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# Luminescence in ophiuroids (Echinodermata) does not share a common nervous control in all species

Y. Dewael* and J. Mallefet

*Laboratory of Animal Physiology, Catholic University of Louvain, Bâtiment Carnoy, 5 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium*

*e-mail: dewael@bani.ucl.ac.be

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**Summary**

**Study of the control mechanisms of light emission in invertebrates shows the involvement of several neurotransmitters. In ophiuroids, only one species (*Amphipholis squamata*) has so far been characterized for luminescence control, which seems to be cholinergic, with an influence of several excitatory and inhibitory neuromodulators (amino acids, catecholamines, neuropeptides S1 and S2, purines). The aim of this work is to investigate the nature of control mechanisms of light emission in three luminous ophiuroid species, *A. filiformis*, *O. aranea* and *O. californica*, in order to see whether or not they share common mechanisms. Luminescence induced by general depolarisation of tissues using KCl (200 mmol $l^{-1}$) shows different patterns, according to species. Only *A. filiformis* emits light in response to acetylcholine. In this species, the involvement of both muscarinic and nicotinic receptors is proposed, since atropine and tubocurarine (at $10^{-3}$ mol $l^{-1}$) inhibited 99 % and 71 %, respectively, of the light emitted. Study of the subtypes of cholinergic receptors involved in photogenesis revealed that several subtypes of muscarinic receptors might be involved. It was also clearly shown that ophiuroids did not share a common mechanism of nervous control of luminescence in all species.**

Key words: echinoderm, ophiuroid, bioluminescence, nervous control, acetylcholine, muscarinic receptor, nicotinic receptor, *Amphiura filiformis*, *Ophiopsila aranea*, *Ophiopsila californica*.

## Introduction

The ability of living organisms to produce light seems to have originated independently several times, perhaps 30 or more, in evolution (Hastings, 1983). This is reflected in the diversity of its phylogenetic distribution, biology, chemistry and control mechanisms. Studies of the nervous control of light emission have shown the involvement of several neuromediators, including adrenaline (Protista, Cnidaria, Chordata), acetylcholine (Ctenophora, Annelida, Echinodermata) and 5-hydroxytryptamine (Arthropoda) (for a review, see Mallefet, 1999). In echinoderms, control mechanisms of bioluminescence have been almost exclusively studied in the small ophiuroid *Amphipholis squamata*. In this species, it has been shown that photogenesis is under nervous cholinergic control (De Bremaeker et al., 1996) and that some neuromediators (amino acids, catecholamines, neuropeptides SALMFamide S1 and S2, purines) were able to modulate light emission positively or negatively (De Bremaeker et al., 1999a,b,c). Furthermore, it was shown that calcium was required for light emission triggered by potassium chloride (KCl) and by acetylcholine (ACh), either in isolated arms or in dissociated photocytes (Mallefet et al., 1994, 1998).

The aim of this work was to investigate nervous control mechanisms of luminescence in three other ophiuroid species (*Amphiura filiformis*, *Ophiopsila aranea* and *O. californica*) and hence to find out whether they share common signalling pathways, leading to light emission.

*A. filiformis* (O. F. Müller 1776) is a rapidly growing suspension feeder brittlestar frequently found on sub-tidal bottoms off the coasts of Europe and of the Mediterranean Sea. This burrowing ophiuroid is a dominant species in the benthic shelf ecosystem, especially in the northeastern part of the North Atlantic region (Josefson, 1995). It has been shown that arms of this species represent an important food source for flatfishes (Duineveld and Van Noort, 1986). Although it has a high predatory rate, *A. filiformis* has a surprisingly long life span (up to 25 years) according to Muus (1981). This can be explained by its ability to rapidly regenerate chopped arms (Wilkie, 1978; Bowner and Keegan, 1983) (J. Mallefet, unpublished results).

Despite numerous eco-ethological investigations on *A. filiformis* (see Josefson, 1995; Loo et al., 1996; Sköld and Rosenberg, 1996; Nilsson and Sköld 1996; Rosenberg and Selander, 2000) nearly nothing is known about its capability to produce light. Emson and Herring (1985) reported the first data on *A. filiformis* bioluminescence: light emission is blue in colour, it appears to be intracellular and the luminous cells, called photocytes, are restricted to the arm spines. No

physiological data are available concerning the control of light emission of *A. filiformis*.

*O. aranea* (Forbes 1843) inhabits the encrusting coralline algae zone (coralligene) in the Mediterranean Sea. Some morphological studies described the luminescence sites as originating from glandular cells located on lateral and ventral plates, and in some spines of the arms, next to the disc (Mangold, 1907; Reichensperger, 1908; Trojan, 1909). Later, Harvey (1952) mentioned a yellowish green fluorescence at the sites of luminescence. The exact nature of luminous cells remains unknown. Mallefet and Dubuisson (1995) described the KCl-induced luminescence as a series of flashes whose maximal intensity increases as a function of KCl concentration.

