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Study the Effect of Alpha $ Beta Adenergic Agonist and Antagonist on Fish Melanophores

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Abstract: ***Isolated scale melanophores of Rasbora elanga were examined for the effect of various specific and non-specific agonists and antagonists prepared in physiological Ringer solution. The responses were recorded as Melanophore Index Number. It was observed that adrenaline, nor adrenaline, phenylephrine and clonidine induced melanosome aggregation in a dose-depended manner. Yohimbine strongly inhibited the melanosome aggregatory effect of adrenaline and clonidine. Hence, it may be concluded that pigment aggregating adrenoceptors are both α_1 and α_2 types but the melanophore membrane in this fish is endowed with α_2-adrenoceptors. The pigment dispersion on treatment with salbutamol and isoxsuprine, β_2-agonists indicate the presence of β_2-adrenoceptors on the melanophores of this species. Atropine, a muscarinic antagonist appears to act directly on the melanophores as its treatment caused rapid melanosome dispersion.***

Keywords: Melanophores, Aggregation Dispersion, Agonist, Antagonist

1. Introduction

The chromatophore unit concept is applicable to the variety of chromatophore associations in the coho salmon, *Oncorhynchus kisutch*. Individual pigment cells of three general types—melanophores, xanthophores, and iridophores—vary structurally according to skin location, age, and physiological state. In growing fish, melanophores have a bimodal size distribution; in adults, they have a normal size distribution. Both melanophores and xanthophores are dendritic cells which respond to potassium and sodium ions by respectively aggregating and dispersing pigment granules. The third cell, the iridophore, is characterized by reflecting platelets of birefringent crystals of guanine of hypoxanthine and has at least two distinct shapes. In the upper dermis of the dark-colored skin, globular iridophores are encompassed by the dendritic arms of subjacent melanophores.

In many teleost species, it has very clearly been demonstrated that melanophores are primarily controlled by ANS and the nerves that function by aggregating the pigment are sympathetic. von Frisch (1911) (Fig.1) showed the melanin aggregating nerves pathway from melanosome-aggregating centre to melanophores in the minnow, *Phoxinus laevis* and this scheme is still applicable to many teleostean species without major modifications (Grove, 1969a, b; Fernando and Grove, 1974a, b; Jain and Bhargava, 1979). As innervations to chromatophores has been thought to be sympathetic post-ganglionic, the peripheral neurotransmitter signalling them was naturally supposed to be adrenergic, being probably norepinephrine (Fujii, 1961; Scheline, 1963; Scott, 1965). Using α-adrenolytics, the transmission was characterized to be α- adrenergic (Fujii and Miyashita 1975). Later Kumazawa and Fujii (1984) actually demonstrated the release of norepinephrine from the nervous elements in response to neural stimuli. Both α_2 (mostly) and α_1 (in same cases) adrenoceptors have been shown functional in melanophores (Fujii, 2000). Kumazawa *et al*. (1986), further detected the apparent liberation of ATP (as a co – transmitter) from chromatic nerves in *Tilapia*, in response to electrical stimulation and they concluded that the co-transmitter is released along with the principal transmitter, norepinephrine, there by proposing the dual transmitter theory for controlling nervously the fish chromatophores. The concurrent release of both the transmitters from fibres to chromatophores was then confirmed in experiments with radio labelled compounds (Kumazawa and Fujii, 1986). The true transmitters NE, acts to induce rapid melanosome aggregation via mediation of α_2 adrenoceptors on the melanophore membrane. ATP released concurrently in the absence of NE (being degraded at the synaptic cleft) is dephosphorylated by AT pase and then by 5-nucleotidase in the synaptic cleft. The resultant nucleoside i.e. adenosine reverses the influence of true transmitter and induces redispersion of pigment via its specific receptors on the membrane of melanophores (Fujii and Miyashita, 1976; Miyashita et al., 1984; Kasukawa et al., 1986; Oshima et al., 1989).

