 min and their responses were photomicrographed. As Ca^{2+} and Na^+ channel blockers, methoxy verapamil (D-600; Sigma) and tetrodotoxin (TTX; Wako Pure Chemical) were used, respectively. Denervated xanthophores pretreated with D-600 or TTX for 5 min were exposed to 50 mM K^+ solution for 4 min in the continued presence of each blocker. After washing with PSS for 30-40 min, cells were stimulated again by K^+ for 4 min and finally by NE (5 μM) for 3 min.

Measurement of fluorescence signal from Ca^{2+}-indicator

Epidermis-free, denervated scales were treated with 5 μM Fluo 3-AM (Sigma) for 60 min in the dark at room temperature; Fluo 3-AM was dissolved in a small quantity of DMSO and diluted with PSS immediately before use. With its reduced polarity, acetoxymethyl ester of Fluo 3, i.e. Fluo 3-AM, passes through the cell membrane with ease, and is hydrolyzed in the cytoplasm by acetylesterase (Minta *et al.*, 1989; Kao *et al.*, 1989). Thus, cells can be loaded with Fluo 3 by incubation with its acetoxymethyl ester. In the cytoplasm, the Ca^{2+}-indicator binds free Ca^{2+}, leading to an augmentation of fluorescence. The treatment with the dye was followed by multiple washings with PSS, and scale preparations were fixed on the bottom of the perfusion chamber.

Fluorescence signals were monitored with an INSIGHTPLUS laser scanning confocal microscope system, equipped with an Olympus inverted microscope IMT-2, (Meridian Instruments). The dye has a peak emission at 525 nm when excited by the 488 nm argon laser line.

Manipulation of intracellular concentration of Ca^{2+}

After epidermis-free, denervated scales were rinsed with CMFS for 5-6 min, they were pretreated with 20 μM calcium ionophore A23187 (Sigma) for 3 min at room temperature. The ionophore solution was prepared by diluting a stock solution of A23187 dissolved in DMSO with CMFS. The final maximum concentration of DMSO in the medium was less than 0.5%. Scale preparations were next incubated in a Ca-buffer (pH 7.4) in the presence of A23187; Ca-buffers were prepared according to the equation given by Imai and Takeda (1967). In the present study, the concentration of EGTA (Dojinndo Laboratories) was fixed at 2.5 mM, and the amount of $CaCl_2$ added was changed.

Effects of calmodulin blocker and adenylate cyclase activator

Denervated xanthophores were pretreated with a specific calmodulin antagonist, N-(6-aminohexyl)-5-chloro-naphthalenesulfonamide hydrochloride (W-7; Seikagaku Kogyo), for 5 min and then exposed to K^+-rich solution (50 mM) for 4 min in the continued presence of the inhibitor. A saline rinse for 30 min was followed by K^+ stimulation for 4 min, and finally 5 μM NE was applied to obtain complete (100%) aggregation of pigment. Xanthophores were also incubated with 50 mM K^+ solution in the presence of forskolin (Wako Pure Chemical), an activator of adenylate cyclase, for 4 min. After a 30 min wash with PSS, responses to K^+ and perfect aggregation evoked by 5 μM NE were photomicrographed.

RESULTS

In physiological saline solution (PSS), xanthophores on scales from denervated medakas maintained a pigment-dispersed state. In isotonic solution containing 10 mM K^+, pigment granules remained dispersed, while about 60% aggregation occurred at 20 mM K^+ (Fig. 1). Thus, the threshold concentration of K^+ must be between 10 and 20 mM in denervated xanthophores. Almost complete aggregation of pigment was induced within 3 min at K^+ concentrations higher than 30 mM, and therefore, a 50 mM K^+ solution was used in subsequent experiments to stimulate xanthophores. K^+ had no effect on denervated melanophores (wild type), amelanotic melanophores (orange-red variety) or leucophores.

