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CONTROL OF MELANOSOME MOVEMENTS IN ISOLATED SKIN MELANOPHORES OF A CATFISH *CLARIAS BATRACHUS* (LINN.)

MOHAMMAD OVAIS

Department of Biosciences,
Barkatullah University,
Bhopal - 462 026

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Abstract: Adrenergic and cholinergic receptors have been studied in isolated skin melanophores of a catfish *Clarias batrachus*. Catecholamines induced a strong aggregatory effect on the melanophores. Melanosome aggregation induced by adrenaline and noradrenaline was partially blocked by alpha adrenergic receptor blockers and a beta receptor blocker. Cholinomimetic drugs aroused a significant dispersion of melanophroes. Atropine effectively blocked the dispersal, responses of melanophores to acetylcholine and carbachol, while, hexamethonium blocked the nocotine induced dispersal responses of the melanophores.

Key words: fish melanophores adrenergic receptors cholinergic receptors

INTRODUCTION

Melanophores of some teleostean fishes have been reported to show centripetal movement of melanosomes in response to catecholamine applications (1-4). This centripetal movement of melanosomes has been attributed mainly to the alpha nature of adrenergic receptors in melanosomes (3-5). While in few fish species aggregation of melanosomes has been reported to be due to activation of beta adrenergic receptors (2, 6, 7). The effects of cholinomimetic drugs on fish melanophores are varied and only a few species respond with a clear dispersion of melanosomes (1, 4, 8).

The control of melanosome movements in catfishes studied by Fujii and his group (9-11) reveals that in these fish species the mechanism of dispersion and aggregation of melanosomes is variedly controlled. In silurid catfishes, the melanosome aggregation has been found under the cholinergic control, while in other catfishes it may be under adrenergic sympathetic postganglionic control (11).

The present work is an attempt to elucidate the nature and control of melanosome movements in an Indian fresh water catfish *Clarias batrachus*.

METHODS

Pieces of skin (3-4 × 10-12 mm) were excised from the lateral sides of a fresh water catfish *Clarias batrachus* (8-18 cm in length) after decapitation. The skin was immediately immersed in 0.7% NaCl solution and smaller pieces of 2-3 mm^2 were cut. The pieces of skin were equiliberated in saline solution for 10-15 minutes. After equiliberation, the pieces of skin were incubated (3-5 minutes) in various drugs freshly dissolved in saline medium. In experiments, where antagonists were employed alongwith agonists, the pieces of skin were first incubated in the antagonist for 5 minutes; then agonist was added and further incubation was carried out for 3-5 minutes. After incubation piece of skin was placed on a microscope slide covered by a cover glass with incubation medium. The melanophores in the skin were measured with the help of ocular micrometer (Erma, Japan) and the mean melanophore size index (MMSI) of the control as well as drug treated skins was calculated, according to the method of Bhattacharyya et al (12). The experiments were conducted on both the sexes during the months of April-June at the room temperature (25-30°C).

The following drugs were used : L-adrenaline bitartrate (C.H. Boehringer Sohn, Germany). L-noradrenaline bitartrate (Fluka, A.G.), Phenylephrine hydrochloride (C.H. Boehringer Sohn, Germany), Isoprenaline hydrochloride (Fluka, A.G.), Phenoxybenzamine (S.K. &. F., Philadelphia),

Propranolol hydrochloride (Cipla, India), Phentolamine (CIBA-Geigy, India), Dihydroergotamine (Sandoz, India), Acetylcholine chloride (Merck, Germany), Carbachol (B. D.H., Bombay), Pilocarpine nitrate, (Sigma, U.S.A), Atropine sulphate (C.H. Boehringer, Sohn, Germany), Physostigmine sulphate (Mac Farlan Smith Ltd., Edinburgh), Nicotine (B.D.H., England), Hexamethonium bromide (Fluka, A.G.). Statistical analysis was carried out using students "t" test.

