Responses Of Buspirone As Agonist And Antagonist To 5-Hydroxytryptamine On Isolated Scale Of Melanophores Of Rasbora Daniconius (Ham.)

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Abstract

The response of buspirone as agonist and antagonist is well studied in dorso-lateral region as well as band region in melanophores of Rasbora daniconius. The melanophore size index (MSI) was employed as a recording parameter for the responses. The responses were both as dispersal as well as aggregation are seen. The dispersal as well as aggregation are both seen is dorso-lateral region while aggregation has been observed in band region melanophores.

Keyword: Melanophores, 5-HT, buspirone

Date of Submission: 27-08-2025 Date of Acceptance: 07-09-2025

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I. Introduction

Buspirone, an anti-anxiety drug that affect chemicals in the brain and is used to treat fear, tension, irritability, dizziness pounding heart beat and other physical symptoms. The main action of buspirone is that its interaction with 5-HTA receptor.

Acharya (2002) on *Oreochromis mossmabica* melanophore, buspirone *per se* was not effective in elicited any kind of response but it potentiated and inhibited the 5-HT induced aggregation according to its concentration employed. Therefore it may be concluded that 5-HT₁ like receptor do exist in fish melanophores but with a difference according to species. Further in the lizard melanophores Ovais *et.al.*, (1994) also indicated the presence of 5-HT₁ like receptors in that animal species. It would be of further interest to note that buspirone has also induced aggregation in frog *Rana cyanophlyctis* melanophores *in vitro* by Pandey (2003). Therefore it can safely conclude that 5- HT₁ like receptors are present in the lower vertebrates right from fishes to the reptiles.

II. Material & Method

Experiments were performed on the isolated scale melanophores from dorso-lateral and band regions of the young fishes of either sex of *Rasbora daniconius* (Ham.), belonging to the Family - Cyprinidae and Order – Cypriniformes were procured from the local water bodies and transported to the laboratory alive. The size of these fishes varied from 5 to 7 cms and weighing from 1.5 to 3 gms. The fishes were acclimatized in laboratory for at least 48 hrs. Care was taken to maintain healthy laboratory conditions for fish. Those having infection or showing slight sluggishness were immediately removed. Water was changed twice in a week during the experiments. For experimental purpose the fish of equal size and weight were taken.

The scales were removed from the dorso-lateral region below the head and band region of the fishes. Care was taken to hold the scales by their basal non-pigmented region. The size of the melanophores of *Rasbora daniconius* is different in dorso-lateral region with the band region. The size of melanophores in band region is larger than the dorso-lateral region.

Initial experiments were performed in fish Ringer. (Ovais, 1976), the results when compared with the experiments in 0.7% NaCl solution, it was found that saline medium gave better results than fish Ringer. Hence all the experiments were performed in saline medium.

The scales were incubated for 10-15 minutes in saline medium with frequent shaking. Different concentrations of drug were prepared for experiments in separate petri- dishes. Drug solutions were also prepared in 0.7% saline. Solution of 10 ml saline taken in different petri dishes. Out of that 0.5 ml saline was removed and drug in equal volume was added to avoid the final volume exceeding 10 ml.

Drugs were dissolved immediately before use. Incubation period for the scales in drug solution is 10 minutes, when antagonists were used along with agonists; scales were first treated with antagonists for 10

minutes in different petri- dishes. Then 0.5 ml solution of antagonists was removed from each petri dish and then equal volume of different concentrations of agonist was added to different petri- dishes. The contact time of agonist was also 10 minutes.

The control as well as treated scales were first placed on a glass slide with dermal side down with a little incubation medium and covered with a glass cover slip.

