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EVIDENCE FOR MELANIN CONCENTRATING HORMONE (MCH) RECEPTORS MEDIATING MELANOSOME AGGREGATION IN *LABEO* MELANOPHORES*

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Abstract : Melanin concentrating hormone (MCH; 5×10^{-12} - 5×10^{-8}M) induced a concentration related, rapid and reversible pigment aggregation in innervated melanophores of *Labeo rohita*. In inducing melanosome aggregation MCH was found to be 10^4 times more potent than norepinephrine. Experiments employing phentolamine and propranolol suggest that MCH acts through its own specific receptors on the melanophores unrelated to adrenoceptors. MCH was able to aggregate the melanosomes even in the absence of extracellular Ca^{2+}.

Key words : melanophores, neurotransmission, melanin concentrating hormone, alpha receptors, beta receptors

INTRODUCTION

Employing *Labeo rohita* (Ham.) an Indian major carp belonging to the group of teleosts, we have recently demonstrated the dual regulation of colour change by hormonal and neural mechanisms (1). Further, nervous mechanism seems to dominate in this species but endogenous hormonal influences are still required to accomplish the phenomenon (2). While data pertaining to involvement of ANS (autonomic nervous system) in bringing about melanosome aggregation leading to a lightening in colour through sympathetic postganglionic fibres and peripheral adrenergic transmission have been presented earlier (2), we now report the results of an *in vitro* study concerning biological actions of melanin concentrating hormone (MCH) which has been isolated in pure form from *Salmon* pituitary only recently (3).

METHODS

Fish : Juveniles (5-7 cm in length) of the Indian major carp (*Labeo rohita*) were obtained locally from Govt. Fish Farm. They were maintained in aerated fresh-water aquaria under natural photoperiodic conditions and were allowed to acclimate for at least a week before use.

Isolated scale-melanophore preparations : Experiments were conducted on the scales excised from the dorsal trunk of the rohu (*L. rohita*). They were immediately immersed in a physiological solution of the following composition (mM): NaCl 128; KCl 2.7; $CaCl_2$, 1.8; D-glucose, 5.6; Tris-HCl buffer, 5.0 (pH 7.4). A divalent cation-free saline was prepared by withdrawing $CaCl_2$ and by adding 2.0 mM EDTA (disodium salt).

For fixing the scale to be observed microscopically the basal portion of the scale inserted between a coverslip (24 mm wide) and a glass fibre (about 400 μm in diameter and 20 mm in length), which had previously been glued on the coverslip with epoxy adhesive at one end. The assembly carrying the scale (fastened epidermis side down) was then turned over and put on a perfusion chamber for

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observation, so as to make glass needle parallel to the narrow trough (8 mm in width and 4 mm in depth).

All solutions were added to the trough so as to bath the skin tissue in the scale and washed thoroughly once before another solution was tested.

Dermal melanophores situated focally in the pigmentary part of the scale (that remain attached to the posterior or exposed part of the scale when the latter is detached from the fish body), as found suitable in our earlier study (2) were screened with various drug treatments.

Drugs used :Noradrenaline bitartrate (Sigma Chemical, St. Louis), phentolamine mesylate (Hindustan Ciba-Geigy Ltd., Bombay), propranolol hydrochloride (Cipla, Bombay) and synthetic salmon melanin-concentrating hormone (MCH) (Peninsula Laboratories, Europe Ltd., Pherseydide, U.K.) were used. All concentrated stock solutions (frozen in case of MCH) of the drugs were diluted with physiological solution or Ca^{2+} free saline.

MCH Bioassay (The subjective melanophore index) : Using magnification (X100-X400), the effects of drugs, *in vitro*, on the dermal melanophores were studied microscopically and the responses were recorded as per Hogben and Slome's melanophore index (4). Proposed initially in 1931 for amphibian melanophore responses, the index is still widely employed to evaluate pigment cell responses to various chemical stimuli in many invertebrate as well as poikilothermic vertebrate species.

