RESEARCH ARTICLE

Pharmacological effect on scals of Rasbora elenga

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ABSTRACT

In the present study it is observed that the histamine *perse* caused dose dependent significant aggregating effects in the dorsal skin melanophores of *Rasbora elenga*. Higher concentrations of histamine caused more sever aggregating effects of the scale melanophores.The isolated scale melanophores of fish *R.elanga* maintained an intermediate state in physiological solution of 0.7 % NaCl. After this, melanophores are pretreated with a specific H_3 Receptor antagonist thioperamide in the concentration of 2×10^{-6} g/ml. They were later incubated in increasing concentrations of histamine in a logarithmic scale of 1×10^{-6} g/ml to 6.4×10^{-5} g /ml. In the lowest concentration of 1×10^{-6} g/ml of histamine it was observed that thioperamide blocked the melanophore aggregation effect of histamine *per se.* Increase in the concentration of histamine with the blocker thioperamide to 3.2×10^{-5} g /ml the teleost melanophores were not able to aggregate in the presence of the antagonist. After that the melanophores of R.elenga were previously bathed in 0.7% Saline in order to bring the melanophores in the neutral state, where melanophores remained in a state of neither aggregation nor dispersion. When such scale melanophores were incubated with H_3 Agonist immethridine it was observed that immethridine induced physiologically significant melanophore aggregation in all concentration. The intial concentration of immethridine *perse* of 1×10^{-6} g/ml caused the melanophores to decrease in size showing aggregation. Increase in the dose concentration of immethridine *perse* to 6.4×10^{-5} g /ml, induced a complete aggregation of all the melanophores

Keywords: *Histamine, Thioperamide, Immethridine, Melanin granules, Aggregation*

INTRODUCTION

The pigment cells of vertebrates (melanophores/ melanocytes) are specialized type of smooth muscle cells which due to their

intracellular movement of melanin granules , control skin hue. Colour change in vertebrates represents some of the most drametic example of adaption to the environment and a scientific interact in this phenomenon can be traced back to the days of Aristotle. Change in or colour patterns are mediated through the activity of integumentary pigment containing cell called melanophores (Parkers, 1948) Physiological colour changes are due to the motile activities of pigment granules within chromatophores (Fuji, 1969, Bagnara and Hadley, 1973 & Fuji & Oshima, 1986). The findings of the present study are very important from pharmacological characterization point of view, as histaminargic receptorss of all the four sub typewhich have been recentaly discovered have been found to be present on the melanophores of *Rasbora. Elenga.*

In the vertebrates several types of chromatophores can be distinguished and these are classified into five categories. According to the type of pigment they contain:

1. Melanophore (Black or brown)
2. Erythropore (Red)
3. Xanthophore (Yellow)
4. Leucophore (white)

Melanophores are spherical or ellipsoidal bodies with an average diameter of about 0.5 µm. Each melanosomes is surrounded by a limiting membrane. The brown or black pigment in the melanosome have been shown to be melanin's, which are highly polymerized studies these melanophores of a indian fresh water carp *Rasbora elanga* have been investigeted to unviel the mechanism involved i.e. aggregation or dispersion. The melanophores of vertebrates are generally controled either neural or hormonal control exist (Fuji, 1969, Bagnara and Hadley, 1973 & Fuji & Oshima, 1986).

***Chromatophores*:**

The word Chromatophore is derived from the Greek word Chroma = colour, phore = to bear. Chromatophore is integumentary coloured cells which contain pigment that can disperse or concentrate thereby changing the colour of the barers. These cells are located in the skin, scales or even in certain deeper tissues of the body.

1. The chromatophores on the basis of the colour pigment present in the have been classified as Black or brown melanophores, containing melanin granules.
2. White reflecting Iridophore containing guanine rich reflecting platelets.
3. Yellow Xanthophore containing caretenoid vesicles pteridine rich pterinosomes.
4. Red Erythropores, also containing caretenoid vesicles and pterinosomes.

