RESEARCH ARTICLE

Studies of Cholinergic and Histaminergic Drugs on Melenophores of a Teleost Fish *Rasbora elanga* (Ham.)

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ABSTRACT

In the present study, it is observed that the acetylcholine show dispersal effect in isolated skin pigment melanophores of *Rasbora elenga*. The cholinergic blocking agent (atropine) decreases the dispersion rate of acetylcholine, while cholinergic receptors increase the rate of dispersion of acetylcholine. In the present investigation clearly indicated that acetylcholine is more govern the dispersion of skin pigment melanophores, while histamine induced the aggregation of melanophores in the fish scale.

Key Words : Acetylcholine, Histamine, Atropine, Dipheniramine, Rasbora *elanga* **.**

INTRODUCTION

Several workers are studying pigmented cells in many vertebrates dermis as a model for intracellular organelle transport. Fish and amphibians possess specialized cells, called melanophores, which contain hundreds of melanin filled pigment granules, termed melanosomes. Melanophores transport their pigment in response to extracellular cause, neurotransmitters in the case of fish and hormonal stimuli in the case of frogs. In both cases, melanosomes dispersion is induced by elevation of intracellular cAMP levels, while aggregation is triggered by depression of cAMP (Reiter, 1985). The pharmacological studies also suggested the presence of cholinergic receptors which mediate dispersion of pigment in the melanophores of fishes. The effect of acetylcholine on the fish melanophores has been a subject of debate for long time (Parker, 1948; Scott, 1965; Miyashita and Fujii, 1973).

Several studies have been conducted on the effects of various hormones, enzymes and pharmacological agents on vertebrate pigments cells and it has been widely accepted that vertebrate melanocytes (melanocytes are cells located in the bottom layer, the basal lamina, of the skin's epidermis and in the middle layer of the eye) are controlled by either nerves alone or by a combination of nervous and endocrine system. (Bagnara and Hadley, 1973; Fuji and Oshima, 1994; Fuji, 2000). In fishes, there are several kinds of chromatic cells each recognized by its color. Few reports are available on the effects of histaminergic on fish scale melanophores.

The integument of freshwater fish *Rasbora elanga* possesses three kinds of chromatophores, *viz.,* melanophores, xanthphores and iridophores. The present account concerns only a study of melanophores pattern in some detail as these are by the far the most numerous chromatophores, which play the most important role in the normal coluor change as well as in the existence of colour pattern of the fish. Therefore, the present paper deals with the mechanism of dispersion and aggregation induced by histamine and acetylcholine on melanophores of freshwater fish *Rasbora elanga* (Ham.).

MATERIALS AND METHODS

The fish *Rasbora elanga* were collected from Saroth reservoir about 25 km from Chhindwara, Madhya Pradesh and kept in large glass aquaria at a room temperature 20^0C to 30^0C, under natural day night lightening, containing dechlorinated freshwater. These fishes are their diet includes algae and plant materials. The experiment is conducted from January, 2012 to February, 2013 (fig.1).

Fig. 1 showing photograph of *Rasbora elanga*

The freshwater teleost fish *R. elanga* was used as the experimental material having body length between 9.5 cm and 10.2 cm and body weight from 10.0 to 12.0 gm were collected and maintain in an aquarium for at least 7 days before being used for the experimental purpose.

The fish scales were removed from dorso-lateral region below the head and lateral sides of the fish according to the method of Spaeth (1913). Scales after removed from the dorso-lateral region were immediately transferred into the glass Petri dishes containing 0.7% NaCl (control) solution in this solution provided the best results in comparison to the other physiological salt solution for isolated scale melanophore preparation for 30 minutes (Ovais and Gorakh, 1988; Masood, 1991). The scales were transferred from Petri dishes in the 10 ml of saline medium solution.

Ten scales of fish were used in various dishes with each dish having a different concentration of drugs. After a constant incubation (07-10 min) period, the MMSI of ten of such treated melanophores from each concentration was recorded. Thus a set of experiment comprised the measurement of responses of about hundred melanophores.

Measurement methods

Individual melanophores were measured with the Ocular-meter (Erma, Japan) in look power microscope and melanophores size index was calculated according to the method of Bhattacharya *et al.* (1976). The observed values have been multiplied by unit of micrometer which was 15µm. Thereafter the mean was calculated and this value was divided by 100 to obtain a value in a digit with three decimal points. This was Mean Melanophores Size Index (MMSI). Statistical analysis of data were conducted according to Cochran (1967).

$$MMSI = \frac{VD \times HD}{100} \times 15$$

Where,
VD-Vertical diamet
HD- Horizontal diameter

Drugs used for experiment:

