Belg. J. Zool. — Volume 125 (1995) — issue 1 — pages 167-173 — Brussels 1995

PRELIMINARY RESULTS OF LUMINESCENCE CONTROL IN ISOLATED ARMS OF *OPHIOPSILA ARANEA* (ECHINODERMATA)

by

JÉRÔME MALLEFET and MARLÈNE DUBUISSON
Université Catholique de Louvain, laboratoire de Physiologie Animale,
5 place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium

SUMMARY

Luminescence induced by KCl applications on isolated arms from *Ophiopsila aranea* (FORBES) is described. Light emission is characterized by a series of flashes whose maximal intensity increases as a function of KCl concentration. None of the neuromediators tested (adrenaline, noradrenaline, 5-HT, acetylcholine, carbachol, gaba, taurine, glutamate) induced the production of light. Our study requires further experiments in order to characterize the nature of the luminescence control mechanism of *Ophiopsila aranea*.

Keywords : Echinoderm, ophiuroid bioluminescence, physiology, isolated arms.

INTRODUCTION

Ophiopsila aranea (Forbes, 1843) is a member of the class Ophiuridea living in the encrusting coralline algae zone (coralligène) in the Mediterranean Sea. The mean size of the disc is 8 mm. The very long arms (more than 10 cm) that show a pattern of bands of different pigments are known to luminesce. Early morphological studies described the luminescence sites originating from glandular cells located on the ventral and lateral plates and also in some spines of the arms next to the disc (MANGOLD, 1907 ; REICHENSPERGER, 1908 ; TROJAN, 1909). In 1952, HARVEY mentioned a yellowish green fluorescence appearing as bright points in U.V. light at the sites of luminescence. The exact nature of luminous cells remained unknown until a more recent study on *Ophiopsila californica* (Brehm and Morin, 1977) suggested a neural origin of luminous cells, termed photocytes.

In luminous ophiuroids, potassium chloride (KCl) is a classical stimulation used to induce a light emission by depolarization of the nervous elements controlling

the luminescence tissues (HERRING, 1974). For example, in *Amphipholis squamata*, the optimal concentration of KCl used to trigger light emission is 200 mM (MALLEFET *et al.*, 1989). Many different neuromediators were detected in various tissues of echinoderms (see COBB 1987, 1988) ; among them acetylcholine represents a major component of the nervous system for which some physiological activities were described. For example, pharmacological studies have identified the presence of a cholinergic system involved in the luminescence control of *Amphipholis squamata* (DEBREMAEKER *et al.*, 1993a,b).

Since no physiological data concerning the luminescence control mechanism in *Ophiopsila aranea* are available, we first tested the responsiveness of isolated articles from *Ophiopsila aranea* arms to depolarization by KCl and tried to identify a putative neuromediator that could trigger light emission.

MATERIAL AND METHODS

Specimens of *Ophiopsila aranea* were collected at the ARAGO biological station (C.N.R.S.) at Banyuls-sur-mer (France) by scuba divers working at 15-20 metres depth. The animals were transported in aerated and running natural sea water and then kept in aquaria filled with aerated and recirculating natural and artificial sea water (ASW) at 12° C. Food was provided to them three times a week.

After anaesthesia of the brittlestars by immersion in 3 % MgCl2 in ASW, arms were cut out and divided into sections of 2 to 4 articles which were then rinsed in ASW of the following composition : NaCl 400.4 mM, KCl 9.6 mM, CaCl2 9.9 mM, MgCl2 52.3 mM, Na2SO4 27.7 mM, Tris 20 mM, pH 8.3.

The articles were placed in small chambers filled with 200 µl of sea water and then stimulated by injection of air saturated solutions. Stock solutions of potassium chloride (Merck), acetylcholine (Sigma), carbachol (Janssen), adrenaline, noradrenaline (Fluka), 6-OHDA (Aldrich), 5-HT, gaba (Merck), taurine (Fluka), L-glutamate (Sigma) were dissolved in ASW just before experiments ; 200 µl of these solutions were injected in the small chambers containing the articles, using a micropipette ; light responses were followed during 10 minutes. Knowing that each isolated arm provides more than 18 articles as preparations, a total of 6 different *Ophiopsila aranea* was used in this work. Each experiment was performed on a different preparation coming from an isolated arm. Light emission was monitored with a PM270D photomultiplier connected via an amplifier to a graphic recorder. The maximum light emission (Lmax) is the parameter used to characterize *Ophiopsila aranea* light emission ; results are expressed in megaquanta per second per

article (Mq/s/art). Statistical analyses were performed using variance analysis (ANOVA 1 criteria) ; each mean value is expressed with its standard error of the mean (mean ± sem) and number of tested preparations (n).

