Evidence for presence of GABA-ergic receptor mediated dispersion in isolated scale melanophores of a carp, Cirrhinus mrigala Ham.

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Effects of GABA-ergic agonists and antagonists were examined on the melanophores of a carp C. mrigala in vitro. GABA and baclofen both induced concentration-related dispersion in fish melanophores. Denervation of the melanophores by reserpine treatment potentiated the sensitivity of the melanophores to GABA. While denervation by cooling treatment inhibited the sensitivity of the melanophores to GABA, atropine, bicuculline and pentylenetetrazole all inhibited the dispersal responses of the melanophores induced by higher concentrations of GABA. 5-aminovaleric acid also significantly inhibited the dispersion of the melanophores induced either by GABA or baclofen. It is concluded that GABA-ergic agonist induced dispersal responses in C. mrigala melanophores are mediated through specific GABA receptors. The presence of both GABA_A and GABA_B receptors in this fish melanophores has been indicated.

Gama-aminobutyric acid (GABA) is an important inhibitory neurotransmitter of mammalian CNS, and is also found in peripheral tissues. It may act as a neurotransmitter or neuromodulator of the autonomic nervous system or function as a hormone or trophic factor in non-neuronal tissues. In the mammalian systems at least three subclasses of GABA receptors, GABA_A, GABA_B and GABA_C which are pharmacologically and structurally distinct, have been identified. In the invertebrate nervous system too, GABA has been detected and found to play a significant role in neurotransmission. In several fish species GABA has been detected both in brain and visceral organs, which indicates that this substance may also have a role in physiological processes of this group of vertebrates.

Effects of a large number of substances like, neurotransmitters, neuromodulators, various ions, hormones, chemicals and drugs etc. have been studied on fish melanophores both in vivo and in vitro. However, no report is available, concerning with the effect of GABA on fish melanophores except one by Miyashita and Fuji10, who found that GABA has no effect on the fish (guppy) melanophores. Hence, its role in the melanosome control mechanism was ruled out. During survey on the effects of various neurotransmitters and neuromodulators on carp, Cirrhinus mrigala, melanophores, has been observed a clear and potent dispersal effect of GABA on this fish melanophores. Therefore, the present study has been undertaken to analyse the nature and site of action of GABA on C. mrigala melanophores in vitro.

Materials and Methods
The fishes Cirrhinus mrigala (Ham.) of either sex, 10-12 cm long and weighing 8-10 g were procured from local fish farms and transported to the laboratory alive. The fishes were acclimatised in the laboratory for at least 48 hr, with normal day and night cycle of the prevailing season at the room temperature between 16 and 25°C during March to May. Scales were removed from dorsal region below the head and lateral sides of the fishes and immediately immersed in 0.7% saline medium. After equilibration period (15-20 min.), scales (3-5) were transferred in glass petri dishes containing 10 ml saline. For each concentration of the drug, separate petri dish was used. Contact time of drugs with the scales was 10 min. When antagonists were used scales were first incubated in it for 10 min followed by addition of agonist, and ten min. further incubation in the medium. The control as well as treated scales were placed on glass slide with a little incubation medium and covered with a glass cover slip. Individual melanophores were measured with an ocular micrometer (Erama, Japan) in low power microscope and mean melanophore size index (MSI) was calculated according to the method described by Bhattacharya et al. The increase or decrease of MSI
from the control value represent dispersion and aggregation of melanophores respectively.

Denervation of the fish melanophores was achieved by reserpine and cooling treatments of the scales. Reserpine was taken in solution according to the method described by Katayama. Denervation of the fish melanophores was achieved by reserpine and cooling treatments of the scales. After completion of incubation in reserpine solution, MSI value was recorded and the scales were transferred in various drug concentrations. Cooling treatment of the scales was done according to the procedure described earlier by Ovais et al. The state of denervation of the melanophores was tested by KCl treatment. No aggregatory response of melanophores to KCl treatment was considered a positive denervation state of the melanophores.

The following drugs and chemicals were used: GABA, baclofen, bicuculline methchloride, pentylentetrazole, 5-aminovaleric acid HCl and atropine sulphate (Sigma Chemical Co., USA), and reserpine (Hindustan Ciba-Geigy Ltd., Mumbai, India).

Statistical analysis was performed with Student’s t-test.

Results

GABA (1 × 10⁻¹¹ M to 1 × 10⁻³ M) elicited dose-related dispersion in the isolated scale dermal melanophores of C. mirigala. The dispersion elicited by GABA was also detectable in the epidermal melanophores, but it was neither dose-related nor consistent (Fig. 1). Therefore, following/foregoing account is given for dermal melanophores only. Denervation of the melanophores by cooling treatment caused a significant inhibition in the sensitivity of this fish melanophores to GABA which is evident by the downward shifting of the dose-response curve of GABA on cooling treated melanophores (Fig. 1). However, the denervation of the fish melanophores by reserpine treatment in vitro potentiated the sensitivity of the melanophores to GABA, consequently the dose-response curve shifted upward (Fig. 1). Baclofen (5 × 10⁻¹¹ M to 5 × 10⁻⁴ M) which is a specific GABAA receptor agonist, elicited dose-related dispersion in C. mirigala melanophores (Fig. 1). It is evident from the dose-response curve of baclofen that the melanophores are more sensitive to baclofen than GABA.

