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Study the Effects of Signal Transduction Pathway in Teleosts Fish Melanophores

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Abstract: ***The fresh water Indian neopterigian teleost fish deals with some aspect concerning chromatic regulatory mechanisms, within the boundary of the two points of view: one of these is the value of colour pattern, as signals to other animals for the purpose of recognition, stimulation or warning or for the purpose of concealment of predator of prey. In this field is included the phenomenon of rapid colour change. The other point of view concerns the pharmacological nature of fiber which controls the activity of melanophores. Theophylline is a competitive nonselective phosphodiasterase inhibitor, which raises intracellular cAMP, activates PKA. Theophylline down regulates inflammatory and immune cell function in vitro in animals with airway inflammation. Theophylline increase the intracellular concentration of cyclic nucleotides in airway, smooth muscle and inflammatory cells by inhibiting phosphodiesterase-mediated hydrolysis.***

Keywords: Melanophores, Aggregation, Dispersion, Adrenaline, Forskolin

1. Introduction

In fish, the bidirectional translocations of melanophores are fast and synchronized in melanophores and are regulated by hormones or direct innervation (Fujii, 1969). The melanophores or other dendritic chromatophores do possess molecular motors that early the eargo (the melanosomes in melanophores) along the cytoskeleton to disperse them throughout the cell or to aggregate them into the cell centre. The skin not only becomes pale but more transparent, when dark melanosomes are aggregated in the cell centre (centripetal movement) and increased body transparency can also contribute to background matching conversally, when melanosomes distribute themselves homogenously in the entire cytosol (centrifugal movement), in response to appropriate signals, the skin becomes dark. Fast long range and bidirectional melanosomes movements are microtubule-dependant in both fish and frog melanophores (Rodinov et al. ,1998 ; Aspengren et al. 2006) but they are better synchronized in fish (Nilsson et al.,2002) and move at a faster speed (0.5 – 1.5µm/s) (Aspengren et al., 2009). Contrasy presence of a pigment dispersing innervations for teleost melanophores had been highly controversial for quite a long time. The antagonistic melanosome dispersing fibres were naturally thought to belong to the parasympathetic system. The normal "resting state" of the teleost melanophores is that of melanosome dispersion. Contrasy presence of a pigment dispersing innervations for teleost melanophores had been highly controversial for quite a long time. The antagonistic melanosome dispersing fibres were naturally thought to belong to the parasympathetic system. The normal "resting state" of the teleost melanophores is that of melanosome dispersion.

Pigment dispersion is activated by an increase in cAMP levels while aggregation occurs when cAMP levels are reduced. Epinephrine binds to a cell surface receptor, which interacts with a G protein. G protein have GTP binding and GTP hydrolysis capabilities. G protein to which epinephrine binds is an inhibitory G protein. when it is activated it inhibits the enzyme adenylate cyclase. Active adenylate cyclase converts APT into cAMP. The cAMP activates cAMP dependent protein Kinase (PKA), cAMP levels drop PKA is inhibited and the pigment granules aggregate. α_1 receptor activation may cause breakdown of phosphate dylinositol in membrane through activation of phospholipase C there by including a change in intracellular Ca^{2+}. Fujii and Fujii (1965) first reported that Ca^{2+} is required for catecholamine release from the sympathetic nerve terminal in the goby, chosmichthys gulosus. Ca^{2+} and cAMP act in opposition to regulate pigment aggregation and dispersion in melanophores. Epinephrine binds on the cell surface receptor causing influx of Ca^{2+} into the cell from the extracellular space.

Recent studies clearly indicate that the parasympathetic system is not involved in the regulation of bidirectional pigmentary movements of chromatophores. Although parker (1948) strongly advocated its involvement in the dispersal of pigment in melanophores and could actually detect the presence of Acetylcholine in the skin of the Cat fish studied. However Fujii & Miyashita (1976) and Fujii et al (1982) could demonstrate cholinergic peripheral transmission to melanophores belonging to sympathetic system in some siluroid cat fishes.

2. Materials and Methods

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 with mean overall length of 5-6 cm. and a mean weight of 5 grams respectively were used in the present study.

The scale slips used in experiments conducted for this study were isolated from the dorsal trunk region of the animal. The anterior unpigmented part of the scale remains under the glass needle and the posterior pigmentary part remains free for observation. They were plucked and immediately perfused with the physiological saline which had the following composition in mm (Nacl: 12.8, KCl: 2.7, CaC_{l2}:1.8, Glucose, 5.6 and HepesNaOH with pH value 7.4). For each individual experiment 25 melanophores from 5

different scales belonging to different animals were observed. All the experiment was performed at room temperature (20 ± 24^{0}C). The animals were maintained in the laboratory on commercial fish diet. Durring the experiment feeding was cut off. The aquria were cleaned regulary with the removal of faecal material and uneaten food by siphoning process. 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.