RESULTS

KCl depolarization

Applications of KCl concentrations ranging from 100 mM to 400 mM on articles from isolated arms of 4 different *Ophiopsila aranea* always induced a light emission characterized by a series of flashes (Fig. 1) whose maximal intensity increased as a function of KCl concentration.

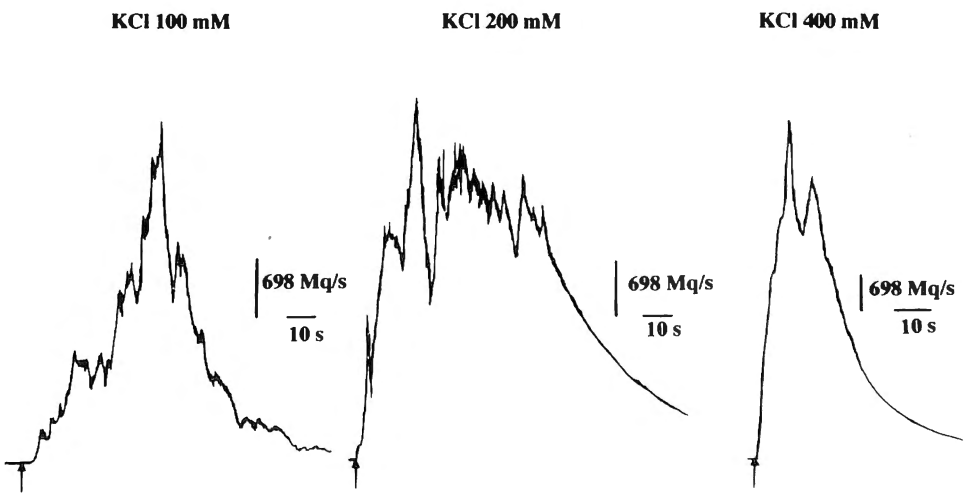

Fig. 1. — Original recordings of *Ophiopsila aranea* isolated arms in responses to different KCl concentrations ; vertical bar : light intensity of 698 Mq/s. ; horizontal bar : time scale of 10 s.

Application of KCl 100 mM triggered after 8,2±0,9 s. a luminescence which reached a maximum level of 1385±125 Mq/s/art (n=72) after 37±1,9 s. At 200 mM KCl, luminescence started after 1,3±0,2 s. and showed a maximal value of 1733±126 Mq/s/art within 32±3,3 s (n=48). In response to KCl 400 mM, light emission started 1,1±0,1 s. after KCl application and developed a maximal value of 3061±140 Mq/s/art after 12,8±1,1 s (n=84). The maximal light output in response to KCl 400 mM is statistically (anova 1 criteria, Fobs : 47.7, $p<0.0001$) higher than those observed with the two other KCL concentrations tested in this study (Fig. 2).

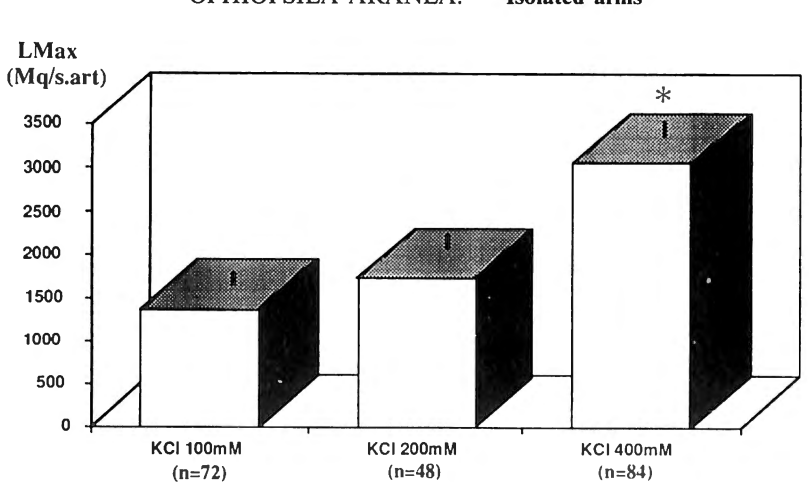

Fig. 2. — Effects of KCl concentration on light emission of isolated arms of *Ophiopsila aranea* ; (mean ± sem, n = number of preparations, * = p < 0,05).

Analysis of the maximal light responses to KCl 400 mM of articles isolated from the 5 different arms of a single animal indicates that there is no significative difference (anova 1 criteria, Fobs : 0.5, N.S.) between the arms (Fig. 3).

