The Journal of Experimental Biology 207, 3749-3756
Published by The Company of Biologists 2004
doi:10.1242/jeb.01209

Synergic effects of tryptamine and octopamine on ophiuroid luminescence (Echinodermata)

C. Vanderlinden* and J. Mallefet

Laboratoire de Biologie Marine, Université Catholique de Louvain, Bâtiment Kellner, Place Croix du Sud, 3, B-1348 Louvain-la-Neuve, Belgium

*Author for correspondence (e-mail: vanderlinden@bani.ucl.ac.be)

Accepted 26 July 2004

Summary

In ophiuroids, bioluminescence is under nervous control. Previous studies have shown that acetylcholine is the main neurotransmitter triggering light emission in *Amphipholis squamata* and *Amphiura filiformis*. By contrast, none of the neurotransmitters tested so far induced luminescence in two other ophiuroid species, *Ophiopsila aranea* and *Ophiopsila californica.* The aim of this work was thus to investigate the putative involvement of two biogenic amines, tryptamine and octopamine, in light emission of three ophiuroid species. *A. filiformis* responds to both tryptamine and octopamine, mainly on its arm segments, while *O. californica* only responds to tryptamine stimulation. By contrast, tryptamine and octopamine do not seem to be involved in *O. aranea* luminescence control since none of these substances induced light emission in this species. The synergic effects of several other drugs with tryptamine and octopamine were also tested.

Key words: *Amphiura filiformis*, Echinoderm, *Ophiosila aranea*, *Ophiopsila californica*, Ophiuroid, octopamine, pharmacology, tryptamine, luminescence.

Introduction

Bioluminescence (the emission of visible light by living organisms) seems to have appeared independently several times (up to 30 or more) during evolution (Hastings, 1983), as illustrated by the diversity of its phylogenetic distribution, biology, chemistry and control mechanisms (Hastings, 1983; Herring, 1987; Campbell, 1989). Several neuromediators such as adrenaline (Protista, Cnidaria, fish), acetylcholine (ACh; Ctenophora, Annelida, Echinodermata) and 5-hydroxytryptamine (5HT; Arthropoda) are involved in the nervous control of bioluminescence (for a review, see Mallefet, 1999). Our laboratory has been focusing on the nervous control of ophiuroid (brittlestar, Echinodermata) luminescence for 15 years. Although it was assumed that control mechanisms would be similar in closely related species, pharmacological experiments have shown a high diversity in luminous control mechanisms among ophiuroid species. (i) In the well-studied dwarf ophiuroid *Amphipholis squamata*, for instance, photogenesis is under nervous muscarinic cholinergic control (De Bremaeker et al., 1996) and some neuromediators (amino acids, catecholamines, neuropeptides SALMFamide S1 and S2, purines) positively or negatively modulate light emission (De Bremaeker et al., 1999a,b,c). (ii) Dewael and Mallefet (2002a) showed that acetylcholine induces luminescence *via* muscarinic and nicotinic cholinergic receptors in *Amphiura filiformis*. (iii) By contrast, photogenetic control mechanisms remain unsolved in two other ophiuroid species studied, *Ophiopsila aranea* and *Ophiopsila californica*, since none of the substances tested so far systematically trigger luminescence.

The aim of the present work was to further investigate the nervous control mechanisms of bioluminescence in ophiuroids by a comparative study on three species: *Amphiura filiformis*, *Ophiopsila aranea* and *Ophiopsila californica.* We studied the putative involvement of two biogenic amines, tryptamine and octopamine, present in trace levels in mammalian nervous systems. These trace amines do not seem to play a main neurotransmitter role in mammals (Borowsky et al., 2001; Premont et al., 2001), but in invertebrates, octopamine serves as a major neurotransmitter/neuromodulator (Roeder, 1999; Premont et al., 2001). Boulton (1976, 1979) had already suggested that amines such as tryptamine and octopamine, with low endogenous concentrations but a rapid turnover, may continuously be released from synaptic terminals and act as modulators of neurotransmission mediated by the metabolically related ‘classical’ amine transmitters. Tryptamine is a serotonin-related indolamine (Ramos et al., 1999), whose importance has been underestimated due to the general assumption that it occurs as a byproduct of 5HT synthesis. Nevertheless, tryptamine is not simply present as an accident of metabolism; indeed, neuropharmacological and electrophysiological data strongly suggest the existence of post-synaptic receptors for tryptamine independent of those for 5HT (Jones, 1982).

Recently, a new family of G protein-coupled receptors

called trace amine (TA) receptors has been described in humans (Borowsky et al., 2001; Yu et al., 2003). Experiments indicated that tryptamine plays some role in mammal neurotransmission (McCormack et al., 1986; Juorio and Paterson, 1990; Mousseau, 1993), but it has never been shown to be a neuromediator involved in any invertebrate physiological process. As a consequence, the present study brings an innovative concept to ophiuroid luminescence control: a neurotransmitter role for tryptamine. Octopamine, on the other hand, is well known to act as a neurohormone, a neuromodulator and a neurotransmitter in various invertebrate species (Roeder, 1999) such as annelids, molluscs and arthropods (Dhainaut-Courtois, 1982). Since its discovery in the salivery glands of the cephalopod *Octopus* (Erspamer and Boretti, 1951), it has been found in most invertebrate tissues studied so far. Several studies indicate that octopamine is a neurotransmitter involved in many invertebrate nervous control mechanisms (for a review, see Roeder, 1999). Octopamine is a multipotent biogenic amine that also acts as a neurohormone, especially in insects where it modulates the physiological state of the insect in a complex way to enable it to cope with energy-demanding situations such as long-term flying (Orchard et al., 1993). Moreover, octopamine is known to control glowing of the firefly lantern (Nathanson, 1986). Testing the effect of octopamine on ophiuroid luminescence is thus entirely justified.

