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EFFECTS OF ADRENALIN ON THE OXYGEN CONSUMPTION AND LUMINESCENCE OF THE PHOTOPHORES OF THE MESOPELAGIC FISH *ARGYROPELECUS HEMIGYMNUS*

BY J. MALLEFET AND F. BAGUET

Laboratoire de Physiologie Générale et des Animaux Domestiques, Université Catholique de Louvain, 5, Place de la Croix du Sud, B-1348 Louvain-la-Neuve, Belgium

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SUMMARY

1. Photophores isolated from the mesopelagic fish *Argyropelecus hemigymnus* consume oxygen at a rate of $1{\cdot}22 \pm 0{\cdot}17$ nmol min^{-1} in saline at 20 °C.

2. In the presence of 5×10^{-4} mol l^{-1} adrenalin the preparations that responded by a long-lasting luminescence showed a significant decrease of their resting respiration rate. After the adrenalin had been washed out, oxygen consumption increased immediately to the previous resting level and was inhibited by 5×10^{-4} mol l^{-1} KCN.

3. It is suggested that the mechanism of light production by isolated photophores of the epipelagic fish *Porichthys* and the mesopelagic fish *Argyropelecus* are different.

INTRODUCTION

The isolated photophore of the epipelagic luminescent fish *Porichthys notatus* increases its oxygen consumption during luminescence (Baguet, 1983; Mallefet & Baguet, 1984). This large increment in oxygen uptake has been interpreted as originating from the oxygen involved in the intracellular light reactions of the photogenic cells (Mallefet & Baguet, 1984). Since the oxygen consumption of marine fish appears to decrease with increasing depth of occurrence (Torres, Belman & Childress, 1979), we decided to investigate the oxygen requirement of photophore luminescence of a deep-sea luminescent fish. The present work is a study of the oxygen consumption of the isolated photophores of the mesopelagic fish *Argyropelecus hemigymnus* at rest and during the light emission evoked by adrenalin, the putative neuromediator (Baguet & Maréchal, 1978). The results show a significant decrease in the oxygen uptake associated with luminescence, suggesting that the rôle of oxygen in the control of light production by the photophores of *Argyropelecus* and *Porichthys* is different.

Key words: Respiration, luminescence, photophores.

MATERIALS AND METHODS

Dissection and mounting the photophores

Fresh specimens of *Argyropelecus hemigymnus* (Fig. 1) were collected in the Strait of Messina as previously described (Baguet & Maréchal, 1976) during 8 days between 05.00 and 06.00 h and brought to the 'Istituto Talassographico' at Messina where they were transferred to glass vessels in cooled sea water from the Strait and stored in a refrigerator at 7 °C.

The ventral row of photophores of each specimen was excised under a binocular microscope with fine scissors and forceps (Dumont no. 5). The ends of the preparation were tied to a thin stainless steel needle and fixed to the lower part of the oxygen electrode by an O-ring as previously described (Mallefet & Baguet, 1984).

Measurement of luminescence

The light-emitting area of the preparation was 10 mm from the end of a fibre-optic light guide (conducting area, 10 mm) connected to a photomultiplier PM 270C (International Light). The signal was displayed on a two-channel strip-chart recorder. The apparatus was calibrated using a tritium irradiated phosphor (Betalight, by Saunders Roe, Nuclear Entreprises Ltd) emitting on an area of 2 mm^2, in the same location as the light organ.

Measurement of oxygen consumption

The oxygen consumption of the isolated light organ was estimated from the oxygen content of the surrounding saline during a given period of time, using a

