THE SYMPATHETIC NEURO-MELANOPHORE TRANSMISSION IN A FRESH-WATER INDIAN MAJOR CARP, \textit{Labeo Rohita} (Ham.)*

SHASHI PATIL AND AJAI K. JAIN**

Pigment Cell Biology Unit,
School of Studies in Zoology,
Jiwaji University, Gwalior - 474 011

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Summary: Adrenaline was effective in aggregating the melanosomes both in innervated as well as denervated melanophores. Isotonic KCl could induce pigment aggregation only in innervated melanophores. Adrenaline-and K+-induced pigment aggregation response in these melanophores was blocked by phentolamine; propranolol failed to do so. It is suggested that the chromatric nerves in the fish, \textit{Labeo Rohita} are adrenergic and via post-synaptic \textalpha\-adrenoceptors, control the melanosome aggregation.

Key words: melanophores alpha receptor
drug effects neurotransmission
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INTRODUCTION

Mechanisms of colour change are complex in teleosts in which humoral factors from the hypothalamus, pituitary gland and the pineal gland and autonomic nervous system combine to regulate not only the motile activities of specialized integumentary coloured cells, the chromatophores (physiological colour change) but also the net amount of pigmentary materials within them (long term morphological colour change) (1). Neural regulation takes a role in adapting fish more rapidly to the environment. The hormonal means may have evolved to control chromatophore motility slowly, since the process inevitably includes the gradual increase or decrease of their titer in the blood. Such dual regulation in \textit{Labeo Rohita} (Ham.), a freshwater Indian major carp has been demonstrated in our laboratory recently (2).

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**Corresponding Author
RESULTS

Melanophores in the scales isolated from the fish totally lost the capacity to aggregate their melanosomes in response to K⁺—rich saline but they responded to adrenaline with a rapid melanosome aggregation (Fig. 1).

![Figure 1: Ineffectiveness of K⁺ but effectiveness of adrenaline (A) (5 x 10⁻⁶M) to arouse rapid melanosome aggregation (decreased melanophore index) in melanophores obtained from a reserpine fish. (n=5; SEM values between 0.04 and 0.10 M.I.-unit).](image)

Type 2 melanophores in the scales from antero-dorsal part (See Methods) had M.I. less than 2 or even fully aggregated (M.I. = 1). At this stage they appear very dark (black). Gradually they start showing dispersion and in approximately 20 min all melanophores attain their maximal dispersion showing brownish tinge with homogenous melanosome distribution, which served as the starting point for further investigations. The pigment-dispersing state persisted unchanged for at least 2 hr. A constancy in the shape, size, and melanin content (visual estimation) rendered the type 2 melanophores suitable for experiments.
The melanophores in isolated scales not only maintained their dispersed state in the physiological solution but in response to K⁺ or adrenaline they achieved pigment aggregation. Fig. 2 illustrates typical responses to K⁺-rich saline (133 mM) and its blockade by phentolamine (10⁻⁴M). High K⁺ solution was able to produce complete melanosome aggregation within 5 min. Recovery from pigment aggregation proceeded rapidly after the withdrawal of K⁺ stimulation and melanophores attained initial state of pigment dispersion within 15 min. High K⁺ solution could repeatedly induce comparable melanosome aggregation.

The K⁺-action was not blocked by propranolol (10⁻⁴M), a β-adrenoceptor blocking agent. Fig. 3 indicates a typical graded-aggregation response to increasing concentrations of adrenaline on the same melanophore population (5 in number) from an isolated scale of a fish.

The melanin aggregation induced by adrenaline was completely antagonized (Fig 4) by phentolamine, (10⁻⁴M; exposure time, 10 min). However propranolol (10⁻⁴M) had no blocking action on the aggregation response to adrenaline (10⁻⁶M). Withdrawal of Ca²⁺ had practically no influence either on the pigment-aggregating action of adrenaline or on the recovery from this effect. Loss of response to K⁺-rich saline and pigment aggregation in response to adrenaline (5×10⁻⁵M; Fig. 1), blockade of response to adrenaline by phentolamine but not by propranolol were similar in denervated and innervated melanophores. After K⁺ stimulation when the scale was reimmersed in the physiological saline for a long period (90 min), a profound dispersion of melanosomes was observed, the phenomenon being more pronounced in the innervated than in denervated preparations.
DISCUSSION