Trace amines have not yet been highlighted in echinoderms but might potentially play some role in luminescence, such as in the firefly mechanism. We therefore compared the effects of both tryptamine and octopamine in three ophiuroid species: (i) *O. aranea* and *O. californica*, where the neurotransmitters responsible for photogenesis are unknown, and (ii) *A. filiformis*, where light emission seems to be under cholinergic control (Dewael and Mallefet, 2002a). Our results from isolated photocytes and arm segments suggest an involvement of octopamine and tryptamine in *A. filiformis* luminescence control and of tryptamine in *O. californica.*

Materials and methods

Animals

Specimens of *Amphiura filiformis* Müller 1776 were collected at the Kristineberg Marine Station (Fiskebäckskil, Sweden) by a Smith–McIntyre mud grab at a depth of 25–40 m. Animals were then kept in circulating natural seawater. Specimens of *Ophiopsila aranea* Forbes 1843 were collected at the ARAGO Biological Station (CNRS, Banyuls-sur-Mer, France) by scuba diving at 20–25 m depth. Specimens of *Ophiopsila californica* Clark 1921 were collected by the same technique at the Marine Sciences Institute of the University of California (Santa-Barbara, CA, USA). All these animals were transported to our laboratory in Belgium in aerated natural seawater and then kept in aquaria filled with natural recirculating seawater. The physicochemical parameters of the water were controlled once a week and during the winter the temperature was maintained around 10°C for *A. filiformis* and 12°C for the two other species. The temperature was steadily raised to 14°C and 16°C, according to the species, in the summer. Food was provided to the ophiuroids once a week, comprising liquid food for invertebrates (Marine Interpret, Liquifry and Co., Dorking, UK) for *A. filiformis* and a mixture of liquid food for invertebrates and *Artemia*, *Mysis*, red plankton, micro-plankton (Frozen Fishfood, Visvoer BV, Schijndel, The Netherlands) for the two other species. Animals kept in captivity were used between 1 week and 3 months after collection. Control stimulations were carried out for each experiment to make sure that no difference in light capabilities appeared as a result of the time spent in captivity.

Experiments on dissociated photocytes

The method used to isolated the luminous cells (photocytes) is based on that described by De Bremaeker et al. (2000). First, the ophiuroids were anaesthetized by immersion in 3.5% $MgCl_2$ in artificial seawater (ASW: 400.4 mmol l^{-1} NaCl, 9.9 mmol l^{-1} $CaCl_2$, 9.6 mmol l^{-1} KCl, 52.3 mmol l^{-1} $MgCl_2$, 27.7 mmol l^{-1} Na_2SO_4, 20 mmol l^{-1} Tris, pH 8.3). Since the arms are the only luminescent body parts of the ophiuroid species studied, they were isolated from the disc and chopped into tiny pieces before enzymatic digestion and differential centrifugation. The enriched luminous-cell fraction was then divided into 100 μl samples.

Experiments on arm segments

After anaesthesia of the animals by immersion in 3.5% $MgCl_2$, arms were isolated from the disc and divided into 20 segments for *A. filiformis*, 8 for *O. aranea* and 5 for *O. californica*, and rinsed in ASW. These different preparations have roughly the same size. Segments of each of the five arms cut at the same distance from the disc produce light of similar intensity (Mallefet et al., 1992; Dewael and Mallefet, 2002b). Given this information, one segment was used for the control stimulation with KCl while the four others were treated with the tested drugs.

Stimulations

A stock solution of 400 mmol l^{-1} KCl was prepared in ASW without NaCl to keep the osmolarity the same as normal ASW. Maximal light emission was triggered by application of 200 mmol l^{-1} KCl (Mallefet et al., 1992). For each experimental protocol, one aliquot part was stimulated in normal ASW, as a control, while the other preparations were first immersed in ASW containing the tested drug for 10 min before stimulation with KCl. For each experiment, recordings were performed during the entire 10 min of the drug treatment for a few aliquot parts. The remaining aliquot parts were only measured for the first 2 min of drug treatments since none of the light emissions observed exceeded those first two recorded minutes. For some experiments, calcium was removed from the ASW (Ca^{2+}-free ASW) by addition of 1 mmol l^{-1} EGTA.

Light emission was measured using a FB12 Berthold

luminometer (Pforzheim, Germany) linked to a personal computer. Injections of corresponding volumes of ASW served as controls before the assays. These controls indicated that luminescence due to mechanical excitability was absent or negligible. Each light response was characterized by its maximal intensity (L_{max}, in Mq s^{-1}) and expressed as a percentage of the control.

Drugs

In this study, we tested 3-[2-aminoethyl]indole (tryptamine; Sigma, Bornem, Belgium) and 1-[*p*-hydroxyphenyl]-2-aminoethanol hydrochloride (octopamine; Sigma) on arm segments and on isolated photocytes of the three studied ophiuroid species. Solutions of tryptamine were dissolved in methanol before dilution in ASW (or Ca^{2+}-free ASW, depending on the experiment), with a maximum of 1% methanol at final concentration. Octopamine was dissolved and diluted in ASW or Ca^{2+}-free ASW. We tested a wide concentration range of tryptamine and octopamine (10^{-11}–10^{-4} mol l^{-1}) on both isolated photocytes and on arm segments of the three species studied. We also tested mixtures of tryptamine with each of the following drugs: acetylcholine chloride (ACh, 10^{-3} mol l^{-1}; Sigma), 2-aminoethylsulfonic acid (taurine, 10^{-3} mol l^{-1}; Sigma) and 5-hydroxytyramine hydrochloride (dopamine, 10^{-4} mol l^{-1}; Sigma) on arm segments of *O. californica*, and mixtures of octopamine (10^{-9} mol l^{-1}) + taurine (10^{-3} mol l^{-1}), tryptamine (10^{-5} mol l^{-1}) + taurine (10^{-3} mol l^{-1}), tryptamine (10^{-5} mol l^{-1}) + octopamine (10^{-9} mol l^{-1}), tryptamine (10^{-5} mol l^{-1}) + ACh (10^{-3} mol l^{-1}), octopamine (10^{-9} mol l^{-1}) + ACh (10^{-3} mol l^{-1}) and tryptamine (10^{-5} mol l^{-1}) + octopamine (10^{-9} mol l^{-1}) + ACh (10^{-3} mol l^{-1}) on arm segments of *A. filiformis.* Fresh solutions were prepared daily.

