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Auto-regulatory Role of Novel Histamine H_3 like Receptor (H_3R) and Subsequent Modulation of Adrenergic Induced Aggregation in the Pigmentary Responses of *Oreochromis mossambicus*

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Abstract: Background and purpose: H_3 receptor plays an essential role in the integration of histaminergic signal transmission in central and peripheral nervous systems. Recently histaminergic innervation and molecular expression of L-histamine decarboxylase has been described in zebrafish brain. Pre-synaptically H_3Rs have been described as auto-receptors, mediating negative feedback of histamine release. Subsequently, it is reported that H_3Rs are not just restricted to histaminergic neurons but also serves as peripheral postsynaptic hetero-receptors, regulating the release of other neurotransmitters. Although, histaminergic innervation of zebrafish CNS resembles that of other vertebrates, little is known about their pharmacological profiles and functional implications. Also, peripheral tissue distribution of heterogeneously distributed H_3Rs in different mammalian species has been described but there is limited information on the presence of H_3Rs in lower vertebrates. Keeping the aforementioned facts we investigated the existence of H_3Rs at the neuro-melanophore junctions of teleost fish *Oreochromis mossambicus*. Understanding the putative role of this monoaminergic system in teleostean pigmentary effector cells; melanophores would be a considerable step to unravel the complex and fragmentary picture of post-synaptic histaminergic system in the process of skin pigmentation and highlight its evolutionary disposition. **Results and conclusion:** The auto-regulation of histamine release via stimulation of H_3Rs in dorsal skin melanophores was studied at the neuro melanophore junction. Histamine emanated a dual response within the cells. At low concentration histamine caused pigment aggregation while high doses resulted in pigment dispersion. Immethridine a specific H_3R agonist caused slight pigment granule dispersion. Thioperamide, a specific H_3R antagonist blocked the dispersion caused by Immethridine. Compound 48/80 also elicited a significant increase in release of histamine that was significantly inhibited by Immithridine. Thioperamde prevented and effectively antagonized the inhibition caused by Immethridine resulting into pigment aggregation. Yohimbine a specific alpha adrenergic antagonist attenuated the aggregation caused by thioperamide suggesting a subsequent involvement of adrenergic receptors. These data suggest that the melanophores may have histamine H_3 receptors and that histamine probably modulates its own release through the stimulation of H_3 receptors and subsequently regulates the liberation of adrenaline which via alpha adrenergic receptors induces the pigmentary responses leading to paling of the skin.

Key words: Histamine receptors, melanophores, pigmentation, *Oreochromis mossambicus*

INTRODUCTION

Central histaminergic functions are not just confined to brain but have been extended in the periphery. The presence of multitudinous histaminergic targets across the periphery makes this system one of the most important regulatory systems that not just controls the activity of brain but other neurotransmitter systems. The functions of histamine and its disposition in the CNS have been well understood in mammals, however the exact target localization of its peripheral concourse is still not completely known. Also the presence of this monoaminergic system in lower vertebrates has been hardly explored. Recently it has been reported that mast cells of most evolutionary advanced fish contains histamine and is involved in the immunological responses (Mulero *et al*., 2007). The primary physiological bearing of histamine as a mediator of immunological response has been well accepted. However its other subsidiary functions in the peripheral tissues have been beginning to emerge. One such function is in skin pigmentation (Salim *et al*., 2011). Skin pigmentation in vertebrates is a very perplexed phenomenon governed by an array of signal transduction pathways. Involvement

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of histaminergic receptors in the pigmentary responses of amphibian and piscean pigment cells, the melanophores have been brought to light by Ali (1983) Ali *et al.* (1993, 1998) Peter *et al.* (1996), and Salim *et al.* (2011) unpublished data. Emergence of histamine as a mediator in skin pigmentation in vertebrates presents this biogenic amine in a very interesting and novel facet.