*O. californica* (Clarck 1921) is a sand-dwelling ophiuroid found along the Californian coast. Previous work has shown that luminescence is used as an aposematic signal (Basch, 1988) and that photocytes, of nervous origin, are located in the arms. Light emission seems to be under nervous control (Brehm, 1977; Brehm and Morin, 1977) and requires the presence of calcium (Brehm, 1977).

These ophiuroid species were chosen for this comparative study since they belong to two different families (Amphiuridae for *A. filiformis*, Ophiocomidae for *O. aranea* and *O. californica*) and they live in two different types of habitat (in mud or sand for *A. filiformis* and *O. californica*, in coralligene for *O. aranea*). Our results show that control mechanisms of light emission differ from species to species; a cholinergic system appears to be involved in light emission of *A. filiformis*, but the nature of luminous control in *Ophiopsila* sp. remains undetermined.

## Materials and methods

### *Animals*

Specimens of *A. filiformis* were collected at the Kristineberg Marine Station (Fiskebäckskil, Sweden) by mechanical grab at 25–40 m depth. Animals were then kept in circulating natural sea water. Specimens of *O. aranea* were collected at the ARAGO biological station (CNRS) of Banyuls-sur-Mer (France) by scuba diving at a depth of 20–25 m and specimens of *O. californica* were collected in the same way at the Marine Sciences Institute of the University of California (Santa-Barbara). All these animals were transported to our laboratory in Belgium in aerated natural sea water and then kept in aquaria filled with recirculating natural and artificial sea water (ASW) at 12 °C. Food was provided to ophiuroids once a week.

### *Experiments on arm segments*

After anaesthesia of the animals by immersion in 3.5 % $MgCl_2$ in ASW, arms were isolated from the disc and divided into segments of 8 articles (*Ophiopsila*) or 20 articles (*Amphiura*), which were then rinsed in ASW (NaCl 400.4 mmol $l^{-1}$, $CaCl_2$ 9.9 mmol $l^{-1}$, KCl 9.6 mmol $l^{-1}$, $MgCl_2$ 52.3 mmol $l^{-1}$, $Na_2SO_4$ 27.7 mmol $l^{-1}$, Tris 20 mmol $l^{-1}$, pH 8.3).

### *Stimulations*

Stimulations were performed by injection of drugs onto arm segments. Light emission was measured with a FB12 Berthold luminometer linked to a PC-type computer. For each experimental protocol, one arm segment was treated with the control stimulus (200 mmol $l^{-1}$ KCl or $10^{-3}$ mol $l^{-1}$ ACh), while the other preparations were stimulated with the tested drug.

### *Drugs*

The following drugs were used in this study: acetylcholine chloride (Sigma), adenosine (Sigma), adenosine 5′-triphosphate (ATP; Sigma), L-adrenaline (Fluka), 4-aminobutyric acid (GABA; Aldrich), 2-aminoethylsulfonic acid (taurine; Fluka), atropine (Sigma), carbamylcholine (carbachol; Janssen Chimica), 4-diphenylacetoxy-*N*-methyl peperidine (4-DAMP methiodide; ICN), 1,1-dimethyl-4-phenyl piperazium iodide (DMPP; ICN) eserine (Sigma), L-glutamic acid hydrochloride (glutamate; Sigma), glycine hydrochloride (Sigma), hexamethonium dichloride (RBI), hydroxylamine hydrochloride (Sigma), 5-hydroxytryptamine (5-HT; Sigma), 5-hydroxytyramine hydrochloride (dopamine; Sigma), McN-A-343 (RBI), L-noradrenaline hydrochloride (Fluka), pirenzepine dihydrochloride (RBI), SALMFamide 1 and SALMFamide 2 (provided by M. Thorndyke's laboratory), sodium nitroprusside (Sigma), tubocurarine chloride (Janssen Chimica). All solutions were diluted in ASW. The concentrations used ranged from $10^{-6}$ to $10^{-3}$ mol $l^{-1}$. These rather high concentrations are commonly used in echinoderms because of the heavy calcification of the ophiuroid arms, which impairs adsorption and penetration to the photocytes.

### *Statistics*

Statistical analyses (ANOVA) were performed using SAS (Statistic Analysis System).

### *Photogenesis characterization*

Different parameters were used in order to characterize the photogenesis. (1) $L_{max}$, the maximum level of light emission expressed as a percentage of the control; (2) *LT*, latency time, the time between stimulation and the beginning of the light emission; (3) $TL_{max}$, the time between onset of light production and maximum light emission.