Statistics

Statistical analyses [analysis of variance (ANOVA), Dunnet and Tukey tests] were performed using SAS/STAT® software (SAS Institute Inc., 1990).

Results

The effects induced by stimulation with tryptamine, octopamine and several drug mixtures on arm segments and isolated photocytes of *A. filiformis*, *O. aranea* and *O. californica* are summarized in Tables 1 and 2. Values for maximal light emission (as shown in the different graphs) are expressed as a percentage of KCl controls. These controls (for the arm segments) reach about 5000 Mq s^{-1} for *A. filiformis*, 25 000 Mq s^{-1} for *O. aranea* and 50 000 Mq s^{-1} for *O. californica*.

Tryptamine

Tryptamine triggers light emissions (~2% of KCl controls) of arm segments in *A. filiformis* at concentrations between 10^{-4} and 10^{-7} mol l^{-1} (Fig. 1). At lower concentrations (10^{-8}–10^{-11} mol l^{-1}), a very weak luminescence (still distinguishable from mechanical stimulation) remains but only reaches approximately 0.5% of KCl controls. On photocytes isolated from *A. filiformis*, tryptamine does not induce photogenesis except at a concentration of 10^{-11} mol l^{-1}, which triggers a light emission representing 2% of the controls.

Table 1. *Summary of light emissions observed with tryptamine and octopamine stimulations on arm segments and isolated photocytes of* A. filiformis, O. aranea *and* O. californica

	A. filiformis		*O. aranea*		*O. californica*	
[Drug] (mol l^{-1})	Tryptamine	Octopamine	Tryptamine	Octopamine	Tryptamine	Octopamine
Arm segments						
10^{-4}	++	0	0	/	0	/
10^{-5}	++	0	0	/	0	/
10^{-6}	++	0	0	/	0	/
10^{-7}	++	+	0	/	++	/
10^{-8}	+	++	0	0	++	+
10^{-9}	+	++	0	0	++	+
10^{-10}	+	++	0	0	++	+
10^{-11}	+	++	0	0	0	+
Isolated photocytes						
10^{-4}	0	/	0	/	+	/
10^{-5}	0	/	0	/	+	/
10^{-6}	0	/	0	/	+	/
10^{-7}	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	+
10^{-9}	0	0	0	0	0	+
10^{-10}	0	0	0	0	0	+
10^{-11}	++	/	0	/	++	+

0, no effect; +, weak luminescence; ++, light production; /, not tested.

Table 2. *Summary of synergic effects observed with different drugs on arm segments of* A. filiformis *and* O. californica

A. filiformis		*O. californica*	
Tryptamine+octopamine	0	Tryptamine+dopamine	0
Tryptamine+taurine	0	Tryptamine+taurine	I
Tryptamine+ACh	0	Tryptamine+ACh	I
Tryptamine+octopamine+ACh	I		
Octopamine+taurine	I		
Octopamine+ACh	0		

Tryptamine (10^{-5} mol l^{-1} for *A. filiformis* and 10^{-7} mol l^{-1} for *O. californica*), octopamine, 10^{-9} mol l^{-1}; taurine, 10^{-3} mol l^{-1}; ACh, acetylcholine, 10^{-3} mol l^{-1}; dopamine, 10^{-4} mol l^{-1}.

0, no effect; I, luminescence increase.

In *O. aranea*, tryptamine does not induce any light emission on either arm segments or isolated photocytes, at all the tested concentrations.

In *O. californica*, by contrast, tryptamine induced light responses (~2.5% of control) on arm segments at concentrations between 10^{-7} and 10^{-10} mol l^{-1}; higher and lower concentrations did not trigger any luminescence (Fig. 2). On photocytes isolated from this species, very weak light productions were observed at 10^{-4}–10^{-6} mol l^{-1} and 10^{-11} mol l^{-1} tryptamine concentrations.

Tryptamine does not modify the KCl-induced luminescence of either preparation types (arm segments and photocytes) in all three species and at all tested concentrations.

Octopamine

Octopamine triggers light emission (~1.7% of controls) of arm segments in *A. filiformis* at concentrations of 10^{-8}–10^{-11} mol l^{-1} (Fig. 3). This was not observed in isolated photocytes.

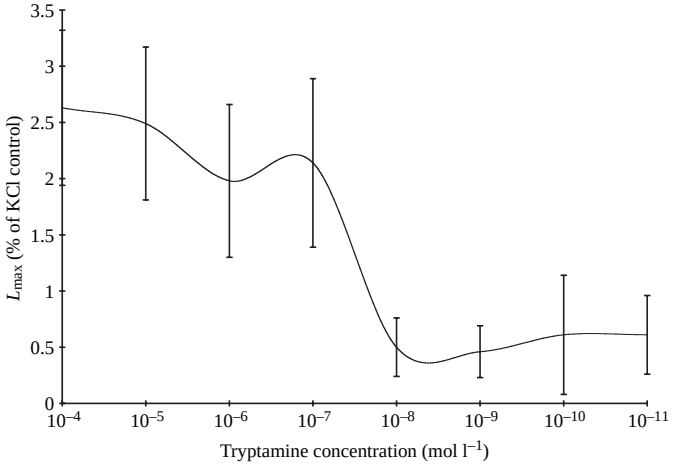

Fig. 1. Maximal light emissions (L_{max}) of *A. filiformis* arm segments induced by tryptamine (10^{-4}–10^{-11} mol l^{-1}). Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. *N*=12 stimulated aliquot parts.

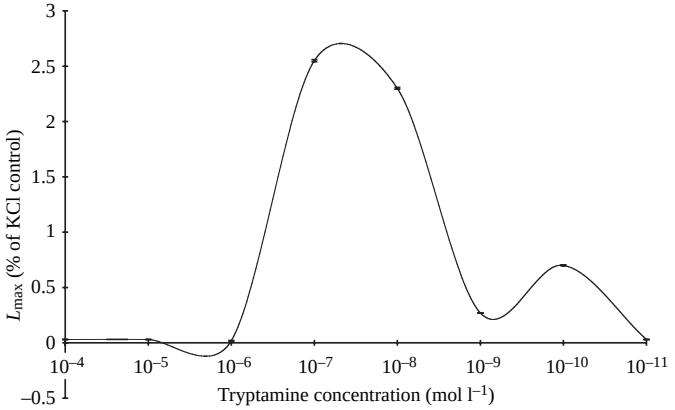

Fig. 2. Maximal light emissions (L_{max}) of *O. californica* arm segments induced by tryptamine (10^{-4}–10^{-11} mol l^{-1}). Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. *N*=8 stimulated aliquot parts.

