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Effect of adrenergic receptors in melanophores of teleosts fish: *Rasbora elanga*

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Abstract
In the melanophores on isolated scale preparation of fish *Rasbora elanga*, adrenaline a non-selective adrenergic agonist produced dose dependent aggregation of melanosomes. The melanosome aggregation response of the amine was completely blocked by Yohimbine (10-5 M), but propranolol (10-5 M) a non-selective β- antagonist failed in blocking such a response of amine, where it produced its normal response.
The results presented suggest that the melanosome aggregation in melanophores of the fish can be mediated by α_2 adrenergic receptors. These receptors are known to couple with Gi proteins that utilize the enzyme adenylate cyclase to decrease the intracellular concentration of cAMP resulting in retrograde transport of melanosomes to be accumulated at the cell centre. β-receptors have also been implicated to mediate melanosome dispersion by stimulating Gs protein in various fish species.

Keywords: Melanophores, aggregation, dispersion, adrenaline, yohimbine, propranolol

1. Introduction
The integumentary colour changes of animals represent some of the most dramatic example of adaptation to environment, its hues and patterns, by which they are able to cope with various ethological encounters. Research in integumentary colour changes and chromatophore physiology among the chordates has been carried out on lampreys, elasmobranchs, teleosts & amphibians and reptiles but the bulk of it has involved the fishes (Fujii 1969, 1993, 2000) [8, 10, 11].

The colouration in fish is labile and can be manipulated at the individual level both by morphological (Sugimoto 2002, Leclercq *et al*. 2010) [20] and physiological colour change (Aspengren *et al*. 2009 a, b). Chromatic responses are of importance for both camouflage and communication. This adaptation is possible because of the ability of these animals to shift pigment in certain special cells in their integument the chromatophores (Parker, 1948; Waring, 1963; Fujii, 1969, 2000; Bagnara and Hadley, 1973) [22, 26, 8, 11, 4].

Several monographs and reviews then were published on animal colour changes (Waring, 1963; Fingerman, 1965; Pickford and atz, 1957; Fujii, 1969, 1993a, b; Fujii and Novales, 1972; Fujii and Oshima, 1986, 1994; Bagnara and Hadley, 1973; Chavin, 1972; Baker, 1991; Iwata and Fukuda, 1973; Kasukawa and Fujii, 1986; Obika, 1986; Abott, 1973; Nery and Castrucci, 1997; Grove, 1994) [26, 7, 23, 8-10, 12-14, 4, 18, 19, 21, 15] mostly in the second half of the 20th century. In many investigation on different species of fishes the results have suggested that the colour changes in teleosts are normally under the control of both endocrine and nervous systems (Fujii 1969, 1993a, b, 2000; Abbott 1973; Bagnara and Hadley 1973; Fujii and Oshima 1986, 1994) [8-11, 1, 4, 13, 14].

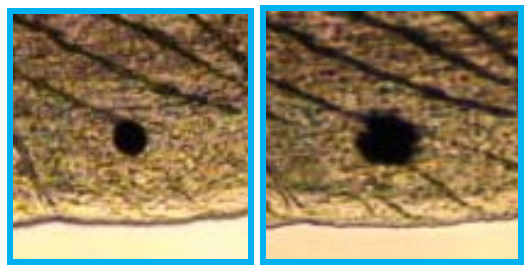
Teleost fishes show great variation in mechanism controlling their colour changes. The time taken by them in accomplishing the changes varies from species to species. The importance of time relation in the study of chromatic responses in teleosts has been realised from the work of Hogbeg (1924). Number of worker published monographs and review on this. Notable among them are Waring (1963) [26], Fingerman (1963) [6], Fujii (1969, 1993a, b, 2000) [8-11], Bagnara and Hadley (1973) [4] and Fujii and Oshima (1986, 1994) [13, 14]. On surveying the studies it was found that some fishes undergo paling at a faster rate than darkening and some others undergo darkening at a faster rate than paling. For example in Crenilabrus (Von Frisch, 1912) in which colour changes are chiefly in the red and yellow, these changes take place in the course of few seconds. Adrenoceptors have been reported are designated as alpha and beta

Adrenoceptors (Ahliqiust, 1948). The division of these adrenoceptors as α_1 α_2 (Langer, 1074) and β_1 β_2 adrenoceptors defined in terms of agonist potencies, β_1 adrenoceptors demonstrated equal affinity for adrenaline and noradrenaline while β_2 adrenoceptors displayed a higher selectivity for nor-adrenaline than for adrenaline.

