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Adrenocorticotropic Hormone and Cyclic Adenosine Monophosphate are Involved in the Control of Shark Bioluminescence

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ABSTRACT

Among Etmopteridae and Dalatiidae, luminous species use hormonal control to regulate bioluminescence. Melatonin (MT) triggers light emission and, conversely, alpha melanocyte-stimulating hormone (α-MSH) actively reduces ongoing luminescence. Prolactin (PRL) acts differentially, triggering light emission in Etmopteridae and inhibiting it in Dalatiidae. Interestingly, these hormones are also known as regulators of skin pigment movements in vertebrates. One other hormone, the adrenocorticotropic hormone (ACTH), also members of the skin pigmentation regulators, is here pharmacologically tested on the light emission. Results show that ACTH inhibits luminescence in both families. Moreover, as MT and α-MSH/ACTH receptors are members of the G-protein coupled receptor (GPCR) family, we investigated the effect of hormonal treatments on the cAMP level of photophores through specific cAMP assays. Our results highlight the involvement of ACTH and cAMP in the control of light emission in sharks and suggest a functional similarity between skin pigment migration and luminescence control, this latter being mediated by pigment movements in the light organ-associated iris-like structure cells.

INTRODUCTION

The lanternsharks *Etmopterus spinax* (Linnaeus, 1758) and *Etmopterus molleri* (Whitley, 1939), two common etmopterid deep-sea sharks, are known to emit an intrinsic blue-green light by using thousands of tiny photophores mainly distributed in the ventral epidermis ((1–4); Fig. 1A). Photophores are composed of a cup-shaped pigmented cell layer, which form a dark granular sheath, enclosing the emitting cells, the photocytes, capped by one or several lens cells. A multilayer cell zone called the iris-like structure (ILS) acting as a shutter of the light emission is present between the lens cells and the photocytes ((1,3); Fig 1B). Various functions of bioluminescence have been suggested in lanternsharks such as counterillumination, aposematism or intraspecific communication (1,5–8).

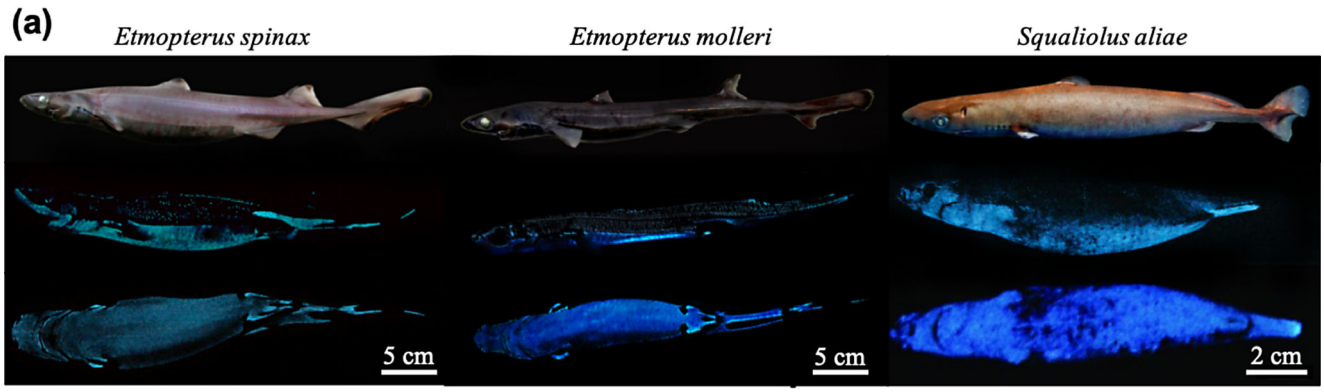
To achieve these specific biological functions, photophore light emission has to be under precise control. While bony fish mainly regulate their luminescence with a fast-nervous control mechanism (9–12), sharks are the only known vertebrates to make use of a slow hormonal bioluminescent control (13). Melatonin (MT) and prolactin (PRL) trigger a low long-lasting and a strong fast light emission, respectively, while α-melanocyte-stimulating hormone (α-MSH) inhibits luminescence in lanternsharks ((13); Fig. 1B,C). Moreover, some neurotransmitters like nitric oxide (NO) and γ-aminobutyric acid (GABA) are able to modulate etmopterid light emission ((14–16); Fig. 1B,C).