In *O. aranea*, no light production was observed after injection of octopamine on either arm segments or photocytes at all the tested concentrations.

Finally, a weak luminescence was observed in *O. californica* for octopamine treatments at 10^{-8}–10^{-11} mol l^{-1} on arm segments and at 10^{-7}–10^{-10} mol l^{-1} on isolated photocytes. Nevertheless, even if this luminescence was slightly different from a mechanical stimulation (injection of ASW), it is difficult to consider it as a proper light response since it only reached 0.2% of KCl controls.

Octopamine does not influence KCl-induced luminescence in any of the studied species.

Tryptamine and octopamine stimulations in Ca^{2+}-free ASW

Tryptamine and octopamine were tested at the concentrations that induced luminescence of *A. filiformis* and

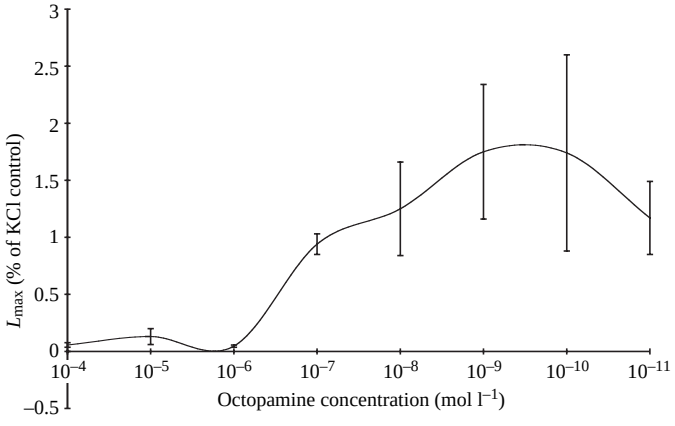

Fig. 3. Maximal light emissions (L_{max}) of *A. filiformis* arm segments induced by octopamine (10^{-4} to 10^{-11} mol l^{-1}). Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. *N*=8 stimulated aliquot parts.

O. californica arm segments in Ca^{2+}-free ASW to see whether light production still remained after Ca^{2+} removal. Fig. 4 shows that in *A. filiformis*, only 4.14% and 16.17% of tryptamine-induced luminescence remained after Ca^{2+} removal at tryptamine concentrations of 10^{-4} mol l^{-1} and 10^{-5} mol l^{-1}, respectively. In *O. californica*, 10^{-7} mol l^{-1} and 10^{-8} mol l^{-1} tryptamine only triggered 10.71% and 0.47% of control (tryptamine 10^{-7} mol l^{-1} and 10^{-8} mol l^{-1} in normal ASW) in absence of external Ca^{2+} (Fig. 5). Octopamine only triggered 13.84% and 1.38% of octopamine controls (10^{-9} mol l^{-1} and 10^{-10} mol l^{-1} in normal ASW, respectively) in Ca^{2+}-free ASW (Fig. 6). In both species, KCl still induces some light responses in absence of Ca^{2+}, reaching about 5% of the control stimulation in normal ASW (results not shown).

Synergic effects of tryptamine, octopamine and other drugs

We tested the effects of other drugs (ACh, dopamine, taurine) on 10^{-7} mol l^{-1} tryptamine-induced luminescence of *O. californica* arm segments. The results show that mixtures of tryptamine + 10^{-3} mol l^{-1} ACh as well as tryptamine + 10^{-3} mol l^{-1} taurine increase the light response in comparison with tryptamine on its own (Fig. 7). By contrast, no increase of light emission is observed with the mixture of tryptamine + 10^{-4} mol l^{-1} dopamine.

In *A. filiformis*, both tryptamine and octopamine alone triggered light emissions, so we tested the synergic effect of both drugs mixed together at concentrations of 10^{-5} mol l^{-1} and 10^{-9} mol l^{-1}, respectively. The results showed no effect of this mixture on light production compared with both drugs administered on their own (Table 2). Synergic effects of 10^{-3} mol l^{-1} taurine + 10^{-9} mol l^{-1} octopamine were clearly seen (Fig. 8), however, since this drug mixture induced about 52% of KCl controls as against only 1% with octopamine on its own. By contrast, the combination of 10^{-5} mol l^{-1} tryptamine + 10^{-3} mol l^{-1} taurine did not potentiate the light response compared with the one triggered by tryptamine alone (Table 2). Finally, the following combinations were tested: 10^{-5} mol l^{-1} tryptamine + ACh, 10^{-9} mol l^{-1} octopamine + ACh and 10^{-5} mol l^{-1} tryptamine + 10^{-9} mol l^{-1} octopamine + ACh. Only the last combination

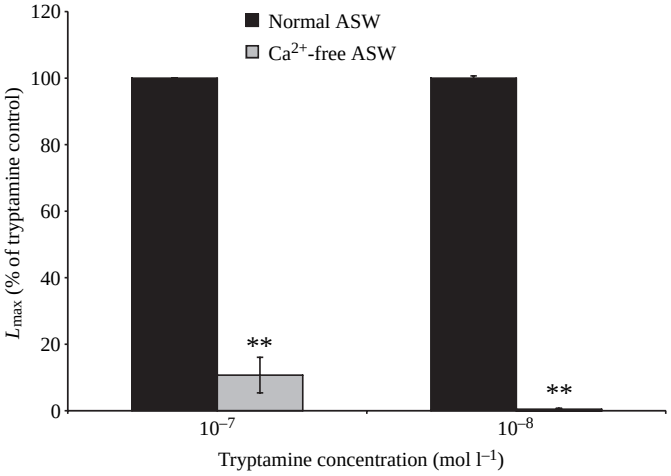

Fig. 5. Differences of maximal light emissions (L_{max}) of *O. californica* arm segments after stimulation by tryptamine (10^{-7} mol l^{-1} and 10^{-8} mol l^{-1}) in normal ASW and in Ca^{2+}-free ASW. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 10^{-7} mol l^{-1} and 10^{-8} mol l^{-1} tryptamine, respectively, in normal artificial seawater. $^{**}P<0.01$; $N=8$ stimulated aliquot parts for each concentration.

