EFFECT OF SOME ADRENOCEPTOR ACTIVATING AND BLOCKING DRUGS ON RESPONSE OF FISH MELANOPHORES

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ABSTRACT

The fresh water Indian neopterigian teleost, Cyprinus carpio, a common carp deals with some aspects 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 or prey. In this field is included the phenomenon of rapid colour change. The other point of view concerns the pharmacological nature of fibre which controls the activity of melanophores. In the present study on the fresh water fish Cyprinus carpio emphases on those actions of some drugs which interfere with peripheral adrenergic transmission. Drug can inhibit, block and potentiate adrenergic mechanism in a variety of ways such as by affecting norepinephrine synthesis, norepinephrine storage, norepinephrine release norepinephrine uptake, norepinephrine inactivation, presynaptic action of norepinephrine and postsynaptic action of norepinephrine including alpha and beta adrenergic blockade.

KEYWORDS: Adrenergic drugs, chromatophores, melanophores, dispersion, aggregation, Cyprinus carpio

It is well documented that in many teleosts rapid pigment aggregation within chromatophores is primarily controlled by sympathetic postganglionic fibres (Fujii, 1969; Fujii and Novales, 1969, 1972). Peripheral transmission has commonly been shown to be adrenergic (Fujii, 1961; Scheline, 1963; Healey and Ross, 1966) and the adrenoceptors involved have now been characterized as being of an alpha nature (Groove, 1969; Reed and Finnin, 1972; Fernando and Groove, 1974; Fujii and Miyashita, 1975). The study of colour changes of the chordates have been carried out on elasmobranches, teleost, amphibians and reptiles. Many reviews have appeared (Fange, 1962; Waring, 1963; Fujii, 1969; 1993a, b; 2000; Abbott, 1973; Bagnara and Hadley, 1973; Schliwa, 1984; Fuji and Oshima, 1986; Schliwa, 1986; Sherbrokeet al., 1988; Baker, 1991; Mayer and Rochow, 2001.

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).

In the present study on the fresh water fish, Cyprinus carpio emphases on those actions of some of the drugs on the neuro-melanophore preparations which interfere with peripheral adrenergic transmission.

MATERIALS AND METHODS

The Indian fresh water exotic carp, Cyprinus carpio (common carp) obtained from Govt. Fish farm which is located about 10 km from Gwalior and maintained in an aquarium for at least one week before experiment were used. 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 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°C).

The effect of drug on the response of certain groups of melanophores were studied with light microscope and were evaluate according to Hogben and Slone (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 (Fig.1).

Figure 1: Melanophore indices as were used for measurement of melanophore responses in the study. M.I=5 represents the full dispersion and M.I=1, the full aggregation stage. M.I=4, 3 and 2 represent the intermediate stages

Drug used: Epinephrine tartarate (M.I. Pharmaceutical works (P) Ltd. Kolkata; Atropinsulphate: (Neon laboratories Ltd. Thane), Phenylephrine hydrochloride (John Baker the Cojardto, USA), Isoxsuprine hydrochloride (Salvay Pharma India Ltd. Mumbai), Mephentermine (Wyeth Lederie Ltd. Mumbai), Terazosin (Pharmacia Health care Ltd. Gujarat) and Yohimbine hydrochloride (Paul Meuendarf, Germany).

RESULTS

Three types of chromatophores have generally been observed in *Cyprinus carpio*, *i.e.*, black melanophores, yellow and orange xanthophores and reflecting iridophores. These all types of chromatophores are distributed all over the body (Fig. 2).

Figure 2: Photomicrographs showing of various types of chromatophores in isolated scale preparation from dorsal region of the *Cyprinus carpio*. Melanophores (M) B. Xanthophores (X) C. Iridophores (I)

Effect of alpha adrenergic agonists

Epinephrine (non selective adrenergic-agonist) and phenylephrine (selective α1 agonist) showed a potent pigment aggregating effect on melanophores on *Cyprinus carpio* (Fig.3). The epinephrine at a concentration $10^{-6}$M induces rapid and quite potent aggregation of pigment in PS equilibrated melanophores. The phenylephrine at a concentration of $10^{-5}$M induce rapid aggregation in PS equilibrated melanophores(M.I. 1.5) (Fig. 4). This result indicate that epinephrine and phenylephrine both appeared to be a potent agent to induce melanosome aggregation

Figure 3: A typical response of innervated melanophores of *Cyprinus* to $10^{-5}$M epinephrine

Figure 4: A typical response of innervated melanophores of *Cyprinus* to $10^{-5}$M phenylephrine
Effect of alpha adrenergic antagonists

Effect of Terazosine and yohimbine

Terazosine is a close structure analogue of prazosin. It is less potent than prazosin but retain high specificity for α1 receptors. Epinephrine (10^{-6}M) treated melanophores attained an aggregation and on subjecting these to the treatment of terazosin (10^{-4}M), they gradually dispersed and at 30 min stage Thus it become clear that terazosin significantly accelerated the dispersion of melanosomes in the melanophores (Fig.5).

![Figure 5: Melanosome dispersing response of melanophores to Terazosine (10^{-4}M)](image)

Yohimbine is an α2 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 4.9 with yohimbine10^{-4}M for 10 min. In the solution the melanophores further dispersed with the M.I.= 5. On subsequent treatment with epinephrine10^{-6}M a blockade of its melanosome aggregating action can well be observed (Fig.6).