The morphology of the pigment cells (with motile pigmentary organelle) as related to the distribution of pigments within the cells can be observed microscopically and assessed numerically on a scale of 1-5, where 1 stands for maximal aggregation of melanosomes and 5 for their maximal dispersion possible in a pigmentary effector cell. A change of one unit or more in the mean M.I. was arbitrarily designated as significant (5). In teleosts, melanophores assume a state of full dispersion (stage 5) representing their resting state, when they are under no stimulation (6) and *Labeo* is no exception. When placed in isotonic physiological saline solution, the melanophores in isolated scale preparations soon equilibrate in their "resting" completely dispersed stage and maintain the same for a considerable time period (about 2 hr). The M.I. values, as recorded in the study, are expressed as means ± S.E.M. All experiments were performed at room temperature between 20 and 22°C (slow drifts, ± 1°C over several hr.)

RESULTS

MCH induced a remarkable uniform pigment aggregation in melanophores. The degree of pigment aggregation was directly proportional to the concentration of the peptide in the medium between 5×10^{-12} M and 5×10^{-8} M (maximum effective concentration) (Fig. 1). Comparing MCH and norepinephrine through bioassay (Jain, unpublished) we found that MCH is about 10^4 time more potent than norepinephrine in inducing melanosome aggregation in *Labeo* melanophores (Fig. 1).

Redispersion of the melanophores pretreated by MCH (5×10^{8}M) for 10 min occurred after several washes with physiological saline but base values could not be achieved upto 30 min.

The presence of phentolamine (an α-adrenoceptor blocker) or propranolol (a β-adrenoceptor blocker) in the medium did not interfere with the melanosome-aggregating action of MCH (Fig. 2) while the action of catecholamines could be blocked by phentolamine (2), indicating that the melanotropic peptide acts through separate receptors, unrelated to adrenoceptors.

It is known that Ca^{2+} is indispensable for the release of norepinephrine from adrenergic chromatic nerve terminals induced by elevated K^+ (7) and that it is definitely required for MSH (melanophore stimulating hormone) induced melanosome dispersion (8). In marked contrast, we have found that Ca^{2+} was not required for the MCH action on the melanophores in the scale skin of the *Labeo*.

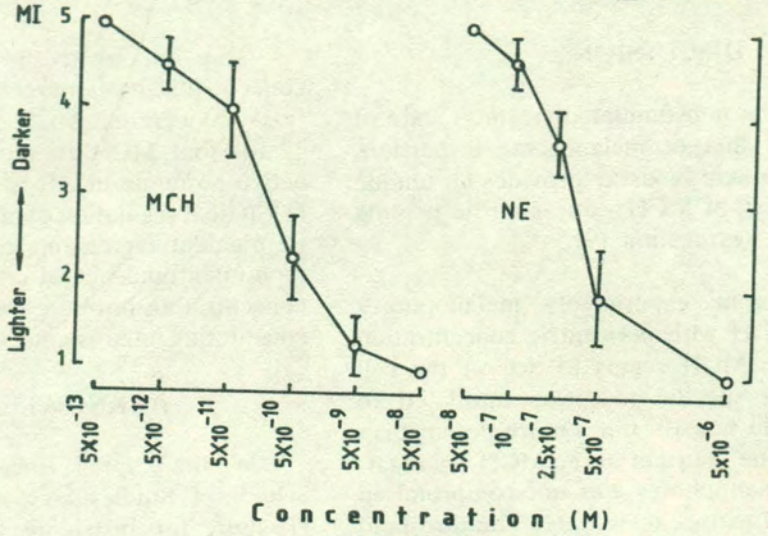

Fig. 2

Fig. 1 : Concentration response curve to melanin concentrating hormone (MCH) and norepinephrine (NE) as determined by melanophore index (MI) of the melanosome aggregating response of rohu melanophores. Solutions of various strengths were applied for 10 min. Abscissa, molar (M) concentration of MCH or norepinephrine (logarthmic scale). Ordinate, magnitude of response. Each point is the mean of 10 measurements on different animals. Vertical lines indicate standard deviations.