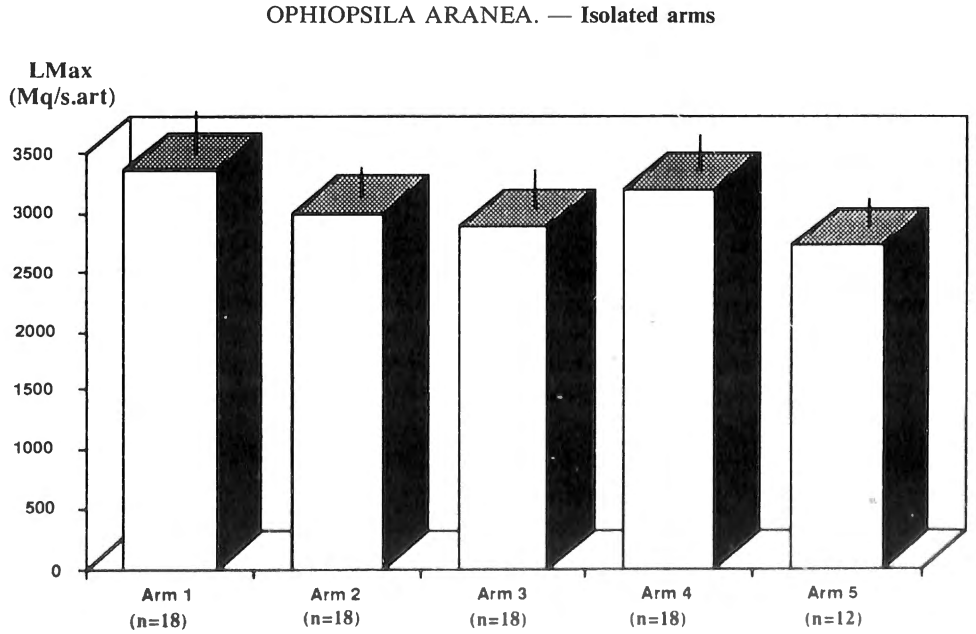

Fig. 3. — Luminescence of isolated arms from one specimen of *Ophiopsila aranea*. in response to 400 mM KCl application ; (mean ± sem, n = number of preparations, * = p < 0,05).

Pharmacology

Each neuromediator was tested on separate preparations from isolated arms of 2 different animals ; articles were bathed for 10 minutes in ASW containing the drugs. The following drugs and concentrations were used : cholinergic compounds — acetylcholine from 10^{-7}M to 10^{-3}M and carbachol at 10^{-3}M ; aminergic compounds — adrenaline, noradrenaline, 5HT and 60HDA at 10^{-3}M ; other compounds — glutamate, taurine and Gaba at 10^{-3}M. Each treatment was tested on 6 different preparations. None of the neuromediators induced the production of light from *Ophiopsila aranea* isolated articles.

DISCUSSION

KCl solutions isotonic to sea water (540 mM) were frequently used to test echinoderm's ability to luminesce (HARVEY 1952 ; HERRING 1978 ; MILLOT 1966 ; BREHM and MORIN 1977) ; in this context the 100 to 400 mM KCl concentrations used in this study are comparable to those utilised to obtain response on various isolated effector organs of echinoderms (PROTAS and MUSKE 1979). We must point out that *Ophiopsila aranea* appears to be very sensitive to KCl since the first light emission was detected at 50 mM KCl (not shown) although at this KCl concentration responses obtained were very erratic.

Comparison of light amplitudes from the 5 arms isolated from one animal, has shown that luminous capabilities are not different from one arm to another. Since we stimulated articles of the first arm before stimulating those from the next arm, this comparison also reveals that maintenance of isolated arms in ASW does not modify their luminous capabilities ; this result indicates that our experimental conditions (anaesthesia, ASW composition...) are sufficient to conserve isolated arms in good physiological conditions.

The presence of multiple flashes may suggest that KCl acts either through a mechanism that progressively activates luminous cells to trigger light emission or through depolarization of some nervous elements implicated in *Ophiopsila* luminescence control. This second hypothesis seems more plausible since it has been shown with *Ophiopsila californica* that luminescence is related to nervous activity in the radial nerve cord (BREHM 1977) ; nevertheless, the nature of the mechanism coupling nerve activity and light emission, as well as the nature of the nervous receptors are unknown.