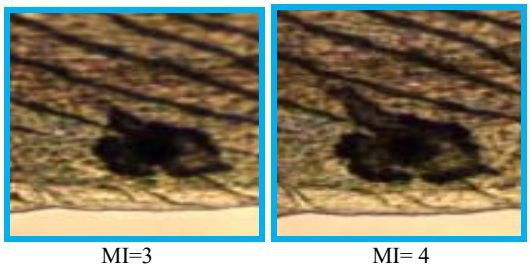
2. Materials and methods

Live specimens of *Rasbora elanga* (Hamilton) of either sex with average total length of 5-8 cm and average weight of 6-9 grams were procured with the help of a local fisherman from Ramsagar reservoir situated in Datia, Madhya Pradesh, India. On the day of their arrival to the laboratory, fishes were treated with water containing $KMnO_4$ to prevent them from infection. They were stocked routinely in transparent glass aquaria (30x30x60 cm.) for a weak at temperature 18-30 ^{0}C under natural photoperiodic condition. They were given a period of a week to get adapted to the given conditions (acclimatization), so as to make them suitable for experimental work.

The scale slips used in experiments conducted for this study were isolated from the dorsal trunk region of the animal. They were plucked and immediately perfused with the physiological saline which had the following composition in mm (NaCl: 12.8, KCl: 2.7, CaCl2:1.8, Glucose, 5.6 and Hepes NaOH with pH value 7.4). For each individual experiment 25 melanophores from 5 different scales belonging to different animals were observed. The effect of drug on the response of certain groups of melanophores were studied with light microscope and were evaluate according to Hogben and Slome (1931) in amphibian melanophores where 1, representing the maximum aggregation and 5,representing maximum dispersion and 2,3,4 as intermediate stage of aggregation dispersion.



MI=1 MI=2



MI=3 MI= 4

MI=5

3. Results

In the present study, concentration dependent response of adrenaline was recorded. The concentration 10^{-9} M was found to be minimal effective concentration to arouse a discernible melanosome aggregation within melanophores. Complete melanophores aggregation (M.I=1) was however, found at the concentration of 10^{-6} M. Recovery to the dispersion of melanosomes, here took about 20 min after drug withdrawal.

Yohimbine is an α_2 adrenergic antagonist. The melanosome aggregation induced by epinephrine on adrenoceptor agonist was antagonized by yohimbine. The antagonism was detected by first treating the dispersed melanophores equilibrated in the physiological saline equilibrated M.I. being 4.8 with yohimbine 10^{-5} M for 5 min. In the solution the melanophores further dispersed with the M.I.= 5. On subsequent treatment with epinephrine 10^{-6}M a blockade of its melanosome aggregating action can well be observed.

Propranolol the β blocker have greater specificity of action, as compared to the α blocker in terms of blocking noradrenergic receptors. Isolated scales pretreated with PS (M.I.=5) were incubated in propranolol (10^{-5} M) for 5 min. in which the melanophores retained the dispersed state of M.I. = 5. Afterwards, the melanophores were treated with adrenaline (10^{-6} M) for 5 min and full aggregation of the pigment with M.I.= 1 was attained. Then adrenaline was replaced by PS and the dispersed state of M.I. = 4.88 was attained in 25 min.

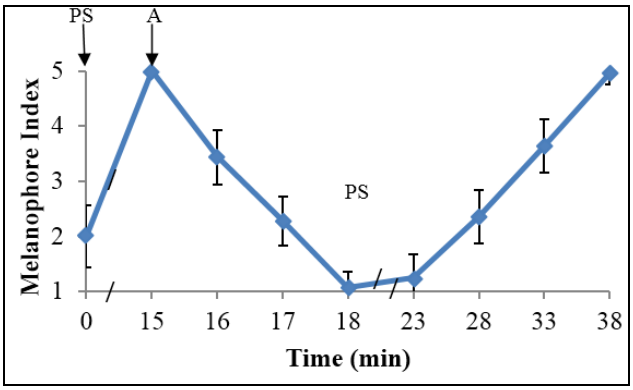


Fig 1: Aggregation of pigment in melanophores by treatment with Adrenaline (10^{-6} M), their recovery in PS after withdrawal of the drug.