The histamine H_3-Receptors (H_3R) are primarily located on the pre-synaptic membranes of histaminergic neurons as autoregulators, negatively regulating the synthesis and release of histamine (Haas *et al.*, 2008). In addition, H_3Rs are also located on non-histaminergic neurons, acting as hetero-receptors to regulate the release of other neurotransmitters such as norepinephrine, GABA, dopamine and serotonin (Yoshimoto *et al.*, 2006). Although the CNS has highest density of H_3 receptors, but in situhybridization studies have revealed that these receptors do exist in dorsal root ganglia, spinal cord and some of the peripheral tissues including skin (Pollard *et al.*, 1993; Heron *et al.*, 2001; Pillot *et al.*, 2002). Interestingly, it has been reported that Zebra fish brain contains a widespread histaminergic system with the existence of histamine H_1, H_2 and H_3 receptor genes in zebra fish. These histamine receptors resemble those of higher vertebrates and they provide a useful model for pharmacological and behavioral studies for characterizing the functions of histamine in more detail (Peitsaro *et al.*, 2007). Also in addition, the actions of histamine in the immune cells of most evolutionary advanced fish has been reported to be mediated through well conserved H_1 H_2 and H_3 receptors (Mulero *et al.*, 2007). The histamine receptor is fishes have been linked to a variety of neurological functions such as the control of arousal, attention, sensory processing and cognition. Histamine also plays a role in pituitary hormone secretion, appetite control and, potentially, regulation of vestibular reactivity (Choich *et al.*, 2004), however the peripheral function of histamine apart from its immunological aspect has still been incognito. In the case of fishes, only a few investigators have explored the role of histamine in the process of skin pigmentation. Interestingly, Ali (1983) had reported that histamine H_1 and H_2 receptors are present in the melanophores of teleost *Channa punctatus* contributing to the substantial involvement of histaminergic receptors in pigmentary responses of teleosts. Besides a few reports there has been no studies undertaken so far, with the revelation of new member receptors to the histamine family. The present study was conducted to reveal the presence of histamine H_3 like receptors in the periphery at the neuro-melanophore junction and their putative involvement in the regulation of skin pigmentary responses in an important member of teleosts; *Oreochromis mossambicus.* The present finding would abet in bringing out the disposition of histaminergic system in lower vertebrates and signify in underscoring their evolutionary implication.

MATERIALS AND METHODS

Oreochromis mossambicus (Peters) commonly known as *Tilapia mossambica* of both sexes, 10-12 cms in length weighing 25-30 g were purchased from the local commercial sources. The fishes were kept in laboratory aquaria for 2-3 days for acclimatization with 12:12 h of light and dark phase with temperatures between to 22-25°C. Fishes with signs of disease were discarded and any chances resulting to cause stress were minimized.

The experiments were conducted on the isolated scale melanophores. The scales were carefully plucked with the help of fine forceps from the dorso-lateral region of the fish according to the method described by Spaeth (1913). Thereafter the scales were immediately immersed into freshly prepared physiological saline solution (PS) of the following concentration (mM): NaCL, 125.3; KCL, 2.7; $CaCL_2$, 1.8; $MgCL_2$, 1.8; D-glucose, 5.6 (pH 7.2).

After 10 min of equilibration in PS the scales containing approx. 50-100 melanophores were incubated with known concentrations of drugs for 7-10 min. All drugs were dissolved freshly in doubled distilled water and their solutions were added to the Petri-dishes containing the PS, the total volume of which was kept constant (10 mL). For experiments using antagonists scales were first incubated in antagonist solution for 10 min and then treated with agonist for 10 min.

Drugs: Histamine dihydrochloride (>98%) was purchased from Sigma Aldrich (USA), Immethridine, Ranitidine hydrochloride and Thioperamide were generous gifts from Dr. Leurs, Netherlands. Mepyramine maleate (>98%) was a generous gift from Dr. Samreen Arshad, Sanofi- Aventis, Bridgewater, NJ (USA), Yohimbine (>98%) was purchased from Sigma Aldrich USA. Compound 48/80 was purchased from Sigma Aldrich (USA).

Responses of melanophores in terms of mean melanophore size index assay: The relationship between concentration of an agonist and the magnitude of the response elicited was studied by exposing the preparation of solutions of various strengths in cumulative and increasing order. The responses of control as well as of those melanophores that were incubated in 10 mL PS containing various concentrations of receptor specific agonists were measured according to the method by Bhattacharya *et al.* (1976) modified by Ali *et al.* (1998)

based on Hogben and Slome (1931) melanophore index. In this modified method, the individual melanophore was measured with the help of Leitz ocular micrometer (Erma, Japan) calibrated by Stage micrometer, by marking the maximum vertical and horizontal diameters. Ten such randomly selected melanophores from each scale were measured. When the melanophores disperse i.e., the melanin pigment granules within the melanophores move to the periphery, the diameter of the cells increases and vice versa. The calculated value is the mean melanophore size index and expressed as MMSI.

Statistical data analysis: Statistical data analyses are presented as Mean±SEM (standard error of the mean) represented by vertical bars and n represents the number of experiments carried on different animals (n = 7). Comparisons were made between treated and control groups by use of Student's t-test (Lewis, 1971). All data were analyzed using Graph Pad Prism software (UK). $p<0.05$ indicates statistically significant difference.

RESULTS

Histamine per se displays dual response in the melanophores of *O. mossambicus*: The melanophores of *Oreochromis mossambicus* showed considerable sensitivity to histamine. Histamine was found to cause pigment aggregation in a dose dependent manner. The threshold dose that could result in aggregation within the melanophores was found to be as low as 1×10^{-8} g mL^{-1}. This effect remained consistent with the increasing dose concentration. The melanophores showed considerable pigment aggregation and appeared significantly aggregated at dose concentration of 4×10^{-5} g mL^{-1}. At this stage the MMSI was observed to be (1.0±0.1241, $p<0.007$). Later, it was observed that subsequent increase in concentration of histamine resulted in gradual pigment dispersion. The melanophores showed progressive pigment dispersion with increasing histamine concentration. The dendritic processes of melanophores sequentially spread out and attained complete reticulated state. This state was observed at dose concentration of 6.4×10^{-4} g mL^{-1} of histamine. The pigment granules within the cells extended towards the peripheral dendrites and the cells appeared expanded with the MMSI measured at (9.98±0.321, $p<0.0001$) (Fig. 1).

