Effect of various drugs on isolated scale Melanophores of fishes-A Review

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ABSTRACT

Fishes are blessed with specialized cells known as melanophore, which holds hundreds of melanin filled pigment granules, termed as melanosomes. Melanophores transport their pigments in reaction to extracellular cause, neurotransmitters either towards or away from the cell centre in fishes. The scrutiny on the captivating aspect regarding animal behaviour that deal with physiological colour changes, physiological responses, nature of chromatic fibers and occurrence of receptors on the dominant melanophores in fishes in response to various drugs has been taken up to review different mechanisms relating to this aspect in different species of fishes.

Key Words: Fish, Melanophore, Pigments, Drugs, Behaviour

INTRODUCTION

Colouration displayed by a variety of living creatures has been a topic of interest and research in biology for well over a century. A considerable number of the higher metazoan has a labile colouration responsible to relevant environmental changes and this is essentially physiological. The physiological colour change is due to the redistribution of pigment granules within the pigment cells of the organism. This ability permits the animal to modify colour to match the background; functioning as an adaptation allowing the prevention of prey detection or concealment of predator and using this for communication with conspecifics. Thus animal colouration may be considered from two different points of view, one is the value of colours and colour patterns to the animals as signals to other animals for the purpose of recognition, advertisement or warning or for the purpose of avoiding attack by predators and to obtain prey more easily for survival. This is due to the phenomenon of rapid colour change. The other point of view concerns the nature of colouring matters i.e., the pigments, their chemistry and physics, origin of colours from food, and fate of these pigments, if they are to be got rid-off etc.

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Aristotle who is often referred to as the "Father of Natural History" described the colour changes of an Octopus and Chameleon in his famous book "History of Animal". Although the most perfect physiological colour change is shown by chameleon, it cannot be matched with some teleost fishes to change colours and pattern to match their environment. Fish and some of their non-piscine predators (birds) have very acute vision and good ability to distinguish colour and pattern. All these rapid changes of colour are due to changes in shape, position, or nature of pigment cells controlled by nervous and endocrine systems.

Considerable number of examples among the higher animals ranging from cephalopods, crustaceans to the poikilothermic vertebrates displays the excellent background-related chromatic adaptations (Parker, 1948; Waring, 1963; Fingerman, 1963; Bagnara and Hadley, 1973). Among the poikilothermic vertebrates, teleost is the most widely studied group of animals with regard to mechanism of adaptation to a particular background (Fujii, 1969, 1993a, b, 2000; Fujii and Oshima 1986, 1994). The chromatic systems evolved in fishes are quite complex; many fish species display variable colours and colour patterns (Voss, 1970; Bhargava and Jain, 1974; Lanzing and Bower, 1974; Kohda and Watanabe, 1982a; Kasukawa and Oshima, 1987). These chromatic phenomena have great importance
in social communication besides providing protection and assistance in the survival of species in their habitats. Lot of attention has been paid by many ethologists for examining this aspect of pigmentation (Bearends and Bearends, 1950) and some of them are further trying to explain the formation and disappearance of those patterns in terms of differential neuronal control of chromatophores in a restricted area of the skin (De Groot et al., 1969; Lanzing, 1977; Bauer and Denski, 1980; Burton, 1980; Douglas and Lanzing, 1981; Naitoh et al., 1985). Muske and Fernald (1987) showed that nervous cues control the very rapid appearance or disappearance of facial stripe, the ‘eyebars’ which signals territorial ownership and aggressive intent in territorial males.

The body colours of fishes are predominantly dependent on presence of pigmentary substances and/or the light-reflecting microstructures within the integuement (Needham, 1974; Fox, 1979; Fujii, 1993a, b). The compounds responsible for generation of colour are generally packed within specialized organelles, the “chromatosomes”, which are produced and stored in pigment cells called “chromatophores” in the skin. Usually chromatosomes are found in the cytoplasm of the chromatophores. However, they are sometimes detectable in the epidermal cells (keratinocytes) where they have been transferred from the epidermal chromatophores. Chromatophores in the skin tissues of fish are mostly present in the dermis and rather sporadically in the epidermis. The colours of the skin are generated by absorption, reflection and/or scattering of light of certain wavelengths by the contained pigment as well as other cellular microstructures. Each chromatophore is a small entity containing a single type of pigmentary materials.