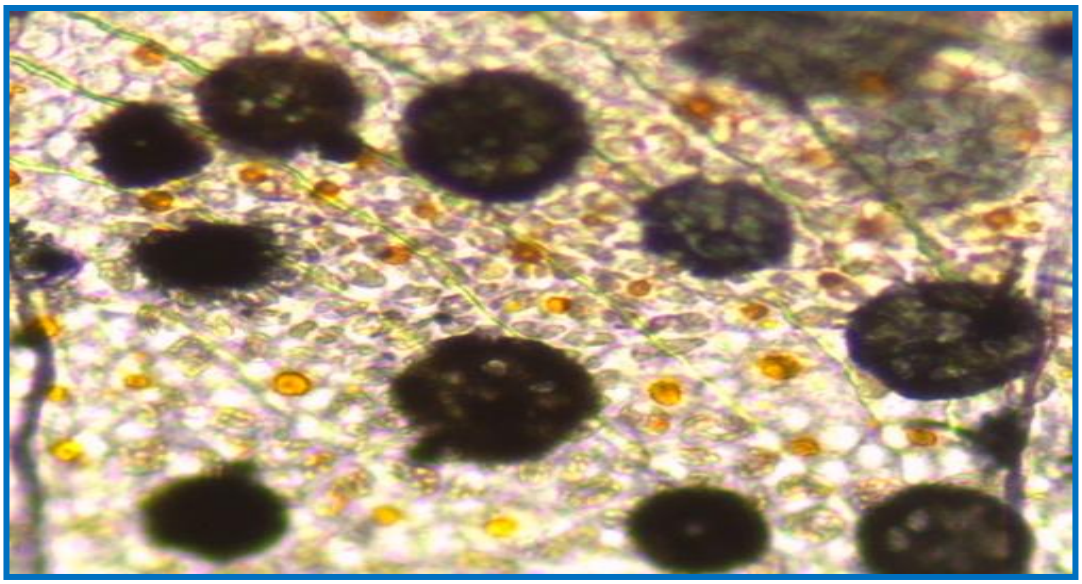

Figure 1: Photomocrograph showing full dispersion stage of Melanophores

3. Results

Effect of Theophylline on the Melanophores

Theophylline also known as dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases such as Asthma. A clinical study reported in 2008 that theophylline was helpful in improving the sense of smell in study subjects with anosmia. Theophylline is a competitive nonselective phosphodiasterase inhibitor, which raises intracellular cAMP, activates PKA. Theophylline down regulates inflammatory and immune cell function *in vitro* and *in vivo* in animals with airway inflammation. Theophylline increase the intracellular concentration of cyclic nucleotides in airway, smooth muscle and inflammatory cells by inhibiting phosphodiesterase-mediated hydrolysis. The freshly isolated scales were equilibrated in PS for 15 min so as to attain full dispersed state of the melanophores. The adrenaline was applied to melanophores of all five fish showed effective aggregation at 10^{-6} M with M.I.=1.08. When the perfusion fluid was changed to theophylline (10^{-5} M) melanophores get redispersed fully at 20 min. incubation time.