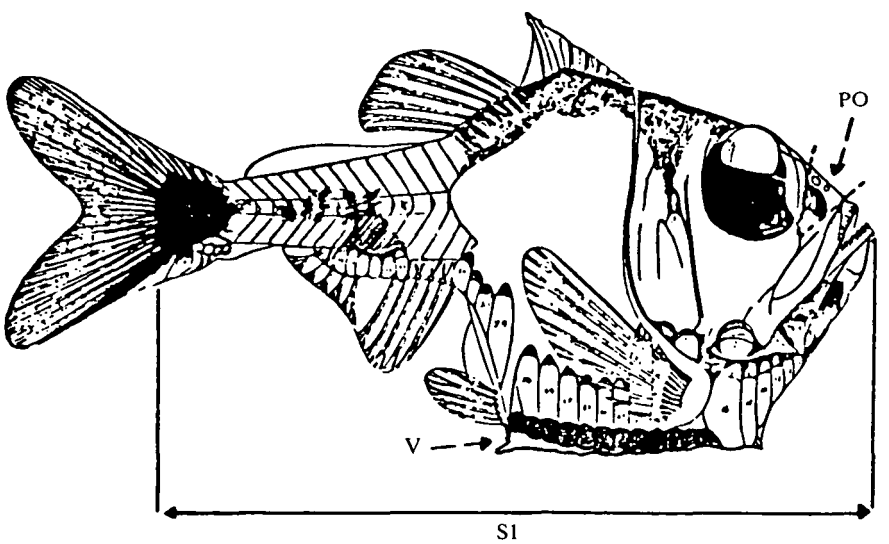

Fig. 1. Diagrammatic side view of *Argyropelecus hemigymnus* showing one ventral row of photophores (V); S1, standard length of the fish; PO, preorbital photophore.

polarographic oxygen electrode as described previously (Mallefet & Baguet, 1984).

Experimental procedure

Each experiment was preceded and followed by: (i) calibration of the oxygen electrode in oxygen-free saline, prepared by adding dithionite ($Na_2S_2O_4$), which eliminates the dissolved oxygen, and in fully aerated saline (20 °C); (ii) measurement of the oxygen consumption of the electrode without the light organ over a period of 15 min.

The preparation attached to the electrode was immersed in 4 ml of fully aerated saline to measure the oxygen consumption and luminescence level for 15 min. After this period, 0·4 ml of air-saturated adrenalin was injected, through a capillary fixed on the side of the electrode, into saline to a final concentration of 5×10^{-4} mol l^{-1}.

Oxygen consumption and luminescence were followed for 40 min. The preparation was then blotted with a filter paper and its weight was estimated with an electrobalance (Metler H1O, accuracy 10 μg).

From the readings in aerated saline and the readings in the known volume of saline containing the preparation, the rate of oxygen consumption could be calculated in nmol min^{-1}. Each mean value is expressed as mean $\pm$ S.E.M. (N = number of preparations). The significance of differences between means was calculated by t-test or analysis of variance.

RESULTS

Oxygen consumption of resting photophores

The resting respiration rate was measured during 8 days on a total of 20 preparations; the experiments started at different periods of the day, ranging from 2 to 13 h after fishing. In order to determine whether the duration of storage in *Argyropelecus* specimens could affect the oxygen consumption of the isolated photophores, we separated the results into three groups according to the time elapsing between the end of the capture and the mounting of the light organs on the oxygen electrode. The length and the weight of the specimens, as well as the weight of the corresponding isolated photophores, were similar in the three groups (Table 1).

For the photophores excised soon after capture of the fish (2–5 h), the mean value of the oxygen consumption ($1{\cdot}22 \pm 0{\cdot}17$ nmol O_2 min^{-1}) did not differ significantly from the values of the photophores that were dissected late (5–9 h: $1{\cdot}18 \pm 0{\cdot}31$ nmol O_2 min^{-1}) or very late (9–13 h: $1{\cdot}30 \pm 0{\cdot}44$ nmol O_2 min^{-1}).

In nine experiments, potassium cyanide (KCN) was added to the saline after adrenalin to a final dilution of 5×10^{-4} mol l^{-1}: the oxygen uptake of the preparations was completely blocked in about 5 min.

Table 1. *Mean values (±S.E.M.) of the respiration rate of photophores isolated from fish at different periods after capture*

	Time after capture (h)		
	2–5	5–9	9–13
Fish length (mm)	28·4 ± 0·5	27·7 ± 1·1	28·0 ± 0·9
Fish weight (g)	522·8 ±32·1	504·8 ±48·2	503·6 ±54·7
Photophore weight (mg)	15·7 ± 1·0	15·4 ± 1·9	15·1 ± 1·7
O_2 consumption (nmol min^{-1})	1·22± 0·17	1·18± 0·31	1·30± 0·44
N, number of preparations.	10	6	3

The mean value of the resting respiration rate of *Argyropelecus* photophores at 20°C and atmospheric pressure calculated on 19 observations was 1·22±0·15 nmol O_2 min^{-1} for a mean fresh weight of 15·64±0·83 mg.