The values are expressed as mean ± SD from five measurements on scales from five different fish.

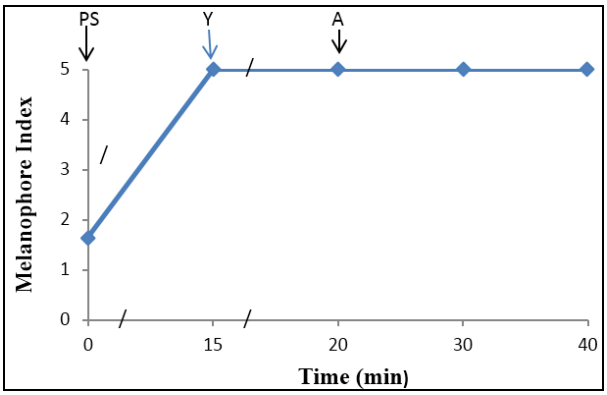


Fig 2: Complete blockade of the effect of adrenaline (10^{-6} M) (aggregation inducing agent) by the pretreatment with α_2 adrenoceptor blocker Yohimbine (10^{-5} M). The values are expressed as mean ± SD from five different fish.

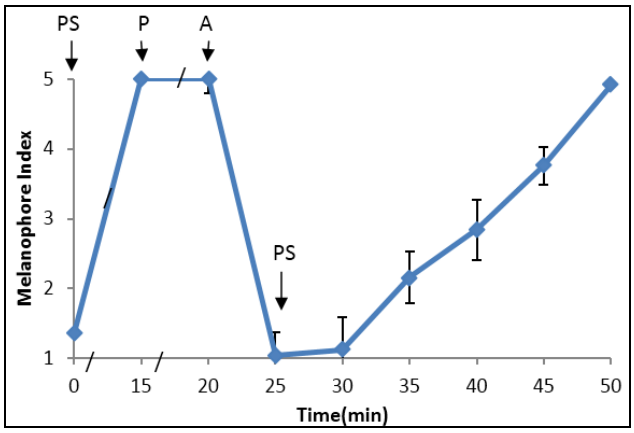


Fig 3: Effect of propranolol (10^{-5} M) β adrenoceptor blocker on the melanosomes aggregatory action of adrenaline (10^{-6} M) on the fish melanophores. The results are shown as mean ± SD from five measurements on scales from five different fish.

4. Discussion

The fibres on excitation due to action potential release a neurotransmitter in synaptic cleft, which is synthesized in the cell body and packaged into vesicles called synaptic vesicles. Here it binds to a receptor on the postsynaptic cell, exciting or inhibiting that cell. The effect to the neurotransmitter is terminated by its degradation in the cleft or by its reuptake into the presynaptic cell. Utilizing selective adrenoceptor antagonist, using an adrenergic blocking agent, yohimbine, it is attempted to characterize the adrenoceptor subtypes on the plasma membrane of melanophores in the fishes. They display high degree of antagonism specifically for α_2-adrenoceptors, as yohimbine completely blocks the pigment aggregating effect of adrenaline in *in vitro* responses (Fig. 2). This confirms the presence of α_2-adrenoceptors on the melanophores. Whether α_1 adrenoceptors are also present on the melanophore membrane (as has been demonstrated for some teleosts) remains to be determined. Propranolol a β – blocking agent was able to significantly reduce this acceleration of dispersion by β stimulants which also support for existence of β- receptors on melanophores (Fig. 3). The result with sympathomimetic drugs and sympatholytic drugs thus provide evidence for the mechanism of aggregation of melanosomes within melanophores of the fish, *Rasbora elanga* through post-ganglionic sympathetic pigment-aggregating nerve fibres with responses being mediated by α_2 adrenoceptors present on the plasma membrane of melanophores of the fish.

5. Acknowledgement

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