***Melanophores*:**

Melanophores are the black or brown pigments cells present in the skin and scales of fish. The melanophores are the best known of all pigment cells and are perhaps the most important of the cell active in colour change since the skin of fish is different from the other "terrestrial vertebrate."

***Origin*:**

A wide histological observation on the embryonic and adult tisseues of every class of vertebrate has has been brought to bear upon the general problem of melanophores origin. After carefully examination of various theories, it has been confirm that the neural crest is the origin of melanophores (Rawles, 1948; Wild CE Jr 1961; Bagnara & Hadley 1973).

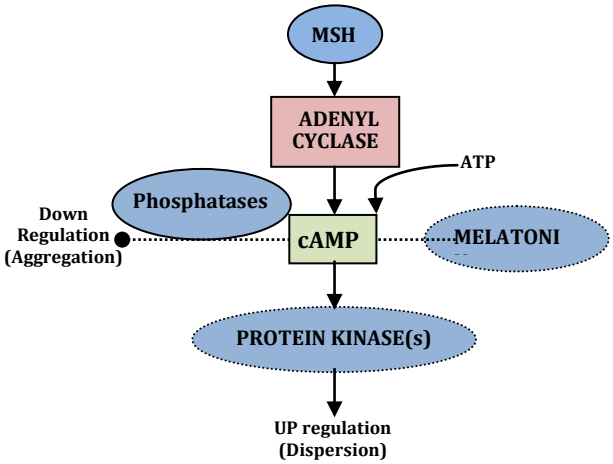
***Morphology*:**

Melanophores are ovoid; astes shaped cells with long dendritic protruding out from a central core, resembling the nerve cells. In fish the melanophores are arranged in definite some in the anterior half of the scales which remain embedded in the skin.

***Melanin and its formation*:**

The melanin pigment is a complex polymer derived from tyrosinase with a high molecular weight and great stability. The synthesis of melanin takes place in specialized vesicles, the melanosomes of melanocyte. The melanin synthesis involve first the oxidation of tyrosinase

to 3, 4 –di hydroxl phenyl alanine and then to dopaquinone. The enzyme concerned is copper containing tyrosinase. Dopaquinone is then polymerized to form melanin which is usually found attached to a protein. . 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).



Types of Melanophores:

Two distinct types of melanophores are generally marked among vertebrates; these are the dermal melanophores and the epidermal melanophores. The dermal melanophores of fishes are involved in the colour changes. The epidermal cells are elongated and are often referred to as spindle shaped.

***Hormonal control of colour change in fishes*:**
Pigment movment withine the melanophores is a highly specialized phenomenon. The translocation of pigment is displayed either by aggregation or dispersion across the cytoplasm, which is controlled by hormone. (Fuji and novales, 1969; baganara and hadley, 1973; Fuji and Oshima, 1994; Fuji, 2000 and salim and Ali, 2011,2012)

***Neural control of colour change in fishes*:**
Chromatophores responsible for the skin colouration are predominantly under nerves control. The autonomic system has two major components, the sympathetic and parasympathetic. System controls effects or response opposite in nature to the other i.e. one excitatory and the other inhibitory depending on the tissues.

The aggregation nerves fiber have been identified to be adrenergic in nature by histochemical methods, when the melanin aggregation fiberd were concluded to belong to the sympathetic division, an assumption was naturally made that the parasympathetic nerve might be involved in dispersing the melanophores.

Pharmacological control of colour change in fish

***Histamine*:**
An amine causing contraction of muscle in hollow organs and dilution of capillaries released by cells in response to injury in allergic and inflammatory reaction.

Receptor:
An organs which capable to respond to an external stimulus such as light, heat or a drug & transmit a signal to sensory nerve.

There are 5 typse o f receptors present in in-vertebrate animal. These are followin g type
1 histaminergic receptors
2 cholinergic receptors
3 Beta adrenergic receptors
4 Gabaergic receptor
5 adrenergic receptors

The Histamine receptor are a class of G-protein coupled receptors with receptor as their endogeneous ligand. There are four known histaminergic receptor H_1, H_2, H_3 & H_4.