In fishes chromatophores can be classified into six groups (Fujii and Oshima, 1986 and Fujii, 2000), as presented below in Table (1):

**Table 1: Types of chromatophores.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chromatophores</th>
<th>Organelle</th>
<th>Pigment</th>
<th>Colour</th>
<th>Nature</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Melanophores</td>
<td>Melanosomes</td>
<td>Melanin</td>
<td>Black/brown</td>
<td>Motile and dendritic in nature</td>
<td>Light absorbing</td>
</tr>
<tr>
<td>2</td>
<td>Xanthophores</td>
<td>Xanthosomes</td>
<td>Carotenoids/pteridines or both</td>
<td>Yellow, orange</td>
<td>Motile and dendritic in nature</td>
<td>Light absorbing</td>
</tr>
<tr>
<td>3</td>
<td>Erythrophores</td>
<td>Erythrosomes</td>
<td>Carotenoids/pteridines or both</td>
<td>Red</td>
<td>Motile and dendritic in nature</td>
<td>Light absorbing</td>
</tr>
<tr>
<td>4</td>
<td>Cynophores</td>
<td>Cynosomes</td>
<td>Yet to be characterized</td>
<td>Blue</td>
<td>Motile and dendritic in nature</td>
<td>Light absorbing</td>
</tr>
<tr>
<td>5</td>
<td>Leucophores</td>
<td>Leucosomes</td>
<td>Guanine/purine crystals/uric acid</td>
<td>White</td>
<td>Motile and dendritic in nature</td>
<td>Light reflecting</td>
</tr>
<tr>
<td>6</td>
<td>Iridophores</td>
<td>Iridosomes (reflecting platelets)</td>
<td>Purines (Guanine/adenosine, hypoxanthines/and uric acid)</td>
<td>Light reflectors</td>
<td>May be motile and dendritic, static and commonly round or polygonal</td>
<td>Light reflecting</td>
</tr>
</tbody>
</table>

With the diversity in the distribution of variety of pigment in the skin the resulting colours appear like a mixture of colours. For survival, fish requires the ability to change their hues and colours rapidly and effectively. The chromatophores thus allow the display of a number of intermediate hues almost at will due to the divisionistic effects made possible by them. Thus pigmentary system in fish has evolved predominantly in the dermis where controlling nerve fibers can more easily reach and control the activity of the chromatophores (Fujii, 1993a). In addition capillaries carry blood through the dermis. If the chromatophores are in epidermis, hormonal influences should be weak and delayed. Thus, both hormonal and nervous commands can move easily and rapidly reach the chromatophores when they are in the dermis.

Chromatophores have common ontogenetic origin as that of nerve cells and are thus fundamentally dendritic cells. Dendritic chromatophores include the melanophores, xanthophores, erythrophores, cynophores and leucophores. Most iridophores lack dendrites, thus they are round, oval or multilateral in shape. The pigmentary organelles (chromatosomes) migrate centripetally (aggregation) or centrifugally (dispersion) in response to various signals received.
by the cells. All types of chromatophores are somewhat controlled differently by actions of the complicated and elaborate endocrine and nervous systems. Thus they enable the fish to adapt to background hues over a wide range and to cope with various ethological encounters by delivering chromatic signals. The process, whereby some species can rapidly change colour through mechanisms which translocate pigment and reorient reflective plates within chromatophores and often use it as a type of camouflage, is referred to as “physiological” (transitory or chromomotor) colour change. In physiological colour change, the total amount of pigment and colour generating cells i.e., chromatophores remain unaltered but the intracellular distribution of the pigmented vesicles, changes. “Chromogenic” changes are the more primitive and widespread. All animals even humans are capable of chromogenic” change, which involves change in number/density of chromatophores and in net amount of pigments materials in the skin. These “morphological” colour changes occur slowly and frequently are regulated by humoral factors which in turn are regulated by biorhythms (lunar or annual periodicities or environmental trigger perceived through specific sense organs such as thermoreceptors, chemoreceptors by stimuli as smell, vision, taste etc.). A prolonged “physiological” colour change may lead to “morphological” colour change (Sugimoto, 2002).

Light, temperature, mechanical stimulus, electric current, pH, ionicity of media, hydrostatic pressure etc. influence the motility of the chromatophores (Fuji, 1969). Among these physical factors light is most important stimulus associated with background–related chromatic response which induces darkening or lightening skin tone to mimic the hue of the immediate environment. Waring (1963) divided melanophore response to light into two categories i.e. the so called primary (nonvisual) and the secondary (visual). The primary or nonvisual responses occur when light acts directly on the chromatophores, these chromatophores acting as independent effectors or the process brought about by light perception through some extrapleptic light receptors like the pineal. Primary colour responses are mainly observable during the embryonic and larval stages. The secondary or indirect responses are the result of perception of light by the lateral eyes and generally depend on the nature of the background (light-absorbing or light scattering). In teleost melanophores it is mediated through neural (rapid) and/or hormonal (slower) mechanisms. It is well established that when chromatophores are denervated or when a blinded or a blindfolded fish is examined, even normal chromatophores respond to light directly (Fuji, 2000).

