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OXYGEN CONSUMPTION AND LUMINESCENCE OF *PORICHTHYS* PHOTOPHORES STIMULATED BY POTASSIUM CYANIDE

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SUMMARY

1. Isolated photophores of *Porichthys notatus*, maintained in saline at 20 °C, do not luminesce and show an oxygen consumption rate of $0{\cdot}07 \pm 0{\cdot}01$ nmol min^{-1} $photophore^{-1}$.

2. In the presence of 10^{-6} M-KCN, the photophores do not luminesce but the resting respiration decreases by about 50 %.

3. In the presence of 10^{-5} M-KCN, some photophores do not luminesce and their respiration rate decreases by about 75 %. Others show a response and resting oxygen consumption slowly increases.

4. At high concentration (10^{-4} and 10^{-3} M), KCN induces a large light emission and increase in oxygen consumption.

5. The stimulatory effect of KCN on the photophore oxygen consumption is tentatively explained by an activation of the luciferin-luciferase system by calcium ions.

INTRODUCTION

The respiratory metabolic inhibitor, potassium cyanide (KCN) induces the luminescence of isolated photophores from the teleost *Porichthys notatus*, either by external application on the light organ (Baguet, 1975) or by direct application on the photocytes, i.e. the photogenic cells, isolated from the photophore (Christophe & Baguet, 1982).

To explain the stimulating effect of KCN, it has been suggested that the photocytes are under control of an inhibitory mechanism (Baguet, 1975). By blocking the cellular respiration and energy supply, KCN should remove the inhibition and induce luminescence of photophores.

The effects of KCN upon simultaneous measurements of the luminescence and oxygen consumption of isolated photophores of *Porichthys notatus* were examined in the present study.

The results suggest that cellular respiration provides energy that prevents the triggering of the light reactions of the photocytes, whilst not being essential to the mechanism of light production.

Key words: Respiration, luminescence, photophore.

MATERIALS AND METHODS

Dissection of the photophores

Four specimens of *Porichthys notatus*, air-shipped by Pacific Bio-Marine Laboratories (Venice, California), were maintained in aerated running sea water from the North Sea (15–17 °C). Fish were anaesthetized by partial immersion in a tray containing sea water and 25 % quinaldine (1·5 ml l^{-1} sea water) at 17 °C. Following anaesthesia, a strip of skin with 3–13 ventral, branchial, mandibular or pleural photophores, using Greene's terminology (1899) was excised. Using fine scissors and forceps (Dumont no. 5) the skin surrounding the photophores was mostly removed.