RESULTS

Adrenaline, noradrenaline, phenylephrine and isoprenaline all caused a marked aggregatory effect on the dermal melanophores of *Clarias batrachus* (Table I). The threshold dose of these amines to elicit a discernible response ranged between 1×10^{-7} - 1×10^{-6} g/ml. The higher dose of these drugs was selected to get consistant results. The aggregation of melanophores caused by these amines was statistically significant in comparison to control values of melanophores in saline. In order to determine the site of action of adrenaline and nor-adrenaline, specific alpha and beta adrenergic receptor antagonists were employed. Phenoxybenzamine partially blocked the aggregatory effects of adrenaline and noradrenaline. However, the blocking effect of phenoxybenzamine on the responses to adrenaline was more pronounced than noradrenaline. Phentolamine and dihydroergotamine also blocked the aggregation of the melanophores in response to adrenaline treatment. The blocking effect of both the alpha adrenergic receptor antagonists to adrenaline induced responses was significant. Phentolamine also partially blocked the responses of melanophores to noradrenaline. However, this was less in comparison to adrenaline. Propranolol an unspecific beta blocker also exerted a blocking effect on the melanophore responses to adrenaline and noradrenaline (Table I).

TABLE I : Effect of adrenergic drugs on the dermal melanophores of *C. batrachus* (Linn.).

S.No.	*Experimental drug*	*Drug concentration in g/ml*	*MMSI ± SE*	
1.	Control (0.7% saline)	—	1.074 ± 0.153	(9)
2.	Adrenaline	1×10^{-5}	0.466 ± 0.049***	(9)
3.	Noradrenaline	1×10^{-5}	0.400 ± 0.065***	(7)
4.	Phenylephrine	1×10^{-5}	0.388 ± 0.034***	(6)
5.	Isoprenaline	1×10^{-5}	0.320 ± 0.073***	(5)
6.	Control (0.7% saline)	—	1.726 ± 0.259	(5)
7.	Adrenaline	1×10^{-5}	0.439 ± 0.056**	(5)
8.	Noradrenaline	1×10^{-5}	0.384 ± 0.072**	(5)
9.	Phenoxybenzamine + Adrenaline	5×10^{-5} 1×10^{-5}	0.880 ± 0.153*	(5)
10.	Phenoxybenzamine + Noradrenaline	5×10^{-5} 1×10^{-5}	0.464 ± 0.032*	(5)
11.	Phentolamine + Adrenaline	5×10^{-5} 1×10^{-5}	1.032 ± 0.041*	(5)
12.	Phentolamine + Noradrenaline	5×10^{-5} 1×10^{-5}	0.743 ± 0.037**	(5)
13.	Dihydroergotamine + Adrenaline	5×10^{-5} 1×10^{-5}	1.379 ± 0.147*	(6)
14.	Propranolol + Adrenaline	2×10^{-5} 1×10^{-5}	0.805 ± 0.113*	(5)
15.	Propranolol + Noradrenaline	2×10^{-5} 1×10^{-5}	0.885 ± 0.122*	(5)

*P < 0.05; **P < 0.01; ***P < 0.001

NS = Not significant

Numbers in parentheses indicate the number of experiments from different fishes.

TABLE II : Effect of cholinergic drugs on the dermal Melanophores of *C. batrachus* (Linn.).

S.No.	*Experimental drug*	*Drug concentration in g/ml*	*MMSI ± SE*	
1.	Control (0.7% saline)	—	1.795 ± 0.086	(6)
2.	Acetylcholine	5×10^{-5}	2.354 ± 0.231*	(6)
3.	Carbachol	2.5×10^{-5}	2.602 ± 0.240**	(6)
4.	Pilocarpine	2.5×10^{-5}	2.504 ± 0.230*	(6)
5.	Control (0.7% saline)	—	1.582 ± 0.114	(5)
6.	Atropine	5×10^{-5}	0.806 ± 0.111**	(5)
7.	Atropine + Acetylcholine	5×10^{-5} 5×10^{-5}	1.462 ± 0.294	(5) NS
8.	Atropine + Carbachol	5×10^{-5} 2.5×10^{-5}	1.063 ± 0.040**	(5)
9.	Physostigmine	2.5×10^{-5}	2.278 ± 0.156*	(5)
10.	Physostigmine + Acetylcholine	2.5×10^{-5} 5×10^{-5}	3.257 ± 0.289***	(5)
11.	Control (0.7% saline)	—	1.534 ± 0.133	(5)
12.	Nicotine	1×10^{-5}	2.050 ± 0.081*	(5)
13.	Hexamethonium	1×10^{-4}	0.526 ± 0.075***	(5))
14.	Hexamethonium + Nicotine	1×10^{-4} 1×10^{-5}	1.706 ± 0.122	(5) NS
15.	Hexamethonium + Acetylcholine	1×10^{-4} 5×10^{-5}	0.932 ± 0.086**	(5)