Bicuculline (1 × 10⁻³ M), a specific GABAA receptor antagonist could not inhibit the dispersal responses of C. mirigala melanophores to lower concentrations of GABA, though it slightly enhanced the responses. However, the responses of the melanophores to higher

![Fig. 1 — Concentration - response curves of GABA and Baclofen on the isolated scale melanophores of C. mirigala. Values are mean ± SE (vertical bars) N = 5. Fig. 1A = dermal melanophores. Fig. 1B = epidermal melanophores.](image-url)
concentration range of GABA (1 × 10⁻⁷ M to 1 × 10⁻⁴ M) were inhibited (Fig. 2). Pentylenetetrazole (1 × 10⁻⁵ M) also slightly inhibited the dispersion of the melanophores induced by only higher concentrations of GABA (Fig. 2). Atropine (1 × 10⁻⁵ M) also potentiated the dispersal responses of the melanophores to low concentration range of GABA (1 × 10⁻¹ M to 1 × 10⁻⁸ M).

The dispersal responses elicited by higher concentration range of GABA (1 × 10⁻⁶ M to 1 × 10⁻⁴ M) were inhibited by atropine (Fig. 2). Bicuculline, pentylenetetrazole and atropine all induced dispersion in the melanophores per se in higher concentrations (1 × 10⁻⁴ M to 1 × 10⁻³ M). 5-aminovaleric acid (1 × 10⁻⁵ M) significantly inhibited the dispersal responses of this fish melanophores to GABA and baclofen (Table I).

Discussion
γ-aminobutyric acid (GABA) induced dispersion in C. mrigala dermal melanophores in a concentration related manner, while in the epidermal melanophores, the responses were quite feeble, inconsistent and not in a concentration related manner. The only available report regarding the effect of GABA on the fish melanophores in vitro, revealed that this neurotransmitter substance has no effect on the guppy (Lebistes reticulatus) melanophores. The present results are contrary to the report of Miyashita and Fujii. The clear, consistent, conspicuous and concentration related dispersion of C. mrigala melanophores elicited by GABA indicates its definite role in controlling mechanism of fish melanophores. The phenomenon may be explained on the basis of species variations. It is well known that few neurotransmitter substances like acetylcholine, adrenaline, histamine, serotonin and melatonin etc., are capable to influence the melanophores of most of the fish species either in vitro or in vivo. However, the melanophores of a few other species of fishes are refractory to any one or more than one of these substances. The present study also reveals that epidermal melanophores are much less sensitive to GABA and hence it may have no significant role in the controlling mechanism of these melanophores.

Denervation of C. mrigala melanophores by two different methods i.e., either by cooling treatment method or by in vitro method of reserpine treatment, produced different kinds of results. The cooling treatment method rendered the melanophores less sensitive than innervated melanophores. However, nature of response did not change. This indicates that the dispersal responses of C. mrigala melanophores to GABA are not indirect in nature but may be direct in nature through specific receptors. The reserpine treatment of the melanophores in vitro, in the present study induced a significant enhancement of the sensitivity of the melanophores to GABA, which is evident from the upward shifting of the dose-response curve of GABA on reserpine treated melanophores. This phenomenon of enhanced sensitivity to drug after

Fig. 2: Concentration - response curves of GABA in absence and in presence of bicuculline, pentylenetetrazole and atropine on the isolated scale melanophores of C. mrigala. Values are mean ± SE (vertical bars.) N = 5. Fig. 2A = dermal melanophores. Fig. 2B = epidermal melanophores.
Table I—Effect of 5-aminovaleric acid on dispersal responses of *C. mrigala*, isolated scale melanophores to GABA and baclofen.

<table>
<thead>
<tr>
<th>Experimental Drug</th>
<th>Concentration of drug (M)</th>
<th>MSI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.7% saline</td>
<td>4.58 ± 0.303</td>
<td>-</td>
</tr>
<tr>
<td>GABA</td>
<td>1 × 10⁻⁴</td>
<td>7.57 ± 0.579</td>
<td>&lt;0.01 (1-2)</td>
</tr>
<tr>
<td>GABA</td>
<td>1 × 10⁻⁶</td>
<td>6.86 ± 0.290</td>
<td>&lt;0.001 (1-3)</td>
</tr>
<tr>
<td>5-aminovaleric Acid</td>
<td>1 × 10⁻⁵</td>
<td>5.18 ± 0.390</td>
<td>-</td>
</tr>
<tr>
<td>5-aminovaleric Acid</td>
<td>1 × 10⁻⁵</td>
<td>5.84 ± 0.128</td>
<td>&lt;0.05 (2-5)</td>
</tr>
<tr>
<td>5-aminovaleric Acid + GABA</td>
<td>1 × 10⁻⁵</td>
<td>5.83 ± 0.108</td>
<td>&lt;0.05 (3-6)</td>
</tr>
<tr>
<td>Baclofen</td>
<td>4.5 × 10⁻⁴</td>
<td>9.24 ± 0.258</td>
<td>&lt;0.001 (1-7)</td>
</tr>
<tr>
<td>Baclofen</td>
<td>4.5 × 10⁻⁶</td>
<td>7.66 ± 0.507</td>
<td>&lt;0.001 (1-8)</td>
</tr>
<tr>
<td>5-aminovaleric Acid + Baclofen</td>
<td>4.5 × 10⁻⁴</td>
<td>5.48 ± 0.309</td>
<td>&lt;0.001 (7-9)</td>
</tr>
<tr>
<td>5-aminovaleric Acid + Baclofen</td>
<td>1 × 10⁻⁵</td>
<td>5.86 ± 0.320</td>
<td>&lt;0.05 (8-10)</td>
</tr>
</tbody>
</table>