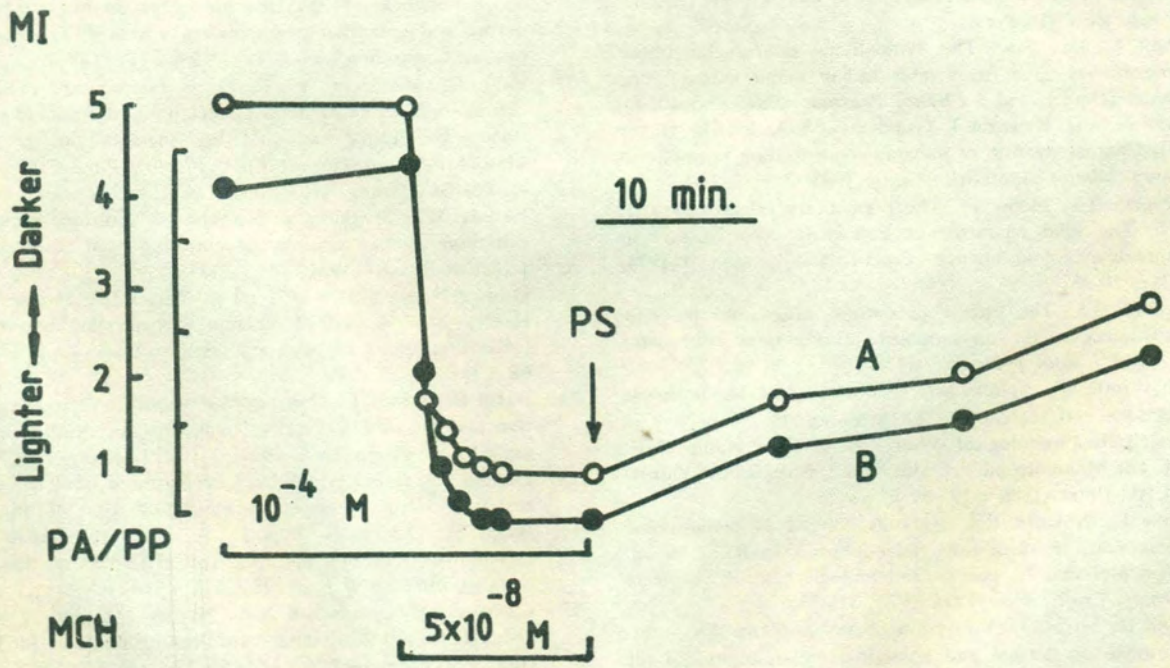

Fig. 2 : Melanosome-aggregating action of MCH (5×10^{-8}M) could not be blocked neither by phentolamine (PA-Curve A) nor by propranolol (PP - Curve B). PS = Physiological saline.

DISCUSSION

In teleosts the nonstimulated (resting) state of melanophores is that of melanosome dispersion. Thus *in vitro* fish skin bioassay provides an unique model for bioassay of MCH - an agonistic peptide for melanosome aggregation (9).

In the present experiments melanophores responded to MCH with pericentric concentration of melanosomes. MCH seems to act on the cell through its own specific receptors unrelated to adrenoceptors and was 10^4 times more potent than the norepinephrine. Furthermore, MCH aggregating action in melanophores was not compromised in the absence of extracellular Ca^{2+}. The threshold concentration found for MCH in this bioassay is of the order of that reported for MSH (10) and melatonin (11), in other fish species.

Thus our results on *Labeo rohita* support the concept that has emerged from various investingations concerning MCH in different teleosts (3, 12-18) that MCH is a physiologically functioning active hormone in teleosts. Our earlier assumption (1) of dual regulation of melanophores by sympathetic pigment-aggregating nerve fibres constituting a mono-neuronal neural control (2) and by melanin concentrating hormone released from the pituitary constituting unihormonal control is thus confirmed.

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