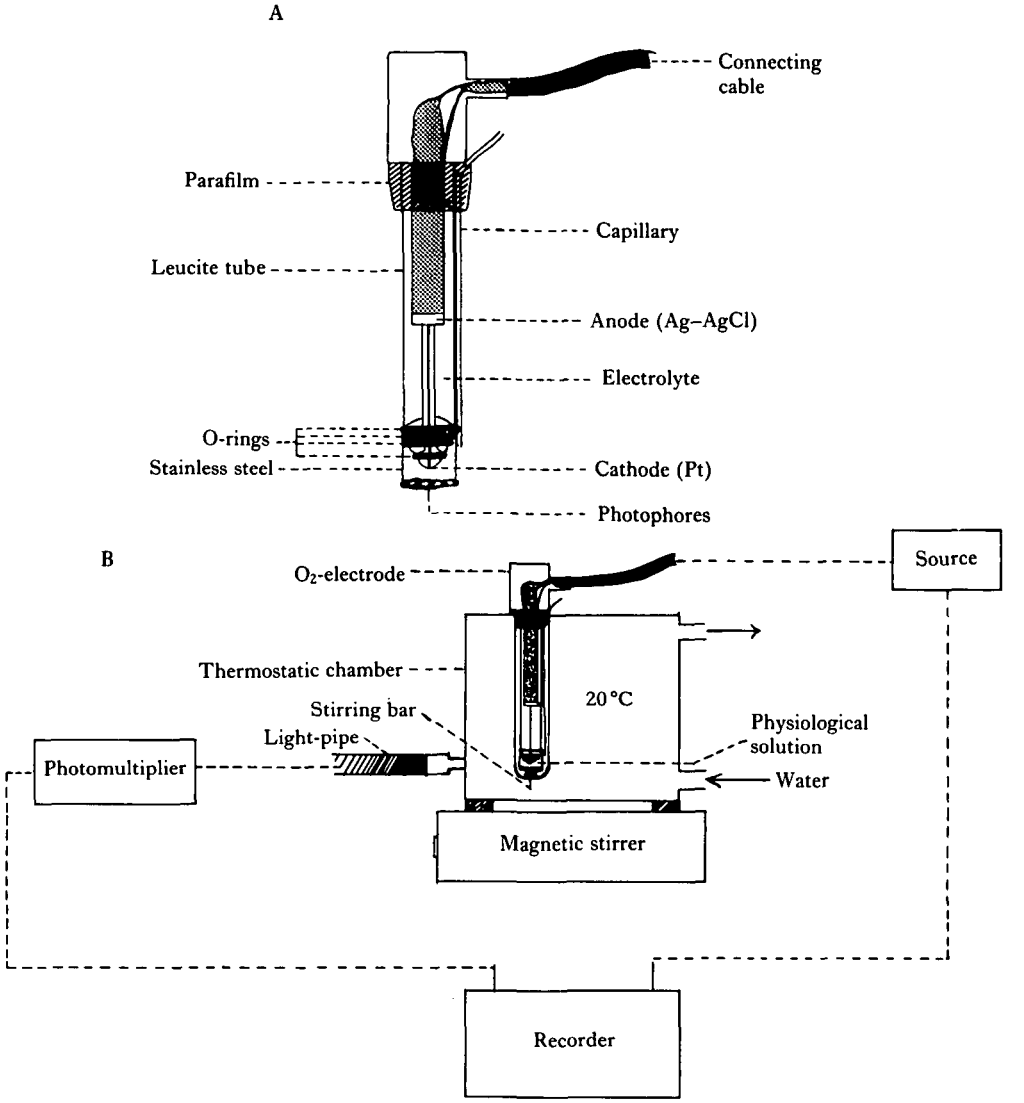

Fig. 1. (A) Drawing of the oxygen electrode with a series of photophores fixed near the cathode. (B) Diagram of the apparatus for simultaneous recording of light production and oxygen consumption of isolated photophores.

Mounting the photophores

A thin piece of cotton thread was fixed to the skin remaining at the ends of the strip; each thread was tied to a thin stainless steel needle, fixed to the lower part of the oxygen electrode by an O-ring (Fig. 1A). In this position, the photophores were 2 mm distant from the plane of the cathode. The oxygen electrode and the photophores were immersed in a glass tube (50 mm length; 14 mm internal diameter) fixed in a temperature-controlled chamber (20 °C); the tube was filled with saline and sealed at the top to the electrode with paraffin. The electrode was filled with 0·1 M-KCl, 0·25 M-KH_2PO_4 and 0·2 M-Na_2HPO_4, pH 7·3.

Measurement of luminescence

The light emitting area of the photophores was 10 mm from the end of a fibre-optic light guide (conducting area, 10 mm) connected to a 1P 21 photomultiplier. The signal was displayed on a two channel strip-chart recorder (Fig. 1B). The apparatus was calibrated using a tritium irradiated phosphor (Betalight, by Saunders Roe, Nuclear Enterprises Ltd) emitting on an area of 2 mm^2, in the same location as the photophore. The spectrum of light emitted by the source showed a peak at 470 nm, corresponding to the peak of the light spectrum observed *in vitro* from the isolated luciferin-luciferase system of *Porichthys* (Cormier, Crane & Nakano, 1967).

Measurement of oxygen consumption

The oxygen consumption of the isolated photophores was estimated from the oxygen content of the surrounding saline during a given period of time, using a polarographic oxygen electrode (Kiel, Eschweiler & Co.) (Fig. 1A). The electrode was separated from the solution to be analysed by a thin (25 μm) Teflon membrane. The membrane was regularly replaced by a spare one that had been kept in the same fluid as the internal solution of the electrode. The saline surrounding the electrode was gently and regularly stirred. Under these conditions, the electrode response was linear with oxygen concentration up to the partial pressure of atmospheric air. The electrode could therefore be equilibrated by taking readings in (i) oxygen-free saline, prepared by adding dithionite ($Na_2S_2O_4$) which reduces the dissolved oxygen, and (ii) fully aerated saline. Using the formula given by Sendroy, Dillon & Van Slijke (1934), which takes into account the temperature and salinity of the solution, it was calculated that fully aerated saline at 20 °C contains 6·05 ml O_2 l^{-1} or 251·6 nmol ml^{-1}.

To measure the oxygen consumption of a preparation, the electric current of the electrode was reduced by 90 % with a back-off system and the remaining signal was amplified. A series of preliminary experiments showed that the oxygen uptake of three photophores and more gave a reading which was above the accuracy of the oxygen measurement system. In this case, repeated measurements of the oxygen consumption on the same preparation under resting conditions agreed to within 1 %.