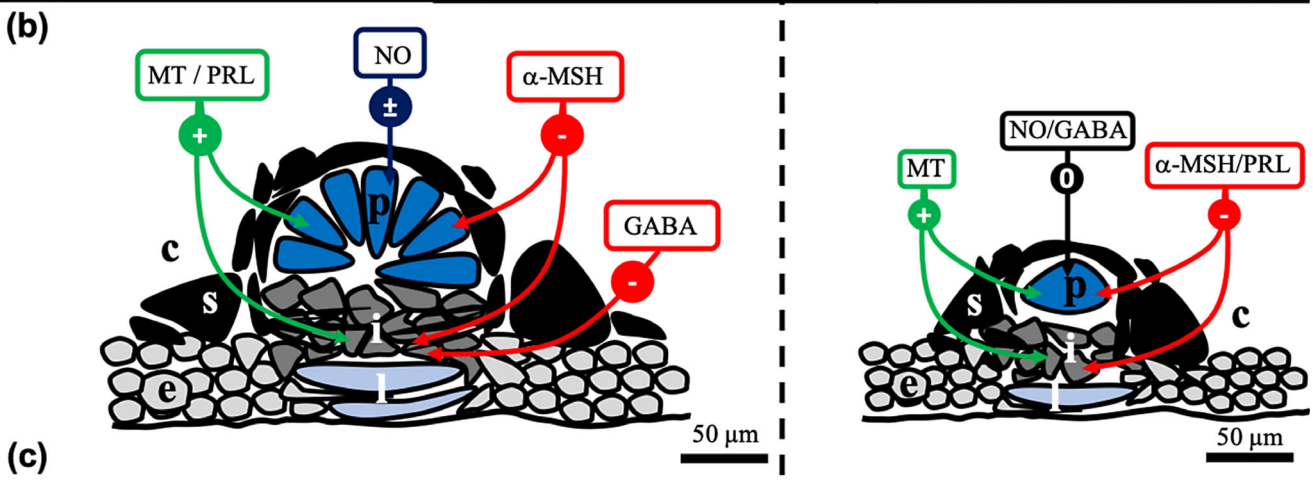
The smalleye pygmy shark *Squaliolus aliae* (Teng, 1959) is the smallest known dalatiid species. Individuals of *S. aliae* harbors thousands of tiny ventral photophores that are smaller and of a simpler structure than in etmopterids, that is one single photocyte surrounded by a pigmented sheath cup and capped by lens cells ((1,17); Fig. 1B). A role in counterillumination has been suggested for pygmy shark bioluminescence since a bluish ventral luminescence matching downwelling light has been observed ((18,19); Fig. 1A). MT and α-MSH have, respectively, the same stimulatory and inhibitory effect on the light emission while PRL inhibits luminescence in *S. aliae* ((19); Fig. 1B,C). Taken together, these observations suggest a common mechanism for the regulation of bioluminescence in luminous sharks in which the opening or closing of the ILS, through pigment movements, is assumed to be involved in altering the light output (13,16,19).

In vertebrates, physiological pigment movement is mainly promoted by bidirectional translocation of dark melanin granules (melanosome) within pigment cells (21–24). PRL, MT, α-MSH, adrenocorticotropic hormone (ACTH), melanin-concentrating hormone (MCH) and catecholamines are the most intensively studied vertebrate pigment cell regulators (25–31). Most of these hormones (α-MSH, ACTH, MCH and MT) interact with a G-protein coupled receptor (GPCR) modulating adenylate cyclase activity, which controls the intracellular levels of cyclic adenosine monophosphate (cAMP) (21,23,25,32,33). Visconti and Castrucci (1993) demonstrated that α-MSH and PRL hormones are both involved in the darkening of the stingray (*Potamotrygon reticulatus*) skin by modulating cAMP levels (34).

To further understand hormone-mediated light emission control in luminous elasmobranchs, the effects of ACTH were

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(c)

	Etmopteridae *E. spinax; E. molleri; E. splendidus (13-16, 20)*	***Dalatiidae*** *S. aliae (19)*
Drugs (mol l^{-1})	*Effect on light emission*	*Effect on light emission*
MT 10^{-6}	+	+
PRL 10^{-6}	+	-
α-MSH 10^{-6}	-	-
GABA 10^{-3}	-	**0**
NO 10^{-3}	±	**0**

Figure 1. Comparative aspects of light emission between Etmopterid and Dalatiid. (a) lateral view (upper), bioluminescent lateral (middle) and ventral view (lower) of the three studied species, *E. spinax* (left), *E. molleri* (middle) and *S. aliae* (right). (b) Schematic representation of the photophore (transversal section) with an emphasis on bioluminescence control in Etmopterid (left) and Dalatiid (right). Colored arrows indicate the targets of the different substances involved in the control of Etmopterid and Dalatiid. Symbols in color circles indicate the effects of these substances on shark bioluminescence. (c) Table resuming the hormonal and neuromodulator control of the light emission in luminous sharks according to literature (13–16,19,20). Effects are symbolized by: +, activatory; -, inhibitory; ±, modulatory; 0, no effects. Substances are abbreviated: GABA, γ-aminobutyric acid; α-MSH, a-melanocyte-stimulating hormone; MT, melatonin; NO, nitric oxide; PRL, prolactin. In photophore structure: c, connective tissue; e, epidermis; i, iris-like structure cells; l, lens cell; p, photocyte; s, pigmented sheath.

investigated in parallel on luminescence and on ILS pigment movement. Based on the assumption that all targeted hormone-GPCRs act through cAMP modulation, this work investigated the intracellular cAMP concentration during the hormonal modulation of light emission.

Using pharmacological assays and luminometry recordings, we demonstrated (1) the inhibitory effect of ACTH on the bioluminescence of two lanternsharks and one pygmy shark species and (2) the modulation of cAMP levels following MT and α-MSH treatments in Etmopteridae.

Interestingly, these observations, combined with previous studies (35), are consistent with the first steps of transduction pathways involved in skin pigment movement, that is GPCRs of MT/ACTH/α-MSH regulating cAMP intracellular levels through modulation of adenylate cyclase activity. This suggests a potential functional shift from general skin color change for countershading *via* skin pigment migration, to specific modulation of ILS cell pigmentation regulating light emitted by photocytes in etmopterid and dalatiid species.

