Role of histamine receptors in the pigmentary responses of the wall lizard, *Hemidactylus flaviviridis*

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Effects of histamine per se along with its specific agonists and antagonists were studied on the skin melanophores of *Hemidactylus flaviviridis* to study the role of histamine receptors in pigmentation. Histamine and 2-methyl histamine induced powerful melanin aggregation effects, leading to paling of the skin through stimulation of H_1 receptors. On the other hand, 4-methyl histamine, a specific H_2 receptor antagonist caused melanin dispersion via activation of H_2 receptors, making the skin appear dark. It is concluded that histaminergic compounds have a considerable role in chromatic physiology of lower vertebrates, possibly as a neurotransmitter substance in melanin dysfunctions such as hyper and hypo pigmentation.

Keywords: *Hemidactylus flaviviridis*, histaminergic receptors, pigmentation, skin melanophores.

LOWER vertebrates have the capacity to rapidly alter their colouration by physiological regulation of the skin pigment cells, majority of which are melanophores. These cells provide an excellent model to study organelle transport in response to the externally applied stimuli[1]. Integumental melanophore changes leading to skin blanching and darkening of the extensively studied American lizard, *Anolis carolinensis* have been found to be mediated through dominantly present alpha and beta adrenoceptors[2–5]. However, no other reptilian species has been studied for the effects of histamine, barring a preliminary study by Ovais and Ali[5], where it was found that histamine mildly aggregated the integumental melanophores of Indian wall lizard, *Hemidactylus flaviviridis*. It has been reported that histamine receptors of H_1 and H_2 type are present in the skin melanophores of some amphibians – *Rana tigerina* and *Bufo melanostictus*, which mediate melanophore aggregation and dispersion respectively[6–8]. In view of this, it was thought worthwhile to study the effects of histamine and its agonists on the integumental melanophores of Indian wall lizard, *H. flaviviridis* in order to find out the nature and role of histamine receptors in its melanophore responses.

The lizard *H. flaviviridis*, Rupell, was procured locally and acclimatized to laboratory conditions prior to experimentation. Dorsal skin from the sacrificed animal was removed and soaked in 0.8% saline (pH 7.4) for 30 min for equilibration in saline. Isolated wall lizard melanophores are admirably suitable as they are uniform in size and are sensitive to pharmacological stimuli, which can be monitored rapidly with a high degree of statistical significance. When skin pieces are removed and soaked in 0.8% saline, they begin to aggregate slightly and after a 30 min pre-incubation they equilibrate. During this intermediate state, the melanophores are neither aggregated nor dispersed and can be tested for their response to various stimuli. The skin pieces, 2–4 mm in length, were transferred to petri dishes and incubated in known concentrations of drugs (agonists/antagonists) in 0.8% saline for 10–15 min with regular aeration. For use of antagonists, the skin pieces were first pre-incubated for 5–8 min in the specific blockers and then treated with varying concentrations of the agonist in a dose-dependent manner for 5–8 min. Melanophore responses were then measured using a previously calibrated Leitz ocular micrometer in low magnification according to the methods of Bhattacharya *et al.*[9]. The actual diameter of 10 randomly selected melanophores from the control and drug-treated skin piece was exactly measured and designated as mean melanophore size index (MMSI). This method is a modified version of the melanophore index of Hogben and Slome[10]. Data were statistically analysed using Student's *t* test following the method of Steel and Torrie[11]. All drugs were freshly dissolved in saline. The drugs used were: histamine acid phosphate (BDH, UK), mepyramine maleate (May & Baker, UK), metiamide, 2-methyl histamine and 4-methyl histamine (Smith Kline & French, UK).

Histamine per se aggregated the dorsal skin melanophores of *H. flaviviridis* in varying doses ranging from 0.32×10^{-7} to 20.8×10^{-6} M. The MMSI decreased from the control value of 3.83 ± 0.0351 to 0.12 ± 0.0101 of 20.8×10^{-6} M (Figure 1 *a* and *b*). This physiologically significant, highly aggregating response of histamine per se was blocked by mepyramine, a specific H_1 receptor blocker and also by metiamide, which is a specific H_2 receptor antagonist in pre-selected doses of 1.99×10^{-6} and 3.12×10^{-6} M respectively. Mepyramine was found to be more effective in blocking the aggregating effect of histamine (Figures 1 *d* and 2).