Experimental procedure

Each experiment was preceded and followed by: (i) calibration of the oxygen electrode in oxygen-free saline and fully aerated saline (20 °C); (ii) measurement of the

oxygen consumption of the electrode without photophores over a period of 30 min. The strip of photophores attached to the electrode was immersed in fully aerated saline to measure the oxygen consumption and the luminescence level for 30 min. After this period, 0·4 ml KCN was injected through a capillary, fixed on the side of the electrode into the saline to a final dilution ranging from 10^{-6}–10^{-3} M. Oxygen consumption and luminescence were followed for 30 min. Afterwards, the volume of saline was determined (3·5–4 ml), the strip was blotted with a filter paper and its weight was estimated with an electrobalance (Cahn, model DTL, 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}.

Statistics

Each mean value is expressed with its standard error (mean ± S.E.M.), and number of preparations, N. Significance of difference between means was calculated by t-test.

RESULTS

Oxygen consumption of resting photophores

Oxygen uptake of isolated strips of photophores in saline (20 °C) remained at a very stable level during 30 or even 60 min provided they were non-luminescent (Fig. 2). Isolated pieces of skin without photophores exhibited a respiration rate of 0·19 ± 0·01 nmol min^{-1} mg^{-1} of tissue ($N = 10$). This value was substracted from the oxygen consumption of 43 resting preparations, each containing 3–10 photophores, to yield an O_2 consumption of 0·07 ± 0·01 nmol min^{-1} for one photophore ($N = 43$). No significant differences were observed between photophores from the four different fishes used or between the photophores from branchial, mandibular, ventral or pleural regions of the same fish.

Effect of potassium cyanide (KCN) on luminescence and oxygen consumption

Oxygen consumption in the absence of light production

In the presence of 10^{-6} M-KCN, none of the 16 preparations tested produced a detectable luminescence. The mean oxygen consumption shown in Fig. 3 did not

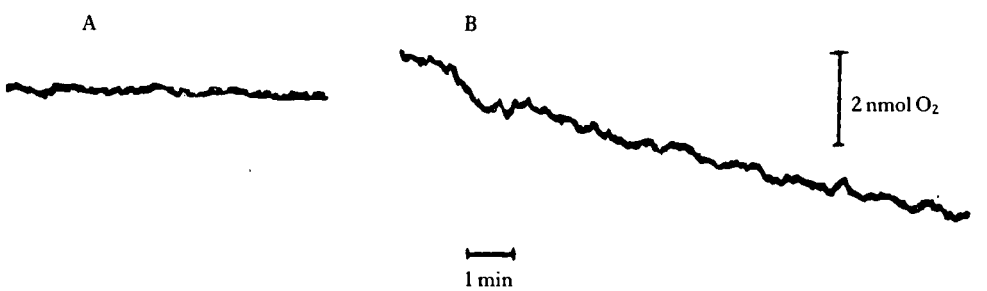

Fig. 2. Original record from the oxygen electrode immersed in saline in absence (A) and in presence of five non-luminescing photophores tied to the holder (B).