## Results

### *Pattern of light emission*

Using KCl (200 mmol $l^{-1}$) to depolarise cells, it was possible to record light emission of arm segments isolated from *A. filiformis* (Fig. 1A), *O. aranea* (Fig. 1B) and *O. californica* (Fig. 1C). Isolated discs never emitted light when stimulated by KCl in any of the three species. In contrast, arm segments always responded to KCl, producing a series of light peaks whose intensity and kinetic parameters are shown in Table 1. Kinetic parameters of *O. aranea* were different from those of

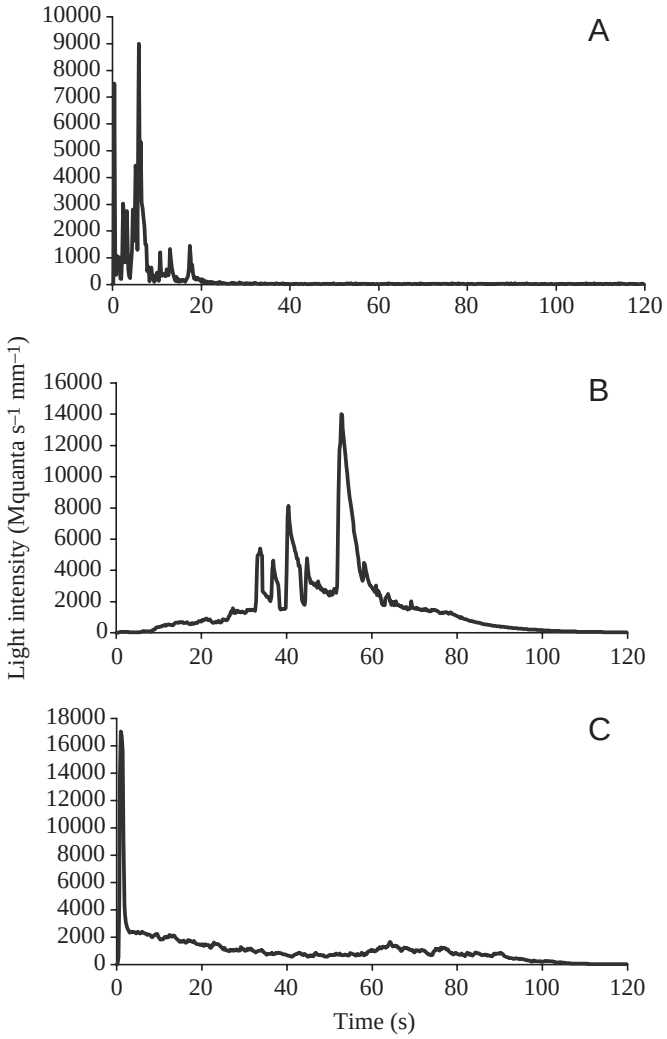


Fig. 1. Representative original recordings of light pattern by an arm segment stimulated by KCl (200 mmol $l^{-1}$). (A) *A. filiformis*, (B) *O. aranea*, (C) *O. californica*. Intensity of light emitted is expressed in Mquanta $s^{-1}$ $mm^{-1}$ arm.

Table 1. *Parameters of light emission induced by 200 mmol $l^{-1}$ KCl in the three ophiuroid species*

| | *A. filiformis* | *O. aranea* | *O. californica* |
|---|---|---|---|
| $L_{max}$ (Mquanta $s^{-1}$ $mm^{-1}$) | 8975±1339 | 13710±2059 | 16138±1947 |
| *LT* (s) | 0.75±0.04 | 2.22±0.40 | 0.50±0.03 |
| $TL_{max}$ (s) | 9.15±0.9 | 55.72±2.70 | 2.07±0.44 |
| *N* | 171 | 113 | 152 |

$L_{max}$, maximal intensity of light (Megaquanta per second and per millimetre of arm); *LT*, latency time; $TL_{max}$, time to reach maximal intensity.

Values are means ± S.E.M.; *N*, number of experiments.

the two other species because the luminous reaction was significantly slower in reaching maximum light intensity and in glowing out.

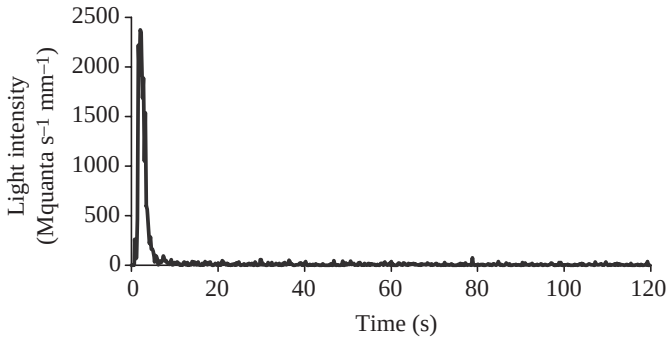


Fig. 2. Representative original recording of the light pattern produced by an arm segment of *A. filiformis* stimulated by acetylcholine (1 mmol $l^{-1}$). Intensity of light emitted is expressed in Mquanta $s^{-1}$ $mm^{-1}$ arm.