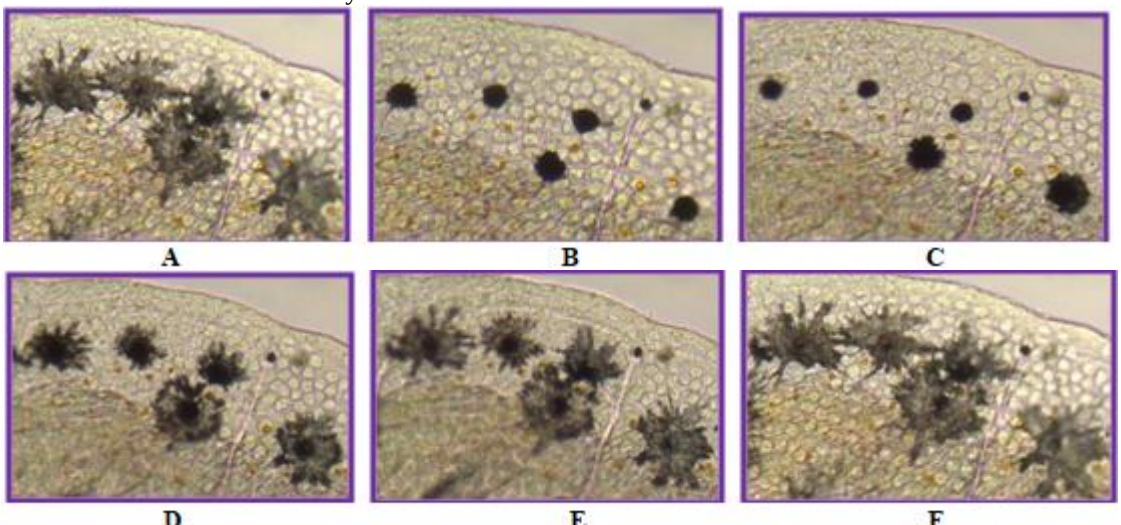

Figure 2 : Photomicrographs showing effects of theophylline (10^{-5} M) on melanophores . (A) Equilibrated in physiological saline (15 min) melanosomes are completely dispersed in the melanophores (B) 5 min after the application of adrenaline (10^{-6} M) melanophores are aggregated (C), (D), (E) and (F), 5, 10, 15, and 20 min after the theophylline treatment melanophores are dispersed. Theophyllone lowers the activity of the nucleotide phosphodiesterase with resultant increase in cAMP leading to pigment dispersion.

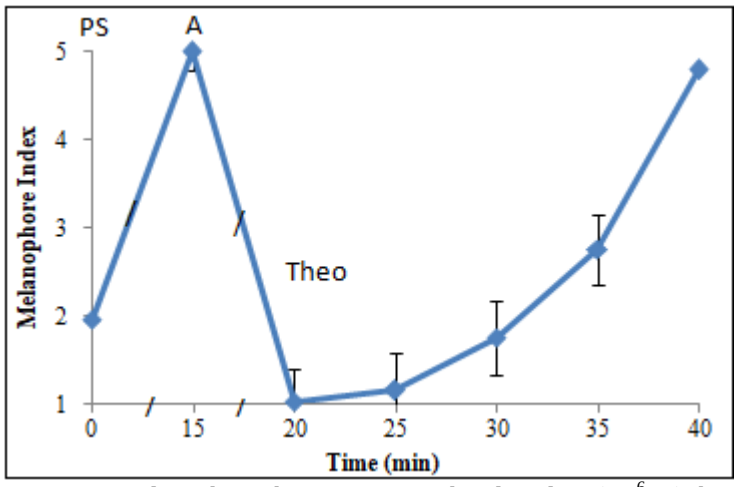

Figure 3: Aggregation of pigment in melanophores by treatment with Adrenaline (10^{-6} M) their recovery in theophylline, after withdrawal of the drug. The values are expressed as mean ± SD from five measurements on scales from five different fish

Effect of Adenosine on the Melanophores

Adenosine plays an important role in biochemical process, such as energy transfer as-adenosine triphosphate (ATP) and adenosine di-phosphate (ADP)-as well as in signal transduction as cyclic adenosine monophosphate(cAMP). It is also an inhibitor neurotransmitter believed to play a role in promoting sleep and suppressing arousal with levels increasing in the brain with each hour an organism is awake.

Adenosine (10^{-5} M) when applied to the melanophores whose pigment had been previously aggregated (M.I.=1) through treatment with adrenaline (10^{-6} M), melanosomes within the cells dispersed fully (M.I.=4.8) with in 20 min. It was observed that adenosine (10^{-5} M) accelerates the melanosome dispersion as observed in adrenaline (10^{-6} M) treated melanophores of the fish.