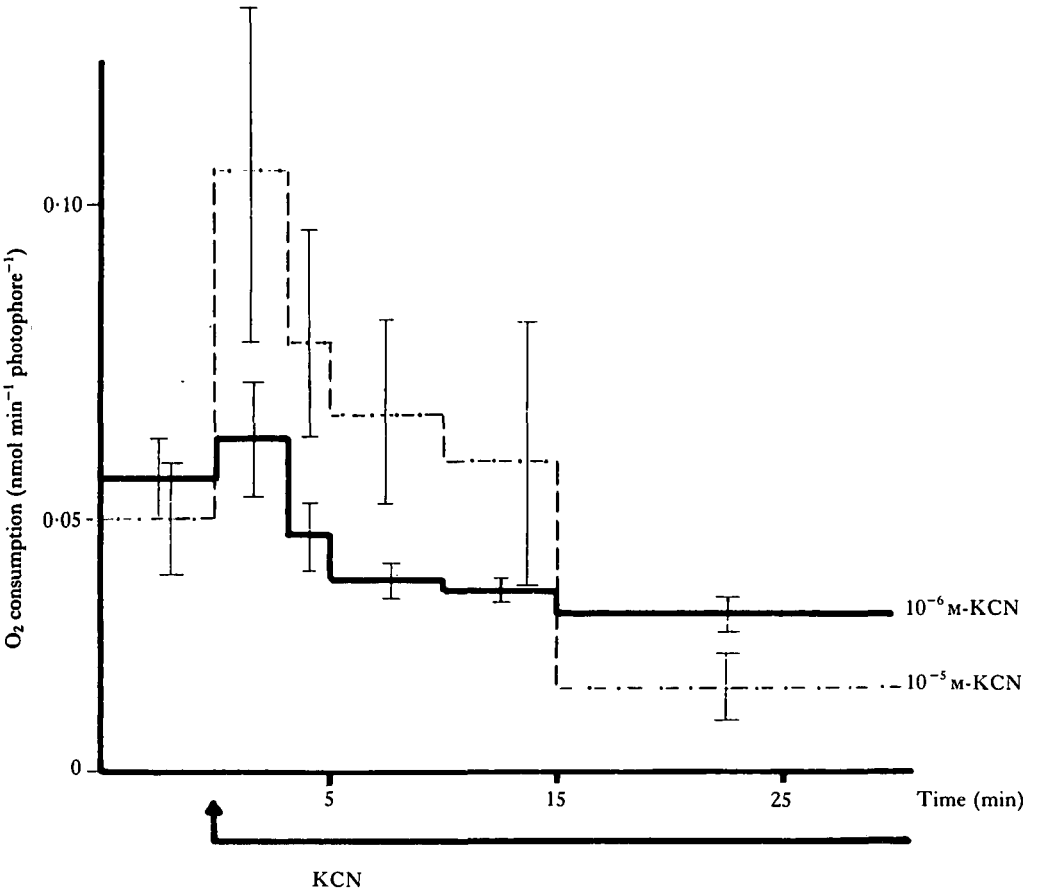

Fig. 3. Mean values (±S.E.M.) of the oxygen consumption of isolated photophores before and after addition of 10^{-6} and 10^{-5} M-KCN.

change significantly from the resting value (0·050 ± 0·010 nmol min^{-1} photophore^{-1}) during the 3 min following application of KCN. The respiration rate began to decrease during the next 2 min, and after 15 min it reached a mean value of 0·028 ± 0·007 nmol min^{-1} photophore^{-1}, which corresponds to about 50 % of the previous resting level. Among the nine preparations treated with 10^{-5} M-KCN, four did not luminesce. The respiration rate of these preparations nearly doubled during the first 3 min and after 15 min decreased to a new level, two-thirds lower than the previous resting level (Fig. 3). In control experiments conducted on isolated pieces of skin neither 10^{-6} M-KCN nor 10^{-5} M-KCN affected the skin respiration rate. It is concluded that the decrement of the oxygen uptake observed on isolated strips is the result of a direct effect of cyanide on the photophores.

Oxygen consumption of glowing photophores

In 10^{-5} M-KCN, five preparations produced a slow light emission beginning 268 ± 53·4 s ($N = 5$) after the application of cyanide and reaching a maximal value after about 22 min (Table 1). In a typical experiment (Fig. 4B) a transient increase

Table 1. *Mean values (± S.E.M.) of different parameters of luminescence of isolated preparations at three different concentrations of potassium cyanide (KCN)*

KCN (M)	LT (s)	TL_{max} (s)	L_{max} (Mq s^{-1} photophore^{-1})	Total light (Mq photophore^{-1})	*n/N*
10^{-5}	268·2 ± 53·4	1345 ± 222	103·6 ± 36·7	76·138 ± 29·582	5/9
10^{-4}	164·3 ± 44·4	445 ± 105	106·7 ± 22·9	51·492 ± 10·028	9/9
10^{-3}	156·0 ± 19·1	359 ± 19	138·0 ± 48·3	72·466 ± 24·938	9/9

LT, latency time (s).
TL_{max}, time elapsing from the application of KCN to the time to reach the peak of the light emission(s).
L_{max}, peak of light emission per photophore (10^6 quanta = Mq).
Total light, total amount of light produced during 30 min.
n/N, number of preparations producing light (*n*) of the total number (*N*) of preparations used.

of the oxygen uptake occurred during the first 3 min, when the photophores had not begun to luminesce. After 5 min, the preparation started to luminesce and simultaneously its oxygen consumption increased to reach a maximal value during the last 15 min of the experimental period. In 10^{-4} and 10^{-3} M-KCN there was a large light response which in both cases showed a similar time course of light production and extinction (Table 1): the photophores started to luminesce within 3 min after addition of KCN and reached the peak of the light response in 6–7 min. The peak of light and the total amount of light emitted during 30 min, calculated from the surface area under the light emission curve, were not significantly different for the three KCN concentrations (Table 1). Oxygen uptake was high and typically reached a maximum between the third and the fifth minute of the incubation, prior to the peak of light (Fig. 4C,D). During this period, the rate in 10^{-4} M-KCN increased from 0·05 to 0·28 nmol min^{-1} photophore^{-1} ($N = 9$) while in 10^{-3} M-KCN it rose from 0·05 to 0·26 nmol min^{-1} photophore^{-1} ($N = 9$). By 10 min oxygen consumption had decreased by 40 % (10^{-4} M) and 60 % (10^{-3} M) of the maximal value. Oxygen consumption then decreased slowly during the next 15 min and reached a steady level similar to (10^{-3} M-KCN) or higher than (10^{-4} M-KCN) the previous resting level.