### *Screening of neuromediators*

Most of the neuromediators classically found in nervous tissue of echinoderms were tested on *A. filiformis*, *O. aranea* and *O. californica* in order to determine the nature of the control mechanism of bioluminescence. We tested 5-HT, ACh, adenosine, adrenaline, ATP, carbachol, dopamine, GABA, glutamate, glycine, hydroxylamine, noradrenaline, SALMFamide 1, SALMFamide 2, sodium nitroprusside and taurine. In *O. aranea*, only taurine triggered a luminescence, which was weak compared to the control induced by 200 mmol $l^{-1}$ KCl, and occurred in only some specimens (Table 2). In *O. californica*, ACh, carbachol, dopamine and taurine triggered weak light emission, which was only a small percentage of the light induced by KCl depolarisation. ACh, carbachol, dopamine, 5-HT and taurine induced a series of light flashes from isolated arm segments of *A. filiformis*. Maximal intensities of these light emissions ranged from less than 1 % (taurine) up to 7.12 % (carbachol) of the control.

### *Effects of acetylcholine in* Amphiura filiformis

Because *A. filiformis* was the only ophiuroid species that emitted light in response to ACh in nearly all trials, we attempted to characterize ACh-induced luminescence in this species. ACh concentrations ranging from $10^{-6}$ to $10^{-3}$ mol $l^{-1}$ were tested on arm segments of *A. filiformis*. The luminescence pattern comprised a monophasic emission that was maximal by 3–4 s and then rapidly decreased in magnitude (Fig. 2). The maximal intensity of light emission ($L_{max}$) was 26 % of the KCl-induced $L_{max}$. Fig. 3 shows the dependence of $L_{max}$ on ACh concentration. All the concentrations tested ($10^{-6}$ to $10^{-3}$ mol $l^{-1}$) triggered light emission. $10^{-3}$ mol $l^{-1}$ ACh triggered a photogenesis of average $L_{max}$ 2400±525 Mquanta $s^{-1}$ $mm^{-1}$. At lower concentrations, the kinetic parameters of luminescence were not modified but maximal intensity gradually decreased. ACh at $10^{-4}$ mol $l^{-1}$, $10^{-5}$ mol $l^{-1}$ and $10^{-6}$ mol $l^{-1}$ induced levels of 49±10 %, 9±2.7 % and 7.5±3.4 % of luminescence induced by $10^{-3}$ mol $l^{-1}$ ACh, respectively. It must be pointed out that the number of arm segments responding to stimulation was gradually lower with decreasing ACh concentration: 225/234

Table 2. *Intensity of luminescence induced by drugs in the three ophiuroid species*

| Drug | Concentration | *A. filiformis* | *O. aranea* | *O. californica* |
|---|---|---|---|---|
| KCl (control) | 200 mmol $l^{-1}$ | 100±0 (171/171) | 100±0 (113/113) | 100±0 (152/152) |
| Acetylcholine | $10^{-3}$ mol $l^{-1}$ | 26.5±5.79 (225/234) | – | 1.68±1.48 (12/12) |
| Carbachol | $10^{-3}$ mol $l^{-1}$ | 7.12±3.77 (11/11) | – | 0.84±0.20 (12/12) |
| Dopamine | $10^{-3}$ mol $l^{-1}$ | 2.31±1.21 (18/27) | – | 0.07±0.05 (7/7) |
| 5-HT | $10^{-3}$ mol $l^{-1}$ | 1.09±0.32 (16/26) | – | – |
| Taurine | $10^{-3}$ mol $l^{-1}$ | 0.74±0.15 (19/23) | 2.08±0.36 (43/57) | 2.49±1.61 (12/12) |

Values are means ± S.E.M. expressed as a percentage of the control value (KCl).
*n*/*N*, number of responses/number of experiments.
–, arm segments did not emit light.

at $10^{-3}$ mol $l^{-1}$, 38/49 at $10^{-4}$ mol $l^{-1}$, 16/25 at $10^{-5}$ mol $l^{-1}$ and 6/20 at $10^{-6}$ mol $l^{-1}$).

### *Effects of anticholinesterase*

The low responsiveness of the arm segments to ACh compared to KCl whole depolarisation could be explained in terms of fast hydrolysis of the neuromediators by endogenous acetylcholinesterase present in the radial nerve cord. To test this hypothesis, we measured the effect of the anticholinesterase drug eserine on ACh-induced luminescence.

In our experiments, arm segments treated for 10 min with $10^{-3}$ and $10^{-4}$ mol $l^{-1}$ eserine were then stimulated by $10^{-3}$ and $10^{-4}$ mol $l^{-1}$ ACh, respectively. Photogenesis was compared to the control, without eserine. Treatment with eserine alone did not trigger luminescence. The results showed no effect of $10^{-4}$ mol $l^{-1}$ eserine on ACh-induced luminescence. However, treatment of the arm segments with $10^{-3}$ mol $l^{-1}$ eserine reduced the ACh-induced light emission by 74 % (data not shown).

### *Effects of cholinergic antagonists*

To identify the receptors involved in the control of light emission, we tested the effects of different cholinergic antagonists, including atropine, which selectively blocks cholinergic muscarinic receptors, and tubocurarine and hexamethonium, which block cholinergic nicotinic receptors.