Physiological and pharmacological studied on the responses of teleost melanophores have indicated that peripheral nerve fibres controlling melanosome aggregation are adrenergic i.e., the transmitter concerned may be norepinephrine (Abbott, 1968; Falk et al., 1969; Fujii and Novales, 1972; Jain and Bhargava, 1979; Fujiiet al., 1980; Anderssonet al., 1984; Patil and Jain, 1989; Amiri, 2009). Karlsson and co-worker have suggested that the post junctional alpha adrenoceptors mediating pigment aggregation in melanophores from several species could be characterized as being of the α2 subtype (Anderssonet al., 1984; Karlssonet al., 1985, 1987, 1988). The presence of α2 adrenoceptors as mediators of melanosome aggregation in teleost has also been confirmed by many worker's such as Miyashita, 1987; Jain and Patil, 1992; Mayo and Burton, 1998; Burton and Vokey, 2000 and Acharya and Ovais (2007).

As pointed by Fujii and Oshima (1986), it may not be difficult for us to understand that the colour changes and the resultant chromatic state including colour patterns should be of an extraordinary importance for the animals lacking the ability of vocal communication in protecting themselves or in the survival of the species. Apparently many of such aquatic animals including fishes use primarily the visual

means of communication between or among individual of the same or different species of animals, even if their chromatic strategies form subject of mere interest for us. 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). In most of fresh water forms the colour change is restricted in a black- grey-white series, where upon the shade of the body surface either gets lighter or darker depending upon the stimulus that the fish receives. Certainly it is the melanophores which are the most conspicuous and dominant among all their collegue, that play a major and most important role in bringing out the desired chromatic response.

2. Materials and Methods

The fresh water Indian teleosts, the *Rasbora elanga* (comman name Bengala barb) of either sex were used as the experimental material. The native habitats of these fish is rivers, pools beels, streams and others. Inhibits rivers throughout Bangladesh (Rahman 1989 and 2005). Found in almost every district of Bangladesh. These are found in India, Bangladesh, Myanmar and Pakistan. Endangered in Bangladesh due to loss of habitats (IUCN-Bangladesh 2000). These fishes were originally described by Hamilton (1822). Body elongate and slender with very small mouth, Single pair of short rostral barbels and body colour is silvery. Lateral line is complete and descends gradually. Fishes are omnivorous. They can be found in large schools feeding at the surface on algae, small aquatic insects, protozoa, mud and sands (Shafi and Quddus 2001). Peoples do not like it because of its nasty habits such as feeding on sputum and living in nasty areas, however some poor rural people take it as food. Fish were procured with the help of a local fisherman from Ram sagar reservoir situated in Datia (M.P.). The fishes were used of either sex with average weight and size. The fresh water teleost fish, the *Rasbora elanga*, with mean overall length of 5-6 cm. and a mean weight of 5 grams respectively were used in the present study. 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 18-30^0c under natural photoperiodic condition.

3. Results

3.1 Effects of adrenergic agonist

Adrenaline is produced by adrenal medulla and account for approximately 80% of catecholamine of adrenal medulla. Chromaffin cells are also the source of adrenaline. Adrenaline is a hormone and a neurotransmitter as well. Its effects on some body systems depend on the concentration of adrenaline as well as the type of receptors. At low concentration β effects are predominant. Because of its properties to produce response in effector cells by directly interacting with α-adrenoceptors it is also referred to as directly acting adrenomimetic drug. The action of nor-adrenaline are carried out via binding to adrenergic receptors ($\alpha_1\alpha_2$ and $\beta_1\beta_2$). Noradrenaline is a catecholamine with dual roles as a hormone and neurotransmitter. It deffers from the adrenaline only by methyl substitution in amino group. Thus it directly acts as an adrenomimetic drug also. It has high affinity for α and β_1 adrenoceptors and low affinity for β_2 adrenoceptors .