Similarly, 2-methyl histamine, a specific H_1 receptor agonist, was also found to aggregate the wall lizard melanophores. Different concentrations of 2-methyl histamine (7.9×10^{-7} to 51.1×10^{-6} M) aggregated the skin melanophores, where MMSI decreased from a control value of 3.75 ± 0.0349 to 1.09 ± 0.0285, as seen by the maximal concentration of 51.1×10^{-6} M. The aggregating response of 2-methyl histamine was antagonized by mepyramine (Figure 3). But the degree of aggregation was more in the case of histamine compared with that of 2-methyl histamine.

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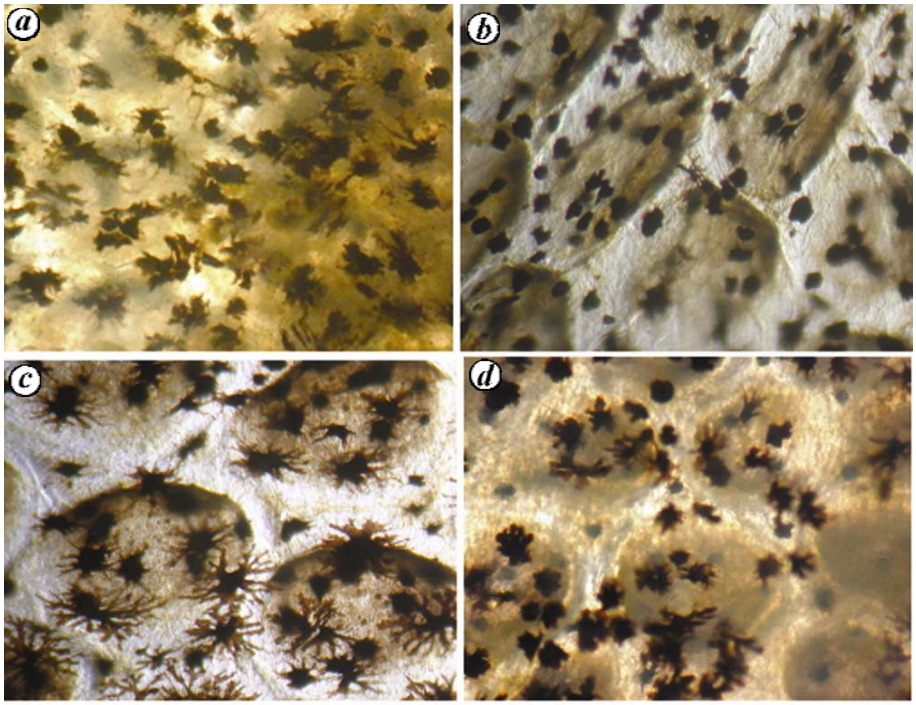

Figure 1. ***a***, Control condition of the isolated integumental melanophores of *Hemidactylus flaviviridis* in 10 ml 0.8% saline. ***b***, Aggregated condition of integumental melanophores by histamine per se. ***c***, Dispersed condition of integumental melanophores by 4-methyl histamine per se. ***d***, Blocking effects of melanin aggregation of histamine by mepyramine in the isolated integumental melanophores of *H. flaviviridis*; magnification: 100 ×.