MATERIALS AND METHODS

Experimental animals. Thirty-one adults of *E. spinax* [41–51 cm total length (TL)] were captured during a field collection in January 2017 by longlines lowered at 250 m depth in the Raunefjord, Norway (60°15′54″ N; 05°07′46″E). Living specimens were kept in a 1 m^3 tank filled with running cold seawater (6°C) and placed in a dark cold room at Bergen University Marine Station (Espegrend, Norway). These sharks were collected in Norway under the "experimental fish care permit" number 12/14048.

A total of 35 adults of *E. molleri* [37–48 cm TL] were collected during field collection in November 2016 and 2017 in the East China Sea near the coast of Sesoko Island, Okinawa, Japan (26°28′94″N; 127°41′20″E). They were captured using a fishing rod equipped with an electric reel at depths ranging from 480 to 510 m. All specimens were transferred to oxygen saturated plastic bags filled with seawater and brought in a refrigerated box to the Okinawa Churaumi Aquarium where they were kept alive in a dark tank filled with running seawater (13°C) until manipulations. Sharks were handled according to Churaumi Aquarium husbandry and veterinary rules for animal experimentations.

A total of 11 adults of *S. aliae* [15.5–22 cm TL] were sampled during a field session in November 2018 in Donggang Harbor, Taiwan (22°24′46″N; 120°24′11″E). They were collected alive by midwater trawls operating at 50–150 m depth as bycatch by local fisherman. Living specimens were brought to the National Museum of Marine Biology and Aquarium (NMMBA; Pingtung, Southern Taiwan) and placed in dark tank filled with seawater (18°C). Sharks were handled following the NMMBA rules for animal experimentations.

All sharks were euthanized by a blow to the head followed by a full incision of the spinal cord at the level of the first vertebrae, following the local rules for experimental vertebrate care. Animals were treated according to the European regulation for animal research handling. Sharks were sexed, measured and weighed (Table 1) before experimentation took place.

Skin tissues were dissected from the ventral luminous zone of each shark. Round shape skin patches were sampled from the skin using a metal cap driller [6 mm diameter] as described in Claes and Mallefet, 2009 (13). These ventral skin patches were washed and kept in a shark saline (5,36) in the dark at 4°C before being used.

Pharmacological studies. In order to document the effect of ACTH drug applications on the shark luminescence, ventral skin patches were placed into wells of 96-well plate allowing to run multiple treatments simultaneously.

Application of MT served as control of the light emission induction since this hormone is known to trigger shark bioluminescence (13). Since α-MSH is an inhibitor of the light emission (13), its application with melatonin served as model of light inhibition. Application of ACTH was made to test the effect of this hormone alone on light emission, shark saline (5,36) being used as nontreatment control. Finally, MT followed by an application of ACTH showed the potential role of the latter hormone on light emission. All drugs were obtained at Sigma, St. Louis, Missouri.

Experiments were first conducted on 16 *E. spinax* specimens. A 96-well plate was filled with 200 µL of either MT, ACTH at 10^{-6} mol L^{-1} or shark saline alone as controls, skin patches were immersed during 60 min. In a second series of experiments, a 96-well plate was filled with 100 µL of MT 10^{-6} mol L^{-1} as the first hormone, then, after 20 min, 100 µL of the second hormone: MT 10^{-6} mol L^{-1}, α-MSH 10^{-6} mol L^{-1} or ACTH was added. In order to perform a dose-dependent response curve, various concentrations of ACTH, ranging from 10^{-8} to 10^{-5} mol L^{-1}, were used. Luminescence of ventral skin patches subjected to the various treatments was measured using a microplate luminometer (Berthold MPL12/Orion; Pforzheim, Germany) calibrated using a standard 470 nm light source (Beta light; Saunders Technology, Hayes, UK). Light outputs were recorded using Simplicity software (Berthold, Pforzheim, Germany) during 65 min with a measurement every minute.

Table 1. Experimental specimens.

Species	*N*	Mean total length (cm)	Mean weight (g)
Etmopterus spinax	25 ♀	46.3 ± 1.1	416.1 ± 23.8
	6 ♂	40.0 ± 1.3	248.1 ± 34.2
Etmopterus molleri	29 ♀	43.8 ± 2.3	242.0 ± 40.0
	6 ♂	40.4 ± 0.8	173.8 ± 15.2
Squaliolus aliae	9 ♀	20.5 ± 1	53.8 ± 9.8
	2 ♂	16.7 ± 1.3	28.5 ± 4.5

N, number of specimens; ♀, female, ♂, male. Measures are expressed with SEM.

For comparative purposes, treatments giving relevant results on *E. spinax* were tested on 13 *E. molleri* specimens (ACTH 10^{-5} and 10^{-6} mol L^{-1}) and 11 *S. aliae* specimens (ACTH 10^{-5} mol L^{-1}).

Luminescence responses were characterized according to Claes and Mallefet, 2009 (13), namely: the maximum intensity of light emission [Lmax, in megaquanta per second (Mq s^{-1})], the total amount of light emitted during this experimentation [Ltot, in Gigaquanta per hour (Gq h^{-1})] and the time to reach the maximum of light intensity [TLmax, in seconds (s)]. Inhibitory action of α-MSH and ACTH was measured as of total amount of light emitted after second drug application [$Ltot_{app}$, in Gq h^{-1}]. All these light parameters were standardized according to the skin patch surface area (in cm^2). The second treatment was added to the first one when the light intensity plateau was reached with the MT application, each timing being species-specific. Results of luminescence decrease were expressed as a percentage of the maximal luminescence value (*i.e.* plateau MT) measured before the second application.