Oxygen consumption related to the production of light

In 10^{-3}, 10^{-4} and 10^{-5} M-KCN, the change in respiration rate showed a similar time course to the change in light production (Table 2). To test the existence of a relationship we must calculate the excess of oxygen consumption induced by luminescence by substracting the resting respiration from the total observed O_2 consumption. To estimate the additional use of oxygen induced by luminescence in 10^{-5} M-KCN, we substracted the resting respiration measured before addition of KCN from the measurements taken during the first 15 min. During the next 15 min, we have assumed that the resting respiration decreased by 75 % as in non-luminescent preparations and we substracted this value from the measurement of the second period of 15 min. The time course of the mean supplementary oxygen consumed in excess of the resting level of luminescent and non-luminescent preparations is summarized in Table 3: it is obvious that during the first 10 min, a similar amount of oxygen is consumed in both types of preparation. During the last 15 min, when the photophore

ere producing most of the light, a large supplementary amount of oxygen was nsumed, corresponding to about 66% of the total extra oxygen consumption. It follows that the oxygen consumption above the resting level could be divided into two parts, one independent of any light emission which occurred immediately on addition of cyanide in non-luminescent and luminescent preparations, another associated with luminescence and exclusively present in luminescent photophores. To estimate the

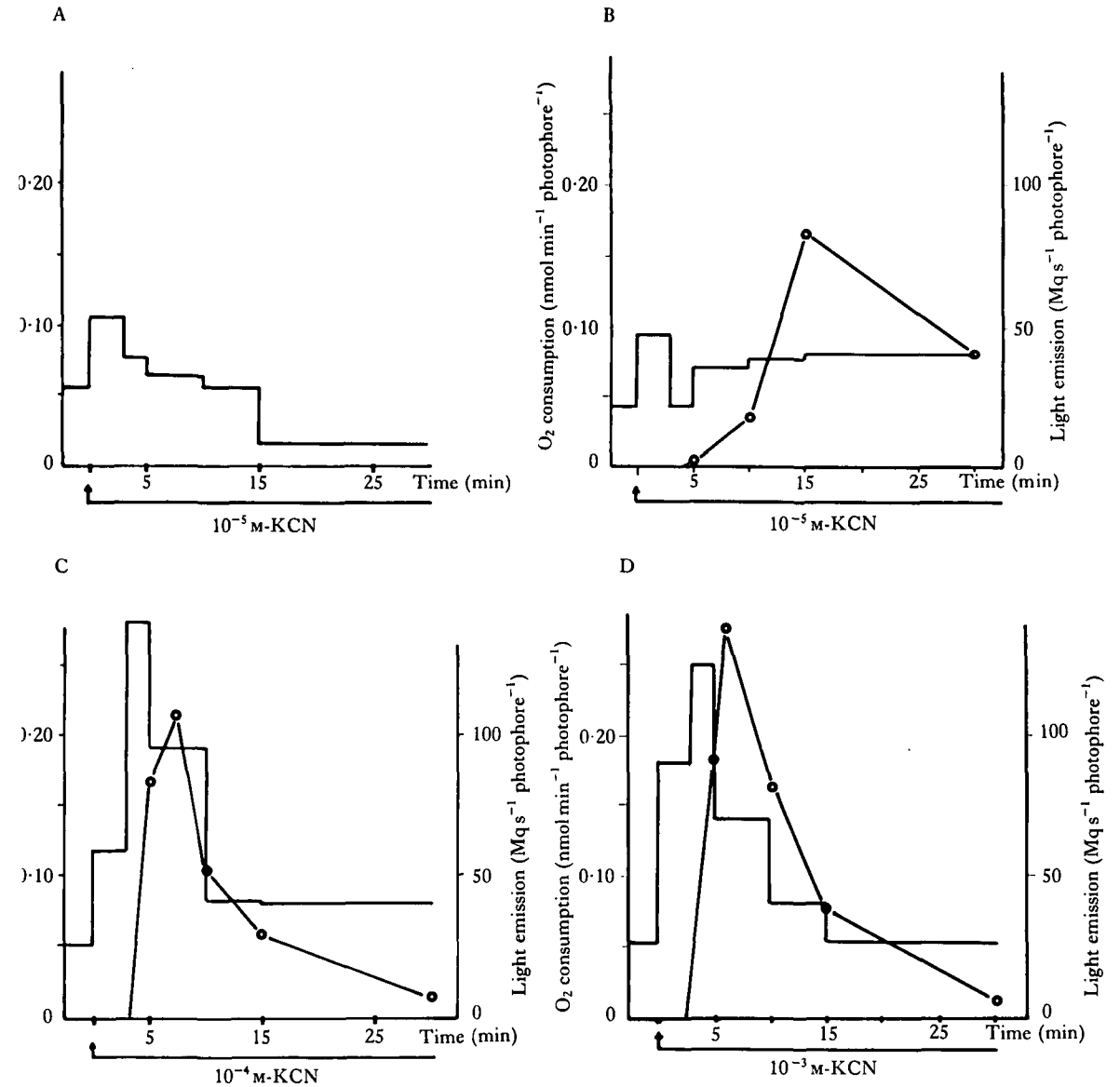

Fig. 4. Typical time course of oxygen consumption and light response of isolated photophores in response to KCN at different concentrations. (A) Effect of 10^{-5} M-KCN *on a preparation which did not luminesce. (B) Slow increase of oxygen consumption and light production in response to* 10^{-5} M-KCN. *(C) and (D) large increase of oxygen consumption and rapid production of light in response to high concentrations of KCN (*10^{-4} *and* 10^{-3} M).

Table 2. *The time course of the mean oxygen consumption induced by 10^{-3}, 10^{-4} and 10^{-5} M-KCN (nmol O_2 min^{-1} photophore^{-1})*

KCN (M)	Resting oxygen consumption (nmol min^{-1} photophore^{-1})	Time after application of KCN (min) 0	3	5	10	15	30
10^{-3}	0·05 ± 0·01	0·19 ± 0·07	0·26 ± 0·04	0·13 ± 0·04	0·08 ± 0·01	0·05 ± 0·01	