Next, responses of xanthophores in primary cell culture were studied. In about half of cultured xanthophores, pigment granules remained dispersed in PSS. Such cells responded within 3 min to 50 mM K^+ by pigment aggregation (Fig. 2), and the responses were reversible. The other half of xanthophores in primary culture assumed a pigment-aggregated state in PSS. After pigment granules were fully dispersed by pretreat-

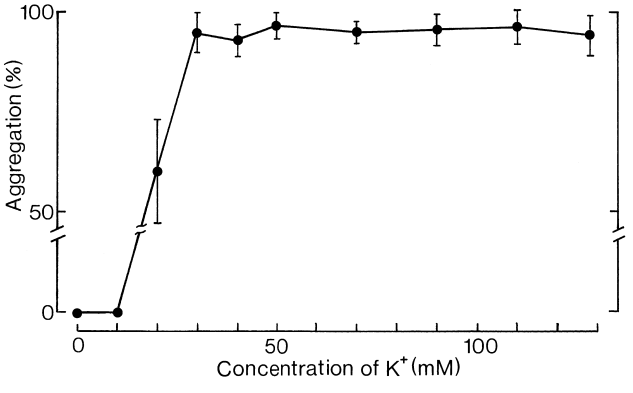

Fig. 1. Relationship of K^+ concentration in the perfusing medium to the magnitude of pigment-aggregating responses of denervated medaka xanthophores. After treatment with K^+-rich saline for 3 min, the cells were exposed to 5 μM norepinephrine (NE) for 3 min to obtain complete (100%) aggregation of pigment. Each data point represents the mean ± SE of 6 measurements on different animals.

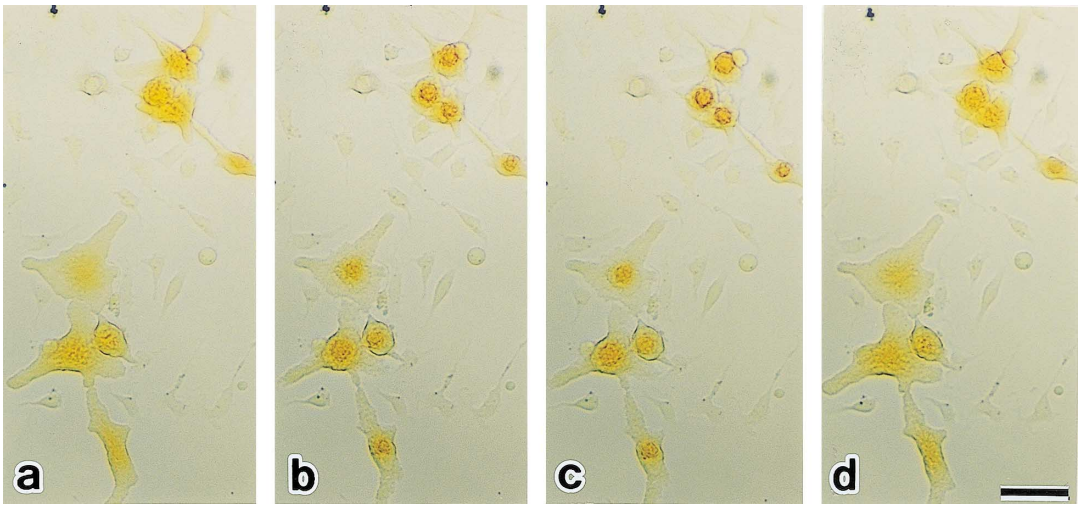

Fig. 2. Photomicrographs showing responses of cultured medaka xanthophores to K^+ (50 mM). (**a**) Xanthophores equilibrated in physiological saline solution (PSS). (**b**) one min after addition of 50 mM K^+ solution. (**c**) 3 min after addition of 50 mM K^+. (**d**) 20 min after the beginning of saline rinse. Bar: 50 μm.