To illustrate the *in vivo* action of ACTH on the light organ, experiments on *E. molleri* were repeated under a video microscope (Keyence VHX2000, Osaka, Japan). Pictures and time-lapse movies were recorded during 20 min after the second drug application.

cAMP assay. Variations of cAMP concentration were calculated according to different treatments for the two lanternshark species (*E. spinax* and *E. molleri*). Ventral skin patches ($n = 4$ specimens for each treatment and both species) were dissected and placed in a small chamber filled with different solutions: saline (control), MT 10^{-6} mol L^{-1}, α-MSH 10^{-6} mol L^{-1} during 20 min. Skin patches were then directly frozen at −80°C. Each skin patch was homogenized according to the manufacturer's instructions, and cAMP concentration was measured using the cAMP-Glo™ Assay kit (V1501, Promega, Madison, WI). Light output was recorded using a Berthold microplate luminometer (MPL12 Orion; Pforzheim, Germany) with simplicity software. cAMP concentrations were calculated using the standard linear regression of cAMP-Glo™ Assay kit, results were then standardized in cAMP concentration per square centimeter (µM cm^{-2}).

Statistical analyses. All analyses [ANOVA, *posthoc* Tukey tests] were performed with the software JMP pro v.14 (SAS Institute Inc., Cary, NC, 1989–2007). The Gaussian distribution condition was obtained for all analyses after logarithm transformation allowing the use of parametric

Table 2. Hormone-induced luminescence parameters (mean maximal light intensity: Lmax; time to reach the Lmax: TLmax) after melatonin (MT) treatments for the three investigated species.

Species	*N*	Treatment	Lmax (Mq s^{-1} cm^{-2})	TLmax (s)
Etmopterus spinax	16	MT	0.85 ± 0.03	1241 ± 156
Etmopterus molleri	13	MT	0.98 ± 0.14	312 ± 170
Squaliolus aliae	11	MT	2.67 ± 0.28	332 ± 166

N, number of specimens, data for Lmax and TLmax are means ± SEM.

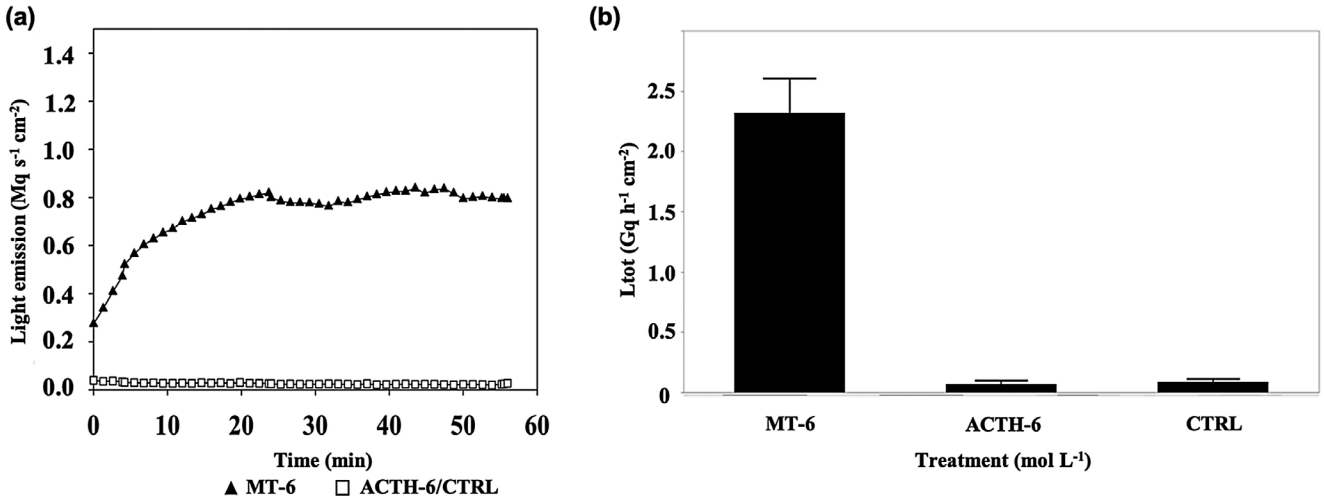


Figure 2. Effects of MT, ACTH and control application on *E. spinax* luminescence. (a) Time course of the mean light emissions (Mq s^{-1} cm^{-2}) from ventral skin patches of velvet belly lanternshark (n = 16). (b) Total amount of light produced (Gq h^{-1} cm^{-2}) under hormonal treatments expressed in 10^{-6} mol L^{-1}. CTRL, shark saline, n = 16, error bars correspond to s.e.m.

tests. ANOVA was used to show significant difference between treatments while *posthoc* Tukey tests allowed the differentiation of clusters.

RESULTS

Pharmacological studies

The effects of ACTH on the ventral skin patches were first tested on *E. spinax*. As shown in the Fig. 2A,B, ACTH alone did not induce a significant light production at all concentrations tested similar to saline controls (P-value < 0.05; Table S1; MT application at 10^{-6} mol L^{-1} triggered a long-lasting luminescence (Fig. 2A,B; Table S1).