Our prime concern in studying K\(^+\)-action on melanophores in *Labeo rohita* was to gather information about the nature of the chromatic fibres. K\(^+\) always induced a rapid and reversible pigment aggregation in innervated melanophores. The reaction time observed was comparable to that reported for other fish species (1, 8). Secondly, in most of the denervated melanophores, the high K\(^+\) stimulation could not induce pigment aggregation indicating that K\(^+\) acts indirectly through a neuronal mechanism. Thirdly, the pigment aggregation by K\(^+\) was not blocked by propranolol, showing that it is independent of \(\beta\)-adrenoceptors. Adrenaline could induce pigment aggregation in denervated melanophores. Adrenaline acts on the nerve endings as well as directly on the melanophore themselves (9). Treating the fish with reserpine, which depletes the neurotransmitter from the adrenergic system makes K\(^+\)-stimulation ineffective, though adrenaline could induce pigment aggregation. This makes it clear that K\(^+\) stimulate the melanin-aggregating adrenergic terminais to liberate the transmitter which then acts to aggregate pigment in melanophores. This is in agreement with reports involving related fish species (5, 7, 10-13).

Yamada et al. (14) looked into the possibility of adrenergic innervation to melanophores in scales of *Oryzias latipes* using \(^3\)H norepinephrine by light microscopic autoradiography. In their work K\(^+\) caused a considerable reduction in the labelling of varicose fibres, which was accompanied by aggregation of melanosome within melanophores.
One step ahead to it, Kumazawa and Fujii (15) using radiolabelled norepinephrine and adenosine clearly demonstrated the actual liberation of the amine and adenine derivatives from the neural elements in response to K⁺ stimulation. As this release was significantly reduced by bretylium they concluded that upon stimulation of the nerve, both the true transmitter, norepinephrine and the co-transmitter, adenine nucleotides, are liberated for the control of chromatophore movements.

From the present results we speculate that high (K⁺)₀ action initially induces a presynaptic membrane depolarization, leading to an increase in Ca²⁺ influx, finally resulting in release of the neurotransmitter from the adrenergic nerve terminals. Such a mode of action of K⁺ in systems other than the pigment cells is supported from the literature (16, 17). For the pigment cells, it has, however, been reported by Kumazawa and Fujii (18) for the first time.

Further, in the present observations, the aggregation of melanosomes due to K⁺ depolarization of the melanosomes was completely inhibited by phentolamine, an alpha-adrenoceptor antagonist which also inhibited the pigment aggregating action of exogenous adrenaline. Propranolol was ineffective against K⁺ or adrenaline. These results provide a strong basis that neurotransmitter responsible for pigment aggregation is norepinephrine and that the cellular receptors involved are α-adrenoceptors.

Adrenaline induced melanosome aggregation was not influenced by the Ca²⁺ deprivation. The response of innervated melanosomes evoked by the adrenaline was dose related between concentrations 5×10⁻⁹ and 5×10⁻⁸M (Fig. 3). It was interesting from a standpoint of comparative physiology of chromatophores that the above concentrations of adrenaline agreed closely with the threshold concentration and the maximally effective reported concentration in other fish species (11, 19 & 20).

Practically all the Indian teleosts tested for the chromomotor-colour changes (2, 21, 22) including one under the present study change their colour quickly when transferred to illuminated black background by dispersing their integumentary melanophores. This agrees with the concept of neural regulation of melanosomal movements described for Tilapia (15). Further the contention of spontaneous release of small amount of ATP in the bathing medium from isolated scale/split-fin preparations during unstimulated period which is responsible for maintenance of melanophores in dispersed condition seems to be true for our findings on the Labeo rohita and other Indian teleosts (22).

Kasukawa et al. (23) while working on Chrysiptera cyanea explained the extraordinary rapid rate of motility of its melanophores. The melanophores from Chrysiptera cyanea exhibit a very rapid motility (23) since they are small (about 50 to 80 μm in diameter) and the diameter of the cellular processes through which the melanosome travel is fairly large. In Labeo rohita, the melanophores are small in diameter but the cellular processes are not so thick as in the fish Chrysiptera, so the motility of the melanophores though rapid is not so high as in Chrysiptera.

We conclude that in Labeo rohita the melanophores are innervated by pigment-aggregating fibres which are adrenergic and melanophores have α-adrenoceptors. Fujii et al. (25) put the loach, Misgurnus anguillicaudatus to a group of fish in which chromatophores are primarily under the control of endocrine systems. In the present study the response to K⁺—stimulation and adrenaline were found to be very rapid, bringing about full aggregation of melanophores, confirming a neural control in Labeo rohita (See, 2).

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