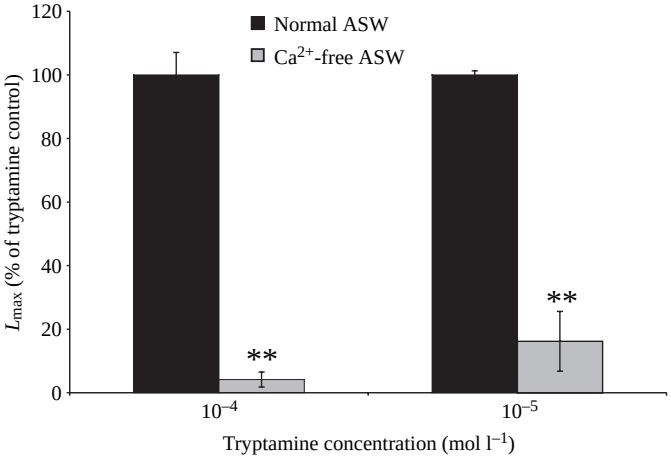

Fig. 4. Differences of maximal light emissions (L_{max}) of *A. filiformis* arm segments after stimulation by tryptamine (10^{-4} mol l^{-1} and 10^{-5} mol l^{-1}) in normal ASW and in Ca^{2+}-free ASW. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 10^{-4} mol l^{-1} and 10^{5} mol l^{-1} tryptamine, respectively, in normal artificial seawater. $^{**}P<0.01$; $N=8$ stimulated aliquot parts for each concentration.

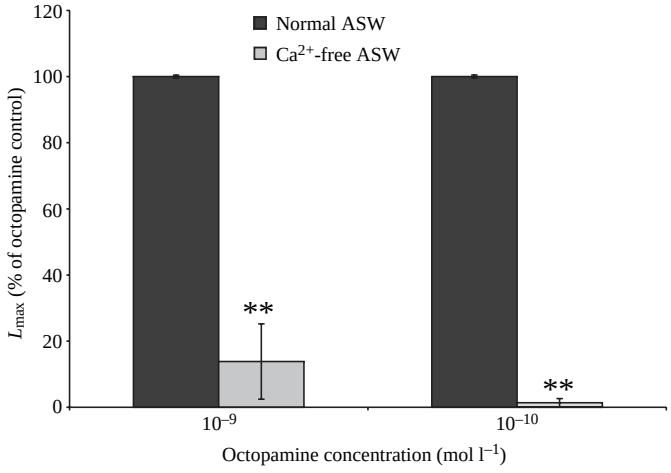

Fig. 6. Differences of maximal light emissions (L_{max}) of *A. filiformis* arm segments after stimulation by octopamine (10^{-9} mol l^{-1} and 10^{-10} mol l^{-1}) in normal ASW and in Ca^{2+}-free ASW. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 10^{-9} mol l^{-1} and 10^{-10} mol l^{-1} octopamine, respectively, in normal artificial seawater. $^{**}P<0.01$; $N=6$ stimulated aliquot parts for each concentration.

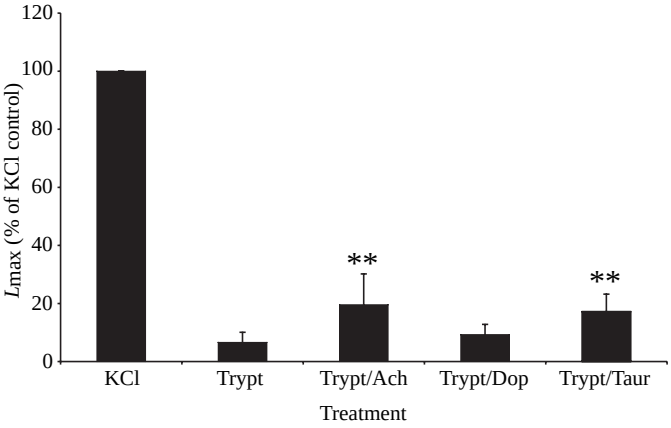

Fig. 7. Synergic effects of 10^{-7} mol l^{-1} tryptamine (Trypt) and other drugs such as acetylcholine (Ach, 10^{-3} mol l^{-1}), dopamine (Dop, 10^{-4} mol l^{-1}) and taurine (Taur, 10^{-3} mol l^{-1}) on maximal light emission (L_{max}) of *O. californica*. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. $^{**}P<0.01$ in comparison to 10^{-7} mol l^{-1} tryptamine on its own; N=8 stimulated aliquot parts per treatment.

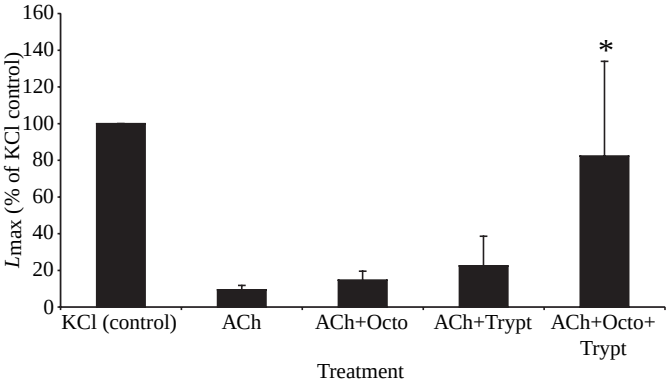

Fig. 9. Synergic effects of 10^{-3} mol l^{-1} acetylcholine (ACh) with 10^{-9} mol l^{-1} octopamine (Octo), 10^{-5} mol l^{-1} tryptamine (Trypt) and with octopamine and tryptamine (Octo+Trypt) on maximal light emission (L_{max}) of *A. filiformis* arm segments. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. $^{**}P<0.05$ in comparison to 10^{-3} mol l^{-1} acetylcholine on its own; N=8 stimulated aliquot parts per treatment.

increased luminescence compared with ACh treatment on its own (Fig. 9, Table 2).

