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Effects of histamine and its new H_1 and H_2 receptor agonists on the isolated scale melanophores of teleost fish, *Rasbora elenga*

Meena Swamy[1], Ram K. Choudhary[2] and Ayesha S. Ali[2]*

[1]*Department of Zoology, Government Autonomous College Chhindwara MP*
[2]*Department of Zoology and Biotechnology, Saifia Science College, Bhopal*

ABSTRACT

All organisms, from simple invertebrates to complex human beings, exist in different colors and patterns, which arise from unique distribution of pigments throughout the body. Histamine mediates its multifarious biological activities stimulated by various immunological and non immunological stimuli via differential expression of its new classes of receptors on effector cells, including the black pigment cells, the melanophores. We here have described the effects of a new class of H_1 and H_2 histaminergic receptor agonists on the isolated scale melanophores of teleost fish *Rasbora elenga* emphasizing the potential physiomodulatory effects of these new pharmacological molecules related to histamine in the phenomenon of skin pigmentation.

KEY WORDS: HISTAMINE H_1 AND H_2 RECEPTORS, MELANOPHORES, RASBORA ELENGA

INTRODUCTION

Melanophores of lower vertebrates are specialized type of smooth muscles cells, derived from the neural crest and are present in the basal layers of the epidermis and dermis (Rawles, 1948), which possess thousands of black melanin pigments, packed in melanosomes (Bagnara *et al.*, 1986; Schliwa, 1976). These specialized pigment containing cells are derived from the neural crest, and are present in the epidermal and dermal layers of cold blooded vertebrates (Rawles, 1948; Arduini *et al.*, 2008). The immense sensitivity of these cells to a variety of

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**Corresponding Author*
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stimuli and their lineage with neurons of the sensory kind has made these cells a subject of great interest amongst researchers (Bagnara and Hadley, 1973; Fujii, 1993, Salim and Ali 2011, Ali *et al*., 2012).

Several extensive studies have been made on physiology and pharmacology of adrenergic and cholinergic drugs on melanophores of fishes and amphibians (Abbott, 1968; Fujii & Hayashi, 1996; Ali, 1983; Ali and Ovais 1993; Ali *et al*., 1998). Though various earlier workers have extensively investigated the fish melanophores, much attention has been directed towards endocrine influences (Waring, 1963; Beaven, 1976; Tomita *et al*., 1988), unfortunately pharmacological characterization of the histamine receptors in fish melanophores remains obscure and it is not yet clear whether histamine receptors plays a role in the physiology and pharmacology of fish melanophore responses. Hence in the present study the main objectives were to study the effects of the new modern and recently available specific agonists of H_1 and H_2 histamine receptors along with their specific antagonists and potentiators on the isolated scale melanophores of the teleost fish, *Rasbora elanga* in order to find out the involvement and nature of histamine receptors at the cellular level.

MATERIAL 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, Pyridyl ethyl amine (Specific H1 agonist) and Amthamine (Specific H2 agonist) along with their specific antagonists, dipheniramine and ranitidine (4×10^{-6} g/mL). 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×breadth 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).

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 significant difference.

RESULTS AND DISSCUSION

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 $6.4\times10\ 10^{-5}$ g /ml. The MMSI decreased from the control value of 4.532±0.1282 to 0.9257±0.05455. After repeated washings and reimmersion (RI) of the histamine treated melanophores in normal saline, it was found that the powerful melanin aggregation effects of the extract completely vanished, as the MMSI became 4.441 ± 0.1138, almost near the control values of 4.532 ± 0.1282 (Figure 1).