To investigate the putative mechanism of this dual response exhibited by melanophores towards histamine and the underlying cellular receptors involved in this process, several receptor specific potent and selective agonists and antagonists were employed. We hypothesized that the dual response exhibited by histamine on pigment cell melanophores could be most likely mediated by histaminergic receptors present at the neuro-melanophore junction that demonstrate an auto regulatory mechanism of action regulating the innervating histaminergic neurons and controlling subsequent histamine release from the encompassing mast cells.

Action of compound 48/80 per se on the pigmentary responses in the melanophores of *Oreochromis mossambicus*: Compound 48/80 displayed dual responses within the melanophores of *O. mossambicus* at low and high concentration respectively. Compound 48/80 exerts mast cell degranulation and releases histamine. The effect of comp 48/80 on melanophores was quite interesting. The immediate response of melanophores to compound 48/80 was seen in the form of pigment aggregation. The minimum dose concentration that resulted in a noticeable pigment translocation towards the centre of cells was found to be (1×10^{-7} g mL^{-1}) with the MMSI (4.71±0.40). Later the aggregating effect was consistent with the increase in concentration of compound 48/80 (Fig. 1). The melanophores showed substantial degree of sensitivity to this compound and confirms the underlying involvement of histaminergic receptors in the process. Later with further increase in concentration of compound 48/80, there was a noticeable change in pigment movement. The pigment granules gradually drifted towards the peripheral processions and showed a considerable expansion in the melanophore index. The MMSI at the highest concentration (6.4×10^{-4} g mL^{-1}) of compound 48/80 was recorded to have elevated to (9.15±0.49, $p<0.0001$) from the control MMSI of (4.92±0.006, $p<0.065$). This dual response that was exhibited by compound 48/80 on the melanophores further reinforces the likelihood of a self regulatory system in the pigmentary responses of melanophores incurring to histamine (Fig. 1).

Antagonistic effects of mepyramine and ranitidine on the action of histamine: Histamine exerts its pleiotropic effect via stimulation of post synaptic cellular receptors. The two major classes of these receptors i.e., H_1R and H_2R have been identified to be present on melanophores of *Oreochromis mossambicus* (Salim *et al*., 2011 unpublished data). The effect of histamine was challenged with H_1R antagonist mepyramine (2×10^{-6} g mL^{-1}) and H_2R antagonist Ranitidine (4×10^{-6} g mL^{-1}). It was observed that histamine in high dose range from 1×10^{-5} g mL^{-1} to 6.4×10^{-4} g mL^{-1} showed discernible pigment dispersion. When this concentration of histamine was challenged by specific H_1R agonist mepyramine (2×10^{-6} g mL^{-1}) and H_2R antagonist ranitidine (4×10^{-6} g mL^{-1}), it was observed that the effect of histamine was feebly attenuated to the

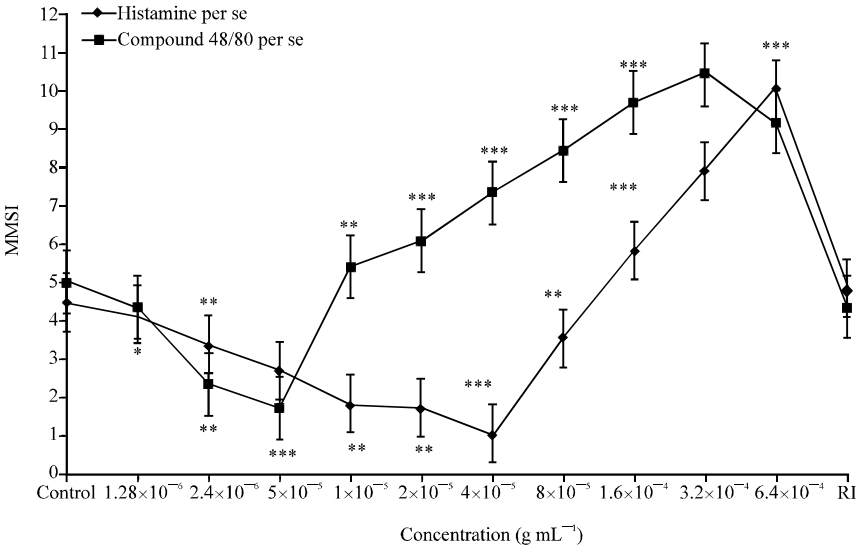

Fig. 1: Dose response curves showing the effect of Histamine per *se* and compound 48/80 per se on the isolated scale melanophores of *Oreochromis mossambicus*. Note the variation in responses with the increase in concentrations of histamine and compound 48/80, respectively. At low dose range the melanophores show a decrease in MMSI, whereas with the shift of concentration towards higher side the MMSI show considerable increase. Abscissa: Concentrations of histamine and compound 48/80 *per se* in g mL^{-1}. Ordinate: Responses of melanophores in terms of MMSI. Vertical bars represent standard error. The p-value signifies level of significance; *p<0.0013, **p = 0.0005,***p = 0.0001