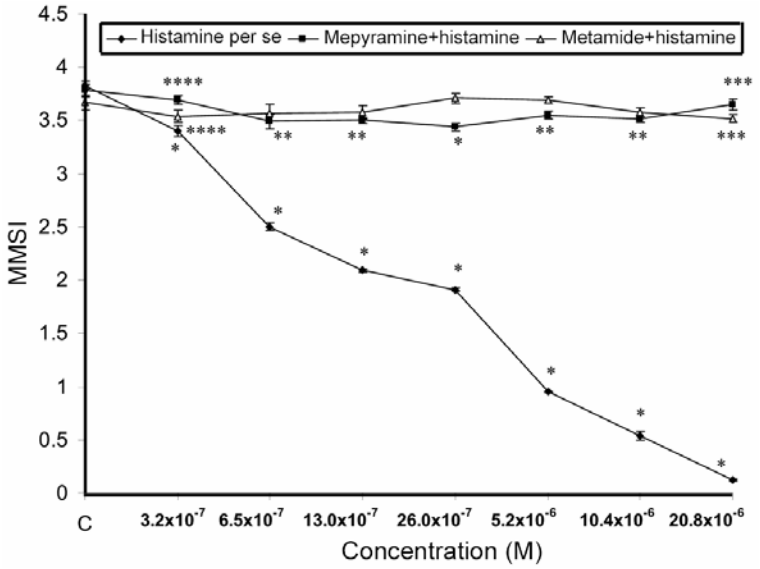

Figure 2. Dose response curve for the melanophore aggregation effect of histamine per se (◆) on the melanophores of *H. flaviviridis*. The complete blocking effects of specific antagonists mepyramine (1.99×10^{-6} M, ■) and metamide (3.12×10^{-6} M, Δ) against histamine aggregation melanophores are also shown. Abscissa: doses of agonist and antagonists in micromolar concentration. Ordinate: responses of melanophores (MMSI). Vertical bars represent the standard error of mean; *P* signifies the level of significance. *P* values: * < 0.001, ** < 0.01, *** < 0.1, ****< 0.2.

4-Methyl histamine, a specific H_2 receptor agonist, surprisingly dispersed the melanophores of *H. flaviviridis* in a dose-dependent manner, where the melanophore processes had become severely extended, showed intermingling and leading to skin darkening. In response to the maximal concentration of 32.3×10^{-6} M of 4-methyl histamine, MMSI showed a value of 13.91 ± 0.019 (Figure 1 *c*) compared to the control value of 3.82 ± 0.0306.

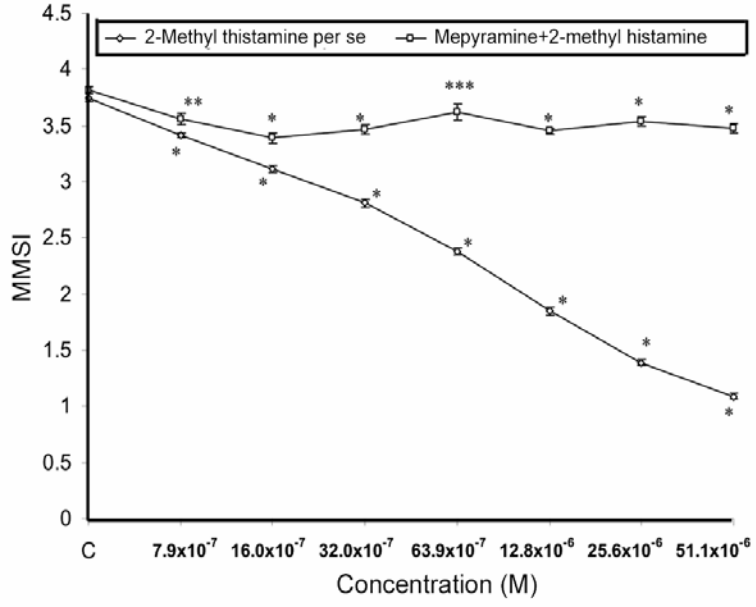

Figure 3. Dose response curve for the melanophore aggregation effect of 2-methyl histamine per se (◇) on the melanophores of *H. flaviviridis*. The complete blocking effects of specific antagonist mepyramine (1.99×10^{-6} M, □) against 2-methyl histamine aggregation melanophores are also shown. *P* values: *< 0.001, **< 0.01, ***< 0.05.