10^{-4}	0·05 ± 0·01	0·12 ± 0·03	0·28 ± 0·08	0·19 ± 0·04	0·08 ± 0·02	0·08 ± 0·02	
10^{-5}	0·04 ± 0·01	0·09 ± 0·04	0·04 ± 0·04	0·07 ± 0·02	0·08 ± 0·02	0·08 ± 0·02	
		Mean light production (Mq s^{-1})					
10^{-3}		82·2 ± 35·0	107·4 ± 35·0	87·0 ± 30·0	60·0 ± 21·0	21·9 ± 7·5	
10^{-4}		79·9 ± 26·7	87·9 ± 25·0	67·3 ± 18·0	40·2 ± 8·0	18·0 ± 3·5	
10^{-5}		0	0·1	8·7 ± 4·0	50·2 ± 20·0	61·7 ± 24·0	

Table 3. *The time course of the supplementary oxygen consumption (mean value) induced by 10^{-5} M–KCN*

		Time after application of KCN (min)				
		0	3	5	10	15	30
Excess oxygen consumed (nmol O_2 photophore^{-1})	L	0·16	0·19	0·33	0·45	1·34
	%	11	14	24	33	100
	NL	0·18	0·24	0·32	0·38	0·38
	%	47	63	84	100	100

L, mean value of the excess oxygen consumed by the preparations producing light in response to 10^{-5} M–KCN.
NL, mean value of the excess oxygen consumed by the preparations that did not produce light in presence of 10^{-5} M–KCN.
%, percentage of the excess oxygen consumed.

amount of oxygen involved in the light emission, we substracted from the mean value of the total oxygen consumption of luminescent photophores (1·34 nmol photophore^{-1}; Table 3) that part of the oxygen which is consumed by non-luminescent light organs (0·38 nmol photophore^{-1}). The production of light, 76·138 ± 29·000 Mq photophore^{-1}, involves an increase of about 1 nmol of oxygen consumption per photophore.

It is not possible, at the present time, to estimate accurately the additional use of oxygen induced by 10^{-4} or 10^{-3} M-KCN: we do not know how far these concentrations affect the resting respiration of the photophores, since it has not been possible, up to now, to block the light response induced by 10^{-4} and 10^{-3} M-KCN. The resting respiration of isolated pieces of skin is completely blocked in 3 min in the presence of 10^{-4} or 10^{-3} M-KCN; assuming a similar blocking effect on the resting respiration of the photophores, the additional oxygen consumption involved in the light response could be 3·3 ± 0·4 nmol min^{-1} photophore^{-1}, in 10^{-4} M-KCN and 2·9 ± 0·5 nmol min^{-1} photophore^{-1}, in 10^{-3} M-KCN. On the other hand if we assume that the effect on the resting respiration is not different from the effect measured with 10^{-5} M-KCN, the values of excess oxygen consumed would be 2·7 ± 0·4 nmol min^{-1} photophore^{-1} in 10^{-4} M-KCN and 1·8 ± 0·5 nmol photophore^{-1} in 10^{-3} M-KCN.