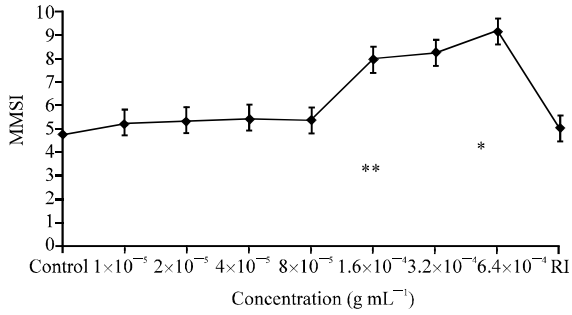

Fig. 2: Dose response curve showing the blocking effect of mepyramine (2×10^{-6} g mL^{-1}) and ranitidine (4×10^{-6} g mL^{-1}) against the action of histamine (at high dose range) on the isolated scale melanophores of *Oreochromis mossambicus*. Note the blockage demonstrated at the first four concentrations of histamine, however with the subsequent increase in histamine concentration the melanophores show considerable dispersion with the increase in MMSI. Abscissa: Concentrations of histamine per se in g mL^{-1}. Ordinate: Responses of melanophores in terms of MMSI. Vertical bars represent standard error. The p-value signifies level of significance; *p<0.0016, **p = 0.0001

first four concentrations, the attenuation in response to histamine was observed by a shift in the dose response curve towards right. However the combined action of mepyramine and ranitidine could not effectively block the dispersion caused by subsequent increase in concentration of histamine. The melanophores showed dispersion with the shift in MMSI from the control value 4.78±0.14 to 9.12±0.20, ($p<0.0001$) at the concentration 6.4×10^{-4}g mL^{-1} of histamine (Fig. 2). The conjecture emanated with this finding whether there is any other receptor class belonging to the histaminergic family that is regulating the pigmentary responses of melanophores.

Specific h_3r agonist immethridine *per se* and combined action of immethridine along with compound 48/80: Specific and potent H_3R agonist Immethridine was employed and its effect was tested on the melanophores of *Oreochromis mossambicus*. It was found that Immethridine *per se* resulted in pigment dispersion in a dose dependent manner. The extent of pigment dispersion in the dose range 1×10^{-6} g mL^{-1} to 6.4×10^{-5} g mL^{-1} was quite feeble with the MMSI recorded at 7.71±0.360 at dose concentration 6.4×10^{-5} g mL^{-1} from the control value 4.84±0.127 (Fig. 3).

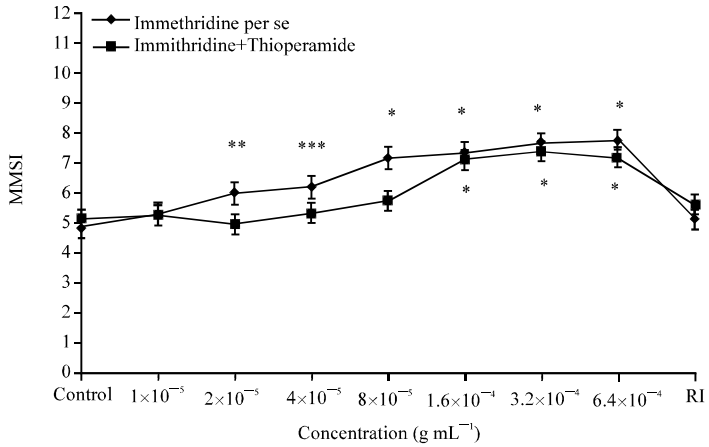

Fig. 3: Dose response curves showing the effect of Immethridine per se and the blocking action of thioperamide against Immethridine on the isolated scale melanophores of *Oreochromis mossambicus*. Note the action of Thioperamide against Immethridine result into a partial attenuation of dispersion exerted by Immethridine only up to first four concentrations. Abscissa: Concentrations of Immethridine *per se* in g mL. Ordinate: Responses of melanophores in terms of MMSI. Vertical bars represent standard error. The p-value signifies level of significance; *p<0.0001, **p = 0.09, ***p = 0.0032