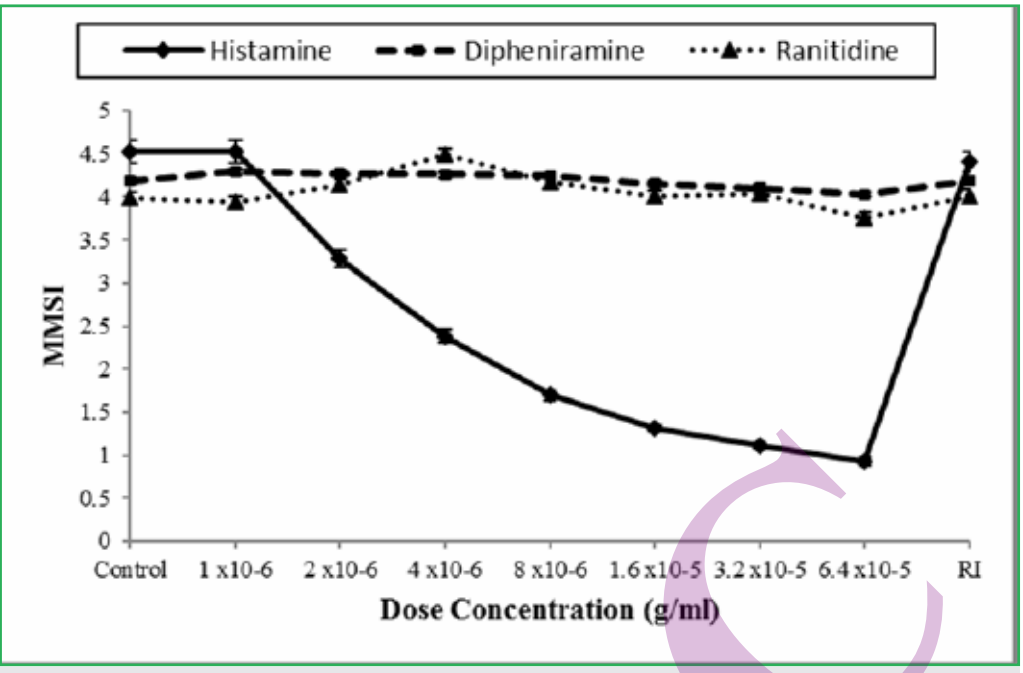

Figure 1: Dose-response curve for the melanophore aggregating effect of Histamine per se and the aggregation blocking effects of the same by the Dipheniramine (H_1 specific antagonist) and Ranitidine (H_2 specific antagonist). RI signifies the MMSI after the reimmersion of tail melanophores in fish ringer saline after repeated washings. Abscissae: Doses Histamine and antagonists in g/mL. Ordinate: responses of melanophores (MMSI). Vertical bars represent the standard error of mean.

The present findings are in contradiction with those of Healy and Ross, (1966) that showed there was no effect of histamine on the melanophores of the *Phoxinus phoxinus*. Later on similar findings were reported by Bhide and Gupta, (1967) who found that histamine liberators like comp 48/80 caused blanching of *R. tigerina* skin via the indirect release of histamine. The workers had also found that histamine *per se* could produce melanin aggregation in the same species, but no receptor study was done by the workers. It was only in 1983, Ali described the presence of histamine receptors in a large number of lower vertibrates (Ali, 1983; Ali *et al.*, 1993; Ali et al 1998; Peter *et al.*, 1996, 2011).

Similarly, Pyridyl ethyl amine, a specific H_1 receptor agonist, was also found to aggregate the fish melanophores. Different concentrations of Pyridyl ethyl amine (1×10^{-6} g/ml to $6.4\times10\ 10^{-5}$ g/ml) aggregated the skin melanophores, where MMSI decreased from a control value of 4.361 ± 0.05872 to 0.8871 ± 0.04980 as seen by the maximal concentration of 6.4×10-5 g/ml (Figure 2).

On other hands ammthamine, a specific H_2 receptor agonist, also aggregate the melanophores of *R. elanga* in a dose dependent manner were the melanophore process had become disappeared, showed almost point shap morphology, leading to lightening of skin. In response to the maximal concentration of 6.4×10^{-5} g/mL of ammthamine, the MMSI of the melanophores became 0.6429 ± 0.04433compared to the control value of 4.532 ± 0.1282. After repeated washings and reimmersion (RI) of the ammthamine treated melanophores in normal saline, it was found that the powerful melanin aggregation effects of the extract completely vanished, as the MMSI became 4.441 ± 0.1138, almost near the control values of 4.532 ± 0.1282 (Figure 3).

