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# Responses of Melanophores in Denervation Experiment of *Rasbora Daniconius* (Ham.) with 5-Hydroxytryptamine

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**ABSTRACT**

The denervation of melanophores with serotonin were examined on the isolated scale of melanophores of *Rasbora daniconius* in its dorso-lateral region and band region. The melanophore size index (MSI) was employed as a recording parameter for the responses of denervated melanophores to serotonin. It was observed that after denervation the sensitivity of the dorso-lateral region was not changed significantly, but in case of band region melanophores sensitivity of melanophores to serotonin was significantly decrease or inhibited.

***Keywords:*** *Fish melanophores, serotonin, denervation*

## INTRODUCTION

Rapport, Green and Page (1948) for the first time described 5-Hydroxytryptamine(5-HT) or serotonin and according to authors its source is serum and its activity is one causing constriction. 5-HT has a wide distribution among the vertebrates and invertebrates and also act as neurotransmitter in many central and peripheral nervous systems of both the groups (Erspamer 1963; Leake and Walker, 1980).

Studies on the effects of 5-HT are few in lower vertebrates in comparison with the effects of adrenergic and cholinergic drugs (Parker, 1948; Bagnara and Hadley, 1973; Fujii and Oshima, 1986, 1994). The results of the studies on the nature of melanophores to 5-HT are rather diverse. Cerletti and Berde (1955) first observed that although it inhibited the melanin dispersing action of D-lysergic acid diethylamide (LSD), 5-HT itself had no effect on guppy melanophores either *in vivo* or *in vitro*. Fujii (1961) noted that 5-HT had no aggregating effect upto $10^{-4}$ M on goby, *Chasmichthys*. Healey and Ross (1966) and Ruffin *et. al,* (1969) presented their results that 5-HT has no significant effect upon *Phoxinus* or *Nannostomus* melanophores, either in aggregating or dispersing melanophores.

Miyashita and Fujii (1973) further observed that reserpine treatment of the live fishes for 4.45 hours potentiated the aggregatory responses of guppy fish melanophores to 5-HT. These authors also mentioned about the effects of alpha and beta adrenergic receptor blocking agents on the guppy melanophore responses to 5-HT. They found that adrenergic blockers induced an inhibitory effect on the aggregatory responses of the fish melanophores to serotonin.

Gaur (1994) worked on the melanophores of *C. mrigala* and found that 5-HT induced aggregation. However, the effect was dose dependent. She also found that denervation of melanophores (either *in vivo* or *in vitro*) increased the sensitivity of melanophores to 5-HT. In an experiment she found that pre-treatment fishes with either PCPA or 5-HTP, which are specific 5-HT synthesis inhibitor and serotonin synthesis potentiators respectively, did not induce any significant change in the sensitivity of this fish melanophores to 5-HT. She concluded from this experiment that 5-HT is not involved directly as neurotransmitters in the pigment translocations of this fish species, but its effect may be mediated through its specific receptors.

## MATERIALS AND METHODS

The young fishes of either sex of *Rasbora daniconius* (Ham.) were procured from local fish farms and transported to the laboratory alive. The fishes were 5 – 7 cms long and weighing 8 -10 gms. The fishes were acclimatized in the laboratory for 48 hrs, with normal day and night cycle of the prevailing seasons at room temperature between $25^0$ to $30^0$C during summer (April to May) and during early winter (October to November). The scales were removed from dorso-lateral region below the head and band region of the fish. The band region is dark black in color which extends from posterior end of the orbit and run upto the tip of tail. The scales were immediately immersed in 0.7 % NaCl (fish saline). The saline medium has been found to give better result than fish Ringer. Hence all the experiment were carried out in the saline medium only. For each concentration of 5-HT separate petri-dish were used. Individual melanophores on scales were measured with an ocular micrometer (Erma, Japan) in low power microscope and mean Melanophore size index (MSI) was calculated according to the method describe by (Bhattachary *et. al.* 1976) and described in detail elsewhere (Ovais *et.al.* 1994). The decrease and increase of MSI from the control value represent aggregation and dispersion of melanophores respectively.

For the denervation experiment reserpine was taken in solution according to method described by Katayama *et. al.,* (1980). 1mM reserpine solution was made in 125mM of citric acid. After incubating the scales in 0.7% saline medium, the scales were incubated for 1 hr 30 min in reserpine solution and MSI was recorded and tested for the state of denervation by KCl treatment. No aggregatory response of melanophores to KCl treatment was considered a positive denervation state of the melanophores. Further experiments were performed only from those fish scales, which responded positively for the denervation test.

Statistical analysis was performed with student's t-test.