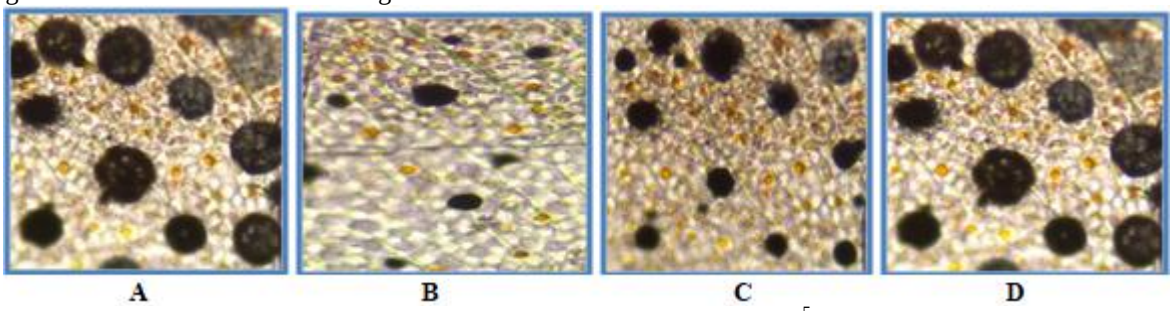

Figure 4: Typical serial photomocrographs showing effects of adenosine (10^{-5} M) on the melanophores × 100. (A) Equilibrated in physiological saline (15 min) melanosomes are completely dispersed in the melanophores (B) 5 min after the application of adrenaline melanophores are aggregated, (C) and (D) 10 and 15 min. after the adenosine treatment melanophores are dispersed. Adenosine induces dispersion by elevating cAMP level in the cells.

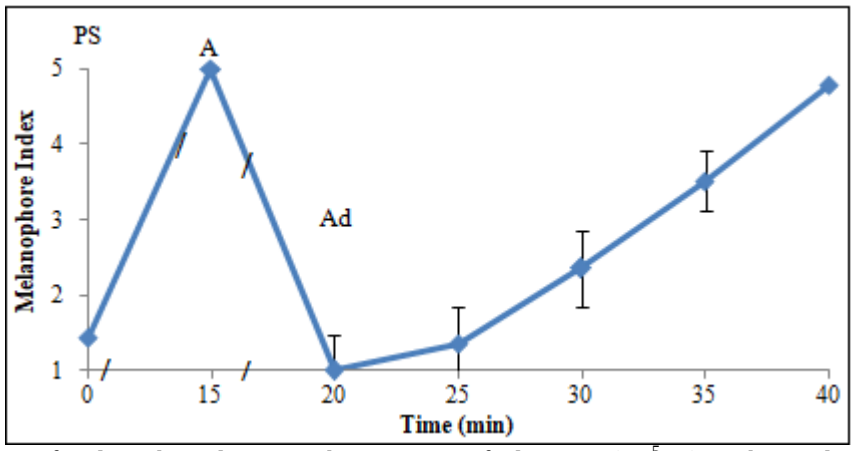

Figure 5: Acceleration of melanophore dispersion by treatment of adenosine (10^{-5} M) as observed in adrenaline (10^{-6} M) treated aggregated melanophores of the fish. The values are expressed as mean ± SD from five measurement on scales from five different fish

Effect of Forskolin on the Melanophores

The principle mechanism by which forskolin exerts its activity is by stimulation of AC, thereby increasing cellular cAMP, which is involved in processes such as glycogen and lipid metabolism. Chemical modification of forskolin at the 6- and 7-positions has led to semisynthetic compounds, with modest selectivity for particular cyclase isoforms, including the cardiac type 5 AC.

Stimulation of AC is thought to be the mechanism by which forskolin relaxes a variety of smooth muscles; however, forskolin has been found to act through other systems, including glucose transport and ion channels.**Seamon 1981**, **Insel 2003**, **Alasbahi 2010**, **Iwatsubo 2003**, **Wessler 2003**

The freshly isolated scales were equilibrated in PS for 10 min so as to attain full dispersed state of the melanophores. The adrenaline was applied to melanophores of all five fish showed effective aggregation at 10^{-6} M with M.I.=1.08. When the perfusion fluid was changed to forskolin (10^{-5} M) melanophores get redispersed fully at 20 min. incubation time.