In order to further validate the presence and role of specific H_1 and H_2 histamine receptors, specific antagonist dipheniramine and ranitidine were used to see the effect on the scale melanophores of *R. elenga*. In a series of experiments it was observed that the presence of dipheniramine completely abolished the melanophore aggregation effects of pyridyl ethyl amine. From the data presented in fig.1 it becomes clear that an even the highest dose of 6.4×10^{-5} g/ml of Pyridyl ethyl amine along with dipheniramine could not aggregate the teleost melanophores where the MMSI remained 4.404 ± 0.07311 which is almost near to the control value of 4.693 ± 0.06342, which without the presence of dipheniramine was 0.8871 ± 0.0498 (Figure 1).

On the other hands pre-incubation of isolated scale fish melanophores in 2×10^{-6} g/ml of ranitidine was followed by the increasing concentrations series of ammathine (1×10^{-6} to 6.4×10^{-5} g/ml) resulted into com-

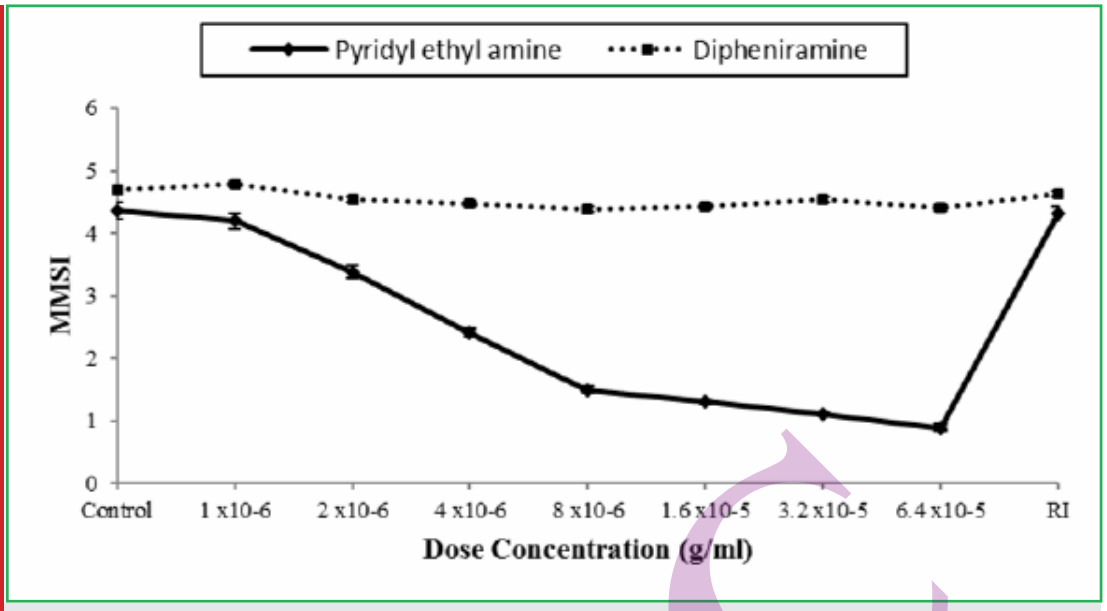

Figure 2: Dose-response curve for the melanophore aggregating effect of Pyridyl ethyl amine (H_1 agonist) *per se* and blocking effects of the same by the Dipheniramine (H_1 specific antagonist). RI signifies the MMSI after the reimmersion of tail melanophores in normal fish ringer saline after repeated washings. Abscissae: Doses Histamine and antagonists in g/mL. Ordinate: responses of melanophores (MMSI). Vertical bars represent the standard error of mean.