Oxygen consumption of glowing photophores

In 5×10^{-4} mol l^{-1} adrenalin, 14 preparations responded with a long lasting luminescence beginning 73·1±18·1 s after the application of adrenalin and reaching a maximal value after 17·1±1·6 min. The kinetics of extinction can be described by a single exponential equation of the type $Y_t = Ae^{-\alpha t}$, where t is the time (min), Y_t is the level of light of the preparation at time t, A is 100 % light, and α is the rate constant of extinction. The mean value of the rate constant was 4·8±1·0 % (N=14).

The time course of the mean oxygen consumption and the mean production of light during the first 40 min of the light response are given in Fig. 2. The oxygen uptake dropped immediately on addition of adrenalin when the preparation had not begun to luminesce and reached the mean value of 0·77±0·13 nmol min^{-1} after 3 min, which corresponds to 60 % of the previous resting level. A t-test showed that this decrease in oxygen consumption was significant (t_{13}=2·67; P=0·02). Three minutes later, when the light level had reached about half of the peak value, the oxygen uptake increased, showing a time course similar to the increase of the light production. At the peak of light and during the first 15 min of the extinction phase, the rate of oxygen consumption did not change significantly, ranging from 1·03 to 1·05 nmol min^{-1}: these values were 20 % lower than the previous resting respiration rate. The oxygen consumption, like the luminescence, decreased slowly during the next 10 min of the experiment.

In a few cases we measured the oxygen consumption until the complete extinction of the light response and after washing out the adrenalin. In a typical experiment (Fig. 3) the oxygen uptake, after a typical transient increase lasting 6 min, dropped to a new level that remained constant until the end of the light extinction phase. After 5 min the preparation was washed and immersed in fresh saline; the rate of oxygen consumption rose immediately to 1·15 nmol min^{-1},

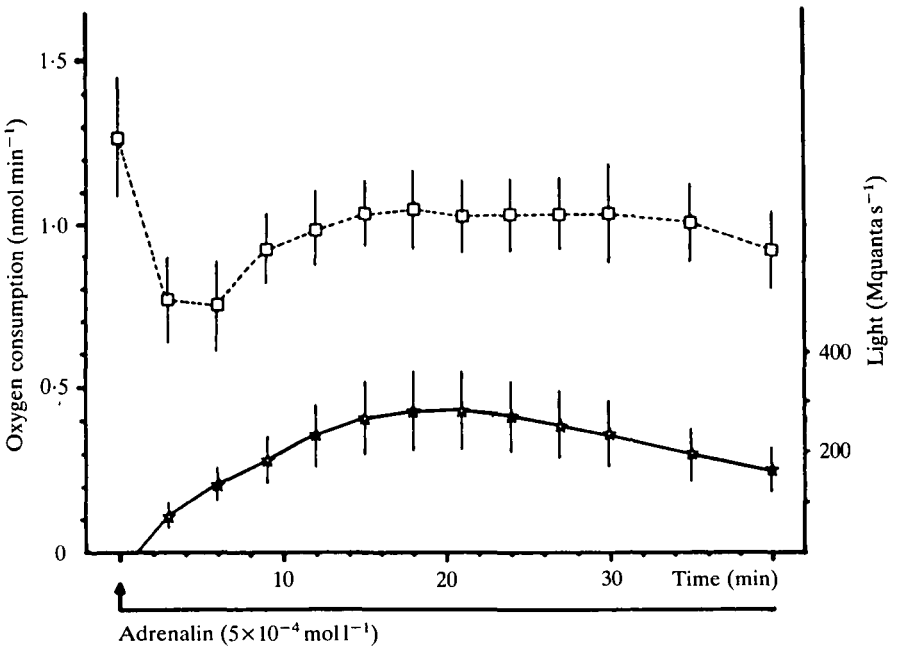

Fig. 2. Time course of the mean values (±S.E.M.) of oxygen consumption rate (□) and light production (☆) of the isolated light organs in response to adrenalin (5×10^{-4} mol l^{-1}).