Histamine Agonist:
Histamine agonist is a drug which causes increased activity at one or more of the four histamine subtype.

Histamine receptor agonist are as follows:

H_1 – Receptor Agonist – Pyridyl ethyl amine

H_2– Receptor Agonist - Amthamine

H_3 – Receptor Agonist - Immethridine

H_4 – Histamine Receptor Agonist – VUF8630

Histamine Ligand Antagonist:

H_1 – Receptor Antagonist – Diphendramine

H_2– Receptor Antagonist – Ranitidine

H_3 – Receptor Antagonist – Thioperamide

H_4 – Receptor Antagonist – JNJ7777120

Drugs used for experiment:

1. H_3- Immethridine (Agonist)
2. H_3- Thioperamide (Antgonist)

MATERIALS AND METHODS

The teleostean fish *Rasbora elenga* has been selected for studying the effects of recent new class of histaminergic drugs on its isolated dorsal scale melanophores, in order to find the nature and role of receptors of histaminergic type in controlling skin pigmentation processes. The fish was selected because of its easy availability, sturdy nature as it can be kept live in laboratory conditions for long periods and the fact that its melanophores are excellent model for *in vitro* studies, and no study has been done on them till now. They were caught with the help of fishermen from various water bodies and transported to the laboratory alive and they were kept in glass aquaria containing 100L of dechlorinated tap water. Experiments were performed in the laboratory conditions having ambient temperature of 25-30° C with a pH of 7.2 to 7.4. Prior to the experiments, the fish were allowed to acclimatize to laboratory conditions for 3 days. Diseased, injured, or lethargic fish were removed and only active, uniformly colored fish were used. For the *in vitro* studies, the fish scales were removed in accordance with the method of Spaeth, (1913) which included the removal of 20–25 scales from the dorsolateral region of live *R. elenga* kept in a wet cloth, held loosely. The scales were removed by forceps from the dorsal lateral pigmented area. These were immediately placed in 0.7% normal saline, containing 700 mg of sodium chloride in 100 mL of double distilled water. They were equilibrated in saline medium for 7–10 min with frequent shaking.

The responses of control as well as of those melanophores that were incubated in 10 mL 0.7% fish saline containing various concentrations starting from 1×10^{-6} to 6.4×10^{-5} g/mL of Histamine, Thioperamide (Specifi c H3 antagonist) and Immethridine (Specifi c H3 agonist) *perse*. Responses of the melanophores were measured in accordance with the method of Bhattacharya *et al.*, (1976) based on Hogben and Slome (1931). In this method, actual diameter (length×width with the processes) of 10 randomly selected melanophores from each scale was measured using a Leitz Occulometer calibrated previously with stage micrometer. The value was then multiplied by the unit of the micrometer 15 µm. Thereafter, the arithmetical mean was calculated and this value was then divided by 100 to obtain the values. This was the mean melanophore size index (MMSI).

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.

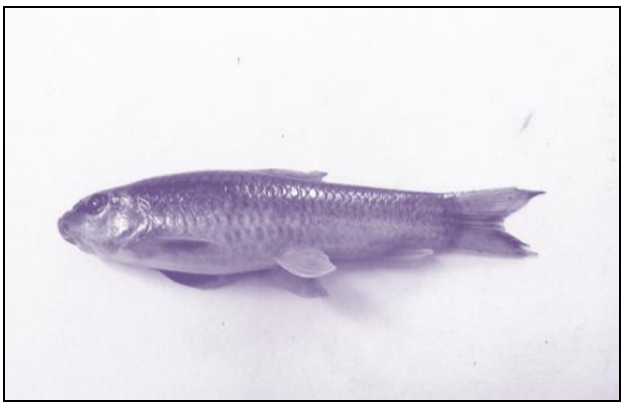


Fig. 1 showing photograph of *Rasbora elanga*

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 was conducted according to Cochran – 1967.