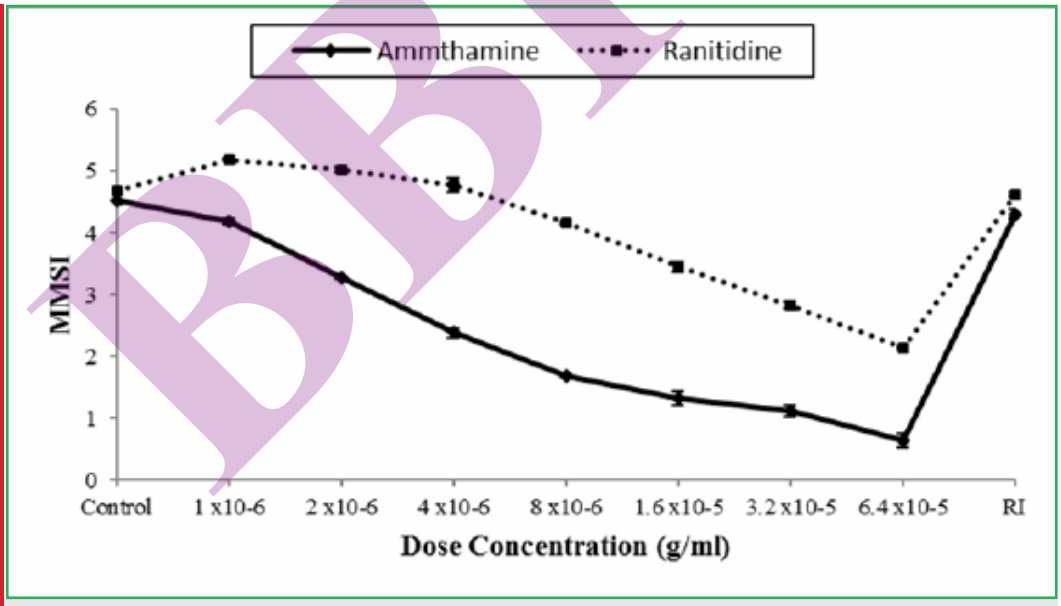

Figure 3: Dose-response curve for the melanophore aggregating effect of Ammthamine (H_2 specific agonist) and blocking effects of the same by Ranitidine (H_2 specific antagonist. RI signifies the MMSI after the reimmersion of tail melanophores in normal fish ringer saline after repeated washings. Abscissae: Doses Histamine and antagonists in g/mL. Ordinate: responses of melanophores (MMSI). Vertical bars represent the standard error of mean.

plete blockade of aggregating effects of ammathine (Figure 3). Where the MMSI remained 5.194 ± 0.04264 in initial dose concentration of 1×10^{-6} g/ml of ammathine, which without the ranitidine was 4.179 ± 0.04546 (Figure 2). This trend continued further and even the concentration of 8×10-6 g/ml of ammathine could not aggregate the melanophores as the MMSI was 4.164 ± 0.03643 whereas, the same without the ranitidine was 1.695 ± 0.0566 (Fig. 2). Even the highest concentration i.e. 6.4×10^{-6} g/ml of ammathine along with ranitidine was not able to exert melanophores aggregation effects. The MMSI remained 2.829 ± 0.09289 at this concentration, otherwise without the ranitidine the value of MMSI was 0.6429 ± 0.04433 (Figure 2). Thus, the present finding suggests that both dipheniramine and ranitidine completely blocked the aggregation effects of pyridyl ethyl amine and ammathine in all concentrations.

CONCLUSION

It is concluded that histamine, induced powerful, dose dependent, physiologically significant melanin aggregation effects in the isolated scale melanophores of *R. elenga*. The use of new specific receptor agonists and antagonists revealed that the skin pigmentary responses owing to histaminergic involvement are mediated by novel H_1 and H_2 subtype of receptors. It is further suggested that the stimulation of H_1 and H_2 receptors brings about pigment lightening. The pharmacological characterization of histamine receptors from our present study has revealed that these receptors are responsible for regulating the pigmentary responses in teleosts with subsequent lightening of skin.