Table 2 summarizes the observed maximum intensity of light (Lmax) and time to reach the maximum intensity (TLmax) for the three investigated species.

A dose-dependent inhibitory effect of ACTH on the light emission of *E. spinax* was demonstrated (Fig. 3). At lower concentrations (10^{-6} to 10^{-8} mol L^{-1}), ACTH did not have any effects on MT-induced luminescence while a strong inhibition of MT-induced luminescence was observed at a concentration of 10^{-5} mol L^{-1} (P-value < 0.05), whereas it was observed at 10^{-6} mol L^{-1} with α-MSH (Fig. 3A,B; Table S2).

In *E. molleri*, MT-induced a long-lasting luminescence while ACTH alone failed to trigger a significant light emission compared with shark saline controls (Table S1). Applications of ACTH after the MT pretreatment induced a rapid decrease of light production (Fig. 4A,B). ACTH 10^{-5} and 10^{-6} mol L^{-1} inhibited light production within <5 min as did α-MSH 10^{-6} mol L^{-1} (Fig. 4A). After MT-induced luminescence, Lto-t_{app} values of α-MSH 10^{-6}, ACTH 10^{-6} and ACTH 10^{-5} mol L^{-1} presented statistical differences compared with

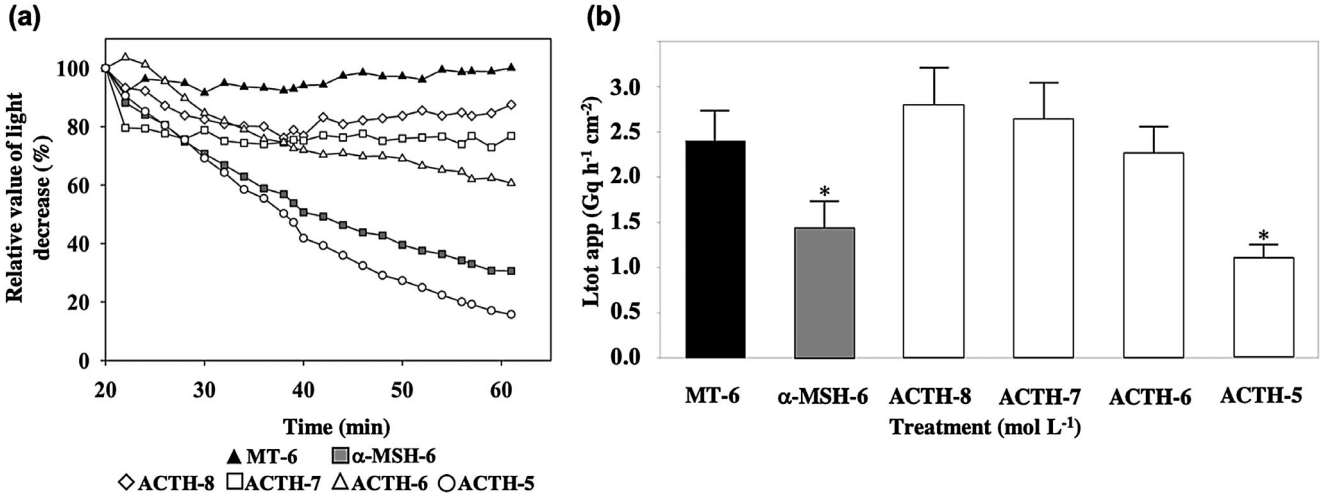


Figure 3. Effects of ACTH and α-MSH on luminescence induced by MT in *E. spinax*. (a) Time courses of the light produced by ventral skin patches; light emission decline was expressed as percentage of maximal MT control value. (b) Effects of α-MSH and ACTH on total amount of light (Gq h^{-1} cm^{-2}) produced after 20-minute MT pretreatment. * corresponds to significant statistical difference from MT-6 control. n = 16, error bars correspond to s.e.m.