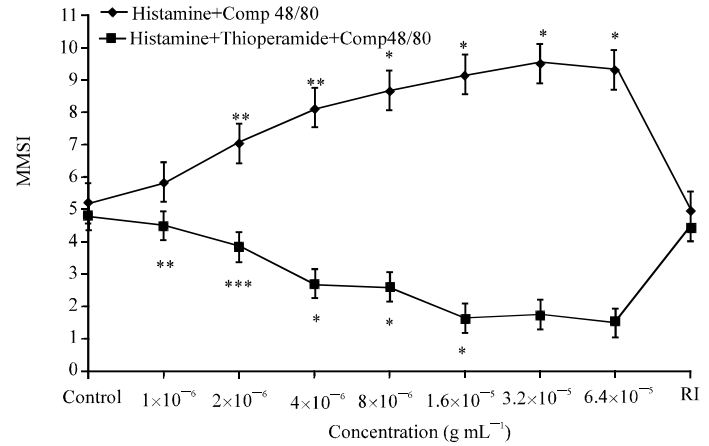

Fig. 4: Dose response curves showing the effect of Immethridine along with compound 48/80. Note the attenuation in the aggregatory response of compound 48/80 on Immethridine pretreated cells. Thioperamide caused considerable reversal in response against Immethridine along with comp 48/80 and resulted into considerable pigment aggregation. Abscissa: Concentrations of compound 48/80 on cells pretreated with Immethridine in g mL^{-1}. Ordinate: Responses of melanophores in terms of MMSI. Vertical bars represent standard error. The p-value signifies level of significance; *p<0.0001, **p = 0.09, ***p = 0.0032

As reported that H_3 agonists modulate the process of mast cell degranulation and henceforth release of histamine (Theoharides, 1998). We investigated if there is any regulatory mechanism involved in the release of histamine from mast cells encompassing the melanophores. Compound 48/80 was employed and found to cause pigment aggregation as discussed in the above section. Cells were pre-incubated with Immethridine (8×10^{-6} g mL^{-1}) and challenged with increasing concentration of compound 48/80. It was found that Immethridine attenuates the action of compound 48/80 (1×10^{-6}-6.4×10^{-5} g mL^{-1}) and blocks the aggregation completely (Fig. 4). Earlier compound 48/80 per se in the dose range 1×10^{-6}-6.4×10^{-5} g mL^{-1} showed a considerable pigment aggregation within the melanophores indicating the involvement of histaminergic receptors. But the marked degree of debilitation displayed by cells pretreated by Immethridne further corroborates the likelihood of H_3R involvement.

Effect of Immethridine and compound 48/80 along with H_3R specific antagonist thioperamide: To affirm the involvement of H_3Rs in the observed pigmentary responses by specific agonist Immethridine we employed a H_3R antagonist thioperamide. Earlier pilot experiments were conducted and it was found that thioperamide showed pigment aggregation *per se* in a dose dependent manner. Thioperamide in dose concentration 8×10^{-6} g mL^{-1} was selected to be the antagonist dose. The cells were pretreated with thioperamide and combined effect of Immethridine along with compound 48/80 was analyzed. It was found that pretreatment of cells with thioperamide resulted into pigment aggregation within the cells (Fig. 4). We found earlier that melanophores of *Oreochromis mossambicus* possess both H_1 and H_2 receptors, with the predominance of H_1 over H_2 (Unpublished data). The observed pigment aggregation therefore might be a result of H_1 (Gq/11) receptor stimulation, that brings about pigment aggregation due to calcium influx within the cells (Oshima *et al*., 1988; Miyashita and Moriya, 1990). To further investigate this contemplation we used specific H_1 and H_2 receptor antagonists and examined the subsequent variance in responses.

Action of Immethridine with H_1R antagonist- mepyramine and H_2R antagonist-ranitidine along with compound 48/80: The effect of compound 48/80 per se showed considerable pigment aggregation. The synergistic actions of combined H_1 and H_2 receptor antagonists; Mepyramine (2×10^{-6} g mL^{-1}) and Ranitidine (4×10^{-6} g mL^{-1}) were examined against cells pretreated with Immethridine against increasing concentrations of compound 48/80. It was quite interesting to observe that the melanophores showed slight pigment dispersion. This effect was dose dependent and increased with increasing concentrations of compound 48/80. The degree of pigment dispersion was noted and the MMSI at the highest dose concentrations (6.4×10^{-6} g mL^{-1}) was found to be 7.92±0.477 ($p<0.0001$). The dispersion observed clearly indicates that Immethridine is resulting into nullifying the aggregatory effects of compound 48/80. The masking of H_1 and H_2 receptors by mepyramine and ranitidine further emphasizes upon the role of histaminergic receptors of H_3 type (Fig. 5).