REFERENCES

Abbott F.S. (1968). The effects of certain drugs and biogenic substances on the melanophores of *Fundulus heteroclitus* L. Can. J. Zool. 46:1149-1161.

Ali S.A. (1983). Physiology and pharmacology of melanophores of teleostean fish *Channa punctatus*. Ph.D thesis, Barkatullah University, Bhopal. 1-203.

Ali S.A., Peter J. and Ali A.S. (1993). Effect of histaminergic drugs on tail melanophores of tadpole, *Bufo melanostictus*. Ind. J. Exp. Biol. 31:440-442.

Ali S.A., Peter J. and Ali A.S. (1998). Histamine receptors in the skin melanophores of Indian Bull frog, *Rana tigerina*. Comp. Biochem. Physiol. 121A:229-234.

Ali S.A., Salim S., Sahni T., Peter J. and Ali A.S. (2012): 5-HT receptors as novel targets for optimizing skin pigmentary responses in Indian bullfrog *Hoplobatrachus tigerinus*. British Journal of Pharmacology **165**; 1515–1525.

Arduini B.L., Gallagher G.R. and Henion P.D. (2008). Zebra fish endzone regulates neural crest-derived chromatophore differentiation and morphology, *PLoS One* 3(7):2845.

Bagnara J.T., Bareiter H.J., Matoltsy A.G. and Richards K.S. (1986). Biology of the integument vertebrates. Berlin: Springer-Verlag. 2:136-49.

Bagnara J.T. and Hadley M.E. (1973). Chromatophores and colour changes. Prentice Hall, Englewood, Cliffs, New Jersey.

Beaven M. (1976). Histamine. The New England Journal of Medicine. 294:30-36.

Bhide N.K. and Gupta I. (1967). Histamine liberators and melanophores of *Rana tigerina*. Journal of Pharmacy and Pharmacology. 19(1), 58-59.

Bovet D. and Staub A.M. (1937). C.R. Soc. Biol., Paris, 138, 99.

Dale H. and Laidlaw P. (1910). The physiological action of Beta- imidazolylethylamine. J. Physiol. 41:318-44.

Fujii R. (1993). Cytophysiology of fish chromatophores. Int. Rev. Cytol. 143:191–255.

Fujii R. and Hayashi H. (1996). Role of nitric oxide (NO) in the control of melanophores in teleosts —I. Effects of NO donors and NO-synthase inhibitors. Zool Sci. 13: 116.

Goodman LS and Gilman A., (2006). The pharmacological basis of therapeutics. McGraw Hill.

Healy E.G. and Ross D.M. (1966). The effects of drugs on the background response of the minnow *Phoxinus phoxinus* L. Comp. Biochem. Physiol. 19:545-580.

Rawles M.E. (1948). Origin of melanophores and their role in the color patterns in vertebrates. Physiol. Rev. 28: 383-408.

Salim S. and Ali S.A. (2011b). Insights into the physio-modulatory role of histaminergic receptors in vertebrate skin pigmentation. J Recept Signal Transduct Res. 31(2):121-131.

Schliwa M. (1976). Fine structure of nerve melanophores contracts in angelfish, *Pterophyllum scalare*. Cell Tissue Research. 171:33-45.

Peter J, Ali AS, Ali SA (1996). Effect of histaminergic drugs on the integumental melanophores of adult *Bufo melanosticus*. Ind J. Expt. Biol. 34:427-430.

Peter J, Meitei KV, Ali AS and Ali SA (2011). Effects of histaminergic compounds on the melanophore responses of the wall lizard, *Hemidactylus flaviviridis*. Current Science 101(2):226-229.

Tomita Y., Maeda K. and Tagami H. (1988). Stimulatory effect of histamine on normal human melanocytes *in vitro*. Tohoku J. Exp. Med. 155:209-210.

Waring H. (1963). Color change mechanism of cold-blooded vertebrates. Academic Press, New York.

Windaus A. and Vogt W. (1907). Ber, dtsch. chem. Ges., 40:3691.