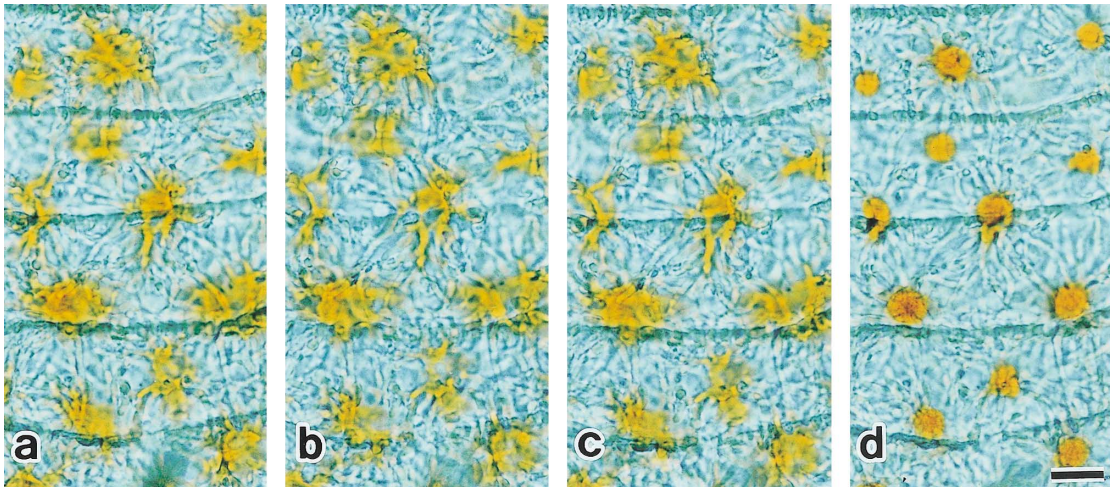

Fig. 3. Photomicrographs showing responses of denervated medaka xanthophores to 50 mM K^+ solution. (**a**) Denervated xanthophores equilibrated in Ca^{2+}- and Mg^{2+}-free saline containing 3 mM EDTA. (**b**) 4 min after addition of Ca^{2+}- and Mg^{2+}-free, K^+-rich solution (50 mM). (**c**) 20 min after the beginning of rinsing with PSS. (**d**) 4 min after addition of 50 mM K^+ solution. A blue filter was used to increase the contrast of xanthophores against the background. Bar: 20 μm.

ment with α-MSH (1 nM) for 8 min, such xanthophores were stimulated with 50 mM K^+, or rinsed with PSS (control). The application of 50 mM K^+ accelerated pigment aggregation; the time required for 50% aggregation was 3.8 min (n = 5), whereas in the control experiment, it took 6.7 min (n = 5) to obtain the same level of aggregation. From these results, it is clear that the pigment-aggregating action of K^+ on medaka xanthophores is direct.

In Ca^{2+}- and Mg^{2+}-free K^+-rich solution (50 mM), pigment aggregation in denervated xanthophores was not detected (Fig. 3), implying that transport of Ca^{2+} through the xanthophore membrane may be indispensable for K^+ action. D-600, a Ca^{2+} channel blocker (Lee and Tsien, 1983), was employed to investigate the role of Ca^{2+} channels on K^+-induced aggregation of pigment. As indicated in Fig. 4, D-600 inhibited pigment aggregation in a dose-dependent manner, and its effect was reversible. On the other hand, TTX, which is one of the most potent channel blockers of Na^+, and which can block Na^+ flux at about 1 μM (Catterall, 1984), had no effect on K^+-induced pigment aggregation, even at 10 or 50 μM. In addition, as shown in Fig. 1, 128 mM K^+ solution prepared by substituting KCl totally for NaCl in standard PSS, aggregated pigment in denervated xanthophores. These results suggest that K^+ acts directly on the xanthophore membrane to bring about Ca^{2+} influx, but not Na^+ influx, to the cytoplasm.