Action of Immethridine with synergistic blocking effects of mepyramine, ranitidine and thioperamide along with compound 48/80: Cells pretreated with specific

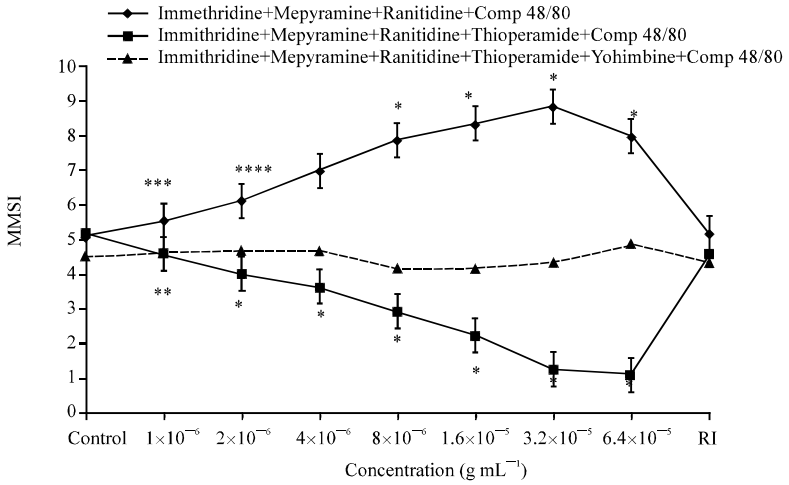

Fig. 5: Dose response curves showing the effect of Immithridine along with mepyramine (2×10^{-6} g mL^{-1}) and ranitidine (4×10^{-6} g mL^{-1}) against compound 48/80. The synergistic blockage demonstrated by reversal in responses exhibited by combined action of mepyramine (2×10^{-6} g mL^{-1}), ranitidine (4×10^{-6} g mL^{-1}) and thioperamide (8×10^{-6} g mL^{-1}) with cells pretreated with Immithridine is shown by decrease in MMSI. Note the complete attenuation to the action of compound 48/80 by synergistic blockage effect of yohimbine (4×10^{-7} g mL^{-1}) along with mepyramine, ranitidine and thioperamide. Abscissa: Concentrations of compound 48/80 on cells pretreated with Immethridine in g mL^{-1}. Ordinate: Responses of melanophores in terms of MMSI. Vertical bars represent standard error. The p-value signifies level of significance; *$p<0.0001$, **$p = 0.0123$, ***$p = 0.00352$, ****$p = 0.0005$

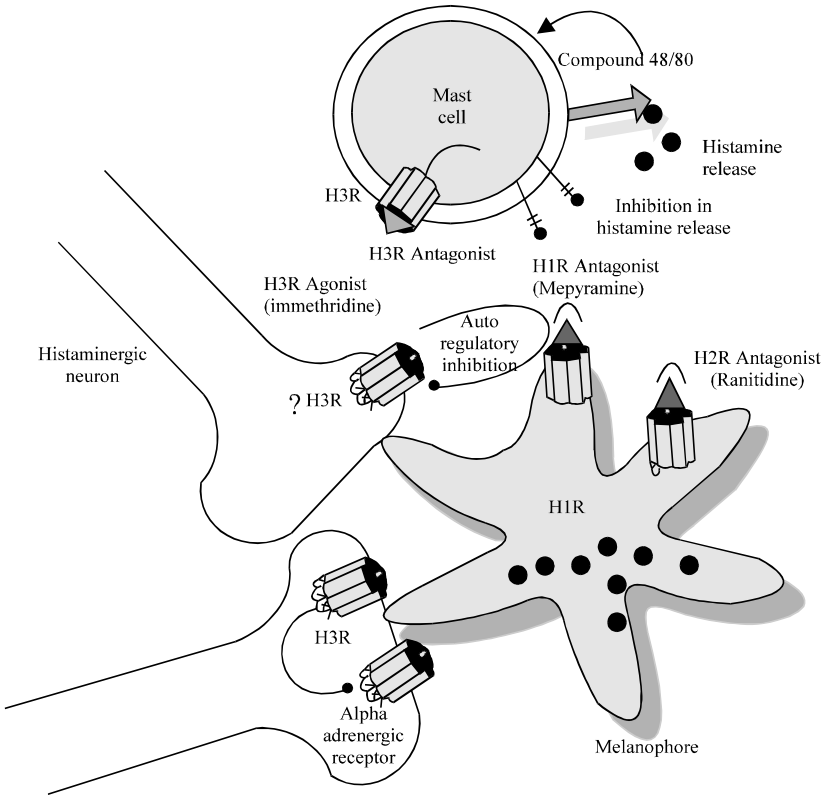

Fig. 6: Putative mechanism of Histamine H3 Receptor in regulation of Pigmentary responses within the melanophores of *Oreochromis mossambicus*. Release of histamine from surrounding mast cells by Comp 48/80 is inhibited by stimulation of H 3R by *Immeth ridine*. The feed back inhibition loop further inhibits the release of adrenaline from encompassing sympathetic nerve terminals and results in subsequent pigment dispersion

antagonists; H_1R- mepyramine (2×10^{-6} g mL^{-1}), H_2R-Ranitidine (4×10^{-6} g mL^{-1}) and H_3R-thioperamide (8×10^{-6} g mL^{-1}) along with Immethridine against comp 48/80 showed a marked decrease in MMSI from the control value. The MMSI recorded at highest dose concentration of compound 48/80 was 0.92±0.105 ($p<0.001$).The marked potentiation in aggregatory responses of melanophores by masking H_1, H_2 and H_3 receptors in presence of immethridine is clearly observed with the pigment movement towards the perikaron and the fall in MMSI (control 5.15±0.160) (Fig. 5). The unexpected exaggeration in pigment aggregation when compared with the dose response curve of compound 48/80 *per se* (Fig. 1) in the same dose range and Immethridine along with H_1 and H_2R antagonists with compound 48/80 implies the likelihood of an auxiliary involvement of sympathetic system. The observed findings indicate that the histaminergic systems further expands in regulating the release of other neurotransmitters most likely adrenaline.