Statistical analysis

Statistical data analyses are presented as mean standard error of the mean (SEM) and n = 7, which represents the number of individual experiments conducted with equal numbers of animals. Comparisons were made between treated and control groups by use of Student's t-test. All data were analyzed using GraphPad Prism software (UK). $P < 0.05$ indicates statistically signifi cant difference.

$$(\text{MMSI}) \frac{\text{VD} \times \text{HD}}{100} \text{ X } 15$$

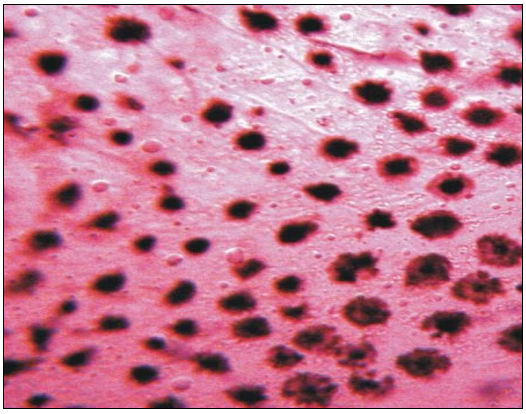
Where,

VD = Vertical diameter

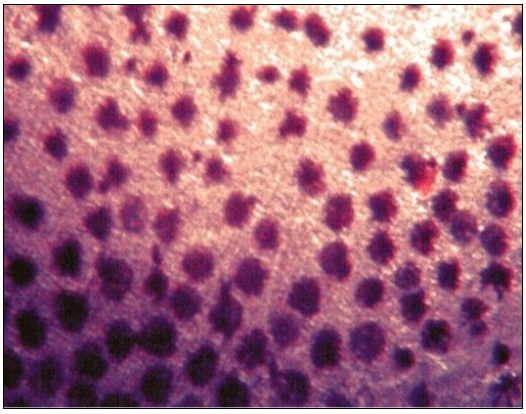
HD = Horizontal diameter

RESULTS AND DISCUSSION

Histamine is an important autacoid biogenic amine present in all biological tissues, and also regarded as a chemical mediator and neurotransmitter on broad spectrum physiological level (Goodman and Gillman, 2006). It is contained in mast cells and basophiles found in all animal and mammalian tissues in both neural and non-neural compartments (Goodman and Gillman, 2006). In the present study histamine *per se* aggregated the dorsal skin melanophores of *R. elenga* in varying doses ranging from 1×10^{-6} g/ml to 6.4×10 10^{-5} g /ml. The MMSI decreased from the control value of 4.532 ± 0.1282 to 0.9257 ± 0.05455 by the highest dose of histamine. The different concentration of histamine used in the present study ranging from 1×10^{-6} g/ml to 6.4×10 10^{-5} g /ml, could gradually and markedly aggregate the scale melanophores, since the MMSI decreased only slightly in lower dose from the control value of 4.532 ± 0.128 to 4.157 ± 0.223 as seen by the first concentration of 1×10^{-6} g/ml of histamine per se. Higher concentrations of histamine caused more sever aggregating effects of the scale melanophores. From these result it becomes clear that histamine *per se* caused dose dependent significant melanin aggregating effects in the melanophores of the fish, *Rasbora elenga* in all concentration used.



Melanin granule aggregation in melanocyte cell (In *Rasbora elenga*)



Melanin granule Dissappearsion in melanocyte cell (In *Rasbora elenga*)

Table 1- Showing the effect of Histamine perse, on the response of *R. elanga* isolated dorsal scale melanophores MMSI.

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.532 ± 0.128	
07	Histamine Perse	**1×10^{-6}**	4.532± 0.128	0.0016
07		**2×10^{-6}**	3.284± 0.098	0.5446
07		**4×10^{-6}**	2.380 ± 0.072	0.1871
07		**8×10^{-6}**	1.695 ± 0.056	0.0669
07		**1.6×10^{-5}**	1.307 ± 0.033	0.0044
07		**3.2×10^{-5}**	1.106 ± 0.027	0.0016
07		**6.4×10^{-5}**	0.925 ± 0.054	0.0565
07		**Reimmersion in 0.7% saline water**	4.441 ±0.113	0.7793