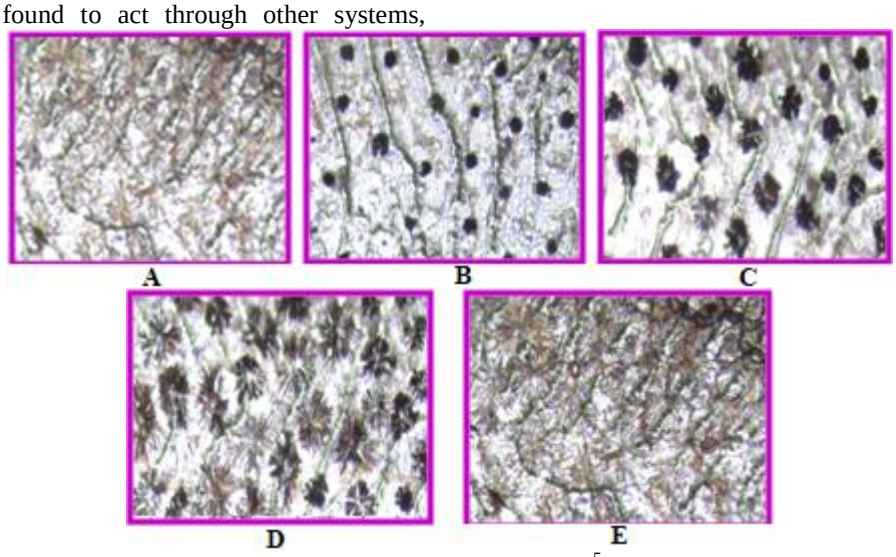

Figure 6: Typical serial photomicrographs showing effcet of forskolin (10^{-5} M) on melanophores x100. (A) Equilibrated in PS (10 min), melanosomes are completely dispersed in the melanophores, (B) 5 min after the application of adrenaline melanophores are aggregated (C) 5 min after the forskolin treatment dispersion are started in melanophores (D) and (E) After 5 and 10 min, melanophores are fully dispersed

4. Discussion

A complex language of colour appears to exist in the fish under study like many other reef fishes. This manner of communication helps in recognizing and keeping mates and other conspecifics, camouflage, predation and territoriality. All are important and have relevance to social complexity. Body colouration of the fish has shown that colour can act as an external signal displaying capacity of an individual are establishing the social rank as well as levels of androgen requires for these behaviours.

Von frisch (1911) and Burton (1964) had estabilished that the spinal chromatic tracts leave the spinal cord in the region of vertebra 12 (in *Phoxinus phoxinus*) and pass into the sympathetic chain. The fibres then pass coudally and in the chain to innervate the skin by way of spinal nerves. Grove (1969) concluded that in this fish acting the fibres leaving the spinal cord are preganglionic and synopse with the post ganglionic adrenergic nerves in the sympathetic ganglia. According to him this synopse is probably cholinergic, since the mammalian ganglionic blocking agents hexamethonium and presidal darken intact white adapted minnows (Healey & Ross, 1966).

Pigment dispersion is activated by an increase in cAMP levels while aggregation occurs when cAMP levels are reduced. Epinephrine binds to a cell surface receptor, which interacts with a G protein. G protein have GTP binding and GTP hydrolysis capabilities. G protein to which epinephrine binds is an inhibitory G protein. When it is activated it inhibits the enzyme adenylate cyclase. Active adenylate cyclase converts ATP into cAMP. The cAMP activates cAMP dependent protein kinase (PKA), cAMP levels drop PKA is inhibited and the pigment granules aggregate. α_1 receptors activation may cause breakdown of phosphatedyl inositol in membrane through activation of phospholipase C thereby inducing a change in intracellular ca^{2+}.

The pigment aggregation activity of melanophore have been shown to be controlled by sympathetic postganglionic fibres where peripheral transmission is proved to be adrenergic and receptors involved are demonstrated to be alpha adrenergic (Grove 1969 a; Reed and Finnin 1972; Fernando and Grove 1974 a; Fujii and Miyashita 1975 ; Fujii et al. 1980 ; Anderson et al. 1984 ; Kumazawa and Fujii 1984; Kasukawa et al. 1986 ; Patil and Jain 1989b ; Nagaishi and Oshima 1989; Zhong and Minnemann 1999).

A fundamental concept of pharmacology is that to initiate an effect in a cell, most drugs combine with some molecular structure or receptor on the surface of cell. Receptors can be classified on the basis of their response to drug that activate or inhibit them. An agonist binds to receptor to stimulate the some or to release a neurotransmitter . An antagonist binds to receptor to inhibit the functioning of the receptor. Two major types of receptors according to receptor and effector function are ionotropic receptors which gates ion channel

directly and metabotropic receptors which gates ion channel indirectly. The result with sympathomimetic drugs and sympatholytic drugs thus provide evidence for the mechanism of aggregation of melanosomes within melanophores of the fish, 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. The results obtained with regard to adenosine induced melanosome dispersion and blockade by phosphodiesterase inhibitor, theophylline do support the concept of release of a catecholamine as a principal transmitter and ATP as a co transmitter from the same nerve terminal (Fig 2) . Fujii and Miyashita (1976) first suggested that adenosine might take part in controlling pigment dispersion in fish chromatophores (Fig 4). They could conclude that pigment dispersing action is mediated by adenosine receptors since those effects could easily be antagonized by methylxanthines, specific blockers of adenosine receptors. ATP has been shown to release from chromatic nerves in response to electrical stimulation. They concluded that ATP is released as a co-transmitter from post-ganglionic sympathetic fibres together with true transmitter, norepinephrine.

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