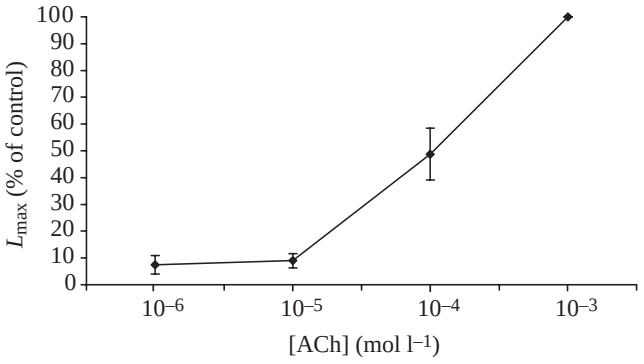


Fig. 3. Effect of actylcholine (ACh) concentration on luminescence of arm segments from *A. filiformis*. Values are means ± S.E.M., expressed as a percentage of photogenesis triggered by 1 mmol $l^{-1}$ ACh.

Arm segments were treated with cholinergic antagonists for 10 min, whereas the controls were immersed in normal ASW. All the segments were then stimulated with $10^{-3}$ mol $l^{-1}$ ACh. Fig. 4 shows the effect of atropine and tubocurarine on luminescence at concentrations ranging from $10^{-6}$ to $10^{-3}$ mol $l^{-1}$. The dose–response curve with atropine showed a gradual decrease of ACh-induced light emission; at $10^{-4}$ mol $l^{-1}$, 26±13 % of the control luminescence remains ($P$<0.05; $N$=8), while a total inhibition of the response occurred at $10^{-3}$ mol $l^{-1}$ ($P$<0.01; $N$=23). With tubocurarine, the dose–response curve showed also a gradual decrease, only 29±9 % remaining at $10^{-3}$ mol $l^{-1}$ ($P$<0.01; $N$=24). A similar decrease of ACh-induced luminescence was observed with $10^{-3}$ mol $l^{-1}$ hexamethonium (result not shown).

### *Effects of specific drugs*

Since atropine had a strong inhibitory effect on light emission, we assumed that muscarinic receptors were involved in the signal transmission pathway leading to photogenesis. Consequently, we attempted to define the sub-type of muscarinic receptors involved in the light response.

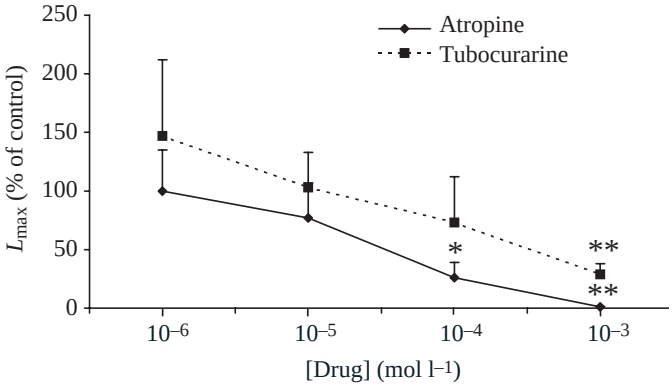


Fig. 4. Dose-dependent inhibitory effect of atropine and tubocurarine on 1 mmol $l^{-1}$ ACh-induced luminescence from arm segment of *A. filiformis*. Values are means + S.E.M. of maximal intensities of light emitted, expressed as a percentage of those measured in control arm segments, not treated with cholinergic antagonists. Asterisks indicate significant differences between control and treated arm segments (*$P$<0.05, **$P$<0.01).

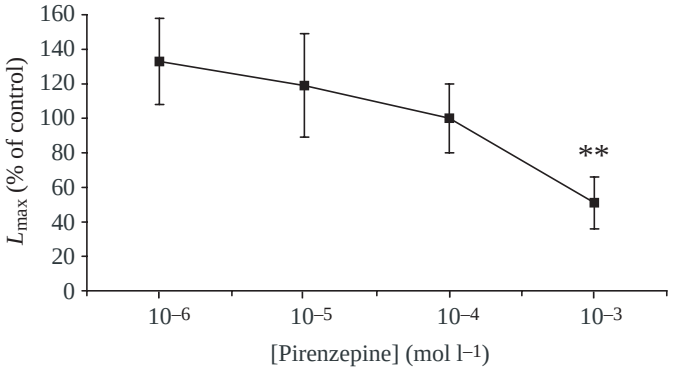


Fig. 5. Dose-dependent inhibitory effect of pirenzepine on 1 mmol $l^{-1}$ ACh-induced luminescence from arm segment of *A. filiformis*. Values are means ± S.E.M. of maximal intensities of light emitted, expressed as a percentage of those measured in control arm segments, not treated with cholinergic antagonists. **Significant difference between control and treated arm segments ($P$<0.01).