Measurement technique

Individual melanophores were measured in low power microscope with an ocular micrometer (Erma, Japan). The melanophore size index (MSI) was calculated according to the method of Bhattacharya *et. al.*, (1976). The maximum vertical and horizontal diameters of five melanophores of a scale were recorded and the MSI was calculated as equal to

Maximum vertical X Horizontal diameter

100

In the present study both dorso-lateral region and band region melanophores were taken under observation separately. The experiments were carried out at room temperature ranging between $20^{\circ}\text{C} - 30^{\circ}\text{C}$.

Composition of fish Ringer solutions:

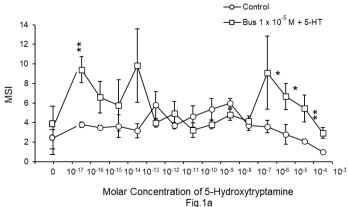
Quantity
5.9 gms
0.2 gms
0.25 gms
0.29 gms
2.00 gms
2.10 gms
0.25 gms
1000 ml.

Both A & B solutions were made in separate beakers containing 500 ml distil water. Solution of beaker B was added slowly in beaker A, which contains the chemicals from NaCl to MgSO₄. The drug Buspirone is belong to Intas Pharmaceutical Ltd., Ahmedabad. Statistical analysis was performed using students "t" test.

III. Observation

To find out the nature and site of action of 5-HT on the two types of melanophores of *Rasbora daniconius*, we employed different non-specific, specific and partial antagonists to block the effects of 5-HT on this fish melanophores. First of all we employed buspirone 1 x 10⁻⁵ M and incubated the melanophores in it for 10 minute followed by the addition of 5-HT and further incubated for 10 minute. This procedure was employed for each concentration of 5-HT. 5-HT in presence of buspirone mostly elicited dispersion in dorso-lateral region melanophores and this dispersal effect was highly significant. In the band region also dispersal effect of 5-HT in presence of buspirone was apparent. It is concluded that 5-HT in combination with buspirone may have elicited the dispersion through the activation of 5-HT receptors (Fig.1a,b).





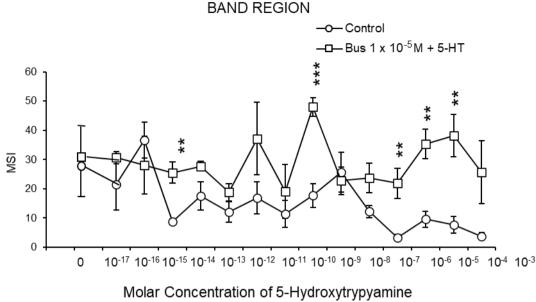


Fig.1b

Fig 1 a,b: Graphs showing concentration-response curves of 5-HT in absence and in presence of buspirone (Bus.) on the isolated scale melanophores of Rasbora daniconius, from dorso-lateral region (a) and band region (b). Abscissa: Molar concentration of 5-HT. Ordinate: Responses of melanophores as melanophore size index (MSI). Each point is the mean \pm SE (vertical bars) from five experiments on different fishes. P values were calculated between concentration-response curves of 5-HT in absence and in presence of antagonist. Symbols not showing SE values represents where they lie within the symbols.

$$* = P < 0.05, ** = P < 0.01, *** = P < 0.001.$$

IV. **Discussion**

In the present study buspirone, the 5-HT agonist elicited aggregatory as well as dispersal responses. These two kinds of responses were significantly different from the control level responses, therefore the presence of 5-HT₁ receptors is strongly indicated. There was an apparent difference of sensitivity of the two regions melanophores to buspirone. The band region melanophore seems more clearly responsive to buspirone then dorso-lateral region.

Buspirone also act as partial antagonist according to Hoyer et.al. (1994), hence we employed it to test where it has any antagonist effects on fish melanophores too. There results indicate that in presences of buspirone 5-HT elicited significant depression, which was obvious in dorso-lateral region and band region melanophores. It is likely that dispersion may be mediated through any other types of 5-HT receptors like 5-HT₄, which we have described earlier or they may be 5-HT₇ type for which a description has been given by Teh & Sugden (2001) in amphibian melanophore.

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