Nervous control of colour change in fish was studied in the last century. Brucke (1852) was first to illustrate the colour changes of lizard, African Chameleon, concluding that the chromatophores are under the direct control of nervous system. For the first time Pouchet (1876), studied the colour change in certain teleosts like turbot and some flatfish which are under the autonomic nervous control and not the cerebro-spinal as originally suggested by Brucke.

Under the histological studies it was demonstrated that a network of nerve fibers are associated with teleostean melanophores (Ballowitz, 1893 a, b; Eberth and Bunge, 1895). These observations regarding the distribution pattern of chromatic nerves was proved by Falck et al., (1969) with histochemical studies on rainbow trout, Salmo gairdneri. They observed a plexus of varicose nerve fibres enclosing intimately the melanophores.


According to von Frisch (1911), in the minnow, Phoxinus phoxinus the pigment-aggregating axons originate from a centre in medulla and pass along the spinal cord to about the level of 15th vertebra, where they emerge and pass through the rami into the sympathetic chain. On entering to the sympathetic chain these fibres run anteriorly and posteriorly to emerge through all spinal (to supply the trunk and tail melanophores) and trigeminal nerves (to supply to head melanophores) to innervate the melanophores. Many other workers proved the anatomical arrangement for pigment-aggregating nerve fibres innervating melanophores in other teleostean fishes. (Adelman and Butcher, 1937; Healey, 1948; Scott, 1965; Wilhelm, 1969; Finnin and Reed, 1970; Khokhar, 1971; Iwata and Fukuda, 1973; Fernando and Grove, 1974a, b; Bhargava and Jain, 1978; Dwivedi, 1978; Jain and Bhargava, 1979; Rajiv and Bhargava, 1990; Kuruvilla, 1991).

However, the level of exit of these fibres from the spinal cord varies depending upon the species.

Parker (1948) claimed that teleost melanophores are inerverted by both pigment-aggregating fibres and pigment-dispersing fibres and postulated that pigment-aggregating fibres were sympathetic (adrenergic) and pigment – dispersing fibres were parasympathetic (cholinergic). Parker et al., (1945) and other workers (Gray, 1956; Healey, 1967; Kinosita and Ueda, 1970), reported that acetylcholine (Ach) could actually be detected in the skin of catfish without identifying chemically the transmitter substance. Visconti and Castrucci (1981) observed in gobioid and cichlid fishes that muscarinic as well as nicotinic blockers could retard the melanosome dispersion. Other workers, however,
have disagreed with Parker's conclusions and have postulated that teleost melanophores are innervated by sympathetic pigment-aggregating fibres only (Healey, 1957; Waring, 1963; Bagnara and Hadley, 1973; Fujii and Oshima, 1986).

The two types of adrenoceptors that have been reported are designated as α and β adrenoceptors (Ahlquist, 1948). Adrenoceptors are defined both on the basis of order of potency of a series of adrenomimetics and on the drug that antagonize sympathetic responses. Many workers have used pharmacological studies, to indicate that the peripheral nerve fibres controlling melanosome aggregation are adrenergic, that the transmitter involved may be norepinephrine (Fujii, 1961; Scheline, 1963; Grove, 1969a, b; Reed and Finnin, 1972; Fernando and Groove, 1974a, b; Fujii and Miyashita, 1975; Jain, 1976; Patil and Jain, 1989; Acharya and Ovais, 2007, Amiri, 2009). From all these studies it has naturally been supposed that adrenergic receptors mediating pigment aggregation in teleost melanophores are of alpha nature. On functional basis α-adrenoceptors have been divided into α1 and α2 subtype (Langer, 1974). Some of the researchers have demonstrated that receptors mediating the sympathetic melanosome aggregation are the α2-adrenoceptors (Andersson et al, 1984; Karlsson et al, 1987, Morishita, 1987; Jain and Patil, 1992; Burton and Vockey, 2000a). Since α2 agonist have been found to be more effective than α1 agonist and transmission is more easily blocked by α2 blockers than by α1 blockers. The α1–receptor activation results in the rise of cytosolic calcium concentration. It has been suggested that α2–receptor activation may cause breakdown of phosphatidyl–inositol in membrane through activation of phospholipase-C, thereby inducing a change in intracellular calcium. This effect does not appear to involve a change in adenylate cyclase activity or cAMP concentration in cells except in certain regions of brain (Hoffman, 1984). The α2 receptors are thought to lower intracellular cAMP by inhibiting adenylate cyclase via Gi stimulation. It was also found, however, in many investigations that a considerable aggregation of pigment occurred in response to α1 agonistic stimuli & α1-type adrenolytics always have inhibitory effects on that process. These observations indicate that in addition to α2–adrenoceptors, α1–adrenoceptor is functional at least in some cases (as reviewed by Fujii & Oshima, 1994 and Fujii, 2000). Further in some species the aggregation of pigment may be triggered by increase in levels of Ca^{2+} ions in the cytosol and in addition