Discussion

The results of the present study show that systematic light responses obtained on arm segments were not reproducible with isolated photocytes, either because the dissociation process alters trace amine receptors present on the photocyte membrane, or these kind of receptors are not located on the photocyte communicating with it. A similar hypothesis was proposed for ACh stimulation in *A. filiformis* (Vanderlinden, 2002). Nevertheless, tryptamine might be involved in bioluminescence control of *A. filiformis* and *O. californica* but not in *O. aranea*. Indeed, in *A. filiformis* and *O. californica*, photogenesis was triggered by different tryptamine concentrations 10^{-4}–10^{-7} mol l^{-1} and 10^{-7}–10^{-10} mol l^{-1}, respectively. Octopamine, on the other hand, only seems to induce luminescence in *A. filiformis* for a concentration range of 10^{-8}–10^{-11} mol l^{-1}. Such low octopamine concentrations are not unusual, since in the firefly photomotor neurons, for instance, octopamine concentrations of 0.03 pmol $cell^{-1}$ have been detected (Christensen et al., 1983) and in vertebrates, the concentrations of trace amines are usually below 100 ng g^{-1} (Roeder, 1999).

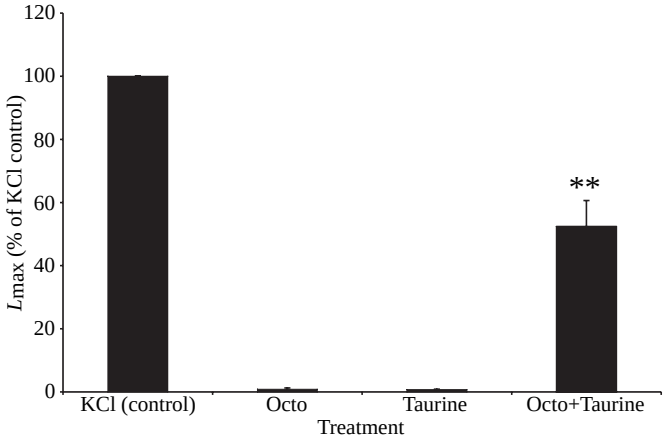

Fig. 8. Synergic effect of 10^{-9} mol l^{-1} octopamine (Octo) and 10^{-3} mol l^{-1} taurine on maximal light emission (L_{max}) of *A. filiformis*. Values (means ± S.E.M.) are expressed as a percentage of photogenesis triggered by 200 mmol l^{-1} KCl in normal artificial seawater. $^{**}P<0.01$ in comparison to 10^{-9} mol l^{-1} octopamine on its own; N=8 stimulated aliquot parts per treatment.

The role of the trace amine tryptamine is not yet well understood in vertebrates and is not documented in invertebrates. However, low levels of a substance do not mitigate against an active function. Tryptamine is now thought to function as a neuromodulator or a neurotransmitter in the mammalian central nervous system (Yu et al., 2003). The neuromodulator role of tryptamine is mainly illustrated by its positive and negative modulation of 5HT transmission, but its action on other systems such as ACh and glutamate cannot be ignored (for a review, see Jones, 1982). Previous studies on the three ophiuroid species studied here have shown that 5HT does not play a neurotransmitter role in photogenesis of those three species (Dewael and Mallefet, 2002a), implying therefore that tryptamine does not modulate 5HT action. Our results are in favour of a neurotransmitter function for tryptamine in *A. filiformis* and *O. californica*, since tryptamine on its own triggers light production in those species. As a neurotransmitter in vertebrates, tryptamine interacts with the G protein-coupled TA_2 receptor (Premont et al., 2001; Yu et al., 2003). This trace

amine receptor is likely to be coupled to conventional signalling pathways as demonstrated for TA_1 (receptor for octopamine; Borowsky et al., 2001), which will be discussed later, and their signalling is probably regulated *via* mechanisms similar to those for other G protein-coupled receptors. Moreover, we have tested the synergic effects of tryptamine with other substances such as ACh, dopamine and taurine that had been shown to trigger weak luminescence in *O. californica* (Dewael and Mallefet, 2002a). Our results highlight that combinations of tryptamine+ACh and tryptamine+taurine both increase light emission in comparison with luminescence induced by tryptamine on its own, suggesting either a synergic effect of several neurotransmitters or a modulatory effect of some substances in luminescence control of this species. Nevertheless, according to classical definitions, one can argue that the light-triggering effect of tryptamine is more in favour of a neurotransmitter role than a neuromodulatory one alone. Other examples of two neurotransmitters coexisting (e.g. 5HT and ACh) are known in invertebrate neurones (Dhainaut-Courtois, 1982).

Octopamine, on the other hand, is a well-known neurotransmitter and neuromodulator in invertebrates, but is only present as a trace amine in vertebrates. Octopamine modulates almost every physiological process in invertebrates studied so far. Indeed, most peripheral organs, sense organs, and numerous targets within the central nervous system are modulated by octopamine. Its presence has been demonstrated in several marine invertebrates such as *Aplysia californica* (Saavedra et al., 1974), *Octopus vulgaris* (Saavedra, 1974), *Tapes watlini* (Dougan et al., 1981), etc. Moreover, octopamine is responsible for glowing of the firefly lantern (Nathanson, 1986). Octopamine is known to exert its physiological actions through a number of G-protein coupled receptors associated to adenylate cyclases (Walker et al., 1996; Roeder, 1999). In the nervous system of the marine snail *Aplysia*, for instance, an octopamine receptor positively coupled to an adenylate cyclase has been identified (Li et al., 1994). In the three ophiuroid species of the present survey, it was shown by Vanderlinden et al. (2003) that the cAMP pathway is involved in bioluminescence control. The results of the present study for *A. filiformis* therefore support the hypothesis that octopamine receptors might be present in the photocyte membrane of this species and stimulate an adenylate cyclase, leading to an increase of cAMP levels inside the photocyte. A similar pathway could be involved in *A. filiformis* and *O. californica* for tryptamine stimulation, but cannot be postulated for *O. aranea* since neither tryptamine nor octopamine triggered any luminescence in this species.