*P < 0.05; **P < 0.01; ***P < 0.001

NS = Not significant

Numbers in parentheses indicate the number of experiments from different fishes.

Acetylcholine, carbachol and pilocarpine all aroused a well marked dispersion of pigment in the *C. batrachus* melanophores (Table II). The melanophores were highly sensitive to carbachol. The order of potency was Carbachol > pilocarpine > Acetylcholine (Table II). The latent period to elicit a discernible response of these drugs ranged between 10-30 seconds. Atropine *per se* elicited an aggregatory response in the melanophores (Table II). However, atropine almost completely blocked the dispersal effects of acetylcholine and carbachol on the melanophores. Physostigmine, the anticholinesterase agent *per se* elicited a significant dispersal effect on the *C. batrachus* melanophores. Pretreatment of the skin with physostigmine resulted in potentiation of dispersal response of melanophores to acetylcholine. It is clear from the data presented in Table II that nicotine caused a significant dispersion of melanophores and this effect was completely blocked by hexamethonium. The pretreatment of pieces of skin in hexamethonium completely abolished the dispersal responses of melanosomes to acetylcholine. However, hexamethonium *per se* caused highly significant aggregatory effect on the melanophores.

DISCUSSION

Catecholamines induced a rapid and complete aggregation in melansomes of *Clarias batrachus*, which was partially blocked by alpha adrenergic receptor blocking agents (phenoxybenzamine, phentolamine and dihydroergotamine) and a beta adrenergic receptor blocker propranolol (Table I). This indicates the partial involvement of both alpha and beta adrenergic receptors in bringing about the melanosome aggregation of *Clarias batrachus*. The view is further supported by the fact that both phenylephrine and isoprenaline which are specific alpha and beta adrenergic receptor agonists respectively (13) induced strong melanosome aggregation in the present studies. Similar results have also been reported from a few earlier studies on fish melanophores (2, 6, 7) which were not the catfishes. However, in

catfishes belonging to the family Siluriidae viz., *Parasilurus* and *Kryptopterus*, the aggregation of melanosomes is mediated through cholinergic muscarinic and dispersion through both alpha and beta adrenergic receptors (9-11).

In the present studies, cholinomimetic drugs aroused a significant melanosome dispersion in *C. batrachus* melanophores. These results are in agreement with those of earlier studies on *Ictalurid* (catfish), *Oryzias, Pleuronectes* and *Bathygobius* (1, 2, 4, 8). However, melanosomes of many fishes respond with feeble dispersion or no effect at all to acetylcholine (3, 5). It has also been demonstrated that in the catfishes belonging to the family Siluriidae, the melanosomes have cholinergic muscarinic receptor of aggregatory in nature (11).

The dispersal effect of acetylcholine in the present study was effectively blocked by atropine and it was potentiated by physostigmine an anticholinesterase agent. These results indicate the presence of cholinergic muscarinic receptors of dispersing nature in the melanophores of *C. batrachus*. Nicotine also elicited a significant dispersal response, which was effectively blocked by hexamethonium. Basing on this, it can be said that cholinergic nicotinic receptors also take part in bringing about the dispersal effect on this fish melanophores. Thus aggregatory effects of atropine and hexamethonium *per se* provide ample evidence to the fact that both cholinergic muscarinic and nocotinic receptors take part in the dispersion of this fish melanophores. This is supported by the observation that both these antagonists blocked the responses of this fish melanophores to acetylcholine and nicotine. The negligible aggregatory effect of acetylcholine in presence of hexamethonium is rather difficult to explain at the moment and need further investigations.

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