Involvement of IP_3 (Inositol 1, 4, 5 triphosphate) in the aggregation of pigment in Tilapia melanophores have also been demonstrated (Fujii et al., 1991).

Miyashita & Fujii (1975) suspected a role for epinephrine in chromatic changes in fish. They thought that it is possibly secreted from the chromaffin cells of adrenal tissues. At physiological concentration the hormone disperses melanosomes in melanophores via β-adrenoceptors. Morishita et al., (1985) identified these receptors in Oryzias to be of β_2 type. Katayama et al., (1999) reported that both β_1 & β_2 adrenoceptors co-exist in marine gobies. The mechanism of dispersing action of epinephrine appears to be cAMP dependent.β_1 and β_2 receptors activation through adenylate cyclase results in increase of the conversion of ATP to cAMP—a second messenger for many hormone receptor interactions (Hoffman, 1984).

In “Orthodox” regulation (Kasukawa et al., 1986), the pigment aggregation activity of melanophores have been shown to be controlled by sympathetic post-ganglionic fibres where peripheral transmission is proved to be adrenergic and receptors involved are demonstrated to be α-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, 1989; Nagaishi and Oshima, 1989; Bylund et al., 1994; Zhong and Minnemann, 1999). β-adrenoceptors on the melanophores are implicated with their dispersion (an opposing response to that of α-receptors) in number of species (Reed and Finnin, 1972; Colley and Hunt, 1974, Miyashita and Fujii, 1975; Patil and Jain, 1991; Bylund et al., 1994; Katayama et al.,1999).

Using specific and non-specific adrenoceptor agonists and antagonists, Acharya and Ovais (2007) pointed out that in Orechromis mossambica α2-adrenoceptors are predominantly involved in the aggregatory responses of melanophores. The same has also been demonstrated in other teleosts by Morishita (1987), Martensson et al., (1990), Martinotti (1991), Fujii (1993a) and Burton and Vockey (2000b).
Fig. Fish *Belaniochelios melanopterus* behaviour

Fig. 1: (A) Photograph of freshwater teleost *Belaniochelios melanopterus*.

Fig. 1: Photograph of freshwater teleost *Belaniochelios melanopterus*.

(A) Fish adapted to White - background.

(B) Fish adapted to Black - background.

(C) Fish adapted to Black - background
Fig. 2: Typical series of photomicrographs of a single melanophore showing Melanophore Index (M.I.) As used for measurement of drug induced *in vitro* melanophore responses in the fish x 100.

(A) M.I.=5  (Fully dispersed state)
(B) M.I.=4
(C) M.I.=3  (M.I.=4,3 and 2 represent the intermediate state)
(D) M.I.=2
(E) M.I.=1  (Fully aggregated state)
Fig. 5: Typical serial photomicrographs showing effect of K⁺-Ringer on melanophores in an isolated scale preparation x 100.
(A) Equillibrated in physiological saline (15 min) melanophores are completely dispersed, (B) 1 (min), (C) 2 (min), (D) 3 (min), (E) 4 (min) and (F) 5 (min) after the application of K⁺-Ringer. (Melanophores are completely aggregated).
Fig. 9: Typical serial photomicrographs showing effect of epinephrine (5 x 10^{-6} M) on melanophores in an isolated scale preparation of the fish x 100. (A) Equilibrated in physiological saline (15 min) melanosomes are completely dispersed in the melanophores, (B) 1 (min), (C) 2 (min), (D) 3 (min), (E) 4 (min) and (F) 5 (min) after the application of epinephrine (5 x 10^{-6} M) Melanophores are completely aggregated.
Melanophores display a radial array of microtubules extending from perinuclear area of the cell where microtubule organizing centre is located and projecting into cell extensions. Microtubule with minus end converges at the cell centre and their plus end points towards periphery. Thus in this arrangement, motor protein kinesin moves towards plus end of the microtubule (away from centrosome) causing dispersion and dyneins which move towards centrosome cause aggregation. Regulation of pigment transport involves changes in level of cAMP which regulate activity of microtubule motors (Nascimento et al., 2003). Rapid transport in melanophores and erythrophores appears to occur in association with microtubules.