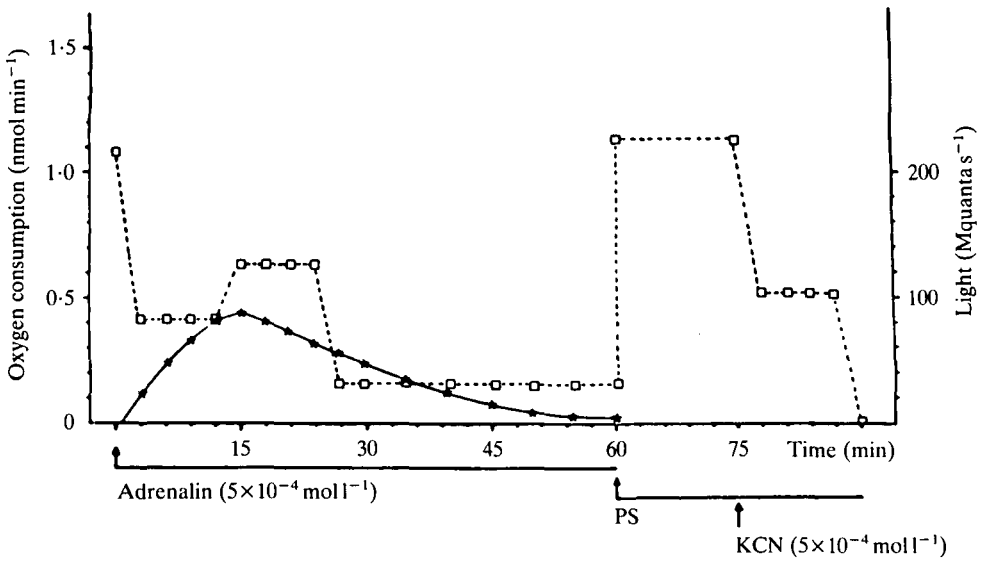

Fig. 3. Typical time course of the oxygen consumption of an isolated light organ during a light response evoked by adrenalin (5×10^{-4} mol l^{-1}). After 60 min, when the preparation was washed out and immersed in saline (PS), the oxygen consumption increased to the level measured before stimulation. KCN (5×10^{-4} mol l^{-1}) blocked the oxygen consumption in two steps.

similar to the value of 1·10 nmol min^{-1} measured before the light response. Moreover, the preparation showed the characteristic sensitivity to the inhibiting effect of KCN (5×10^{-4} mol l^{-1}) on the resting respiration rate as previously described. It is concluded that the decrement of oxygen uptake observed during the light emission evoked by adrenalin (5×10^{-4} mol l^{-1}) is completely reversible.

Oxygen consumption in the absence of light production

Five of the 19 preparations stimulated by adrenalin did not produce any detectable luminescence. We calculated a mean resting oxygen uptake of $1{\cdot}14 \pm 0{\cdot}29$ nmol min^{-1} ($N=5$). On addition of adrenalin, the oxygen consumption decreased for 3 min and reached a steady level of $0{\cdot}92 \pm 0{\cdot}32$ nmol min^{-1}, which corresponds to about 80 % of the previous resting level. This drop was not significant ($t_4 = 2{\cdot}12$; $P = 0{\cdot}2$). These results might suggest that the application of adrenalin has a specific inhibitory effect on the resting respiration rate of the preparation. A statistical comparison between resting oxygen uptakes of the luminescent and non-luminescent photophores failed to display a significative difference ($t_{17} = 0{\cdot}99$).

Oxygen consumption related to light production

Comparison of the time course of oxygen uptake of the glowing and non-glowing preparations shows that, during the first 6 min following the application of adrenalin, the reduction of the oxygen consumption is greater in glowing than in non-glowing preparations.

Moreover, the rate of oxygen consumption and light production show a similar time course (Fig. 2). To test this relationship, we calculated the total amount of oxygen consumed in excess of the minimum level and the total amount of light produced. On average, the amount of oxygen consumed was $8{\cdot}74 \pm 2{\cdot}13$ nmol for a total light production of $31{\cdot}77 \pm 8{\cdot}31 \times 10^{10}$ quanta ($N=12$). A statistical analysis shows that there is no significant correlation ($r = 0{\cdot}3$) between these two phenomena.

