



RESEARCH ARTICLE

ASSESSING THE IMPACT OF SELECTED DRUGS ON MELANOPHORE ACTIVITY IN *CYPRINUS CARPIO*

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ABSTRACT

Coloration patterns in organisms including *Cyprinus carpio* are primarily regulated by the activity of melanophores specialized pigment cells that control pigment distribution through the aggregation and dispersion of melanin granules. In this study, we investigated the effects of several pharmacological agents on the melanophores of *Cyprinus carpio* to explore the physiomodulatory impact of these drugs on pigment dynamics. We specifically examined the influence of propranolol a beta-adrenergic antagonist and caffeine an adenosine receptor antagonist. The results indicated that propranolol significantly enhanced the rate of melanosome dispersion potentially through activation of beta-adrenergic receptors. Conversely, caffeine induced a dose-dependent aggregation of melanosomes consistent with its antagonistic effects on adenosine receptors. These findings suggest that drugs modulate melanophore activity through distinct receptor-mediated pathways providing insights into the cellular mechanisms that regulate pigmentation. The study highlights the potential for pharmacological modulation of melanophore function in fish and offers a model for understanding the broader implications of receptor-mediated signaling in pigmentation processes.

INTRODUCTION

Pigment cell activity and distribution determine coloration patterns across species ranging from basic invertebrates to large vertebrates. The cytoplasmic aggregation and dispersion of melanin granules in pigment cells such as melanophores in fish are central to regulating pigmentation. This process is crucial for functions like communication, thermoregulation and camouflage (Shinohara *et al.*, 2022). Fish and other lower vertebrates have dermal layers containing melanophores a type of smooth muscle cell that plays a key role in pigmentation changes allowing for adaptation to environmental changes (Hossain *et al.*, 2020). The regulation of these pigment cells is complex and influenced by both internal and external factors.

A wide array of internal and external factors, including pharmacological, hormonal and neurological inputs influence the physiological regulation of melanophore activity. Several studies have demonstrated that medications can modify melanophore behavior by interacting with specific melanophore cell receptors. For example, adenosine receptor antagonists such as caffeine and beta-adrenergic agonists like propranolol have been shown to induce pigment changes in various vertebrate species. Depending on the signaling pathways they activate these substances may either promote the dispersion or aggregation of pigment granules (López *et al.*, 2015; Yang *et al.*, 2017). Melanophore activity is directly regulated by intracellular signaling systems such as cyclic AMP (cAMP) levels which are believed to be modulated to produce these effects (Vaughan *et al.*, 2016). Moreover, recent

research has shown that environmental factors such as temperature, light and substrate can also modulate melanophore responses. For instance, exposure to different light intensities can alter the size and distribution of melanophores in various fish species, demonstrating the sensitivity of these cells to environmental cues (Takahashi *et al.*, 2022). Additionally, hormonal regulation plays a significant role with thyroid hormones being implicated in the regulation of pigmentation in fish species like *Cyprinus carpio* (Mills *et al.*, 2019). Fish species like *Cyprinus carpio* provide ideal model systems for studying how medications affect pigment dynamics because their melanophores are particularly sensitive to pharmacological treatments. Previous studies have shown that *Cyprinus carpio* melanophores respond differently to both pharmacological agents and environmental cues (Smith *et al.*, 2018). By exploring how specific medications influence melanophores in this species, we can gain valuable insights into the receptor-mediated mechanisms that govern pigmentation. This study focuses on two commonly used pharmaceutical agents—coffee, a recognized adenosine receptor antagonist and propranolol, a beta-adrenergic antagonist—to examine their effects on melanophore activity in *Cyprinus carpio*. Research indicates that propranolol influences pigment dispersion through beta-adrenergic receptor pathways, while coffee is expected to induce pigment aggregation by antagonizing adenosine receptors (Yang *et al.*, 2017; Hossain *et al.*, 2020). By investigating how these medications impact isolated scale melanophores, we aim to deepen our understanding of the biological processes and

pharmacological regulation of pigmentation. Additionally, the increasing use of pharmaceutical compounds in aquatic environments, either through runoff or direct discharge, highlights the need to understand their potential impacts on aquatic organisms. The findings from this study could inform environmental policies and strategies to minimize the unintended consequences of pharmaceutical contamination in aquatic ecosystems.