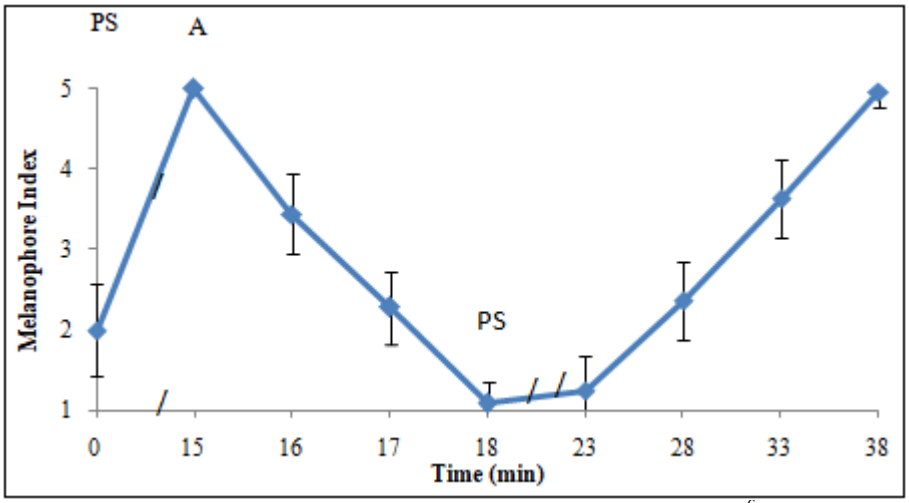

Figure 1: Aggregation of pigment in melanophores by treatment with Adrenaline (10^{-6} M), their recovery in PS after withdrawal of the drug. The values are expressed as mean ± SD from five measurements on scales from five different fish

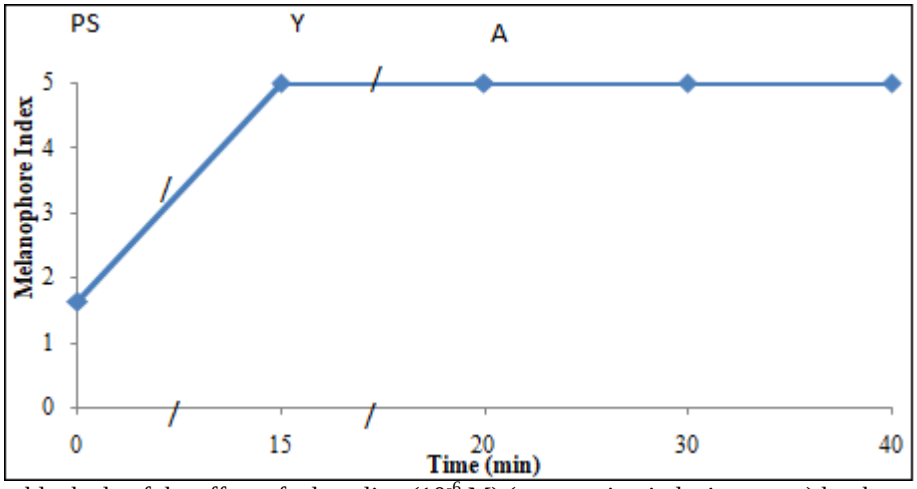

Figure 2: Complete blockade of the effect of adrenaline (10^{-6} M) (aggregation inducing agent) by the pretreatment with α_2 adrenoceptor blocker Yohimbine (10^{-5} M). The values are expressed as mean ± SD from five different fish.

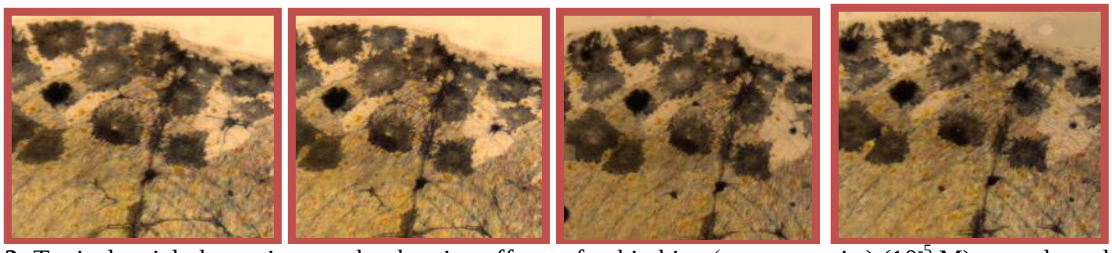

Figure 3: Typical serial photomicrographs showing effects of yohimbine (α_2 antagonist) (10^{-5} M) on melanophores in isolated scale preparation from fish × 100. (A). Equilibrated in Physiological saline (15 min) melanosomes are completely dispersed in the melanophore (B) 5 min (C) 10 min (D) 20 min after the application of adrenaline (10^{-6} M)

Adrenaline induces aggregation of pigment by activating α adrenoceptors (α_2 adrenoceptor activation lowers cAMP level in the cell).

Phenylephrine

Phenylephrine was first studied by Barger and Dale (1910). It is a powerful alpha-1 receptor stimulant. It has only little effect on the beta-receptors of the heart. A direct action on receptors account for the greater part of its effects. Only a small being due to its ability to release NA. It is not a catechol derivative so it is not inactivated by either COMT or MAO and has a much longer duration of action than the catecholamines.