DISCUSSION

Resting respiration

In the absence of any detectable light emission, an isolated photophore of the teleost *Porichthys notatus* shows a respiration rate of 0·07 ± 0·01 nmol min^{-1}.

The mean fresh weight of a photophore is 0·47 ± 0·04 mg ($N = 43$) and the estimated respiration rate is 140 nmol g^{-1} min^{-1}. In order to get a better appreciation of the resting metabolism of a photophore, it would be interesting to compare different types of light organ with respect to their oxygen consumption. Unfortunately, data are not available for comparison with our measurements. As an effector organ, respiration of the photophore may be compared with that of muscle. The respiration rate of a striated muscle, the frog sartorius, in the resting state (20 °C) is 30 nmol g^{-1} min^{-1}; the respiration of smooth muscle of both vertebrates and invertebrates is about

70 nmol g^{-1} min^{-1} (Baguet & Gillis, 1967). The still larger resting respiration of the photophore is probably related to the particularly well-developed surface membrane of the photocytes (Strum, 1968) which increases the surface-to-volume ratio of the photogenic cells.

An adult specimen of *Porichthys* bears approximately 800 functional dermal photophores, which means that all the light organs would consume 56 nmol min^{-1}. Although the oxygen consumption of *Porichthys notatus* has not been measured, it has been reported that the resting respiration rate of *Porichthys porosissimus*, a related luminescent species living in the Atlantic Ocean, is 1700 μmol h^{-1} kg^{-1} (Moore, 1970). If we assume a similar resting metabolism for *Porichthys notatus*, one specimen (about 100 g) would consume 170 μmol h^{-1}. The oxygen consumption of the 800 photophores would be 3·4 μmol h^{-1} corresponding to about 2 % of the resting respiration rate of the fish. This fraction would increase in importance when the photophores were luminescing.

Effects of KCN

The present results indicate a dual effect of KCN on isolated photophores: (i) at those concentrations that do not evoke a luminescent response (10^{-6} and 10^{-5} M), cyanide reduces the photophore respiration and (ii) when KCN induces the production of light, it simultaneously enhances the oxygen consumption of the photophore.

As there is good evidence that cyanide blocks the mitochondrial respiration of fish in the same way as in mammals (Gumbmann & Tappel, 1962), it is likely that the excitatory effect on the photophore can be explained by an inhibition of the mitochondrial respiration of the tissues. The effect increases from 10^{-6} to 10^{-5} M KCN and we could reasonably expect a complete inhibition of respiration of photophores at 10^{-4} or 10^{-3} M as was observed on isolated pieces of skin surrounding the light organs. It is hardly credible that the stimulating effect of cyanide, observed exclusively on luminescing organs, could be explained by an increase in mitochondrial respiration. Instead, since the biochemical mechanism for luminescence in *Porichthys* photophores consists of a luciferase-catalysed oxidation of luciferin by oxygen (Tsuji, Haneda, Lynch & Sugiyama, 1971; Tsuji *et al.* 1977), we suggest that the increase of oxygen consumption with light originates from the oxidation of luciferin by molecular oxygen. Thus, cyanide would act as a classic inhibitor of cellular respiration, the effect being only visible on non-luminescing preparations, and concealed in luminescing photophores.

Photophores treated with 10^{-6} M cyanide for 30 min respond to adrenalin or norepinephrine by a light response which is similar to the response of untreated photophores (Baguet, 1983). This suggests that mitochondrial respiration is not essential for the process of light generation.

One possible explanation for the oxygen uptake increase would be a direct influence of cyanide on the luciferin–luciferase system. Luminescence produced by crystalline luciferin and purified luciferase from the luminescent ostracod *Cypridina hilgendorfii* is inhibited by cyanide, the effect being strongly dependent upon luciferin concentration (Johnson, Shimomura & Saiga, 1962). Since the luminescent system of *Porichthys* photophores appears to be similar (if not identical) to the luminescent system of *Cypridina* (Cormier *et al.* 1967; Tsuji *et al.* 1971), we can reasonably exclude a direct

stimulating effect of KCN. It must be elucidated why KCN does not inhibit the light reactions.