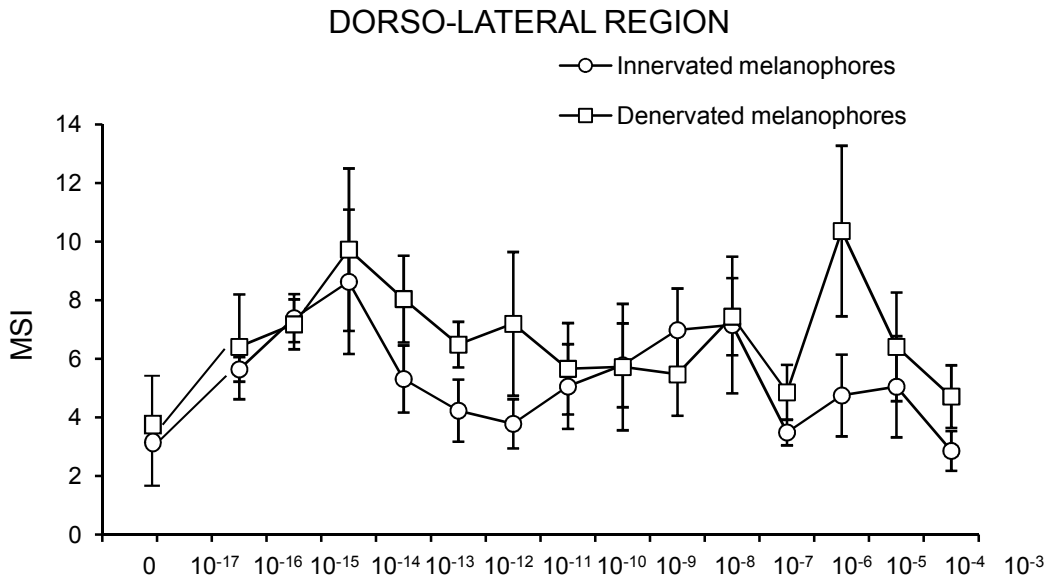


Fig.1a

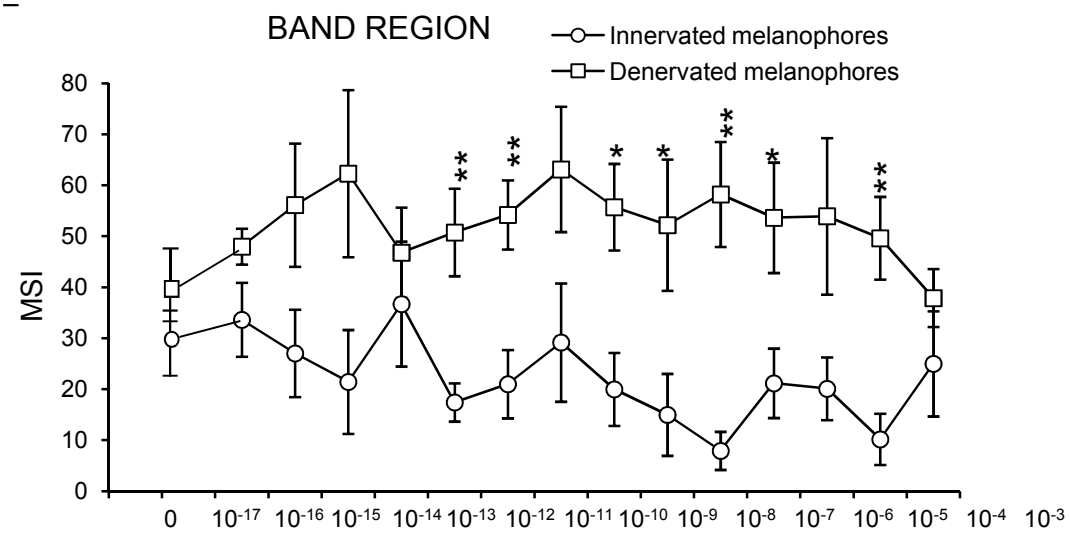

Fig.1b

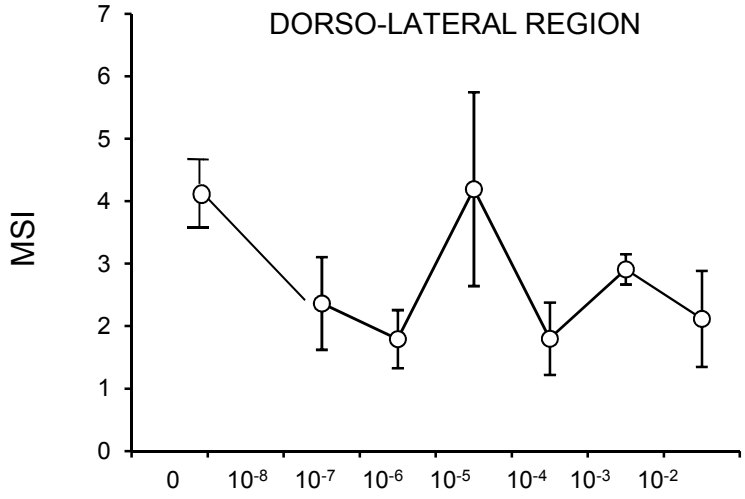

Fig. 2a

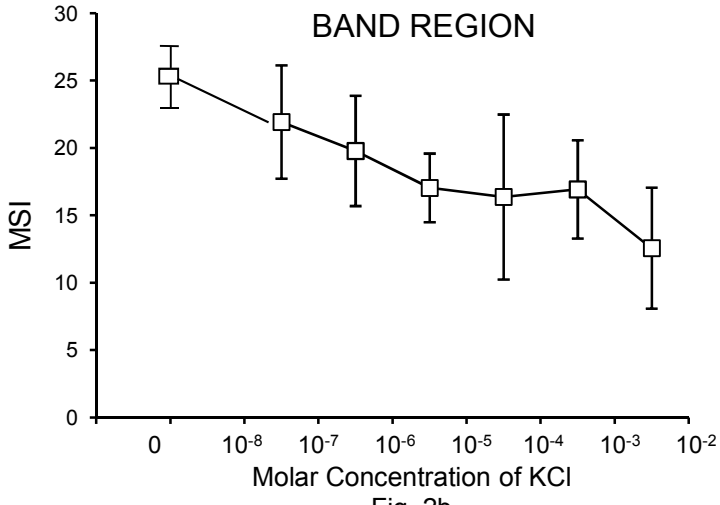

Fig. 2b

## RESULT

The melanophores of *Rasbora daniconius* (Ham.) were of typical star shaped in both the regions i.e. dorso-lateral region and band region. The dorso-lateral region melanophores were smaller in size (MSI 4.43 ± 0.725), while in band region melanophores were larger in size (MSI 25.98 ± 3.609). In both the regions epidermal melanophores were few, hence the present study was performed on dermal melanophores only. Denervation of the *Rasbora daniconius* melanophore was performed as described in the material and methods. On the denervated melanophores the effect of 5-HT was investigated by plotting the concentration-response curves of 5-HT on the innervated and denervated melanophores together. After denervation the sensitivity of the dorso-lateral region melanophores was not changed significantly. However, in a few concentrations the responses were inhibited in comparison with the innervated once in the same concentration of 5-HT (Fig. 1a).

It is concluded from the comparison of the concentration-response curves of innervated and denervated melanophores to 5-HT that some change occurred i.e. an inhibition in the sensitivity of the melanophores after denervation was appeared (Fig. 1a). In the case of band region melanophores sensitivity of the melanophores to 5-HT was significantly decreased or inhibited which is apparent from the shifting of concentration-response curve of 5-HT on denervated melanophores (Fig. 1b). In order to investigate the nature of innervation of this fish melanophores we employed KCl, KCl induced aggregation in a dose range of $1.341 \times 10^{-7}$ to $1.341 \times 10^{-3}$M. All the concentrations