This drug is a directly acting adrenomimetic amine that exerts its effect primarily through on action on alpha-1 adrenoceptors, although it has weak beta adrenoceptors activity as well. Phenylephrine was tested at varying concentrations ranging from 10^{-7} to 10^{-5} M. The drug induced concentration related aggregation of pigment in the melanophores. The responses were not found at the concentration of 10^{-8} M. The concentration of 10^{-7}, 10^{-6} and 10^{-5} M was 4.4 , 2.6 and 1.3 was attained respectively (Table 7) (fig.17.). It reflects that PE aroused weak responses at low concentrations and at 10^{-5}M, maximum aggregation was observed. The full recovery of the melanophores, aggregated by treating with phenylephrine (10^{-5}M), was observed in 20 min after treating with PS.

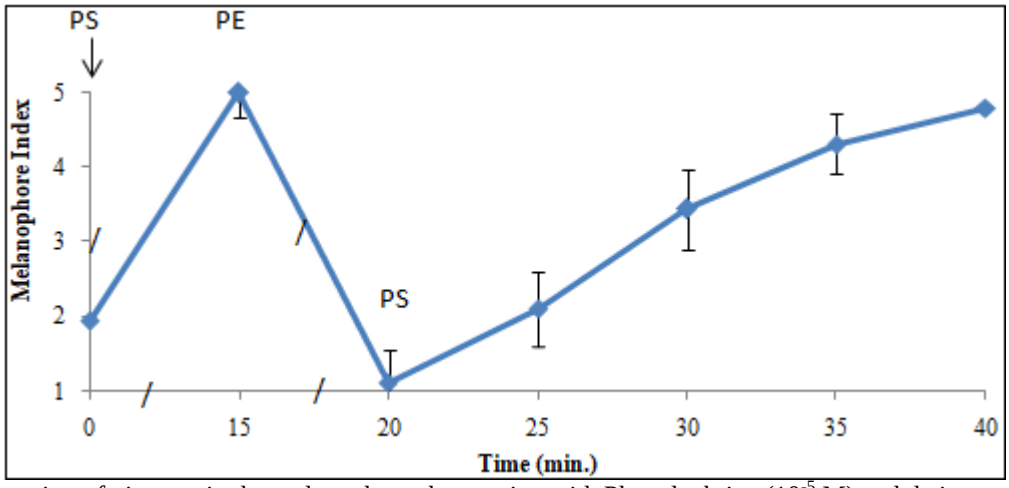

Figure 4: Aggregation of pigment in the melanophores by treating with Phenylephrine (10^{-5} M) and their recovery in PS. The results are shown as mean ± SD from five measurements on scales from five different fish

Clonidine

Clonidine was introduced for the treatment of hypertension in the United States in 1974. It is an imidazoline derivative that is structurally related to the alpha adrenoceptor antagonists, Phentolamine and tolazoline however, it is an alpha agonist acting directly on the receptors. It acts selectively on alpha-$_2$ adrenoceptors.

The melanophores of isolated scale preparation were perfused with PS for 15 min. to attain full dispersion (M.I.=5). These melanophores were then treated with Clonidine (10^{-7} to 10^{-4} M) and the response was found to be dose dependent. Minimum aggregation was aroused at 10^{-7} M. At the concentration 10^{-6} M and 10^{-5} M the M.I. values were 4.8 and 3.7 respectively. Maximum aggregation was observed at 10^{-4} M with M.I.=1.16 (Table 9) (fig. 19). The full recovery of melanophores aggregated by treating with Clonidine was observed in 25 min. after treating with PS.