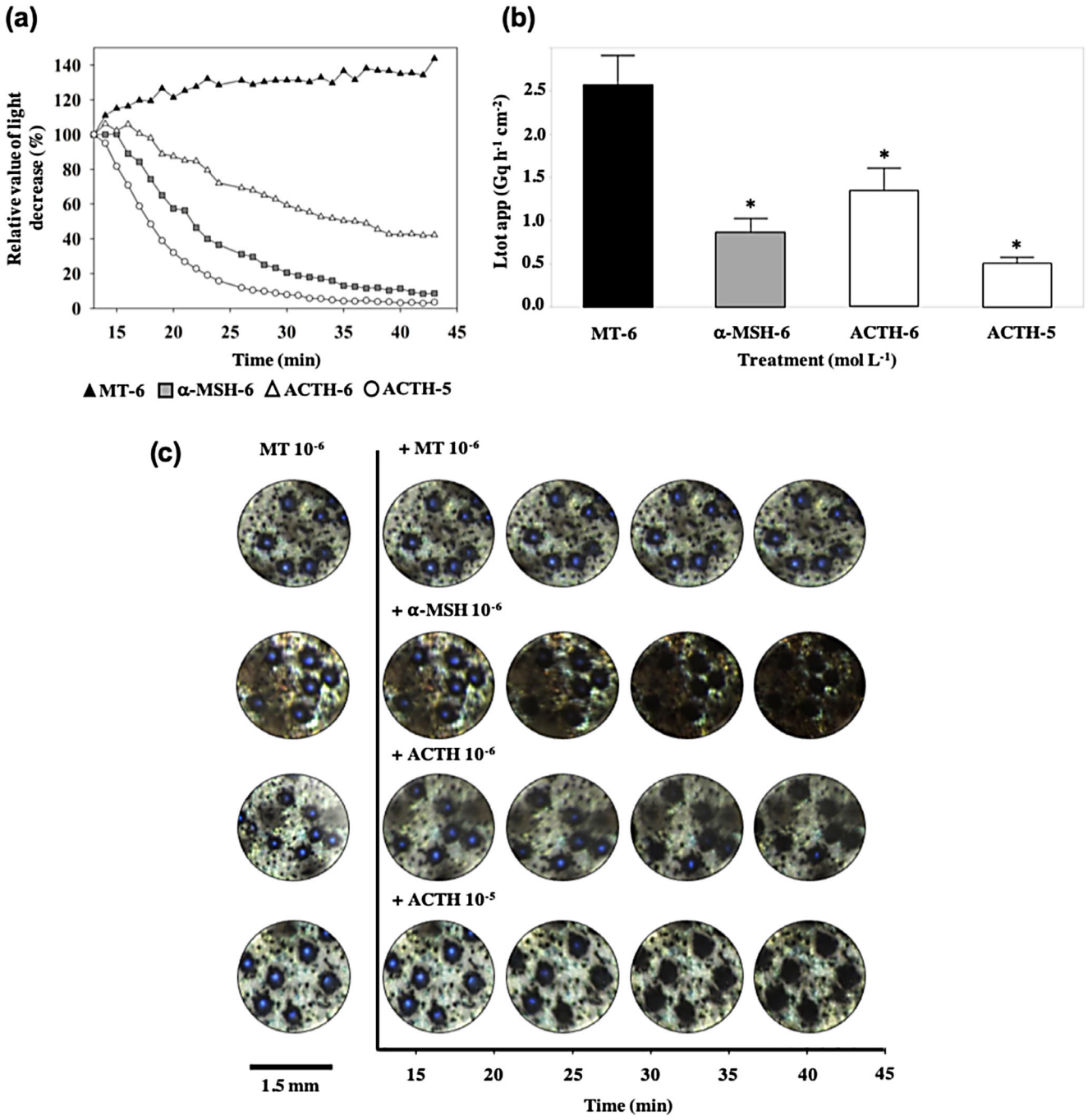


Figure 4. Inhibitory effect of ACTH and α-MSH on luminescence induced by MT in *E. molleri*. (a) Time course of light produced from ventral skin patches of slendertail lanternshark. Results are expressed as percentage of maximal light value recorded for MT pretreatment. (b) Effects of α-MSH and ACTH on the total amount of light (Gq h^{-1} cm^{-2}) produced after 13-minute pretreatment with MT. * corresponds to significant statistical difference from MT control. n = 13, error bars correspond to s.e.m. (c) Time-lapse of pigment movements in skin patches of *E. molleri* pretreated with MT 10^{-6} mol L^{-1}; second application: MT 10^{-6} mol L^{-1} (ctrl); α-MSH 10^{-6} mol L^{-1}; ACTH 10^{-6} and 10^{-5} mol L^{-1}.

MT 10^{-6} mol L^{-1} controls (Fig. 4B; P-value < 0.05; Table S2). Additionally, time-lapse images of skin patches clearly showed that after 13 min, all preparations exhibited a bluish reflection resulting from photophore-associated ILS opening triggered by MT pretreatment (Fig. 4C, Movie S1). A second application of α-MSH or ACTH induced a closure of the ILS, as the photophores became very dark within 20 min (Fig. 4C, Movie S1). Furthermore, the whole skin patch appeared nearly black in response to α-MSH (Fig. 4C).

ACTH 10^{-5} mol L^{-1} was the only concentration tested in *S. aliae*, at this concentration, ACTH alone did not induce light emission since the total amount of light remained significantly below the control level (*i.e.* spontaneous light occurring in shark saline; P-value < 0.05; Table S1). Time courses of controls and total light after treatment are presented in Fig. 5A,B. ACTH 10^{-5} mol L^{-1} rapidly inhibited MT-induced light emission within 4 min, faster than the inhibition observed with α-MSH 10^{-6} mol L^{-1}. Measured $Ltot_{app}$ for α-MSH 10^{-6} and ACTH

10^{-5} mol L^{-1} were statistically different from MT 10^{-6} mol L^{-1} controls (Fig. 5B; *P*-value < 0.05; Table S2).

cAMP concentration assay

In the two etmopterids, *E. spinax* and *E. molleri*, the cAMP assay revealed concentration changes within the photogenic epidermis after treatment. As shown in Fig. 6, ventral skin patches of both species treated with 10^{-6} mol L^{-1} MT showed a significant decrease in cAMP levels compared with untreated controls (*P*-value < 0.05). In *E. spinax,* the mean cAMP concentration was 0.053 ± 0.009 μM cm^{-2} and 0.020 ± 0.003 μM cm^{-2} for the untreated and the MT-treated skin patches, respectively (Fig. 6A). In *E. molleri,* the mean concentration was 0.112 ± 0.006 μM cm^{-2} and 0.075 ± 0.010 μM cm^{-2} for untreated and MT treatments, respectively (Fig. 6B). In contrast, α-MSH has no effect on cAMP levels in *E. spinax* (0.055 ± 0.007 μM cm^{-2}) but it triggered a small increase of cAMP concentration in *E. molleri* (0.132 ± 0.006 μM cm^{-2}) (Fig. 6). However, α-MSH treatments did not produce any significant difference compared with controls (*P*-value > 0.05) for both shark species.