Using Fluo 3 (a Ca^{2+}-indicator)-loaded xanthophores in scales from the denervated medaka (orange-red variety), the fluorescence signal was monitored. Figure 5 shows the fluorescence intensity by means of pseudo-color, 0.3, 3.3, 7.3 and 47.3 sec after application of 50 mM K^+. In the area sur-

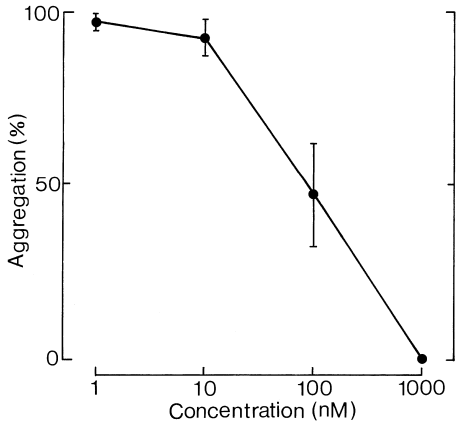

Fig. 4. Relationship of D-600 concentration to the magnitude of K^+-induced aggregation of pigment in denervated medaka xanthophores. Pretreatment with D-600 for 5 min was followed by application of 50 mM K^+ solution for 4 min in the presence of the channel blocker. After saline rinse for 30-40 min, the cells were stimulated by 50 mM K^+, and finally by 5 μM NE to obtain 100% aggregation. Each data point represents the mean ± SE of 5 measurements on different animals.

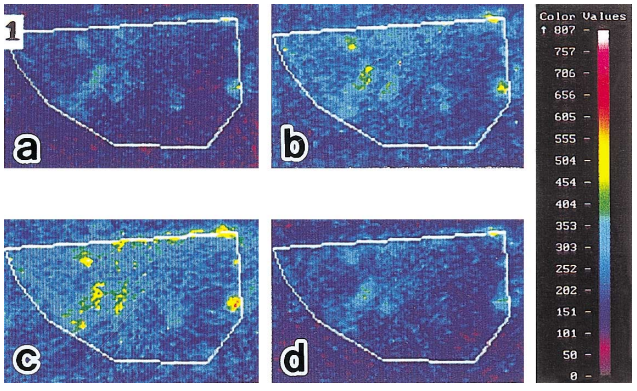

Fig. 5. Fluorescence signal by means of pseudo-color in skin tissue on scale preparations from denervated medaka (orange-red variety). In the area surrounded by the white line, a number of xanthophores were present. (**a**) 0.3 sec after addition of 50 mM K^+ solution. (**b**) 3.3 sec. (**c**) 7.3 sec. (**d**) 47.3 sec.

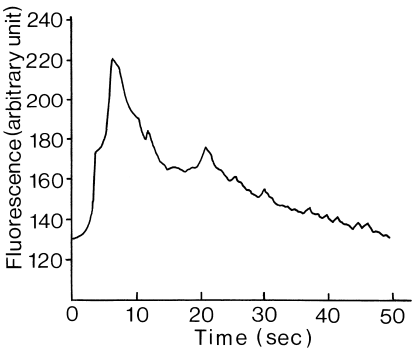

Fig. 6. Changes in fluorescence intensity averaged on the area surrounded by the white line in Fig. 5, resulting from application of 50 mM K^+ solution.

rounded by the white line, many xanthophores were present. The continuous change in the fluorescence intensity averaged on that area is shown in Fig. 6. A temporary increase in intensity occurred immediately after K^+ application, and about 6.5 sec later, the level reached the maximum. After measurements of fluorescence intensity, xanthophores were confirmed to be able to assume the pigment-aggregated state.

The experimental elevation of cytosolic Ca^{2+} concentration actually caused pigment aggregation in medaka (wild type) xanthophores. When denervated scale preparations pretreated with 20 μM A23187 were exposed to 10 nM Ca-buffer in the presence of the ionophore, pigment aggregation within xanthophores occurred, and that aggregation progressed even further in 100 nM Ca-buffer (Fig. 7). In melanophores, however, an aggregation response was not observed.

To analyze the role of cytosolic Ca^{2+} in pigment aggregation within xanthophores, the effect of W-7 (10 and 50 μM), a specific calmodulin inhibitor, was examined. At either concentration of W-7, pigment-aggregating responses of xanthophores were not inhibited. In the presence of 50 μM W-7, the mean value of the magnitude of pigment aggregation by K^+ was 94.8 ± 2.1% (n = 5), whereas it was 97.0 ± 1.3% (n = 5) in the absence of the antagonist. There was no significant difference between those values (t-test, $P > 0.05$).