Action of Immethridine and antagonistic effect of alpha 2 adrenergic blocker; yohimbine (4×10^{-7} g mL^{-1}), mepyramine, ranitidine and thioperamide along with comp 48/80: The unexpected potentiation in aggregatory responses exhibited by the cells on blocking the H_1, H_2 and H_3 receptors along with compound 48/80 emanated the likelihood of adrenergic involvement in pigment responses. We assumed that the association of pre-junctional H_3 like receptors at the neuro-effector alliance might be further modulating the release of adrenaline. Adrenergic innervation has been earlier reported to be present in teleosts (Xu and Xi, 2011; Jacobowitz and Laties, 1968) with the presence of alpha 2 adrenergic receptors in *Oreochromis mossambicus* (Acharya and Ovais, 2007). The effect of compound 48/80 when examined against cells pre-treated with mepyramine, ranitidine, thioperamide resulted into pigment aggregation. When the cells were again preincubated with H_1, H_2 and H_3 receptor antagonists along with yohimbine an alpha adrenergic blocker, it was found that the earlier

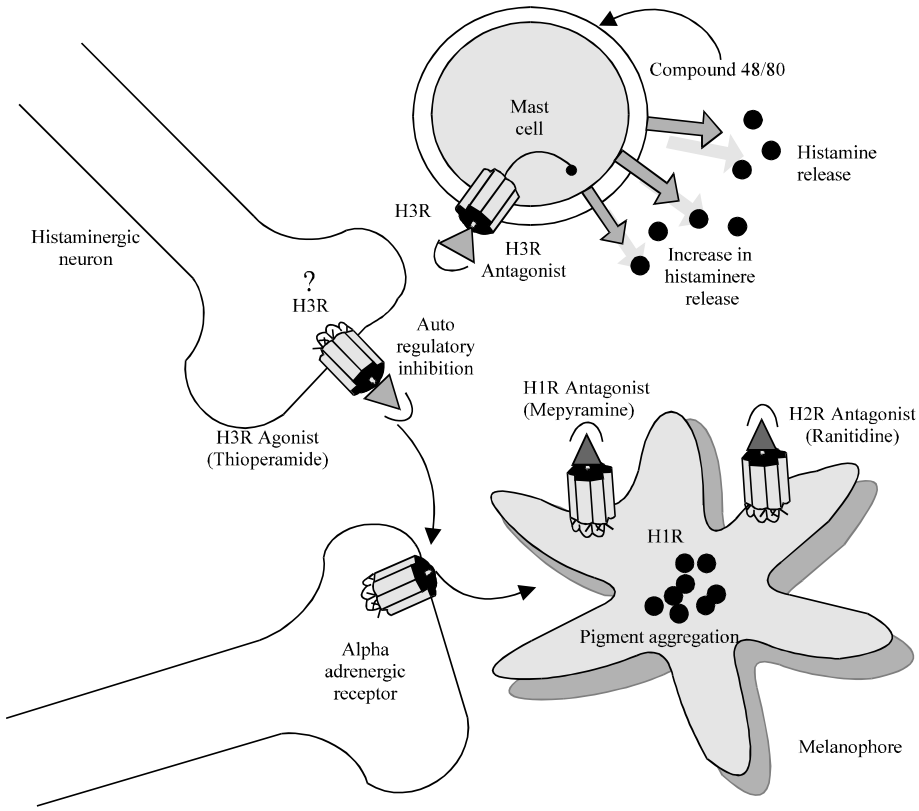

Fig. 7: The blockage of H3R with specific antagonist thioperamide resulted in pigment aggregation most likely due to subsequent stimulation of alpha adrenergic receptors present at the neuro melanophore junction

aggregatory response is completely abolished and blocked (Fig. 5). The dose response curve of yohimbine treated cells showed a shift towards right, almost in line with the control, demonstrating considerable blockage of aggregatory response. This finding confirms that the histaminergic system is involved in modulating the pigment motility within the cells and post synaptic H_3 like receptor is playing a regulatory role in controlling the regulation of adrenergic receptors.

DISCUSSION

The characterization of histaminergic receptors in fish by other workers has revealed that histamine receptors are linked to numerous neurological and neuro behavioral responses (Santos *et al*., 2003; Romaguera and Mattioli, 2008; Giusi *et al*., 2010). Also, the endocrinal employment of histamine extends into thermo regulation and gastric acid secretion (Green and Lomax, 1976; Holstein, 1986). In addition, the fish brain is well characterized structurally and the existence of the H_3-like receptor has been documented recently in zebrafish (Peitsaro *et al*., 2007). Also phylogenetic information on the central histaminergic system in the brain of a teleost, jack mackerel (*Trachurus trachurus*) has been reported (Inagari *et al*., 1991). However, to date there is little information about specific tissue localization and functioning of this histamine receptor in fish. The functional aspect of H_3 receptors in mammalian system has been explored quite significantly; nonetheless the exact localization of this receptor in lower vertebrates in different peripheral systems is still necessitated.