DISCUSSION

Investigations of various luminous shark species strongly suggest that shark bioluminescence is under hormonal control. While a common regulation mechanism core is present in Etmopteridae and Dalatiidae, these two families are characterized by opposite PRL effects on bioluminescence (13,19). Luminescence appears to be mediated by pigment movement within the ILS cells of the light organ (13,19,37).

Among the numerous hormones, MT, PRL, α-MSH and ACTH are known to control pigment migration in vertebrates through the modulation of cAMP concentrations (25–31,38). Melanocortins (*i.e.* MSH and ACTH) are main initiators of the pigment dispersion pathway. According to current understanding of vertebrate pigment dispersion, pathway is thought to unfold as follows: (1) activation of Gs-linked GPCR through a specific ligand (*e.g.* ACTH, α-MSH) which (2) stimulates adenylate cyclase activity leading to (3) a rise of intracellular cAMP levels and, consequently, (4) the activation of the catalytic subunit of cAMP-dependent protein kinase A (PKA) (5) and finally the phosphorylation of a specific protein allowing plus-end cellular motor kinesin to ferry out pigment toward the melanophore digit extensions (39–

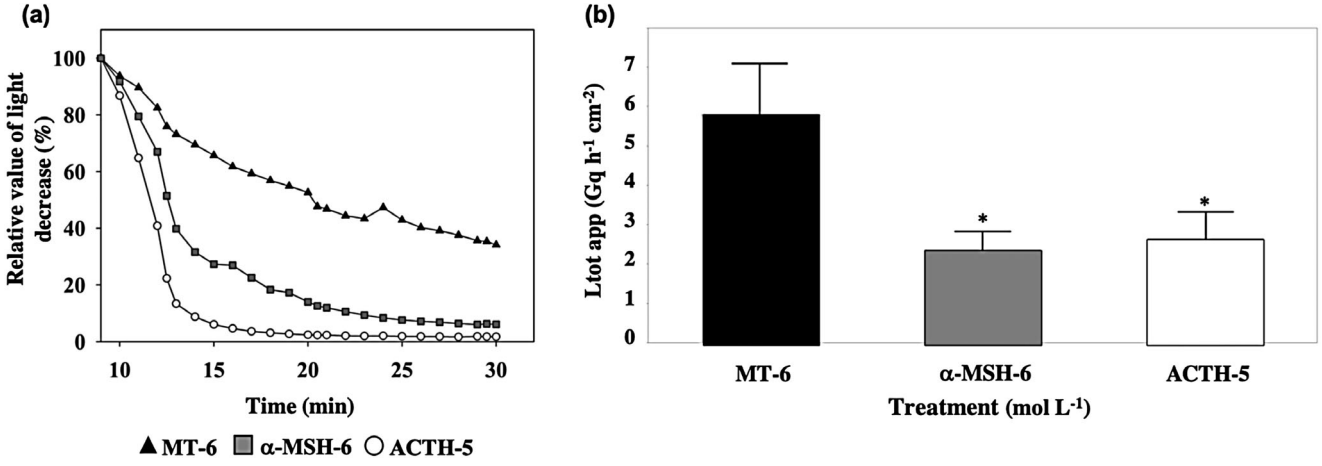


Figure 5. Inhibitory effect of ACTH and α-MSH on luminescence induced by MT in *S. aliae*. (a) Time course of light produced from ventral skin patches of smalleye pygmy shark. Results are expressed as percentage of maximal light value recorded for MT pretreatment. (b) Effects of α-MSH and ACTH on the total amount of light (Gq h^{-1} cm^{-2}) produced after 8-minute pretreatment with MT. * corresponds to significant statistical difference from MT control. $n = 11$, error bars correspond to s.e.m.

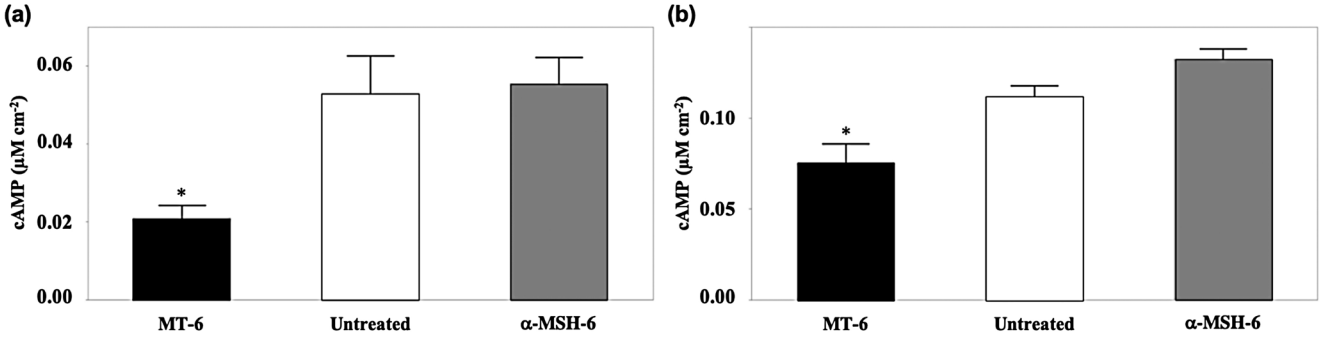


Figure 6. Effect of hormonal treatments on cAMP concentrations in skin patches of *E. spinax* and *E. molleri*. Mean cAMP concentrations in *E. spinax* (a) and *E. molleri* (b); MT at 10^{-6} mol L^{-1}; untreated, shark saline (control); α-MSH at 10^{-6} mol L^{-1}. $n = 4$ for each treatment, error bars correspond to s.e.m. *corresponds to significant statistical difference from shark saline control.