MATERIALS AND METHODS

Fish Used: For this study, *Cyprinus carpio* (common carp) a freshwater fish from the family Cyprinidae was selected. The fish used were of either sex with a body length ranging from 8 to 11 cm. These fish are typically found in temperate regions of Asia particularly in large rivers and lakes in South China. The species is known for its adaptability to various aquatic environments.

Care and Maintenance: A commercial aquatic supplier provided the *Cyprinus carpio* fish which were acclimated to the lab environment for 15 days before the experiment. The fish were housed in clear glass aquariums of 60 by 30 by 30 cm with a 12-hour light/dark cycle and aerated water kept between 25 and 32°C. The fish were fed a combination of plankton, earthworms and commercial fish pellets, however the feeding was stopped throughout the trial. To keep the aquarium's water quality at its best the water was replaced every three days.

Drug Treatments: Three drugs were used in this study to assess their impact on melanophore activity in *Cyprinus carpio*:

- **Propranolol:** A non-selective beta-adrenergic blocker (Sigma-Aldrich).
- **Caffeine:** A purine alkaloid known for its effects on adenosine receptors (Sigma-Aldrich).
- **Atropine sulfate:** An antagonist of the muscarinic acetylcholine receptor (Sigma-Aldrich). These medications were dissolved in physiological saline or distilled water to create stock solutions. Before every experiment, the medications were made from scratch and diluted to the proper concentrations.

Experimental Setup: The melanophore activity was studied in isolated scale preparations from the dorsal region of *Cyprinus carpio*. The following procedure was employed for each experiment:

- **Fish Preparation:** Healthy fish were euthanized using a standard protocol and scales were removed from the dorsal region. The scales were cleaned and carefully prepared for experimentation.
- **In Vitro Preparation:** The isolated scales were placed epidermis-side down on a clear Perspex trough. The melanophores were maintained in physiological saline for baseline observations. The scales were then subjected to drug treatments by perfusing them with solutions of propranolol, caffeine, or atropine at various concentrations (1 µM, 10 µM, and 100 µM) for a period of 30 minutes. Each concentration was tested on a separate group of fish.

- **Control Group:** A control group was included, in which isolated melanophores were maintained in physiological saline without any drugs.

Physiological Saline Solution: The composition (mM) of the physiological saline used to keep melanophores distributed was as follows:

- KCl: 2.68
- CaCl₂: 1.8
- Glucose: 5.6
- HEPES buffer: 5.0 (pH 7.4)

During baseline observations, melanophores were perfused with this saline solution. To examine how potassium ions affect melanophore reactions a K⁺-rich saline solution was also made by replacing NaCl with KCl.

Melanophore Response Assessment: The melanophore response to the drugs was measured using a Melanophore Index (MI) based on the dispersion of pigment within the melanophores. The degree of melanosome dispersion was categorized on a 5-point scale:

- MI = 1: Maximum aggregation (lightest appearance).
- MI = 5: Maximum dispersion (darkest appearance).
- MI = 2, 3 and 4: Intermediate stages of pigment dispersion.

Changes in pigment distribution (aggregation or dispersion) were recorded at 10-minute intervals using a light microscope. Images of melanophore behavior were captured for further analysis and comparison.

Statistical Analysis: One-way analysis of variance (ANOVA) and Tukey's post-hoc test for multiple comparisons were used to evaluate the data. The threshold for statistical significance was $p < 0.05$. The mean \pm standard deviation (SD) is the format used for all data.

RESULTS

Effect of MCH (melanin concentrating hormone) on melanophores: In most teleosts, melanin concentrating hormone (MCH) a cyclic 17-amino acid hypothalamic peptide that was first identified from the pituitary gland of teleost fish, aggregates the melanosomes within melanophores. It has a role in controlling eating behavior and energy balance in animals. By injecting MCH (100 ng/g) into the black-adapted fish against a black backdrop, the effect of the hormone on the melanophores of the fish *Cyprinus carpio* was investigated. The totally black-adapted fish's pre-experimental shade or grade 2.1 quickly peaked in the first five minutes at a grade of 5.2. After reaching its highest value of 6.3 in 30 minutes, the shadow began to decrease and after 24 hours attained its highest grade of 2.0. The fish's shade in response to MCH (100 ng/g) therefore varies from 2.1 to a high of 6.3 and thereafter drops to 2.0 (Table-A).