Cyclic AMP activates protein kinase A, which is involved in the regulation of receptors and the opening and closing of ion channels. It can, among other effects, increase Ca^{2+} influx (Kennedy, 1994). A previous study has, indeed, shown that calcium movements are required in the photogenesis of all three ophiuroid species (Dewael and Mallefet, 2002b). In the absence of extracellular calcium, luminescence is strongly inhibited. Eventually, the intracellular increase of Ca^{2+} concentration would lead to the triggering of the light reaction, through a mechanism not yet understood. Moreover, calcium could also act on octopamine release from nerve terminals, since when lobster nerves are depolarised, octopamine is liberated by a Ca^{2+}-dependent process (Axelrod and Saavedra, 1977). Our results obtained with octopamine stimulation on *A. filiformis* arm segments and with tryptamine stimulation on *A. filiformis* and *O. californica* arm segments, in Ca^{2+}-free ASW, highlight the necessity for extracellular Ca^{2+}. As a matter of fact, almost no luminescence remained when arm segments were stimulated with octopamine and tryptamine in the absence of Ca^{2+}. Although, some very weak luminescence could still be observed in Ca^{2+}-free ASW following octopamine, tryptamine and KCl stimulation, this observation may be due to the intracellular calcium stores that most excitable cells usually maintain (Triggle, 1989), even in the absence of extracellular calcium. Moreover, some calcium could originate from the preparation itself since it is part of most echinoderms' framework (Hernandez et al., 1987).

Furthermore, the combination of 10^{-3} mol l^{-1} taurine, which has been shown to trigger a very weak luminescence in *A. filiformis* (0.74% of KCl control; Dewael and Mallefet, 2002a), + 10^{-9} mol l^{-1} octopamine increases light production (52% of KCl control) compared with octopamine administered on its own (1% of KCl control), suggesting a synergic effect of those two transmitters. Finally, since acetylcholine is known to act as a neurotransmitter involved in *A. filiformis* luminescence control (Dewael and Mallefet; 2002a), we tested the effects of tryptamine and octopamine combined with ACh. The results show that only the combination of all three drugs potentiates light emission compared to luminescence induced by Ach alone. These observations might imply either a modulatory effect of both tryptamine and octopamine on Ach, or that all these substances, including taurine, act as neurotransmitters working in synergy to trigger photogenesis.

In conclusion, this study shows that tryptamine and octopamine might be involved in the luminescence control of some ophiuroid species. In *A. filiformis*, both tryptamine and octopamine trigger photogenesis and the combinations of octopamine + taurine as well as octopamine + tryptamine + ACh increase luminescence. In *O. californica*, tryptamine in synergy with acetylcholine and taurine, induces light production. In *O. aranea*, on the other hand, neither tryptamine nor octopamine seem to be involved in photogenesis. In this latter species, none of the neurotransmitters tested so far trigger light emission; further experiments will be done in order to clarify the luminescence control of *O. aranea*. These experiments, once more, confirm that the control mechanisms of photogenesis differ between ophiuroid species, as shown in other studies (Dewael and Mallefet, 2002a; Vanderlinden et al., 2003). Our results show for the first time that tryptamine and octopamine may be proper neurotransmitters in Echinoderms.

We thank Prof. F. Baguet for his useful comments about this manuscript and we acknowledge financial support from an EEC ARI at Kristineberg Marine Station, Fiskebäckskil,

Sweden, FNRS at Arago Laboratory, Banyuls-sur-Mer, France. Special thanks to Prof. J. Case, S. Anderson and D. Divins for invaluable help during scuba collections at UCSB Marine Sciences Institute (USA). Part of this work was also supported by EEC (ARI to Kristineberg) and FRFC grants no. 2.4573.00 and 2.4516.01. J.M. is a Research Associate of the National Fund for Scientific Research (FNRS, Belgium). The research was supported by a FRIA grant for C.V. Contribution to the Cibim and CBR.

References

Axelrod, J. and Saavedra, J. M. (1977). Octopamine. *Nature* **265**, 501-504.

Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek, K. L., Durkin, M. M., Lakhlani, P. P., Bonini, J. A., Pathirana, S. et al. (2001). Trace amines: Identification of a family of mammalian G protein-coupled receptors. *Proc. Natl. Acad. Sci. USA* **98**, 8966-8971.

Boulton, A. A. (1976). Cerebral aryl alkyl aminergic mechanisms. In *Trace Amines in the Brain* (ed. E. Usdin and M. Sandler), pp. 21-39. New York: Marcel Dekker,

Boulton, A. A. (1979). Trace amines in the central nervous system. *Int. Rev. Biochem.* **26**, 179-206.

Campbell, A. K. (1989). Living light: biochemistry, function and biomedical applications. *Essays Biochem.* **24**, 41-80.

Christensen, T. A., Sherman, T. G., McCaman, A. and Carlson, A. D. (1983). Presence of octopamine in firefly motor neurons. *Neurosci.* **9**, 183-189.

De Bremaeker, N., Baguet, F. and Mallefet, J. (1999a). Characterization of acetylcholine induced luminescence in *Amphipholis squamata* (Echinodermata: Ophiuroidea). *Belg. J. Zool.* **129**, 353-362.

De Bremaeker, N., Baguet, F., Thorndyke, M. C. and Mallefet, J. (1999b). Modulatory effects of some amino acids and neuropeptides on luminescence in the brittlestar *Amphipholis squamata. J. Exp. Biol.* **202**, 1785-1791.

De Bremaeker, N., Baguet, F., Thorndyke, M. C. and Mallefet, J. (1999c). Modulatory effects of some amino acids and neuropeptides on luminescence in the brittlestar *Amphipholis squamata. J. Exp. Biol.* **202**, 1785-1791.

De Bremaeker, N., Dewael, Y., Baguet, F. and Mallefet, J. (2000). Involvement of cyclic nucleotides and IP_3 in the regulation of luminescence in the brittlestar *Amphipholis squamata* (Echinodermata). *Luminescence* **15**, 159-163.