45). Conversely, the MT pathway leads to the inhibition of adenylate cyclase activity and the downregulation of the aforementioned kinesin cellular motor activity through receptor-linked Gi protein (25,46). cAMP intracellular concentration plays a central role on the pigment dispersion movements. Furthermore, other studies highlight the implication of the MT in the pigment aggregation pathway (47,48). Some studies have also demonstrated the role of opsins in the light sensitivity of melanophores; this local response to light can also impact the movements of pigmented granules (49,50). Photoreception of the light emitted [*i.e*. through the encephalopsin (51,52)] could, therefore, act on ILS pigment movements, and thereby, photoreceptive pigment cells could play a role in bioluminescence feedback regulation.

Here, pharmacological tests demonstrated that ACTH, like α-MSH (13,16,19), has a kinetic inhibitory effect on shark ventral luminescence. These two phylogenetically related proteins seem to play an analogous role in luminescence inhibition, but whether they act synergistically or additionally remains to be determined. In *E. molleri*, at the tested concentrations, α-MSH darkens both skin and ILS melanophores, while ACTH acts only at the ILS melanophore level. This observation suggests a differential expression of the melanocortin receptors (*i.e*. MC1-5R) or/and a differential specific affinity for the melanocortin peptides (*i.e*. α-, β-, γ-MSH or ACTH) to one receptor. Further studies are suggested to decipher the exact involvement of each melanocortin on the ILS/skin melanophores.

All these hormones (MT, α-MSH, ACTH, except PRL) bind to GPCRs which activate the release of a G-protein that modulates adenylate cyclase activity (32,33). To evaluate hormonal actions on cAMP levels in lanternshark, cAMP concentration assays were performed. MT treatment induced a decrease in cAMP concentration leading to light triggering and ILS cell pigment aggregation in photophores (13). These results are consistent with the coupling of the MT receptor with a Gi protein that inhibits adenylate cyclase activity (25,46). This cellular pathway exists both in the mechanical control of light emission by ILS cells in bioluminescent sharks and in the modulation of skin pigment movement in shallow-water sharks (53). As skin pigment motion regulation pathways generally involve other molecules such as IP_3, Ca^{2+} and PKA signaling in vertebrates (42,54–58), the role of intracellular IP_3 and Ca^{2+} in ILS cell modulation could be tested to reveal a putative effect on both light emission and pigment motility. In the same perspective, testing cAMP-dependent PKA activation could also be considered.

The pharmacological studies and the cAMP concentration assays suggest the existence of a functional switch between the control of skin pigment movement and the luminescence control mechanism *via* regulation of ILS cell pigment movement. Luminous counterilluminating sharks, as aforementioned, need to finely control their bioluminescence in order to camouflage themselves in the oceanic twilight zone. Further studies need to be conducted to precisely determine the transduction pathways involved in light emission control. In particular, it involves an examination of the intracellular motor carrying melanin granule (kinesin and dynein) and/or skin photoreceptive pathways (encephalopsin). Since *E. spinax*, *E. molleri* and *S. aliae* display specific melanophore cells (ILS cells) overlying photocytes, a specific control of pigment movements could represent an essential step in counterillumination control that is crucial for lanternshark survival.

This work combined with previous studies lead to assume that two interconnected controls exist in the luminous shark light organ: (1) The first control, operating directly at the photocyte level, triggers light emission through an unknown biochemical reaction (*i.e*. unknown luciferin/luciferase system or photoprotein) and (2) The second, the mechanical control of the ILS-associated melanophore pigment motion to regulate the amount of light transmitted. Hormones, neuromodulators and even extraocular opsins being able to act separately or simultaneously on both controls (13–16,19,20,51,52).

The ancestral mechanism of skin color change through skin pigment migrations, involved in countershading to camouflage the body and mimic the background in shallow-water sharks, may have been adapted to specifically regulate ILS cell pigment movements, thus creating favorable conditions for the development of counterillumination mechanisms in deep-sea living velvet belly lanternshark, slendertail lanternshark, smalleye pigmy shark and possibly luminous sharks in general.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Table S1. Total amount of light emitted (Ltot) for each treatment in *Etmopterus spinax* (n = 16), *E. molleri* (n = 13) and *Squaliolus aliae* (n = 11) integrated on 65, 45 and 30 min, respectively.

Table S2. Total amount of light emitted after the second drug application ($Ltot_{app}$) for each treatment in *Etmopterus spinax* (n = 16), *E. molleri* (n = 13) and *Squaliolus aliae* (n = 11) integrated on 45, 32 and 22 min, respectively.

Movie S1. Video recording (accelerated 80 times) of photophore closure after ACTH application on a skin patch of *E. molleri* pretreated with MT.

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