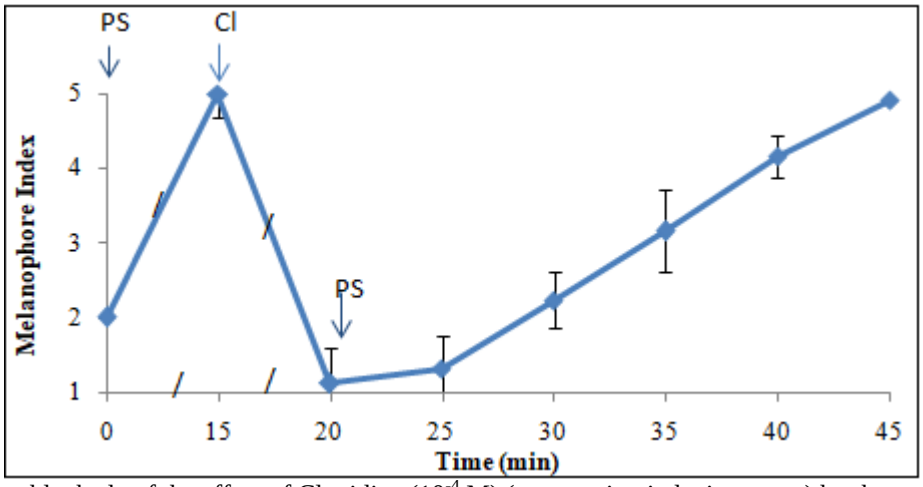

Figure 5: Complete blockade of the effect of Clonidine (10^{-4} M) (aggregation inducing agent) by the pretreatment with α_2 adrenoceptor blocker Yohimbine (10^{-5} M).The values are expressed as mean ± SD from five different fishes

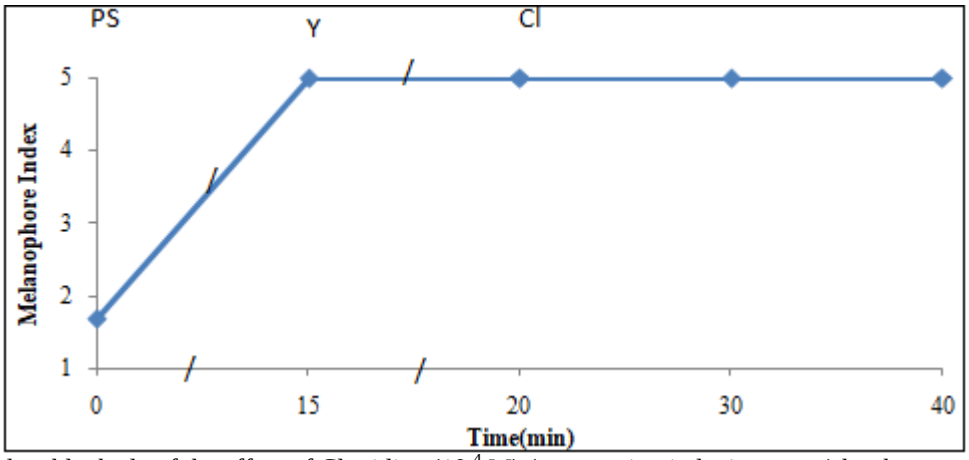

Figure 6: Complete blockade of the effect of Clonidine (10^{-4} M) (aggregation inducing agent) by the pretreatment with α_2 adrenoceptor blocker Yohimbine (10^{-5} M).The values are expressed as mean ± SD from five different fishes.

Effects of adrenergic antagonists

PrazosinIn the present study it was found that prazosin is ineffective to block the melanosome aggregating effect of phenylephrine. The agonist treated and PS equilibrated dispersed melanophore remain unaffected when treated with the antagonists prazosin (10^{-4} M) and the subsequent treatment of preparation with phenylephrine (10^{-5} M) induces rapid aggregating response demonstrating the failure of the blocking effect of the antagonist on these preparation.

The blocking behavior of Yohimbine was detected by first treating the dispersed melanophores (M.I.=5) equilibrated in the physiological saline with Yohimbine (10^{-5} M) for 5 min. In this solution the melanophores remain dispersed retaining the M.I. value of 5. When these melanophores were treated with adrenaline (10^{-6} M) / Clonidine (10^{-4} M) a complete blockade of melanosomes aggregating action was well observed.