Pirenzepine, an M1 muscarinic antagonist, and 4-DAMP, an M2-M3 muscarinic antagonist, were applied using the same experimental protocol as above. Fig. 5 shows the effects of pirenzepine at different concentrations on luminescence triggered by $10^{-3}$ mol $l^{-1}$ ACh; pre-treatment of the arm segments with pirenzepine did not induce spontaneous luminescence. Although a progressive inhibition of light emission was observed, only $10^{-3}$ mol $l^{-1}$ pirenzepine significantly inhibited photogenesis ($P$<0.01; $N$=30). In the case of 4-DAMP, luminescence was induced before ACh injection in all trials, from $10^{-6}$ mol $l^{-1}$ to $10^{-3}$ mol $l^{-1}$. As shown in Fig. 6, $10^{-3}$ mol $l^{-1}$ 4-DAMP triggered a light emission not significantly different from the control (83.5±18.0 %; $N$=23) while $10^{-6}$ mol $l^{-1}$ 4-DAMP-induced luminescence was only 7.66 % of the control ($N$=10). The further photogenesis induced by $10^{-3}$ mol $l^{-1}$ ACh was equally

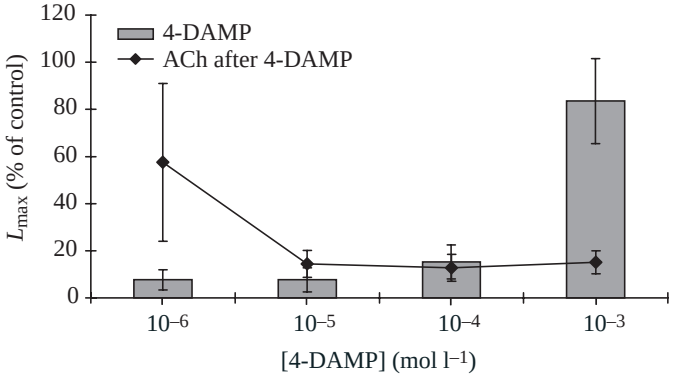


Fig. 6. Dose-dependent effect of 4-DAMP on 1 mmol $l^{-1}$ ACh-induced luminescence from arm segment of *A. filiformis*. Bars show the average intensity of maximal light emitted after injection of 4-DAMP. Diamonds show the average intensity of light emitted induced by ACh. Maximal intensities of light emitted are expressed as a percentage of those measured in control arm segments, not treated with 4-DAMP.

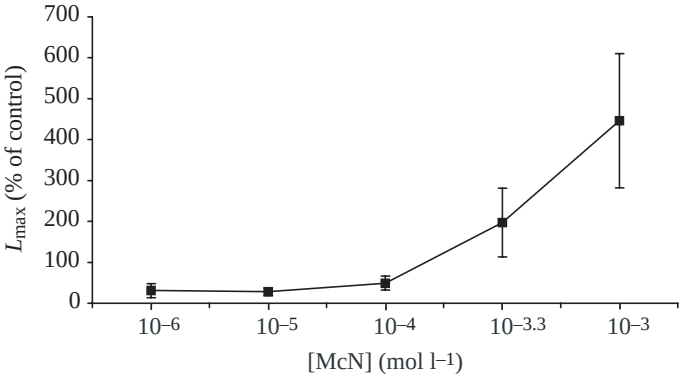


Fig. 7. Dose-dependent emission of light from arm segment of *A. filiformis* in response to McN. Values are means ± S.E.M. of maximal intensities of light emitted, expressed as a percentage of those measured in control arm segments, stimulated with 1 mmol $l^{-1}$ ACh.

inhibited by $10^{-5}$–$10^{-3}$ mol $l^{-1}$ 4-DAMP. With $10^{-6}$ mol $l^{-1}$ 4-DAMP, 57.5±33.5 % of the control photogenesis was produced ($N$=10), but this decrease in amplitude was not significantly different from the control.

We further investigated the nature of the muscarinic subtype of receptors involved in the luminescence control, by testing the M1 muscarinic agonist McN. Fig. 7 shows that $10^{-3}$ mol $l^{-1}$ McN triggered a more intense luminescence than $10^{-3}$ mol $l^{-1}$ ACh. This was also the case with $5\times10^{-4}$ mol $l^{-1}$ McN. This increase of $L_{max}$ was not significant, due to the great variability of the results. $10^{-6}$–$10^{-4}$ mol $l^{-1}$ McN triggered photogenesis of stronger intensity than that induced by $10^{-6}$–$10^{-4}$ mol $l^{-1}$ ACh (21±17 % at $10^{-6}$ mol $l^{-1}$, 28±9 % at $10^{-5}$ mol $l^{-1}$, 49±17 % at $10^{-4}$ mol $l^{-1}$, 197±84 % at $5.10^{-4}$ mol $l^{-1}$ and 446±164 % at $10^{-3}$ mol $l^{-1}$; Fig. 7).