De Bremaeker, N., Mallefet, J. and Baguet, F. (1996). Luminescence control in the brittlestar *Amphipholis squamata*: effect of cholinergic drugs. *Comp. Biochem Physiol.* **115C**, 75-82.

Dewael, Y. and Mallefet, J. (2002a). Luminescence in ophiuroids (Echinodermata) does not share a common nervous control in all species. *J. Exp. Biol.* **205**, 799-806.

Dewael, Y. and Mallefet, J. (2002b). Calcium involvement in the luminescence control of three ophiuroid species (Echinodermata). *Comp. Biochem. Physiol.* **131C**, 153-160.

Dhainaut-Courtois, N. (1982). Les neurosécrétions aminergiques chez les invertébrés. *J. Physiol.* **78**, 523-531.

Dougan, D. F. H., Duffield, P. H. and Wade, D. N. (1981). Occurrence and synthesis of octopamine in the heart and ganglia of the mollusc *Tapes watlingi. Comp. Biochem. Physiol.* **70C**, 277-280.

Erspamer, V. and Boretti, G. (1951). Identification and characterization by chromatography of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of *Octopoda* and in other tissue extracts of Vertebrates and Invertebrates. *Arch. Intern. Pharmacodyn. Ther.* **88**, 296-332.

Hastings, J. W. (1983). Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. *J. Mol. Evol.* **19**, 309-321.

Hernandez, M., Morales, M., Smith, D. S. and del Castillo, J. (1987). Barium spikes are generated in the spines of the sea urchin *Diadema antillarum*. *Comp. Biochem. Physiol.* **86A**, 355-359.

Herring, P. J. (1987). Systematic distribution of bioluminescence in living organisms. *J. Biolum. Chemilum.* **1**, 147-163.

Jones, R. S. G. (1982). Tryptamine: A neuromodulator or a neurotransmitter in mammalian brain? *Prog. Neurobiol.* **19**, 117-139.

Juorio, A. V. and Paterson, I. A. (1990). Tryptamine may couple dopaminergic and serotoninergic transmission in the brain. *Gen. Pharmacol.* **21**, 613-616.

Kennedy, M. B. (1994). Seconds messagers et fonction neuronale. In *Introduction à la Neurobiologie Moléculaire* (ed. Z. W. Hall), pp. 207-246. Paris: Médecine-Sciences Flammarion.

Li, X.-C., Giot, J.-F., Hen, R., Weiss, K. R. and Kandel, E. R. (1994). Molecular cloning and characterization of serotonin and octopamine receptors of *Aplysia*. *Soc. Neurosci. Abstr.* **20**, 1160.

Mallefet, J. (1999). Physiology of bioluminescence in echinoderms. In *Echinoderm Research 1998* (ed. M. D. Candia Carnevali and F. Bonasoro), pp. 93-102. Rotterdam: Balkema.

Mallefet, J., Vanhoutte, P. and Baguet, F. (1992). Study of *Amphipholis squamata* luminescence. In *Echinoderm Research* (ed. L. Scalera-Liaci and C. Canicatti), pp. 125-130. Rotterdam, Balkema.

McCormack, J. K., Beitz, A. J. and Larson, A. A. (1986). Autoradiographic localization of tryptamine binding sites in the rat and dog central nervous system. *J. Neurosci.* **6**, 94-101.

Mousseau, D. D. (1993). Tryptamine: a metabolite of tryptophan implicated in various neuropsychiatric disorders. *Metab. Brain Dis.* **8**, 1-44.

Nathanson, J. A. (1986). Neurochemical regulation of light emission from photocytes. In *Insect Neurochemistry and Neurophysiology* (ed. A. B. Borkovee and D. B. Gelman), pp. 263-266. Clifton, New Jersey: Humana Press.

Orchard, I., Ramirez, J. M. and Lange, A. B. (1993). A multifunctional role for octopamine in locust flight. *A. Rev. Entomol.* **38**, 227-249.

Premont, R. T., Gainetdinov, R. R. and Caron, M. G. (2001). Following the trace of elusive amines. *Proc. Natl. Acad. Sci. USA* **98**, 9474-9475.

Ramos, A. J., Tagliaferro, P., Saavedra, J. P. and Brusco, A. (1999). Tryptamine, serotonin and catecholamines: an immunocytochemical study in the central nervous system. *Int. J. Neurosci.* **99**, 123-137.

Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* **59**, 533-561.

Saavedra, J. M. (1974). Enzymatic-isotopic method for octopamine at the picogram level. *Anal. Biochem.* **59**, 628-633.

Saavedra, J. M., Brownstein, M. J., Carpenter, D. O. and Axelrod, J. (1974). Octopamine presence in single neurons of *Aplysia* suggests neurotransmitter function. *Science* **185**, 363-365.

SAS Institute Inc. (1990). *SAS/STAT User's Guide*, Version 6, Fourth Edition, Volume 2. SAS Institute Inc., North Carolina, USA.

Triggle, D. J. (1989). Drugs active at voltage dependent calcium channels. *Neutransmissions* **555**, 1-2.

Vanderlinden, C. (2002). Implication des seconds messagers dans la régulation de la bioluminescence chez *Amphiura filiformis* (O. F. Müller 1776) et *Ophiopsila aranea* (Forbes 1843). Mémoire de licence, Université catholique de Louvain. 83pp.

Vanderlinden, C., Dewael, Y. and Mallefet, J. (2003). Screening of second messengers involved in photocyte bioluminescence of three ophiuroid species (Ophiuroidea: Echinodermata). *J. Exp. Biol.* **206**, 3007-3014.

Walker, R. J., Brooks, H. L. and Holden-Dye, L. (1996). Evolution and overview of classical transmitter molecules and their receptors. *Parasitol.* **113**, S3-S33.

Yu, A., Granvil, C. P., Haining, R. L., Krausz, K. W., Corchero, J., Küffer, A., Idle, J. R. and Gonzalez, F. J. (2003). The relative contribution of monoamine oxidase and cytochrome P450 isozymes to the metabolic deamination of the trace amine tryptamine. *J. Pharmacol. Exp. Ther.* **304**, 539-546.