Table no.2 Showing the effect of H3 antagonist Thioperamide on the response of *R. elanga* melanophore MMSI

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.680 ± 0.074	
07	Thioperamide Perse (antagonist)	**1×10^{-6}**	3.990 ± 0.038	0.1254
07		**2×10^{-6}**	4.140 ± 0.038	0.1353
07		**4×10^{-6}**	4.847 ± 0.049	0.3476
07		**8×10^{-6}**	4.393 ± 0.015	0.0012
07		**1.6×10^{-5}**	4.006 ± 0.083	0.7962
07		**3.2×10^{-5}**	3.990 ± 0.038	0.1254
07		**6.4×10^{-5}** **Reimmersion in 0.7% saline water**	3.719 ± 0.044 4.659 ± 0.089	0.2343 0.6775

Note :- (concentration of antagonist **2×10^{-6}**, value of MMSI– 4.433 ± 0.052, P -value 0.4048)

Table no.3 Showing the effect of H3 agonist Immethridine on the response of *R. elanga* isolated melanophore. MMSI

No. of exp.	Experimental drugs	Dose in µg/ml	MMSI ± SE	P-value
07	Control	**0.7% saline**	4.543 ± 0.124	
07	Immethridine Perse (Agonist)	**1×10^{-6}**	4.157 ± 0.027	0.002
07		**2×10^{-6}**	3.284 ± 0.098	0.5976
07		**4×10^{-6}**	2.380 ± 0.072	0.2130
07		**8×10^{-6}**	1.695 ± 0.056	0.0779
07		**1.6×10^{-5}**	1.307 ± 0.033	0.0052
07		**3.2×10^{-5}**	1.157 ± 0.037	0.0106
07		**6.4×10^{-5}**	1.524± 0.184	0.3565
07		**Reimmersion in 0.7% saline water**	4.441 ± 0.113	0.8405