DISCUSSION

Resting respiration

The ventral photophores of the mesopelagic fish *Argyropelecus hemigymnus* remain functional and exhibit a very stable resting respiration even when they are isolated from specimens stored for 12 h. This confirms that the specimens we collected in the Strait of Messina have photophores in a good physiological state.

The isolated ventral light organ of *Argyropelecus* showed a respiration rate of $1{\cdot}22 \pm 0{\cdot}14$ nmol O_2 min^{-1} at 20 °C. The mean fresh weight of the preparation was $15{\cdot}64 \pm 0{\cdot}88$ mg and the estimated respiration rate was 78 nmol O_2 g^{-1}. In the same experimental conditions, the resting respiration rate of the isolated photophore of the epipelagic teleost *Porichthys notatus* is 335 nmol O_2 g^{-1} min^{-1} (J. Mallefet & F. Baguet, in preparation). According to Torres *et al.* (1979), the

oxygen consumption of midwater fish decreases with increasing depth: the difference in oxygen consumption rate between a fish living at the surface and a fish living at 1000 m approaches two orders of magnitude. Most of the decline appears to be due to a decrease in muscular enzyme activity (Siebenaller & Yancey, 1984) and probably in aerobic metabolism per unit of muscle. Our results suggest that the lower aerobic metabolism of photophores of a mesopelagic fish might be an inherent characteristic of deep-living species.

Oxygen consumption and luminescence

The biochemical mechanism for luminescence in *Porichthys* photophores consists of a luciferase-catalysed oxidation of luciferin (*Cypridina*-type) by oxygen (Tsuji *et al.* 1977). It has been suggested that the increase of oxygen consumption with light production originates from the oxidation of luciferin by molecular oxygen (Mallefet & Baguet, 1984). Since the production of light by *Argyropelecus* photophores is not associated with increased oxygen consumption, we suggest that the chemical nature of the light reaction is different from that occurring in the *Porichthys* photophore. Several years ago, a new type of light compound was described ('photoprotein') in the jellyfish *Aequora* (Shimomura, Johnson & Saiga, 1962). Light production is simply triggered by the addition of calcium and oxygen is not required. This apparent lack of oxygen for the light reaction is explicable: there is a prior reaction of oxygen with coelenterazine (coelenterate luciferin) to form a stable peroxide intermediate, the photoprotein (Hastings & Morin, 1969). Shimomura, Inoue, Johnson & Haneda (1980) reported the occurrence of a trace of coelenterazine in the light organ of *Argyropelecus hemigymnus*. Assuming that coelenterazine is involved in light production by the *Argyropelecus* photophore, we could expect that no oxygen would be required if it is stored as a peroxide intermediate. In this case no oxygen would be consumed during the light production of a photophore; this could explain why we found no significant relationship between the oxygen consumed and the light produced in response to 5×10^{-4} mol l^{-1} adrenalin. Our results reveal that the application of adrenalin leads to a decrease in the oxygen consumption of the photophores whether they produce light or not. However, in the non-luminous photophores, there is a decrease of only 20 % of the resting rate, whereas the luminous ones show a 40 % decrease. This suggests that the triggering of luminescence is associated with a specific inhibition of the aerobic metabolism starting before the first detectable luminescence. In the case of the non-luminous photophores, the inhibition should not be strong enough to trigger the mechanism leading to light production. Although the functioning of an effector organ is not usually associated with a reduction of its metabolic activity, the present case is not unique: the resting respiration rate of the isolated vertebrate retina decreases when stimulated by light that liberates Ca^{2+} ions from intracellular stores (Sickel, 1972). By blocking the transmembrane Na^{+}-channels, Ca^{2+} indirectly involves an arrest of the Na-K pumps and a decrease in energy expenditure. Any attempt to explain in a similar way the decrease of the resting

respiration of the *Argyropelecus* photophore is purely speculative since we do not know about the role of the membrane in the control of the photocytes' light processes. Moreover, in the retina, the reduction of the respiration rate of the photoreceptive cells follows illumination by external light, while in the photophore, the diminution of the oxygen consumption of the photoemitting cell occurs prior to the production of light. We suggest that the decrease of oxygen consumption with light might be due to the inhibition of a mechanism that should prevent the spontaneous luminescence of the photophore.