It might be argued that KCN does not act directly on the photocytes, but indirectly on the neural processes of the photophore. Radioenzymatic assays and light microscope radiographic studies have demonstrated the uptake and storage of ^{3}H-norepinephrine in the nerve fibres innervating *Porichthys* photophores (Anctil, Brunel & Descarries, 1981). Cyanide should impair the energy supply at the neuro-photocyte junction: run-down ionic pumps would cause depolarization of nerve terminals and trigger release of norepinephrine. We have shown in *Porichthys* photophores that the KCN luminescence cannot be explained by a release of norepinephrine from nervous elements: (i) phentolamine, an α-adrenergic antagonist, does not affect the light response to KCN, neither on photophores, nor on isolated photocytes (Christophe & Baguet, 1982). (ii) $MnCl_2$ (40 mM) does not affect the KCN light response while it depresses by 80 % the slow light responses to norepinephrine (Christophe & Baguet, 1982). (iii) The detergent Triton X-100 (10 % concentration) applied on isolated photophores or isolated photocytes, suppresses any response to norepinephrine without impairing the light response to KCN which should act primarily on intracellular sites (Christophe & Baguet, 1982; 1983). Moreover, the time course of oxygen consumption associated with the light response evoked by 10^{-4} and 10^{-3} M-KCN is different from the time course of oxygen consumption observed during the response to epinephrine or norepinephrine (Baguet, 1983).

We suggest that the stimulating effect of cyanide could be interpreted in terms of an activation of luciferase by calcium ions. This hypothesis is supported by the demonstration that the activity of *Cypridina* luciferase, a metalloenzyme, increases with Ca^{2+} concentration (Lynch, Tsuji & Donald, 1972).

By inhibiting the processes of cellular respiration, the mechanisms sequestering calcium would be inhibited and the increase of free Ca^{2+} could stimulate the catalytic oxidation of luciferin by luciferase, and stimulate oxygen consumption of the photophore.

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REFERENCES

ANCTIL, M., BRUNEL, S. & DESCARRIES, L. (1981). Catecholamines and 5-hydroxytryptamine in photophores of *Porichthys notatus*. *Cell Tissue Res.* **219**, 557–566.

BAGUET, F. (1975). Excitation and control of isolated photophores of luminous fishes. *Prog. Neurobiol.* **5**, 97–125.

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

BAGUET, F. & GILLIS, J. M. (1967). The respiration of the anterior byssus retractor muscle of *Mytilus edulis* (ABRM) after a phasic contraction. *J. Physiol., Lond.* **188**, 67–82.

CHRISTOPHE, B. & BAGUET, F. (1982). Potassium cyanide stimulation of isolated photocytes from a luminescent fish. *Archs int. Physiol. Biochim.* **90**, P10–P11.

CHRISTOPHE, B. & BAGUET, F. (1983). Luminescence of isolated photocytes from *Porichthys photophores*: adrenergic stimulation. *J. exp. Biol.* **104**, 183–192.

CORMIER, A. J., CRANE, J. M. & NAKANO, Y. (1967). Evidence for the identity of the luminescent system of *Porichthys porosissimus* (fish) and *Cypridina hilgendorfii* (Crustacean). *Biochem. biophys. Res. Commun.* **29**, 747–752.

GREENE, C. W. (1899). The phosphorescent organs in the toad fish *Porichthys notatus* Girard. *J. Morph.* **15**, 667–696.

GUMBMANN, M. & TAPPEL, A. L. (1962). The tricarboxylic and cycle in fish. *Archs Biochem. Biophys.* **98** 262–270.

JOHNSON, F. H., SHIMOMURA, O. & SAIGA, Y. (1962). Action of cyanide on Cypridina luciferin. *J. cell. comp. Physiol.* **59**, 265–272.

LYNCH, R. V., TSUJI, F. I. & DONALD, D. H. (1972). Evidence for a calcium requirement in the Cypridina bioluminescence reaction. *Biochem. biophys. Res. Commun.* **46**, 1544–1550.

MOORE, R. H. (1970). Diurnal variations in the activity and metabolism of the atlantic midshipman *Porichthys porosissimus*. *Contr. Mor. Sci.* **15**, 33–44.

SENDROY, J., DILLON, R. T. & VAN SLIJKE, D. D. (1934). Studies of gas and electrolyte equilibrium in blood. XIX. The solubility and physical state of uncombined oxygen in blood. *J. biol. Chem.* **105**, 597–632.

STRUM, J. M. (1969). Fine structure of the dermal luminescent organs, photophores, in the fish, *Porichthys notatus*. *Anat. Rec.* **164**, 433–462.

TSUJI, F. I., HANEDA, Y., LYNCH, R. V. & SUGIYAMA (1971). Luminescence cross-reactions of *Porichthys* luciferin and theories on the origin of luciferin in some shallow-water fishes. *Comp. Biochem. Physiol.* **40**A, 163–179.

TSUJI, F. I., DELUCA, M., BOYER, P. D., ENDO, S. & AKUTAGAWA, M. (1977). Mechanism of the enzyme-catalyzed 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.