Adenosine and adenine nucleotides have been considered to take part in the control of effector activities (Sattin and Rall, 1970; Stone, 1981). In particular, the concept that some neuron which are non-cholinergic and non-adrenergic, might release a purine compound as a neurotransmitter, has become a matter of common knowledge (Burnstock, 1972). The presence of such a system has also been suggested in the neural control of chromatophore movements in fish (Fujii and Miyashita, 1976b). It has been shown that some purine compounds disperse pigment in chromatophores in at least some fish species (Fujii and Miyashita, 1976a; Ozato, 1977). Accumulated evidence indicates that adenosine or a related nucleotide is not a hormone but that it is released from neural elements either as a true transmitter or as a co-transmitter which modifies the former’s action (Miyashita et al., 1984). Later work by Kumazawa et al., (1984) has established the dual transmitter theory with a substantial difference, in terms of action, between the true transmitter (norepinephrine) and the co-transmitter (ATP). According to them, the effect of NE disappears very quickly while that of ATP lasts much longer. After the cessation of neuronal excitation, the latter can effectively reverse the action of former.

The melanotropic peptides play a major role in the control of vertebral integumentary pigmentation. The two physiologically important melanotropins are α-MSH (α-melanocyte stimulating hormone) and MCH (melanin concentrating hormone). In lower vertebrates like fishes, MSH is released from the intermediate lobe of pituitary gland. The action of MSH on fish melanophores is mediated by receptors that are specific to peptide. (Fujii & Miyashita, 1980; Sharma, 1990). Extracellular Ca²⁺ ions are required for this action of MSH (Fujii, 1993a). MCH—secreted from the posterior lobe of pituitary induces colour changes in fishes, and it was originally isolated from salmon pituitary (Kawauchi et al., 1983).

MCH was shown to elicit pigment aggregation in melanophores of almost all teleostean species when present at low concentrations (Wilkes et al., 1984; Oshima et al., 1985; 1986; Nagai et al., 1986 Jain and Patil, 1990), and was shown to act via MCH receptors on the cell membrane (Oshima et al., 1985). In frog and lizard melanophores, however, this peptide had no melanin-aggregating effect, but instead showed weak melanin-dispersing activity (an α–MSH–like activity), being approximately 600 – fold less potent than α– MSH on these melanophores (Wilkes et al., 1984). On the other hand cyclic melanotropin (Cys4, Cys 10) α–MSH has been reported to be a potent agonist of tetrapod melanophores, leading to skin darkening (Sawyer et al., 1982). Since MCH is also a cyclic peptide, Wilkes et al. (1984), thought that melanin dispersion in response to MCH at higher concentration might be caused through the activation of MSH receptors by MCH.

Work done in India with regard to fish chromatophores and colour change includes aspects like integumentary colour pattern (Bhargava and Jain, 1974; Dwivedi, 1978; Dandegoankar, 1980; Dubey and Jain, 1983; Anand, 1985; Dubey 1991), transitory colour change (Bhargava and Jain, 1978; Dandegoankar, 1980; Prabhakar, 1988), quantitative colour change (Dwivedi, 1976; Sharma, 1990) and circadian colour change (Bhargava and Jain, 1981), effect of pollutant on melanophores and colour change (Anand, 1985; Kulshrestha and Arora, 1988) and effects of other autonomic drugs on various fresh-water fish species that have been investigated from India include Nandus nandus (Jain, 1976), Rasbora daniconius (Dwivedi, 1978), Heteropneustes fossilis (Bhargava and Jain, 1978; Gupta 1988; Rajiv and Bhargava, 1990; Shrivastava, 1991; Gupta, 1992), Labeo rohita (Jain and Patil, 1990, 1992), Puntius conchonius (Khare, 1990), Puntius sophore (Dwivedi, 1995), Cyprinus carpio (pigment cell types i.e., melanophores from the fresh water fish, Balantiocheilos melanopterus, belonging to family cyprinidae).

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