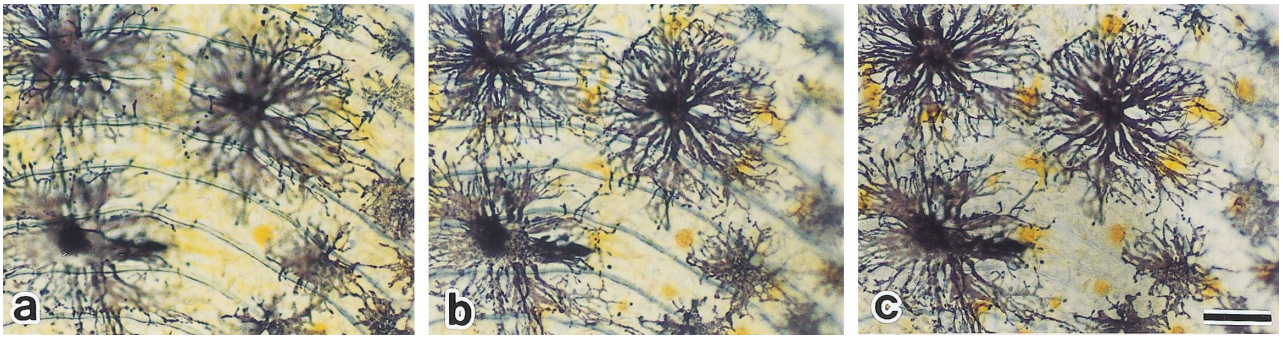

Fig. 7. Photomicrographs showing responses of medaka (wild type) xanthophores exposed to Ca-buffers. Cells pretreated with 20 μM A23187 for 3 min were incubated in 10 nM Ca-buffer for 5 min (**b**), and successively in 100 nM Ca-buffer for 5 min (**c**) in the continued presence of the calcium ionophore. Note that melanophore response did not occur. (**a**) Chromatophores equilibrated in PSS. Bar: 50 μm.

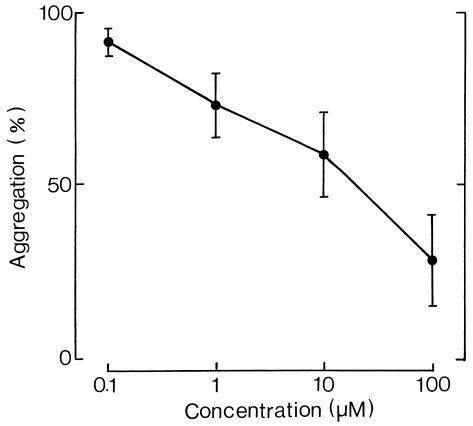

Fig. 8. Relationship of forskolin concentration to the magnitude of K^+-induced aggregation of pigment in denervated medaka xanthophores. Pretreatment with forskolin for 4 min was followed by application of 50 mM K^+ solution for 4 min in the presence of the adenylate cyclase activator. After a saline rinse for 30-40 min, cells were stimulated again by K^+ and recovery from the effects of forskolin was confirmed. The level of aggregation is expressed as a percentage of the full (100%) aggregation attained by 5 μM NE at the end of each series of measurements. Each data point represents the mean ± SE of 5 measurements on different animals.

Therefore, it is likely that calmodulin is not involved in this signal transduction pathway.

When denervated xanthophores were exposed to 50 mM K^+ in the presence of forskolin, an adenylate cyclase (AC) stimulating agent, pigment aggregation was depressed in a dose-dependent manner (Fig. 8). For example, the level of K^+-induced aggregation in the presence of 10 μM forskolin was only 58.8% of the complete aggregation evoked by 5 μM NE. Therefore, Ca^{2+} that influx into xanthophores through Ca^{2+} channels may inhibit the AC activity, leading to a decrease in the cytosolic level of cAMP, which in turn may cause pigment aggregation in medaka xanthophores.