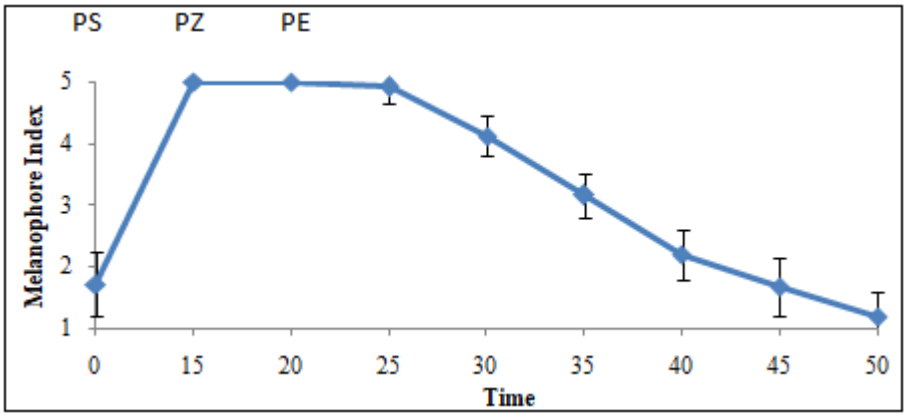

Figure 7: Effects of Prazosin (10-4 M) an α -1 adrenergic antagonist on melanosome aggregating effects of Phenylephrine (10-5 M) in PS- equilibrated (15 min.) melanophores of the fish. The values are expressed as mean ± SD from five measurements on scales from five different fish

The freshly 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.= 4.8 was attained in 25 min. Thus on comparing the effect of adrenaline with and without the pre-treatment with propranolol, it is evident that the drug in equimolar concentration aggregates the pigment not only faster but the melanophores also take less time to recover from the effect of the catecholamine in the adrenoceptors blocked pretreated scales.

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Propranolol It is a nonselective beta adrenergic antagonist without intrinsic sympathomimetic activity. It antagonizes catecholamines at both β_1 and β_2 adrenoceptors. It was the first β blocker introduced by synthesis in the U.S.A. The β blocker have greater specificity of action, as compared to the α blocker in terms of blocking noradrenergic receptors.

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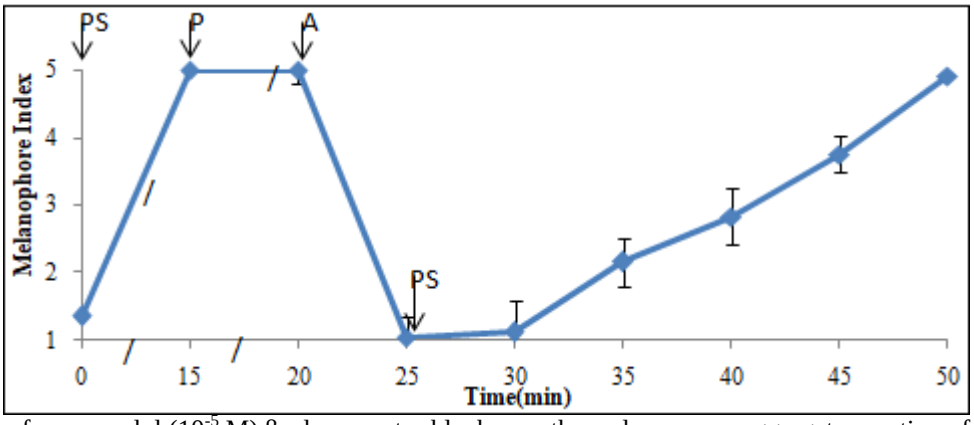

Figure 8: Effect of propranolol (10^{-5} M) β adrenoceptor blocker on the melanosomes aggregatory action of adrenaline (10^{-6} M) on the fish melanophores. The results are shown as mean ± SD from five measurements on scales from five different fish

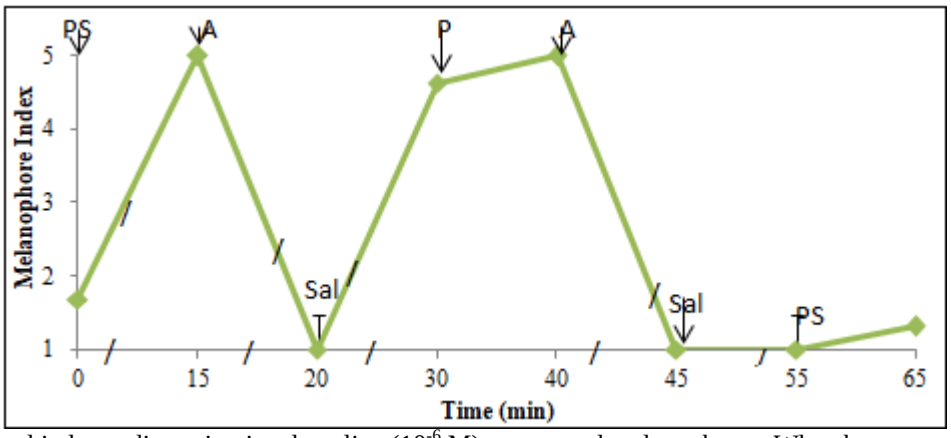

Figure 9: Salbutamol induces dispersion in adrenaline (10^{-6} M) aggregated melanophores. When beta receptors were blocked by treatment with propranolol (10^{-5} M) salbutamol failed to produce its effect. The results are shown as mean ± SD from measurements on five scales from the five different fish