of KCl employed induced aggregation in dorso-lateral as well as band region melanophores except one concentration of KCl i.e. $1.341 \times 10^{-5}$ M and which induced dispersion in dorso-lateral region melanophores (Fig. 2a). In the band region the effect of KCl was concentration related and consistently induced aggregation (Fig.2b). It is concluded that the responses of band region melanophores to 5-HT may be partially mediated through the release of some neuro-transmitter substance by the 5-HT itself.

## DISSCUSION

In the present study denervation experiment was performed to investigate the nature of melanophore responses after depleting the neurotransmitter substances in the melanophore endings. It was observed that no significant inhibition in the sensitivity of the dorso-lateral region melanophores occurred after denervation to 5-HT, while in the case of band region melanophores a significant inhibition occurs, therefore it is concluded that 5-HT induced responses on the dorso-lateral region melanophores are directly mediated through receptors, while in the case of band region melanophores 5-HT induced responses or partially mediated through the 5-HT receptor directly. Gaur (1994), observed that denervation potentiated the sensitivity of *C.mrigala* melanophore to 5-HT. In another study on *O.mossambica* by Acharya (2002) a significant inhibition in the sensitivity of the melanophore to 5-HT was apparent after denervation treatment. In an earlier study by Scheline (1963) on *Labrus ossifagus* melanophores denervation by reserpine treatment completely abolished the aggregatory responses to 5-HT. In *Labrus ossifagus* melanophore it seems that the effects of 5-HT was wholly indirectly mediated through the release of neurotransmitter substance by 5-HT itself.

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