DISCUSSION

Based on the observation that denervated fish melanophores were unresponsive to K^+, Fujii (1959) concluded that K^+ does not act directly on melanophores themselves, but on adrenergic nerves innervating them to release the transmitter, norepinephrine (NE). In medaka xanthophores, however, depolarization of the cell membrane elicited by increases in extracellular K^+ concentration may also trigger aggregation of pigment. The present results imply that voltage-dependent Ca^{2+} channels present in the xanthophore membrane are opened to allow Ca^{2+} to penetrate into the cytoplasm, a phenomenon which resembles the K^+-contracture of musculature. Luby-Phelps and Porter (1982) further reported that cultured erythrophores of *Holocentrus ascensionis* responded to 60 mM K^+ solution by pigment aggregation. In that paper, the authors questioned whether the earlier conclusion of Fujii (1959) was right. Now, we can easily understand that characteristics of brightly pigmented cells (erythrophores and xanthophores) of some fish species are very different from those of melanophores. There may be few voltage-dependent Ca^{2+} channels on the melanophore membrane, because cultured (Oshima *et al*., 1988) and denervated melanophores (present data) do not respond to K^+.

Up to the present, it has not been confirmed whether chromatophore membranes are actually depolarized by certain endogenous mechanisms. However, if such mechanisms are functional, differential responses of melanophores and brightly pigmented cells triggered by membrane depolarization might contribute to revelation of subtle body colors of fish. Using *Fundulus*, Fries (1931) found that, on a blue background, melanophores were generally dispersed while xanthophores were aggregated, whereas both types were dispersed on a black background.

In permeabilized melanophores of the Nile tilapia, exogenously applied inositol 1,4,5-trisphosphate (IP_3) caused pigment aggregation (Fujii *et al*., 1991). The application of NE was accompanied by an increase in cytosolic Ca^{2+} in platyfish (Oshima *et al*., 1988) and tilapia (Toyohara and Fujii, 1992) melanophores. In tilapia cells, the cytosolic level of Ca^{2+} was doubled (about 200 nM) when melanin aggregation was elicited by NE. Further, W-7, a calmodulin antagonist, inhibited NE-induced aggregation in a dose-dependent manner in blue damselfish (Oshima *et al*., 1990) and in tilapia melanophores (Oshima and Wannitikul, 1996). Thus, in melanophores of at least some teleost species, a Ca/calmodulin complex (calmodulin activated by Ca^{2+}) may be involved in signal transduction in the pigment-aggregating response to NE. Activated calmodulin is thought to stimulate protein phosphatase 2B, leading to pigment aggregation (Thaler and Haimo, 1990; Oshima and Wannitikul, 1996). In medaka xanthophores, however, K^+-induced aggregation was not blocked by W-7, suggesting that calmodulin may not play an important role in intracellular events. Since aggregation was inhibited by forskolin, Ca^{2+} supplied from outside the cells through Ca^{2+} channels may regulate the activity of adenylate cyclase (AC). DeBernardi *et al*. (1991) and Yoshimura and Cooper (1992) reported that low concentrations of Ca^{2+} (submicromolar Ca^{2+}), independently of calmodulin, inhibited AC (type V) activity in mammalian cells. If also true in medaka xanthophores, decreases in cytosolic cyclic AMP concentration caused by decreases in AC activity may induce pigment aggregation *via* decreases in activity of protein kinase A (Fujii and Oshima, 1986; Oshima and Wannitikul, 1996), although the type of AC in membranes of medaka xanthophores has not yet been examined.

W-7 also had no inhibitory effect on pigment aggregation by NE in melanophores of the medaka (Oshima, unpublished data). Further, medaka melanophores, different from those of other fish species, were refractory to elevated intracellular Ca^{2+} concentration shown in Fig. 7. Therefore, Ca^{2+} and calmodulin may not signal the pigment aggregation response in medaka melanophores.

ACKNOWLEDGMENTS

We thank to Dr. R. Fujii of Toho University for his valuable discussion. This study was supported in part by a grant from the Biomolecular Science Award Foundation of Toho University.