The significance of oxygen consumption

The photophores of luminescent fish are mainly arranged along the ventral surfaces; Frazer (1962) and Clarke (1963) have suggested that by generating a downwardly directed light, these animals might obliterate the shadows which they would otherwise cast and so make themselves difficult to see from below. In this counterlighting hypothesis it is assumed that the photophore can luminesce continuously, the intensity being adjusted and modulated to match that of the background twilight. In the epipelagic fish *Porichthys* continuous prolonged luminescence is unlikely because of the high oxygen consumption associated with the luminescence of the photophores. On the other hand, our results show that in *Argyropelecus hemigymnus* it is more economical to maintain the photophores luminescent instead of non-luminescent. Direct observations of *Argyropelecus* aboard the submersible *Forel* (Baguet, Picard, Christophe & Maréchal, 1983) provide evidence that the fish emitted a sustained luminescence.

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REFERENCES

BAGUET, F. (1983). Light production and oxygen consumption of isolated photophores of the epipelagic fish *Porichthys*. In *Abstracts of the 15th Pacific Science Congress*. University of Otago, Dunedin, New Zealand, p. 11.

BAGUET, F. & MARÉCHAL, G. (1976). Bioluminescence of bathypelagic fish from the Strait of Messina. *Comp. Biochem. Physiol.* **53**C, 75–82.

BAGUET, F. & MARÉCHAL, G. (1978). The stimulation of isolated photophores (*Argyropelecus*) by epinephrine and norepinephrine. *Comp. Biochem. Physiol.* **60**C, 137–143.

BAGUET, F., PICCARD, J., CHRISTOPHE, B. & MARÉCHAL, G. (1983). Bioluminescence and luminescent fish in the Strait of Messina from the mesoscaph 'Forel'. *Mar. Biol.* **74**, 221–229.

CLARKE, W. D. (1963). Function of bioluminescence in mesopelagic organisms. *Nature, Lond.* **198**, 1244–1246.

FRAZER (1962). *Nature Adrift 'The Story of Marine Plankton'*. London: G. T. Foulis & Co. 184 pp.

HASTINGS, J. W. & MORIN, J. G. (1969). Calcium-triggered light emission in *Renilla*. A unitury biochemical scheme for coelenterate bioluminescence. *Biochem. biophys. Res. Commun.* **37**, 493–498.

MALLEFET, J. & BAGUET, F. (1984). Oxygen consumption and luminescence of *Porichthys* photophores stimulated by potassium cyanide. *J. exp. Biol.* **109**, 341–352.

SHIMOMURA, O., INOUE, S., JOHNSON, F. H. & HANEDA, Y. (1980). Widespread occurrence of Coelenterazine in marine bioluminescence. *Comp. Biochem. Physiol.* **65**B, 435–437.

SHIMOMURA, O., JOHNSON, F. H. & SAIGA, Y. (1962). Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequora*. *J. cell. comp. Physiol.* **59**, 223–240.

SICKEL, W. (1972). Retinal metabolism in dark and light. In *Handbook of Sensory Physiology*, Vol. II/2, (ed. M. F. G. Fourtes), pp. 667–718. New York: Springer-Verlag.

SIEBENALLER, J. F. & YANCEY, P. H. (1984). Protein composition of white skeletal muscle from mesopelagic fishes having different water and protein contents. *Mar. Biol.* **78**, 129–137.

TORRES, J. J., BELMAN, B. W. & CHILDRESS, J. J. (1979). Oxygen consumption rates of midwater fishes as a function of depth of occurrence. *Deep-sea Res.* **26**A, 185–197.

TSUJI, F. I., DE LUCA, M., BOYER, P. D., ENDO, S. & AKUTAGAWA, M. (1977). Mechanism of the enzyme catalysed oxidation of *Cypridina* and firefly luciferins studied by means of $^{17}O_2$ and $H_2{}^{18}O$. *Biochem. biophys. Res. Commun.* **74**, 606–613.