Interestingly, there is a well conserved 60% amino acid similarity between fish and mammalian homologues for H_1, H_2 and H_3 receptors (Peitsaro *et al*., 2007). Teleost fish are phylogenetically closer to the basic vertebrate blueprint than higher vertebrates and appear to have a simpler histaminergic system in terms of fiber density, area of innervation (Inagari *et al*., 1991). Our findings clearly implicate that the peripheral H_3 receptors exist on certain types of sensory nerves that innervate the melanophores and the activation of these receptors regulates/inhibits histamine release. Thereafter, subsequent downstream signaling impulses result in pigment translocation governing the pigmentary responses of the fishes. Although, histamine is not stored in neurons outside of

CNS but mast cell derived histamine can modify peripheral sensory nerve function (Hough and Leurs, 2006). Most importantly, profound pigmentary responses within the animals might result due to acute states of stress, immunological reactions or peripheral nerve cell injury suggesting a definite participation of mast cell histamine in the process (Hough and Leurs, 2006). The present finding indicates upon the putative involvement of mast cells derived histamine in the process of cellular pigmentary responses. The presence of these receptor classes in lower vertebrates and the possible role therein might clarify some inexplicable concepts about the evolution of these receptors classes from lower to higher vertebrates.

Activation of the prejunctional histamine H_3 receptor modulates sympathetic control in smooth muscles in mammals (McLeod *et al*., 1993). The sympathetic control of melanophores has already been reported in a number of species including *Oreochromis mossambicus* (Acharya and Ovais, 2007) where the melanophores respond by pigment aggregation (Yamada *et al*., 1984). It is known that mast cells are distributed on either side of pigment cell layer (Roberts *et al*., 1971) and compound 48/80 is a liberator of histamine from mast cells. In our present study it was observed that compound 48/80 released histamine that elicited a dual response within the melanophores on a concentration dependent manner. This finding entailed a prospective engagement of a signaling route pertaining to histamine that may possess a distinct mechanism of action, most likely self regulatory in operation. Henceforth we examined the effect of Immethridine a specific H_3R ligand and found that Immethridine nullifies the action of compound 48/80, indubitably connoting the involvement of H_3 receptors in the process (Fig. 6). The total attenuation expected with the employment of thioperamide against Immethridine was fairly ineffective; rather a surprising potentiation in the aggregatory response was observed within the melanophores. This finding gave an interesting direction in the study and presented the plausible adrenergic nexus in the course (Fig. 7). We later employed yohimbine a specific alpha adrenergic blocker and found that the aggregation caused by compound 48/80 along with Immethridine and thioperamide is completely and effectively blocked by the synergistic attenuation with yohimbine. The distinguishing feature of H_3 receptors mediating the regulation of other neurotransmitter systems; adrenaline in this regard has been clearly noticed. It is quite possible that there is a cross interaction between the histaminergic receptors inhibiting the innervating neurons at the neuro melanophore junction that are regulating the release of adrenaline. The release of adrenaline and subsequent stimulation of adrenoceptors resulting into pigment aggregation could be significant point crucial to the resultant cascade of signals preceded by histaminergic signaling.

CONCLUSION

It is concluded that there is a plausible role of histaminergic H_3 like receptors in the melanophore responses of the teleost, *Oreochromis mossambicus* at the neuro-melanophore junction. The presence of distinct class of H_3 like receptors on certain nerve endings innervating the melanophores is categorically determined. The engagement of these receptors seems to be more of self regulatory in operation depending upon the concentration of histamine at the encompassing milieu. Since histamine as a neurotransmitter may be associated to a plethora of neurobiological and neurobehavioral aspects, the consequential stimulation of histaminergic receptors may result into a cascade of signaling pathways leading to distinct pigmentary responses in the animal. The characteristic attribute of H_3 receptors mediating the regulation of other neurotransmitter systems; adrenaline in this regard has also been divulged. The release of adrenaline and subsequent stimulation of adrenoceptors resulting into pigment aggregation has been a sequential switching point in the entailing cascade preceded by histaminergic signaling. The presence of histamine receptors at the neuro-melanophore junction has been a very interesting finding. The central operation of histaminergic neurons towards mediation of auxiliary neuro transmitter systems needs to be further investigated. Since vertebrate melanophores have been reported to have a numerous classes of metabotropic receptors (Salim and Ali, 2011) and that vertebrate skin pigmentation in an incredibly complex mechanism, the probable association of other receptor classes may answer some unexplained apprehensions in the phenomenon of skin pigmentation.

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