1. Acetylcholine (Perse):

0.20µg/ml, 0.80µg/ml, 3.20µg/ml and 6.40 µg/ml

2. Atropine (Agonist):

0.20µg/ml, 0.80µg/ml, 3.20µg/ml and 6.40 µg/ml

3. Histamine (Perse):

0.20µg/ml, 0.80µg/ml, 3.20µg/ml and 6.40 µg/ml

4. Dipheniramine (Agonist):

0.20µg/ml, 0.80µg/ml, 3.20µg/ml and 6.40 µg/ml

RESULTS AND DISCUSSION

The isolated scale melanophores of fish *R. elanga* maintained an intermediate state in physiological solution of 0.7% NaCl. After this, melanophores are treated with series of various concentrations of acetylcholine (perse) i.e., 0.2×10^{-6} µg/ml, 0.8×10^{-6} µg/ml, 3.2×10^{-6} µg/ml, and 6.4×10^{-6} µg/ml for 10 to 20 minutes. In present study, melanophores shows less dispersion on 0.2×10^{-6} µg/ml and MMSI at this stage was reported 4.96 ± 0.14, while concentration of 6.4×10^{-6} of acetylcholine was observed higher dispersion in melanophores and the MMSI value of melanophores has been observed at this concentration was 7.26 ± 0.61. The concentration of 6.4µg/ml of acetylcholine, melanophores show higher mean dispersion are held in increasing order with increase of dose which shown in photographs (fig. 2) and MMSI (table 1). Further increase in the dose of acetylecholine upto 6.4µg/ml shows a clear dispersion in the melanophores. In

present study, the acetylcholine varied its effect with deferent concentrations on fish melanophores. At lower concentration of acetylcholine, it had not produced a significant effect but recent study, higher concentration of acetylcholine shown more dispersion of melanophores. Acetylcholine and other related agonist induced dispersion in *R. elanga* melanophores. These studies have result in characterization of adrenoceptors in this fish species in other fishes too similar results are reported by Spaeth (1913), Parker (1948), Scott (1965), Miyashita and Fujii (1973), Hayashi and Fujii, (1993). Ovais and Gorakh (1988) have also studied on *Cirrhinus mrigala* and observed that the acetylcholine induced the dispersion in the fish melanophores of *C. mrigala.*

Atropine blocked the dispersion of melanophores of scales in different concentration 0.2×10^{-6} (MMSI 4.37 ± 0.12), 0.8×10^{-6} (MMSI 4.32 ± 0.12), 3.2×10^{-6} (MMSI 4.30 ± 0.12) and 6.4×10^{-6} (MMSI 4.12 ± 0.12) to find out the effect of atropine on the response of acetylcholine. Atropine was incubated in a few experiment in the same concentration maintained and acetylecholine was then added, it was observed that acetylcholine produced no effect in the present of Atropine. The melanophores showed a less dispersion response in the presence of atropine to acetylcholine which observable from decrease in the MSI value from the control value.

Histamine induced an effective aggregation of all the melanophores of fish skin. The effects of histamine on the melanophores of *R. elanga* have been given in table 1 and fig. 3. In present investigations, the concentration of Histamine (6.4×10^{-6} µg/ml) produced comprehensive aggregation of all the melanophores.

Table 1- Showing the effect of drugs on MMSI of *R. elanga* scale skin melanophores.

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI+SE	Level of significance
07	Control Acetylcholine *perse*	0.7% saline	4.33 ± 0.13	-
07		0.2×10^{-6}	4.96 ± 0.14	0.0023
07		0.8×10^{-6}	5.54 ± 0.26	0.0004
07		3.2×10^{-6}	6.51 ± 0.53	0.0008
07		6.4×10^{-6}	7.26 ± 0.61	0.0002
07	Control Atropine ($0.2x10^{-6}$)	0.7% saline	4.33 ± 0.11	-
07		0.2×10^{-6}	4.37 ± 0.12	0.385
07		0.8×10^{-6}	4.32 ± 0.12	0.493
07		3.2×10^{-6}	4.30 ± 0.12	0.433
07		6.4×10^{-6}	4.12 ± 0.12	0.107
07	Control Histamine (perse)	0.7% saline	4.53 ± 0.22	-
07		0.2×10^{-6}	3.28 ± 0.10	N.S.
07		0.8×10^{-6}	1.70 ± 0.06	N.S.
07		3.2×10^{-6}	1.11 ± 0.03	N.S.
07		6.4×10^{-6}	0.93 ± 0.05	N.S.
07	Control Dipheniramine ($0.2x10^{-6}$)	0.7% saline	4.19 ± 0.05	-
07		0.2×10^{-6}	4.28 ± 0.04	0.221
07		0.8×10^{-6}	4.25 ± 0.04	0.371
07		3.2×10^{-6}	4.11 ± 0.04	0.160
07		6.4×10^{-6}	4.03 ± 0.05	0.018

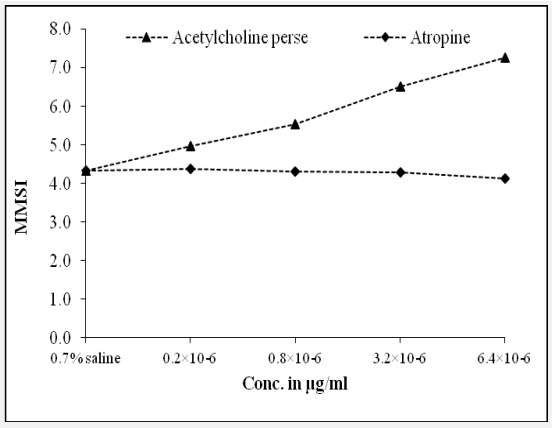


Fig.1: Showing the effect of acetylcholine *perse* and Atropine on the responses of isolated melanophores (MMSI) of *R. elanga*