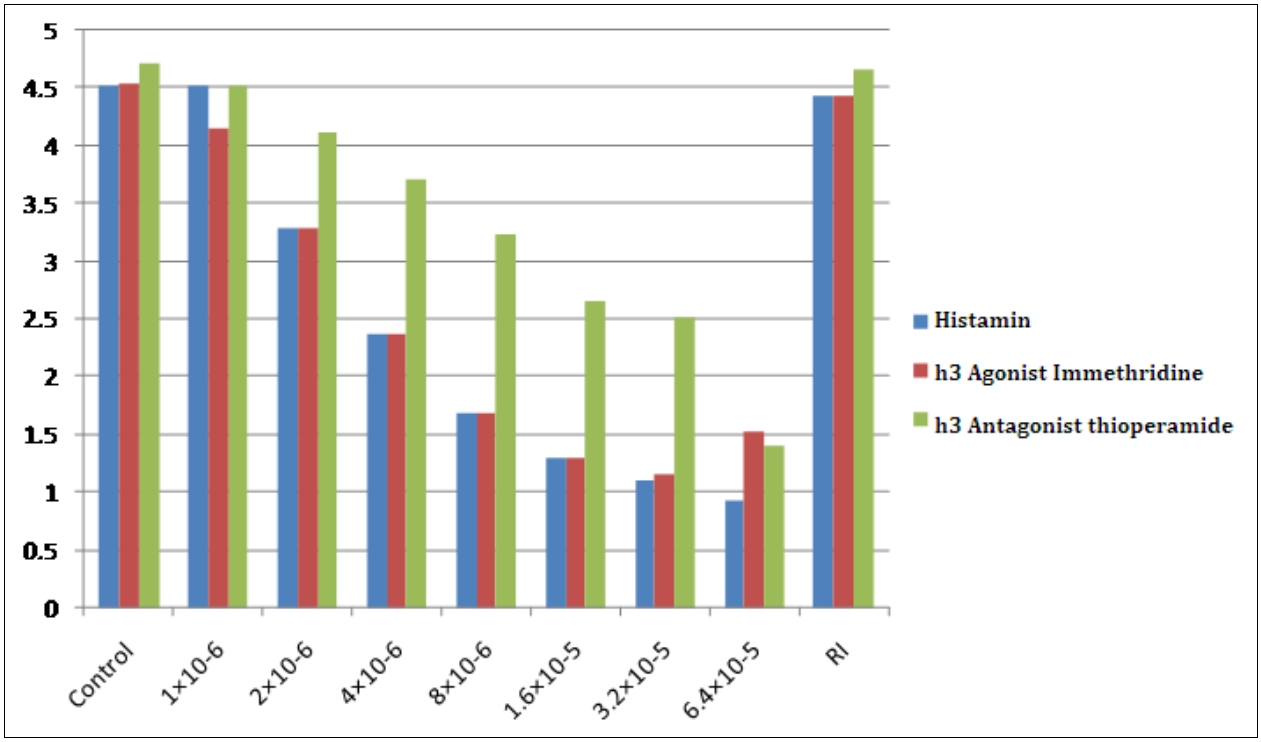


Fig. 2: Showing the effect of Histamine perse, specific H_3 receptor Antagonist Thiperamide, H3 agonist Immethridine on the response of MMSI of *R. elanga* scale skin melanophores

The isolated scale melanophores of fish *R.elanga* maintained an intermediate state in physiological solution of 0.7 % NaCl. After this, melanophores are pretreated with a specific H_3 Receptor antagonist thioperamide in the concentration of 2×10^{-6} g/ml. They were later incubated in increasing concentrations of histamine in a logarithmic scale of 1×10^{-6} g/ml to 6.4×10^{-5} g /ml. In the lowest concentration of 1×10^{-6} g/ml of histamine it was observed that thioperamide blocked the melanophore aggregation effect of histamine *per se.* The MMSI at this stage remained 3.990 $\pm$ 0.0380 which is almost near the MMSI at the control melanopores of 4.680 $\pm$ 0.07464. Increase in the concentration of histamine with the blocker thioperamide to 3.2×10^{-5} g /ml the teleost melanophores were not able to aggregate in the presence of the antagonist and MMSI remained at 3.990 $\pm$ 0.1254. The blocked of the histamine melanophore aggregating effect by thioperamide contined even when the highest concentration of histamine i.e. 6.4µg/ml was employed, where no aggregation was observed and the MMSI recorded as 3.719 $\pm$ 0.014453. In the absence of antagonist thioperamide, the effect of histamine was highly aggregating and the MMSI was 0.9257 $\pm$ 0.0545.

After that the melanophores of R.elenga were previously bathed in 0.7% Saline in order to bring the melanophores in the neutral state, where melanophores remained in a state of neither aggregation nor dispersion. When such scale melanophores were incubated with H_3 Agonist immethridine in the concentrations ranging from 1×10^{-6} g/ml to 6.4×10^{-5} g /ml, it was observed that immethridine induced physiologically significant melanophore aggregation in all concentration.

The intial concentration of immethridine *perse* of 1×10^{-6} g/ml Caused the melanophores to decrease in size showing aggregation and the MMSI at this stage was reduced a control value of

4.543 ± 0.1240 to 4.157 ± 0.027. Increase in the dose concentration of immethridine *perse* to 6.4×10^{-5} g /ml, induced a complete aggregation of all the melanophores thus making the melanophores appear ball like or punctate, where the MMSI was found to be 1.524 ± 0.1844 from a control value of 4.543 ± 0.124 .

It was later found that when the highest concentration i.e. 6.4×10^{-5} g /ml immithridine treated scale melanophores were washed repeatedly with teleost 0.7% saline and re-immersed for 15-20 minutes, the melanophores aggregation effect completely dissappeared and the melanophores returned to their control state of neither aggregation nor dispersion. At this stage of the MMSI of the melanophores had become 4.441 ± 0.1138 which is almost near the control value of 4.543 ± 0.124.

In the present investigation clearly indicated that histamine and immithridine induced the aggregation of melanophores in the fish scale while thioperamide blocked the melanophore aggregation effect of histamine *per se* 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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