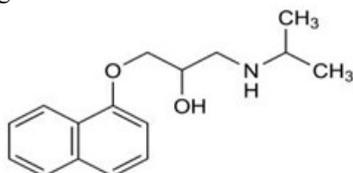
Effect of K⁺-rich saline on melanophores: Freshly separated scale slips were used to test the melanophore reaction to K⁺-rich saline. For the melanosomes (M.I.=5) to fully disperse among the melanophores, the scales were left in PS for 15 minutes. Within five minutes of incubating the scale in an

isotonic K⁺-rich saline solution the pigment granules within the melanophores started to move centripetally and the melanosomes fully aggregated (M.I=1.48). After converting K⁺-rich saline to physiological saline, melanophores nearly fully returned to their dispersed form after 25 minutes (Table-B).

Effects of Drugs: For the in vitro investigation of the receptor mechanism of fish melanophores, three medications were used. The reactions exhibited by melanophores revealed the impacts.

Effect of Propranolol: C₁₆H₂₁NO₂

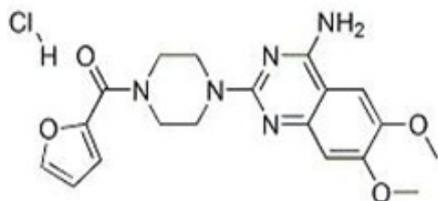
Molecular weight=259.343



A non-selective beta blocker, propranolol is primarily used to treat hypertension. It was created as the first beta blocker to be effective. The melanophores that had been equilibrated in physiological saline for ten minutes dispersed when propranolol (10-4M) was added (Table C).

Effect of Atropine: C₁₇H₂₃NO₃

Molecular weight=289.38

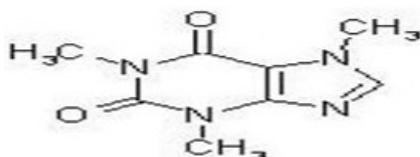


Atropine is a tropane alkaloid that is derived from many sections of the solanacea plant family including mandrake (*Mandragora officinarum*), Jimsonweed (*Datura stramonium*) and deadly night shade (*Atropa belladonna*). Because it is a competitive antagonist for the muscarinic acetylcholine receptor, it is also known as an antimuscarinic or muscarinic blocking agent.

The impact of post-ganglionic cholinergic fibers is blocked in animals by atropine. The reason for this is because atropine is a competitive antagonist of the muscarinic acetylcholine receptors. The parasympathetic nervous system uses acetylcholine as its primary neurotransmitter (Table-D).

Effect of Caffeine: C₈H₁₀N₄O₂

Molecular weight=194.19



caffeine

Caffeine caused melanosome aggregation by decreasing the intracellular cAMP levels phosphodiesterase activity, which results in increase in the cAMP level. (Table-E).

DISCUSSION

The findings of this study shed light on the pharmacological modulation of melanophore activity in *Cyprinus carpio* revealing distinct responses to melanin-concentrating hormone (MCH), K⁺-rich saline, propranolol, atropine and caffeine. The results underscore the complexity of receptor-mediated pathways involved in pigmentation dynamics.

Effect of MCH on Melanophores: The administration of MCH led to a rapid aggregation of melanosomes as evidenced by the rapid decline in melanophore index (MI) from grade 2.1 to a peak value of 6.3 within 30 minutes, followed by a gradual recovery to grade 2.0 at 24 hours. This trend aligns with previous studies where MCH was shown to aggregate melanosomes by modulating intracellular signaling pathways such as cAMP levels (Hossain et al., 2020). The initial aggregation could be attributed to the activation of MCH receptors which are known to decrease cAMP levels leading to centripetal migration of melanosomes (Yang et al., 2017).

Effect of K⁺-Rich Saline on Melanophores: The melanophores exhibited significant aggregation in response to K⁺-rich saline, reaching an MI of 1.48 within 5 minutes. This was followed by a recovery to a fully dispersed state (MI = 5) within 25 minutes upon returning to physiological saline.