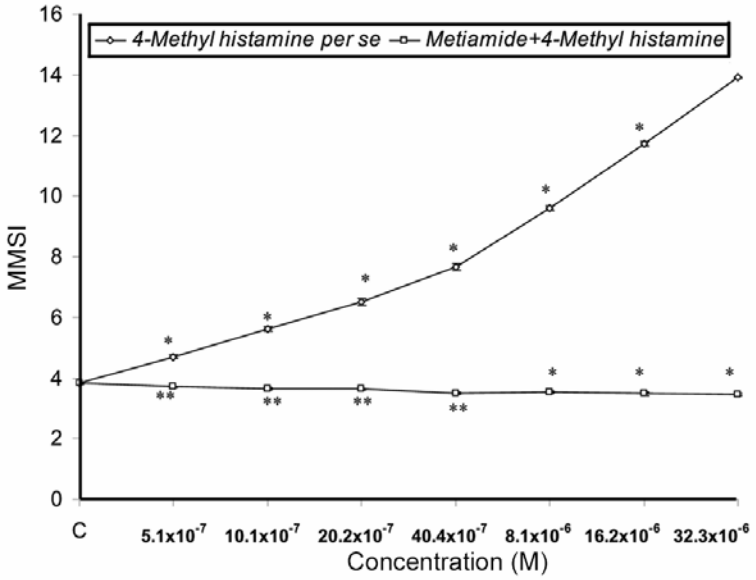

Figure 4. Dose response curve for the melanophore dispersion effect of 4-methyl histamine per se (◇) on the melanophores of *H. flaviviridis*. The complete blocking effects of specific antagonist metamide (3.12×10^{-6} μM, □) against 4-methyl histamine aggregation melanophores are also shown. *P* values: *< 0.001, **< 0.01.

This powerful melanin dispersion effect of 4-methyl histamine was totally blocked by metiamide, a specific H_2 receptor antagonist, where the melanophores remained in a control state of neither aggregation nor dispersion (Figure 4).

Anolis carolinensis is perhaps the only reptile whose melanophore responses have been studied extensively. It has been found to possess a mosaic population of adrenoceptors along with MSH receptors on its melanophores. The aggregation of melanin granules within the pigment cells of this species is regulated through alpha adrenergic receptors, whereas dispersion is through beta adrenoceptors of the melanophores[2–5].

Melanophores of no other reptilian species have been studied for the effects of histamine and its related compounds. In the present study, histamine and 2-methyl

histamine induced severe melanin aggregation within the melanophores of wall lizard, *H. flaviviridis* through dominantly present H_1 histaminergic receptors, as suggested by the data of the specific agonist and antagonist. The specific H_2 receptor agonist, 4-methyl histamine markedly dispersed the integumental melanophores of the lizard, leading to darkening of the skin. Metiamide, a specific H_2 receptor antagonist effectively blocked this melanin dispersal response, confirming the presence of H_2 type of histamine receptors which mediate melanin dispersal. In the past, melanophores of the studied reptilian species have been known to possess even α_2 and β_2 adrenergic receptors, which have been shown to induce melanosome dispersion by stimulation of adenylyl cyclase and melanophore aggregation by its inhibitors[4].

In the present study, the aggregation of melanophores is by H_1 receptor stimulation, possibly through inhibition of adenylate cyclase by histamine or by the release of endogenous catecholamines. Similarly, the dispersal of melanophores by H_2 receptor stimulation is either by the stimulation of adenylate cyclase leading to cyclic AMP elevation, or by a direct involvement of histamine as a neurotransmitter substance. We have recently demonstrated that in amphibian melanophores, histamine aggregated the melanophores by H_1 and H_2 receptor stimulation and the dispersal response was by the stimulation of H_2 receptors[6–8]. These findings are in fairly good agreement with those of Arrang *et al.*[12], where it has been reported that a variety of cell types such as smooth muscles, neurons, endocrine or exocrine glands, and other cells respond to histamine by increasing the intracellular levels of signals generated by either the phosphatidyl inositol cycle or adenyl cyclase system. Similarly, the present data showing the involvement of histamine H_2 receptors in pigment dispersion leading to skin darkening in *H. flaviviridis* corroborate with those of Yoshida *et al.*[13], where it has been demonstrated that histamine is involved in ultraviolet B-induced pigmentation and that famotidine suppressed the pigmentation by the prevention of histamine binding to H_2 receptors in guinea pig melanocytes. These findings are similar to those of the present study, where we demonstrate involvement of histamine H_2 receptors in skin darkening response of the wall lizard. The data also suggest the evolutionary significance of the lower vertebrate melanophores with those of mammalian melanocytes from the point of view of histamine receptor involvement. Currently, we are focusing on the role of histamine as a neuro-transmitter substance with its receptor induction in melanin dysfunctions, such as hyper and hypo pigmentation.

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