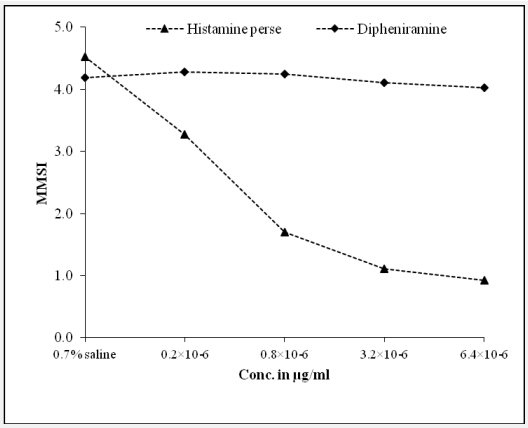
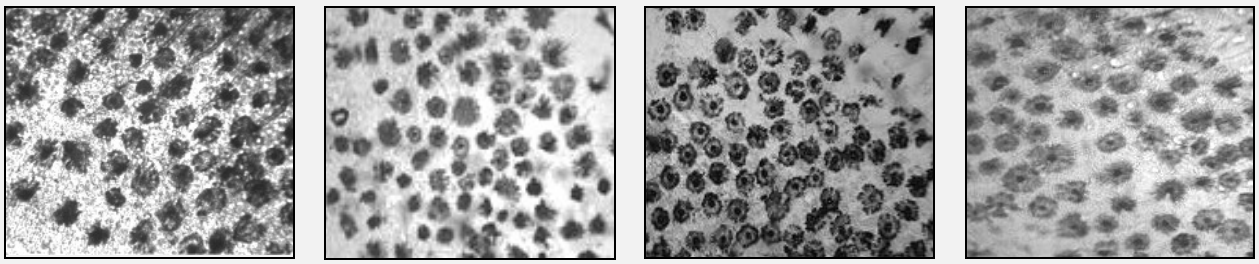
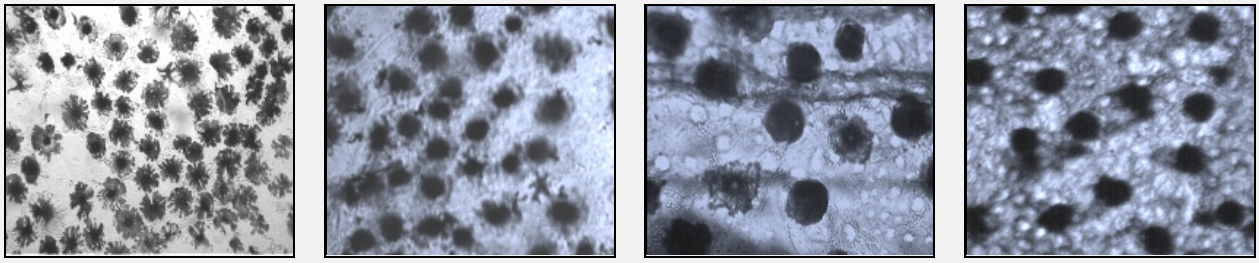


Fig.2: Showing the effect of Histamine *perse* and Dipheniramine on the responses of isolated melanophores (MMSI) of *R. elanga.*



(A) **(B)** **(C)** **(D)**

Fig. 1 :Serial photographs showing the effects of Acetylcholine in a isolated scale melanophores of *R. elanga;* **(A)** Control (0.7% saline solution);**(B)** 0.8 µg/ml; **(C)** 3.2 µg/ml ,and **(D)** 6.4 µg/ml the melanophores was totally dispersed.



(A) **(B)** **(C)** **(D)**

Fig. 2: Serial photographs showing the effects of Histamine in a isolated scale melanophores of *R. elanga*; **(A)** Control (0.7% saline solution); **(B)** 0.8 µg/ml; **(C)** 3.4 µg/ml, and **(D)** 6.4 µg/ml the melanophores was totally aggregated.

However, MMSI at this stage was 0.93±0.05. An increase in the dose of histamine from 0.2×10^{-6} to 6.4×10^{-6} µg/ml caused a slight aggregation of melanophores, which is not statically significance. Earlier findings the data of the present investigation clearly demonstrate the presence of histaminergic receptors of Histamine type in the melanophores of *R. elenga*, which may control melanin aggregation. Similarly, higher concentration of Dipheniramine (6.4×10^{-6} and MMSI 4.03±0.05) inhibited completely aggregation of melanophores induced by histamine responses of fish melanophores.

In the present investigation clearly indicated that acetylcholine is more govern the dispersion of melanophores, while histamine induced the aggregation of melanophores in the fish scale. These data have been considerable significance in relation to the species diversity, which is not only found in genus level of this species.

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