Many different neuromediators were detected in various tissues of echinoderms (PENTREATH and COBB 1972, COBB 1987, 1988, SLOLEY and JUORIO 1990), among them acetylcholine represents a major component of the nervous system for which some physiological activities were described (SHELKONIKOW *et al.*, 1977, MORALES *et al.*, 1989). Although the present pharmacological results are preliminary, they failed to reveal any activity of this neuromediator ; this observation is in contradiction with the recent finding showing that a muscarinic cholinoreceptor is implicated in the control of another luminescent ophiuroid, *Amphipholis squamata* (DE

BREMAEKER *et al.*, 1993a,b). Our study requires further experiments in order to characterize the nature of the luminescence control mechanism of *Ophiopsila aranea*.

ACKNOWLEDGEMENTS

We thank the director of Arago laboratory (Pr. Guille) for providing us with necessary information and bench space. Specials thanks to Dr J.P. Feral and T. Smith who helped us to collect the animals ; to Aqualung and Scubapro for logistic supports. This work was supported by FRFC n° 6.231.85 and FDS-UCL n° 92.766. Contribution to CIBIM. JM is research associate of FNRS of Belgium.

REFERENCES

BREHM, P. (1977) — Electrophysiology and luminescence of an ophiuroid radial nerve. *J. exp. Biol.* **71** :213-227.

BREHM, P. and J.G. MORIN (1977) — Localization and characterization of luminescent cells in *Ophiopsila californica* and *Amphipholis squamata* (Echinodermata : ophiuroidae). *Biol. Bull.* **152** :12-25.

DE BREMAEKER, N., J. MALLEFET and F. BAGUET (1993a) — Involvement of a cholinergic control in the *Amphipholis squamata* (Echinodermata) luminescence. *Arch. int. Physiol. Bioch.* **101**(3) : 30.

DE BREMAEKER, N., J. MALLEFET and F. BAGUET (1993b) — Nature of cholinergic receptors involved in the luminescence control of *Amphipholis squamata* (Ophiuroidea). *Abstr. 8 th I.E.C.* : 16.

COBB, J.L.S. (1987) — Neurobiology of the echinodermata. In *Nervous systems in invertebrates, M.A. Ali* (ed.), 483-517. NATO ASI series A :141.

COBB, J.L.S. (1988) — Neurohumors and neurosecretion in echinoderms : a review. *Comp. Biochem. Physiol.* **91C** : 151-158.

HARVEY, N. (1952) — Echinodermata. In *Bioluminescence.* HARVEY, N. (ed.), AC Press, 472-479.

HERRING, P.J. (1974) — New observations on the bioluminescence of echinoderms. *J. Zool. Lond.* **172** : 401-418.

HERRING, P.J. (1978) — Echinoderms. In *Bioluminescence in action,* HERRING P.J. (ed.), A.C. Press : 231-236.

MALLEFET, J., F. BAGUET and M. JANGOUX (1989) — Origin of the light emission of *Amphipholis squamata* (echinodermata) to potassium chloride. *Arch. int. Physiol. Biochem.* **97** : 37.

MANGOLD, E. (1907) — Leuchtende schlangensternne und die flimmerbewegung bei *Ophiopsila. Arch. ges. Physiol. Pfügler, Bd.* CXVIII : 613-640.

MILLOT, N. (1966) — Light production. In R.A. *Boolotian* (ed.) : 487-501.

MORALES M., J. DEL CASTILLO and D.S. SMITH (1989) — Acetylcholine sensitivity of the spine test articular capsule of the sea urchin *Eucidares thibuloides. Comp. Biochem. Physiol.* **94C** : 547-554.

PENTREATH, V.W. and J.L.S. COBB (1972) — Neurobiology of echinodermata. *Biol. Rev.* **47** : 363-392.

PROTAS, L.L. and G.A. MUSKE (1979) — The effects of some transmitter substances on the tube foot muscles of the starfish, *Asterias amurensis*. *Gen. Pharma.* **11** : 113-118.

REICHENSPERGER, A. (1908) — Die drüsengebilde der ophiuren. *Zeit Wiss Zool.* **91** : 304-350.

SHELKOVNIKOV, S.A., L.A. STARSHINOVA and E.V. ZEIMAL (1977) — Two kinds of cholinoreceptors on the on-visceral muscle of some echinodermata. *Comp. Biochem. Physiol.*, **58C** : 1-12.

SLOLEY, B.D. and A.V. JUORIO (1990) — Biogenic amines in the nervous and other tissues of several species of starfish — presence of relatively high levels of tryptamine and low levels of 5 hydroxytryptamine. *Biogenic Amines.* **7** : 341-349.

TROJAN, A. (1909) — Leuchtende Ophiopsilen. *Arch. mikr. Anat.* **73** : 883-912.