4. Discussion

Melanophores of the fish differed in their response to different backgrounds i.e., becoming dispersed on a black background (black adapted) and becoming pale on a white background (white adapted). The Munsell Colour Index that is based essentially on the lines of the Derived Ostwald Index (D.O.I.) method proposed by Healey (1967) has been adopted for the *in vivo* studies such as background responses in the fish, keeping in mind the chromatic behavior of the fish during handling. The Melanophore-Index (M.I.) method derived from Hogben and Slome (1931) was used to record *in vitro* responses i.e., studies utilizing the isolated scales of the fish, *Rasbora elanga* to see the effects of various chemicals on the melanophores of the fish. The melanophore Index scale was prepared for the fish beforehand by aggregating the fully dispersed melanophores with an appropriate agent selecting 3 intermediate stages between fully dispersed (stage 5) and fully aggregated (stage 1) states of melanophores.

The body shade of the fish over a white and a black background ranges from M.C.I. 3.1 to 7.3 (fig. 1). Thus these are the grades between which the shade fluctuates during background adaptation of the fish. The response appears to be completed in two phases an initial faster one (about 15 min) (fig. 1) and then a prolonged slower one (about 3 to 5 hours) until a maximum response (24 hours) is achieved. The initial faster response indicates a predominant neural regulation. The hormonal control is synergestic being slow that takes time to set its effect.

The effects of various drugs and hormones know for their pharmacological and physiological effects in mammals and tested in varius groups of fishes as well, were examined for their *in vitro* studies using mlanophores on isolated scale preparations taken from dorso-lateral trunk region of the fish determine the nature of chromatic nerves, the nature of neuromelanophore transmission and to characterize the receptors involved in the neural and hormonal control of colour change mechanism in the fish, *Rasbora elanga*.

The results clearly indicated that the melanophores of the fish are innervated by sympathetic postganglionic pigment-aggregating nerve fibres only (Thus supporting mononeuronic hypothesis- Hogben, 1924; Waring, 1963; Bagnara and Hadley, 1973; Fujii, 1969) and that these fibres are adrenergic in nature.

All the sympathomimetic drugs, catecholamines (adrenaline, noradrenaline), clonidine tested induced aggregation of melanosomes within melanophores the catecholamines were most potent than other drugs and the results obtaines with these do suggest that either of them could serve as a chemical transmitter in an adrenergic system controlling the centripetal movement (aggregation) of the melanosomes (fig.2). The aggregating activity of sympathomimetic amines as well as adrenergic agonists (α_2- agonist clonidine) support the existence of sympathetic pigment aggregating fibres of adrenergic character controlling the active rapid aggregation of melanophores in the fish, which is mediated through the α-adrenoceptors.

On the different α-adrenergic antagonist (prazosin and yohimbine) yohimbine- the α_2- adrenoceptor antagonist has been found to be most effective drug in blocking the aggregation responses induced by α-adrenoceptor, specially of α_2- subtype. Prazosin an α_1 adrenolytic agent produced a weak response as comparaed to full blockage by α_2 antagonist – adrenoceptors on the yohimbine which certainly point that nervously evoked melanophore aggregation largely depends on activation of α_2 plasma membrane of melanophores (fig.6 and fig.7). However a mixed population of both α_1 &α_2 adrenoceptors in this study can not be denied with α_2 as dominant receptor types and study with more adrenergic agents can reveal the participation of α_1-receptors in inducing melanosome aggregation & resultant paling in the fish.

β- adrenoceptors mediating pigment dispersion has been reported in some fishes. The β- stimulants, isoprenaline in the present study well able to induce acceleration of dispersion over control by physiological saline indicating some role for β- receptors in the present fish. 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.

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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