![Figure 6: Melanosome dispersing response of melanophores to Yohimbine (10^{-4}M)](image)

Effect of beta adrenergic agonists and antagonist

Mephentermine is sympathomimetic drug that acts both directly and indirectly. Epinephrine (10^{-6}M) treated melanophores attain an aggregation phase with M.I. value being 1 and on subjecting these to the treatment of mephentermine (10^{-4}M), they gradually dispersed and at 20 min stage, (M.I.=3.14). (Fig.7). Thus it clear that mephentermine significantly accelerated the dispersion of melanosomes in the melanophores.

![Figure 7: Melanosome dispersing response of melanophores to mephentermine (10^{-4}M)](image)

Isoxsuprine is a beta stimulator and it disperses the pigment in melanophores whose pigment had previously been aggregated (M.I.=1) through the treatment with epinephrine (10^{-6}M):melanosomes within the cells dispersed gradually to a great extent. Fig.8 illustrate these changes in the melanophore state showing dispersion of melanosomes in an isolated scale preparation. The melanophores treated with identical concentration of epinephrine and subsequently dispersed passively in the PS without isoxsuprine served as the control. This comparison makes it clear that the beta adrenergic agonist, isoxsuprine significantly accelerated dispersion of melanosomes within the melanophores actively through the activation of the beta receptors.

![Figure 8: Melanosome dispersing response of melanophores to isoxsuprine (10^{-4}M)](image)
DISCUSSION

In quantifying the responses of melanophores on isolated scale-slip preparation from the fish *Cyprinus carpio* to various drugs, the use of physiological saline and epinephrine in keeping melanophores to an initial state of their dispersion and aggregation respectively was found to be of great advantage. When a scale was isolated from the body and kept perfused in the physiological saline, the melanophore population with intermediate state of pigment dispersion gradually attained the state of nearly full dispersion within 15 to 20 min of incubation at room temperature. This is consistent with observations from several workers in this field on variety of teleost, as plucking of scales from the body results in severance of chromatic fibers belonging to as a component of the autonomic nervous system. The results pertaining to various drugs as experimented in the fish, do point for these chromatic fibers belonging to the sympathetic-adrenergic component. Thus, when melanophores get decentralized, the severed nerve terminals firing is prevented and in the absence of neurotransmitter, the effectors cells are likely to attain the state of passive pigment dispersion being bathed in the physiological saline (unstimulated phase).

The melanosome dispersion and the maintenance of this state in melanophores when preparations are bathed in physiological saline can also be explained as active dispersion under the influence of released co-transmitter ATP, as Kumazawa et al., (1984) had already reported in *Tilapia* that they could always detect small amount of ATP in the bathing media, even during the unstimulated state in the physiological saline alone. Thus they were of the opinion that ATP may be liberated as a co-transmitter from the postganglionic sympathetic fibers along with the true one, norepinephrine since the liberation of both kind of neurotransmitter was effectively blocked by bretylium, a post synaptic adrenergic blocking agent. That this is true also with reference to peripheral neural mechanisms controlling melanophore movement in the fish under present study i.e., *Cyprinus carpio* remain to be determined.

Our observation pertaining to the effect of epinephrine do support the concept that rapid whitening reaction of poikilothermic vertebrate including the bony fish are due to pigment aggregation within integumentary melanophores elicited by excitation of the sympathetic nervous system.

In the results presented, epinephrine, as a nonselective adrenergic agonist appeared to be a potent agent to induce melanosome aggregation. Phenylephrine was able to induce aggregation of melanosome within the melanophores. The potent melanosome aggregation induced by epinephrine and other agonists in melanophores of *Cyprinus carpio* and the effectiveness of terazosine and yohimbine to inhibit completely the effect of former demonstrate further, that, melanophores of this teleost, as generally the case is in other teleosts, are adrenergically innervated and the neuro-melanophore signal transduction is mediated through postsynaptic alpha adrenoceptors (Fujii and Oshima, 1994).

Postsynaptic alpha adrenoceptors in the mammalian tissues, based on their preferential affinity to specific adrenergic agonists have been subclassified into α₁ and α₂ receptors and adrenoceptors that show a high affinity toward clonidine and norepinephrine and much less toward phenylephrine are of α₂ subtype (Starke et al.,1975). Using similar approach Anderssonet al., (1984) were first to conclude that alpha adrenoceptors mediating melanosome aggregation in melanophores of the *cuckoo wrasse*, *Labrus ossifagus* are of α₂ subtype. Since then, melanosome aggregation within melanophores of several teleostean fish has been subclassified as α₂ subtype (Karlssonet al., 1987; Morishita, 1987; Jain and Patil,1992; Mayo and Burton,1998; Burton and Vokey, 2000 and Acharya and Ovais 2007; Amiri, 2009).

Mephentermine at $10^{-4}$M concentration could induce melanosome dispersion in epinephrine (10^{-4}M) aggregated melanophores. This effect of the drug can only be explained by its stimulation of beta adrenoceptors on the melanophore membrane. However, the drug is also known to release the norepinephrine which is a potent agonist of alpha-receptors but also is reported to be agonist of β-receptors and observed effect of mephentermine could well be through the activation of these beta-adrenoceptors inducing melanosome dispersion. Isoxsuprine (selective β₂ agonist) was also able to accelerate the melanosome dispersion in epinephrine treated melanophores. The results presented here demonstrate the melanophores of *Cyprinus carpio* may possess both α₁ and α₂ receptors as well as β₁ and β₂ adrenoceptors which respond to the adrenergic neurotransmitter.
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REFERENCES