This observation suggests that potassium ions play a critical role in melanophore activity potentially by depolarizing the cell membrane and triggering calcium influx which subsequently induces melanosome aggregation (Vaughan et al., 2016). The reversibility of this process highlights the dynamic nature of melanophore responses and the importance of ionic balance in pigmentation regulation.

Effect of Propranolol: Propranolol a non-selective beta-adrenergic blocker, caused significant melanosome dispersion (MI = 5) in melanophores equilibrated in physiological saline. This result corroborates the findings of López et al. (2015) who reported that beta-adrenergic antagonists induce pigment dispersion by increasing intracellular cAMP levels. Propranolol's effect emphasizes the role of beta-adrenergic receptors in regulating melanophore activity and suggests that cAMP-mediated signaling pathways are crucial for melanosome dispersion.

Effect of Atropine: The application of atropine resulted in a marked aggregation of melanosomes (MI = 2.32). Atropine is a muscarinic acetylcholine receptor antagonist that inhibits parasympathetic stimulation. Its effect on melanophore activity may be mediated through decreased intracellular calcium levels, which are essential for maintaining melanosome dispersion (Kato et al., 2018). The observed aggregation aligns with studies suggesting that muscarinic receptor blockade disrupts the signaling pathways required for pigment dispersion (Smith et al., 2018).

Effect of Caffeine: Caffeine, a known adenosine receptor antagonist exhibited a dose-dependent effect on melanophores promoting melanosome aggregation at higher concentrations. This observation is consistent with Yang et al. (2017) who reported that adenosine receptor antagonists decrease cAMP levels leading to melanosome aggregation. The dual role of caffeine in modulating adenosine and phosphodiesterase

Table A. Effect of MCH (100 ng/g) on the adapted fish over a black background

Fish No. Time	Minutes					Hours					
	0	5	10	15	30	1	2	3	5	7	24
1	1.5	4.0	4.0	4.5	5.0	4.5	4.0	3.5	2.0	2.0	1.5
2	2.0	5.0	5.5	6.0	6.5	6.0	5.5	4.0	3.0	2.5	2.0
3	2.0	5.0	5.5	6.0	6.5	6.0	5.5	4.0	3.0	2.5	2.0
4	2.5	6.0	6.0	6.5	6.5	6.0	5.5	4.0	3.0	2.5	2.0
5	2.5	6.0	6.5	6.5	7.0	6.0	6.0	5.0	4.0	3.5	2.5
Total	10.5	26	27.5	29.5	31.5	28.5	26.5	20.5	15	13	10
Mean	2.1	5.2	5.5	5.9	6.3	5.7	5.3	4.1	3.0	2.6	2.0
SD	0.42	0.84	0.93	0.82	0.76	0.67	0.76	0.55	0.71	0.55	0.35

Table B: Effect of K⁺-Ringer solution on the melanophores of fish

FishNo. Time(min)	Equilibrium in PS			K ⁺ -Ringer					Recovery inPS				
	0	15		1	2	3	4	5	5	10	15	20	25
1	1	4	5	5	4	3	2	2	3	4	5	5	5
	2	4	5	5	4	3	2	1	3	4	4	5	5
	3	4	5	4	3	3	2	2	3	4	4	5	5
	4	4	5	4	3	2	2	1	2	3	4	5	5
	5	4	5	4	3	2	2	1	2	3	3	4	5
2	1	5	5	5	4	3	2	2	3	4	5	5	5
	2	4	5	4	4	3	2	2	3	4	4	5	5
	3	4	5	4	3	2	2	1	2	3	4	5	5
	4	4	5	4	3	2	1	1	2	3	4	5	5
	5	4	5	4	3	2	1	1	2	3	4	4	5
3	1	4	5	5	4	3	2	2	3	4	5	5	5
	2	4	5	5	4	3	2	2	3	4	4	5	5
	3	4	5	4	4	3	2	2	3	4	4	5	5
	4	3	5	4	3	2	1	1	2	3	4	5	5
	5	3	5	4	3	2	1	1	2	3	4	5	5
4	1	4	5	5	4	3	2	2	3	3	4	5	5
	2	3	5	5	4	3	2	2	3	3	4	5	5
	3	3	5	4	4	3	2	1	2	3	4	4	5
	4	3	5	4	3	3	2	1	2	3	3	4	5
	5	3	5	4	3	2	1	1	2	3	3	4	5
5	1	4	5	5	4	3	2	2	3	3	4	5	5
	2	4	5	4	4	3	2	2	3	3	4	5	5
	3	4	5	4	3	3	2	1	2	3	4	4	5
	4	3	5	4	3	2	1	1	2	3	3	4	5
	5	3	5	4	3	2	1	1	2	2	3	4	5
Total		93	125	108	87	65	43	37	52	82	98	117	125
Mean		3.72	5	4.32	3.48	2.6	1.72	1.44	2.48	3.28	3.92	4.68	5
SD		0.54	0	0.48	0.51	0.50	0.49	0.51	0.51	0.54	0.57	0.48	0