## Discussion

### *Effects of potassium chloride*

200 mmol $l^{-1}$ KCl isotonic to seawater has been frequently used to test the ability of echinoderms to emit light (Harvey, 1952; Brehm and Morin, 1977; Herring, 1978; Mallefet and Dubuisson, 1995; De Bremaeker et al., 1996). Our results clearly show that luminescent tissues of arm segments isolated from *A. filiformis*, *O. aranea* and *O. californica* produce light in response to external application of 200 mmol $l^{-1}$ KCl. The presence of multiple flashes suggests that KCl acts either through depolarisation of nervous elements involved in the luminescence control, or through a mechanism that progressively activates luminous cells to trigger light emission. The kinetics and the amplitude of photogenesis differ from species to species. *A. filiformis* and *O. californica* emit light with a short latency time and the time to reach maximal intensity is quite fast. The luminous reaction in *O. aranea* is slower, and the total time of the luminous response is also longer. Since nothing is known about the function of luminescence in *A. filiformis* and *O. aranea*, hypotheses to explain the differences in kinetics of the light reaction are purely speculative. Nevertheless, one possible

explanation for evolution of this type of response is found in the living habitats of the ophiuroids: *A. filiformis* and *O. californica* live buried in mud or in sand, whereas *O. aranea* burrows in the encrusting coralline algae zone (coralligene). The requirements of bioluminescence might be different in these contrasting habitats. Another hypothesis is the involvement of different nervous fibre types in the luminous control, conducting nerve impulses at different speeds. Cobb and Stubbs (1981a,b) have shown the existence of giant fibres in ophiuroids, which conduct nerve impulse at high speed compared to other nerve fibre types.

Differences in amplitude of light emission might reveal either a variation in the amount of luminescent tissue in the arms, or a variation in the quantity of substrate for the light reaction in the luminescent cells. Because light emission is measured from all the photocytes of the entire arm segment taken together, it is impossible to distinguish between these two hypotheses.

### *Screening of neuromediators*

ACh triggered a significant amount of light in *A. filiformis*, but only very weak photogenesis was produced in *O. californica* and none in *O. aranea*. The difference in intensity between KCl- and ACh-induced luminescence suggests that other neuromediators or neuromodulators are involved in luminescence control of *A. filiformis*. Moreover, since ACh did not trigger systematic luminous responses in *O. aranea* and *O. californica*, we tested several neuromediators, commonly found in echinoderms, to try to identify the nature of the luminous control mechanisms. In *A. filiformis*, taurine, 5-HT and dopamine occasionally triggered a weak luminescence, whose intensity did not exceed 3 % of that induced by KCl. This might reinforce the hypothesis that ACh is not the only neuromediator and that some drugs could act as neuromodulators of the luminous response. These neuromodulators could either act directly on the photocyte, or lead to activation of ACh release from the nervous system. Similar observations have been made in the ophiuroid *Amphipholis squamata*, where ACh is the main transmitter and some neuromodulators (GABA, glycine, catecholamines, ATP, adenosine) either increase or decrease the light emission (De Bremaeker et al., 1999a,b,c). In *Ophiopsila* species, only taurine (for *O. aranea*) and ACh, carbachol, taurine and dopamine (for *O. californica*) triggered a weak light emission. Luminescence intensity is so low, compared to that induced by KCl, that these drugs could not be considered as main neuromediators, but perhaps act synergically with another still-unidentified compound. Further experiments are in progress to try to identify the main neurotransmitter(s) involved in the luminous control of *O. aranea* and *O. californica*.

### *Effects of acetylcholine*

Only arm segments from *A. filiformis* responded to ACh stimulation by emitting light in nearly all trials. The pattern of the light emission evoked by ACh was different from that evoked by KCl: both latency time and time to reach maximal intensity of light were smaller with ACh. Moreover, the amplitude of the KCl response was much higher than the response to ACh. To explain these differences, it could be assumed that cholinergic luminescence is mediated through cholinergic receptors, whereas KCl luminescence is due to a general depolarisation of the photogenic cells and of the nervous tissue controlling the photocytes. This was also the case with *A. squamata*, another luminous ophiuroid belonging to the same family (Amphiuridae). In this species, $10^{-3}$ mol $l^{-1}$ ACh triggered light emission about 10- to 100-fold lower than with KCl, according to the colour variety of the ophiuroids (De Bremaeker et al., 1996). In *A. filiformis*, $10^{-3}$ mol $l^{-1}$ ACh triggered a light emission whose intensity reached 26 % of the KCl response. Moreover, ACh at concentrations of $10^{-6}$–$10^{-4}$ mol $l^{-1}$ also initiates luminescence in *A. filiformis*. At these lower concentrations, both the intensity and the number of arm segments responding decreased. The difference in intensity between KCl- and ACh-induced photogenesis could be explained by their mechanism of action. The KCl peak of light may result from simultaneous recruitment of a large number of photocytes, by general depolarisation of the photogenous tissues and of the nervous tissues controlling photocytes, while ACh may diffuse progressively through the surrounding tissues. The concentration of ACh reaching the photogenic tissue may be low and, as a consequence, trigger a weaker intensity of flashes. A similar phenomenon has been observed in the ophiuroid *Amphipholis squamata* (De Bremaeker et al., 1996) and in the starfish *Asterias rubens*, where ACh contraction of the tube feet was about 1000-fold stronger on tube feet whose epithelium was removed, thus lowering diffusion distance, compared to 'intact' tube feet (Protas and Muske, 1980). Moreover, ACh acts through cholinergic receptors, which are subject to positive and negative neuromodulation. Another hypothesis to explain the low response to ACh compared to KCl, is that exogenous ACh might be quickly hydrolysed by endogenous acetylcholinesterase before reaching the photocytes. This phenomenon has been observed in *A. squamata*, where pre-treatment of the arm with the anticholinesterase drug eserine significantly increased, by up to 100-fold, the maximal amplitude of light emitted by ACh (De Bremaeker et al., 1996). But this hypothesis was not supported by results in *A. filiformis* since pre-treatment of arm segments by eserine (at $10^{-4}$ mol $l^{-1}$) did not affect or even (at $10^{-3}$ mol $l^{-1}$) inhibit the ACh-induced luminescence. This inhibitory effect of $10^{-3}$ mol $l^{-1}$ eserine could be due to increased ACh availability for a putative inhibitory receptor. This unexpected effect of eserine has not been reported in the literature.