MSI = Melanophore size index

Number in parentheses indicate the serial numbers of the table between which P value was calculated.

reserpine treatment in various smooth muscles of mammals is very well known and is called as supersensitivity. In the mammalian smooth muscles the development of supersensitivity by reserpine treatment may be of specific or unspecific nature i.e., the sensitivity of the muscle may be enhanced to a particular drug or to variety of drugs. In the present study supersensitivity of *C. mrigala* melanophores to GABA seems to be of unspecific nature as this type of reserpine treatment in *C. mrigala* melanophores has been shown to induce similar phenomenon to a variety of substances like adrenaline, nor-adrenaline, 5-HT, melatonin etc. Therefore, the present studies indicate that development of supersensitivity like phenomenon which seems to be unspecific in nature in fishes may be due to species variation, as fishes occupy the lowest position in the ladder of vertebrate evolution. In this regard the present studies differ from mammalian species, where supersensitivity like phenomenon to GABA is unknown to the best of our knowledge. While, the mechanism of this phenomenon in fishes may be similar to that, as proposed by Taylor and Green for mammalian species. In the present study the purpose of employing two different methods of denervation was to ascertain whether the effect of GABA is mediated directly through specific receptors or indirectly through the release of some neurotransmitter substance which in turn activates the receptors, resulting in the dispersion of the melanophores. Similar effect of GABA on the innervated and denervated melanophores of *C. mrigala* ruled out the indirect effect of this substance on the fish melanophores. Therefore, the present results suggest that the dispersal responses of *C. mrigala* melanophores to GABA are mediated through specific GABA receptors.

Baclofen, the specific GABA<sub>B</sub> receptor agonist elicited the concentration related dispersion in the *C.mrigala* melanophores. The threshold dose of baclofen to elicit a discernible response was lower than GABA and the magnitude of dispersal responses of the melanophores in comparison with GABA was also higher to baclofen. This indicates the presence of GABA<sub>B</sub> receptors in *C. mrigala* melanophores and the activation of these receptors results in the dispersion of the melanophores.

Atropine, the cholinergic muscarinic receptor antagonist and bicuculline, the specific GABA<sub>A</sub> (ref. 3) receptor antagonist both potentiated the dispersal responses of *C. mrigala* melanophores induced in low...
dose-range of GABA, while dispersal responses caused by high-dose range of GABA were inhibited \((P < 0.01\) and \(P < 0.001\)). Similarly, pentyleneetetrazole also inhibited the dispersion of this fish melanophores induced by higher concentrations of GABA. Bicuculline is a specific \(\text{GABA}_A\) receptor antagonist, while pentyleneetetrazole is a Cl channel blocker. It is also well known that \(\text{GABA}_A\) receptors are linked directly to Cl channels and rapidly inhibit cellular excitability through bicuculline sensitive mechanism. Therefore, the inhibitory effect of these two antagonists indicates the presence of \(\text{GABA}_A\) receptors in the \(\text{C. mirgala}\) melanophores. GABA has been implicated as a neuromodulator of cholinergic, parasympathetic nervous system in mammals. Hence the partial blocking effect of atropine on GABA induced dispersion of \(\text{C. mirgala}\) melanophores indicates that in fishes too GABA may have some physiological role.

To confirm the presence of \(\text{GABA}_B\) receptors in \(\text{C. mirgala}\) melanophores specific \(\text{GABA}_B\) receptor antagonist, 5-aminovaleric acid was also employed. It was observed that this antagonist significantly inhibited the dispersal responses of \(\text{C. mirgala}\) melanophores to both GABA and baclofen. Therefore, it is concluded that \(\text{GABA}_B\) receptors are also present in this fish melanophores along with \(\text{GABA}_A\) receptors.

It could be of interest to note that in an amphibian \(\text{Xenopus laevis}\), Verbur van Kenenade et al. have demonstrated a GABA-ergic regulation of MSH release from pars intermedia, while in a mammalian system GABA has been shown to inhibit the release of MSH.

**Acknowledgement**

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**References**