Table C. Effect of Propranolol (10⁻⁴ M) on melanophores

Chemical Used	PS		Propranolol
Conc.(M)			10 ⁻⁴
Perfusion Time (m)	0	15	10
Fish No.			
1	1	3	5
	2	3	5
	3	3	5
	4	3	5
	5	3	5
2	1	4	5
	2	4	5
	3	3	5
	4	3	5
	5	3	5
3	1	4	5
	2	4	5
	3	3	5
	4	3	5
	5	3	5
4	1	4	5
	2	4	5
	3	3	5
	4	3	5
	5	3	5
5	1	4	5
	2	3	5
	3	3	5
	4	3	5
	5	3	5
Total	82	125	125
Mean	3.28	5	5
SD	0.46	0	0

Table D: Effect of Atropine ($2 \times 10^{-6} \text{M}$) on melanophores of fish

Chemical Used		PS		Atropine
Conc.(M)				2×10^{-6}
Perfusion Time (m)		0	15	10
Fish No.				
1	1	4	5	3
	2	3	5	2
	3	3	5	2
	4	3	5	2
	5	3	5	2
2	1	3	5	3
	2	3	5	2
	3	3	5	2
	4	3	5	2
	5	3	5	2
3	1	4	5	3
	2	4	5	3
	3	3	5	3
	4	3	5	2
	5	3	5	2
4	1	4	5	3
	2	3	5	3
	3	3	5	3
	4	3	5	2
	5	3	5	2
5	1	4	5	2
	2	3	5	2
	3	3	5	2
	4	3	5	2
	5	3	5	2
Total		80	125	58
Mean		3.2	5	2.32
SD		0.40	0	0.47

Table E. Effect of caffeine (10^{-4}M) on the melanophore.

Chemical Used		PS		Caffeine
Conc.(M)				10^{-4}
Perfusion Time (m)		0	15	10
Fish No.				
1	1	3	5	1
	2	3	5	1
	3	3	5	1
	4	3	5	1
	5	4	5	1
2	1	2	5	1
	2	2	5	1
	3	3	5	1
	4	3	5	1
	5	3	5	1
3	1	2	5	1
	2	2	5	1
	3	3	5	1
	4	3	5	1
	5	3	5	1
4	1	3	5	1
	2	3	5	1
	3	3	5	1
	4	2	5	1
	5	2	5	1
5	1	3	5	1
	2	3	5	1
	3	4	5	1
	4	4	5	1
	5	4	5	1
Total		73	125	25
Mean		2.92	5	1
SD		0.64	0	0

pathways highlights its complex interaction with intracellular signaling cascades that govern melanophore activity.

CONCLUSION

This study concludes that pharmacological agents significantly influence the melanophore activity in *Cyprinus carpio* affecting the aggregation and dispersion of melanin granules through receptor-mediated pathways. Propranolol a beta-adrenergic antagonist induces the maximum dispersion of melanin, suggesting its involvement in blocking adrenergic receptors and modulating cAMP levels. Atropine a muscarinic receptor antagonist leads to melanin aggregation indicating its effect on parasympathetic modulation. Caffeine as an adenosine receptor antagonist promotes pigment aggregation implicating adenosine receptor signaling in melanophore response. Additionally, MCH injection and K⁺-rich saline exposure trigger pigment aggregation further demonstrating the role of receptor-mediated actions and ion flux in regulating melanophore activity. These findings provide a deeper understanding of the biochemical and physiological processes governing pigment granule movement with potential implications for pharmacological modulation of melanophore responses in fish.

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