### *Effects of cholinergic drugs*

Both muscarinic and nicotinic antagonists inhibited light emission of *A. filiformis*. Consequently, it seems that both cholinergic muscarinic and nicotinic receptors are involved in the photogenesis of *A. filiformis*. Muscarinic receptors might be predominant since light emission was inhibited more strongly by the muscarinic antagonist atropine than by tubocurarine and hexamethonium. Similar observations have

been reported in other tissues of echinoderms: tube foot muscle of the starfish *Asterias amurensis* (Protas and Muske, 1980), viscosity of the body wall of the sea cucumber *Holothuria leucospilota* (Motokawa, 1987) and longitudinal muscle of the body wall of the sea cucumber *Sclerodactyla briareus* (Devlin et al., 2000) are controlled by both nicotinic and muscarinic drugs. On the contrary, only muscarinic receptors are involved in the luminescence control of the ophiuroid *Amphipholis squamata* (De Bremaeker et al., 1996).

The results obtained with M1 muscarinic agonists and antagonists suggest that ACh luminescence is partially mediated through the activation of M1 subtype muscarinic receptors in *A. filiformis*. The systematically higher intensity of light emitted by $10^{-6}$–$10^{-3}$ mol $l^{-1}$ McN, compared to $10^{-6}$–$10^{-3}$ mol $l^{-1}$ ACh, seems to bring out the existence of an inhibitory modulation, using another subtype of cholinergic receptor. The M2/M3 muscarinic antagonist 4-DAMP gave unexpected results since it triggered photogenesis itself, at concentrations ranging from $10^{-6}$ to $10^{-3}$ mol $l^{-1}$. Although there is no mention in the literature of any agonist effect of 4-DAMP, some drugs can act either as agonists or antagonists, according to the animal species studied. Baguet and Marechal (1978) showed that propranolol, a common β-adrenergic antagonist, triggered light emission by isolated photophores from *Argyropelecus hemigymnus*, and an antagonistic effect of synthetic α-adrenoceptor agonists has been shown on isolated artery strips from *Gadus morhua* (Johansson, 1979). Moreover, 4-DAMP inhibited ACh-induced luminescence with the same efficiency at concentrations from $10^{-5}$ to $10^{-3}$ mol $l^{-1}$. This inhibition could be due to the former light emission triggered by 4-DAMP, leading to a partial exhaustion of the luminous capabilities, or it could suggest that 4-DAMP blocks all M2/M3 receptors, even at concentrations as low as $10^{-5}$ mol $l^{-1}$. The remaining light emitted may be produced by the stimulation through another subtype of cholinergic receptor. It appears then that M2/M3 muscarinic receptors might also be involved in the luminous control of *A. filiformis*. Further experiments are planned in order to confirm the inhibitory effect of 4-DAMP on ACh-induced luminescence, using another specific M2/M3 antagonist that does not trigger light during the pre-treatment.

It must be pointed out that specific muscarinic antagonists or agonists used in this study have been demonstrated to have specific effects on mammalian tissues. We cannot rule out the possibility that muscarinic receptors in invertebrates, such as ophiuroids, are somewhat different from those encountered in mammalian tissues. Onai et al. (1989) have shown that invertebrate genes from *Drosophilia melanogaster* coding for muscarinic receptors showed only 60 % homology with the five vertebrate subtypes. Therefore, we have to be cautious in extrapolating pharmacological results from mammalian to invertebrate tissues.

In conclusion, we propose that ACh is the main transmitter controlling the luminescence in *A. filiformis*. Both nicotinic and muscarinic receptors seem to be involved. In *Ophiopsila* species, other mechanisms might act to trigger light emission. Therefore, we can postulate the absence of a common signal transmission pathway, leading to luminescence in all ophiuroid species.

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