REFERENCES

Catterall WA (1984) The molecular basis of neuronal excitability. Science 223: 653–661

DeBernardi MA, Seki T, Brooker G (1991) Inhibition of cAMP accumulation by intracellular calcium mobilization in C6-2B cells stably transfected with substance K receptor cDNA. Proc Natl Acad Sci USA 88: 9257–9261

Fries EFB (1931) Color change in Fundulus, with special consideration of the xanthophores. J Exp Zool 60: 384–426

Fujii R (1959) Mechanism of ionic action in the melanophore system of fish I. Melanophore-concentrating action of potassium and some other ions. Annot Zool Jpn 32: 47–59

Fujii R, Oshima N (1986) Control of chromatophore movements in teleost fishes. Zool Sci 3: 13–47

Fujii R, Wakatabi H, Oshima N (1991) Inositol 1,4,5-trisphosphate signals the motile responses of fish chromatophores. I. Aggregation of pigment in the tilapia melanophores. J Exp Zool 259: 9–17

Fujii R, Oshima N (1994) Factors influencing motile activities of fish chromatophores. In "Advances in Comparative and Environmental Physiology Vol 2" Ed by R Gilles, Springer-Verlag, Berlin, pp 1–54

Iga T (1969) The action of potassium ions on the xanthophores of the teleost, *Oryzias latipes*. Mem Fac Lit & Sci Shimane Univ Nat Sci 2: 67–75

Iga T (1978) The mode of action of potassium ions on the leucophores of a freshwater teleost, *Oryzias latipes*. J Exp Zool 205: 413–422

Imai S, Takeda K (1967) Action of calcium and certain multivalent cations on potassium contracture of guinea-pig's taenia coli. J Physiol 190: 155–169

Iwata KS, Watanabe M, Nagao K (1959) The mode of action of pigment concentrating agents on the melanophores in an isolated fish scale. Biol J Okayama Univ 5: 196–206

Kao JPY, Harootunian AT, Tsien RY (1989) Photochemically generated cytosolic calcium pulses and their detection by Fluo-3. J Biol Chem 264: 8179–8184

Lee KS, Tsien RW (1983) Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. Nature 302: 790–794

Luby-Phelps K, Porter KR (1982) The control of pigment migration in isolated erythrophores of *Holocentrus ascensionis* (Osbeck). II. The role of calcium. Cell 29: 441–450

Minta A, Kao JPY, Tsien RY (1989) Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. J Biol Chem 264: 8171–8178

Oshima N, Fujii R (1984) A precision photoelectric method for recording chromatophore responses *in vitro*. Zool Sci 1: 545–552

Oshima N, Kasukawa H, Fujii R (1985) Effects of potassium ions on motile iridophores of blue damselfish. Zool Sci 2: 463–467

Oshima N, Suzuki M, Yamaji N, Fujii R (1988) Pigment aggregation is triggered by an increase in free calcium ions within fish chromatophores. Comp Biochem Physiol 91A: 27–32

Oshima N, Hayakawa H, Sugimoto M (1990) The involvement of calmodulin in motile activities of fish chromatophores. Comp Biochem Physiol 97C: 33–36

Oshima N, Makino M, Iwamuro S, Bern HA (1996) Pigment dispersion by prolactin in cultured xanthophores and erythrophores of some fish species. J Exp Zool 275: 45–52

Oshima N, Wannitikul P (1996) Signal transduction of MCH in melanophores of the tilapia, *Oreochromis niloticus*. Zool Sci 13: 351–356

Thaler CD, Haimo LT (1990) Regulation of organelle transport in melanophores by calcineurine. J Cell Biol 111: 1939–1948

Toyohara J, Fujii R (1992) Simultaneous recording of the changes in intracellular levels of Ca^{2+} and the motile responses of melanophores in tilapia. Zool Sci 9: 1229

Yoshimura M, Cooper DMF (1992) Cloning and expression of a Ca^{2+}-inhibitable adenylyl cyclase from NCB-20 cells. Proc Natl Acad Sci USA 89: 6716–6720

(Received April 27, 1998 / Accepted May 26, 1998)