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METABOLIC CONTROL OF SPONTANEOUS GLOWING IN ISOLATED PHOTOPHORES OF *PORICHTHYS*

BY J. F. REES AND F. BAGUET

Laboratoire de Physiologie générale et des animaux domestiques, Université Catholique de Louvain, Place de la Croix du Sud, 5, B-1348 Louvain-la-Neuve, Belgium

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SUMMARY

1. Isolated photophores of the epipelagic fish *Porichthys notatus* become spontaneously luminescent after a few hours in saline at 20°C. This luminescence is not significantly affected by the adrenergic antagonists propranolol and phentolamine.

2. The addition of glucose, mannose, fructose or galactose in saline inhibits glowing according to their respective rates of metabolism in fishes.

3. Glycolytic metabolites, such as phosphoenolpyruvate and pyruvate, prevent or abolish the spontaneous glowing. Monoiodoacetate (IAA), the classical inhibitor of glycolysis, reverses the inhibitory effect of glucose but not of pyruvate.

4. We suggested that glycolysis provides an energy supply to an inhibitory mechanism that keeps the *Porichthys* photophore in a non-luminescent state.

INTRODUCTION

Some photophores isolated from the epipelagic luminescent fish *Porichthys* show a feeble spontaneous luminescence after dissection and immersion in saline. After 2 h at room temperature, all isolated photophores in saline are luminescent. This spontaneous luminescence can impair the responsiveness of the isolated organ to electrical stimulation (Baguet & Case, 1971) and restricts the duration of the experimental period. Since dinitrophenol (DNP) and potassium cyanide (KCN) induce a bright luminescence when applied to isolated photophores, it is likely that these metabolic inhibitors block an inhibitory mechanism that prevents luminescence in the resting photophore (Baguet, 1975). Spontaneous luminescence might be due to a defect of this inhibitory mechanism caused, for example, by the absence of an energy source.

As glucose is one of the most fundamental fuel substances for living cells, we investigated its possible role as an energy source. We attempted to prevent or abolish the spontaneous luminescence of isolated photophores by adding different sugars in saline. As glycolysis is the principal metabolic pathway of glucose, we investigated how glycolytic metabolites and the glycolytic inhibitor monoiodoacetic acid (IAA)

Key words: glycolysis, luminescence, fish, photophore.

can affect the development and extinction of the spontaneous luminescence. Our results strongly support the view that glycolysis could be an important metabolic pathway in the *Porichthys* photophore and may control the luminescence in the isolated organ.

MATERIALS AND METHODS

Dissection of the photophore

Five specimens of the midshipman fish *Porichthys notatus* shipped by Pacific Bio-Marine Laboratories (Venice, CA) were kept in aquaria provided with aerated running sea water. After anaesthesia with quinaldine (0·037 % w/v) in sea water, a strip of six photophores was excised and immersed in saline.

Mounting the photophores

The six photophores were isolated under a binocular microscope and laid in six small cylindrical chambers drilled in a circular plastic plate; the chambers were filled with 180 μl of saline. The plate rotated around a central axis fixed on the side of the box covering the photomultiplier (IP21). Thus, each photophore was successively positioned above the photocathode by rotating the plate.

Recording the light emission

The light-emitting area of the photophore facing a circular opening was 2 cm from the photocathode. The signal was amplified and displayed on a strip chart recorder. The apparatus was calibrated using a tritium-irradiated phosphor light source (Betalight, Nuclear Enterprises Ltd) emitting on an area of 2 mm^2, in the same location as the photophore.

Solutions

D-sugars (Merck), L-amino acids (Merck), (−)phentolamine (Ciba), (±)propranolol (Sigma), phosphoenolpyruvate (Sigma), pyruvate (Merck) and iodoacetic acid (Sigma) were dissolved in buffered physiological saline containing (in $mmol\,l^{-1}$): NaCl, 150; KCl, 7·5; $CaCl_2$, 3·5; $MgCl_2$, 2·4; pH 7·3 with Tris-HCl, 20, just prior to use.

Experimental procedure

To study the effects of a given substance on photophore extinction, we measured the half-extinction rate (ER_{50}), i.e. the rate of the luminescence decay from the maximal intensity to half the maximal intensity (see Fig. 1) after injecting 20 μl of the dissolved substance at the time of maximal glowing of the photophore; the sensitivity was estimated by comparing the values of ER_{50} measured on several series of six photophores, each one treated with a different concentration ranging from 10^{-6} to $10^{-2}\,mmol\,l^{-1}$. ER_{50} was expressed as a percentage of ER_{50} measured when $5{\cdot}5\times10^{-3}\,mol\,l^{-1}$ glucose was applied to one of the six photophores of the experimental series.

Each mean value is expressed with its standard error (S.E.M.) and number of preparations, N. Significance of difference between means was calculated using Student's t-test.

RESULTS

The time course of the spontaneous luminescence

Fig. 1 gives a typical example of the glowing produced by an isolated photophore immersed in glucose-free saline after dissection. We defined four parameters to characterize the shape of the light curve: the latency time (TL), i.e. the time elapsing between the end of the dissection and the first detectable luminescence produced by the isolated preparation; TL_{max}, the time elapsing from the end of TL until the maximal intensity of light (L_{max}) was produced; and the half-extinction time, i.e. the time necessary to return to 50 % of the maximal light emission ($T_{1/2,ext}$). The values of these parameters measured on 13 preparations showed that the emission of light was a very slow phenomenon: a preparation began to luminesce after a mean time of $76{\cdot}8 \pm 8{\cdot}9$ min following immersion in glucose-free saline. After this long latency, it took $58{\cdot}0 \pm 6{\cdot}0$ min to reach a maximal light emission of $182{\cdot}6 \pm 19{\cdot}7$ Mquanta s^{-1}. The half-extinction time was $37{\cdot}1 \pm 3{\cdot}1$ min, which corresponds to a half-extinction rate of $1{\cdot}35\,\%\,L_{max}\,min^{-1}$. The low variance for the means of the different parameters suggests that the phenomenon is rather constant among the different organs from the same fish and even among photophores of different fishes. The emission latency time was the only parameter that fluctuated among the photophores from different fishes; it could vary from 20 to 120 min.

It might be argued that an isolated photophore becomes luminescent due to the liberation of neuromediators associated with the progressive alteration of nerve

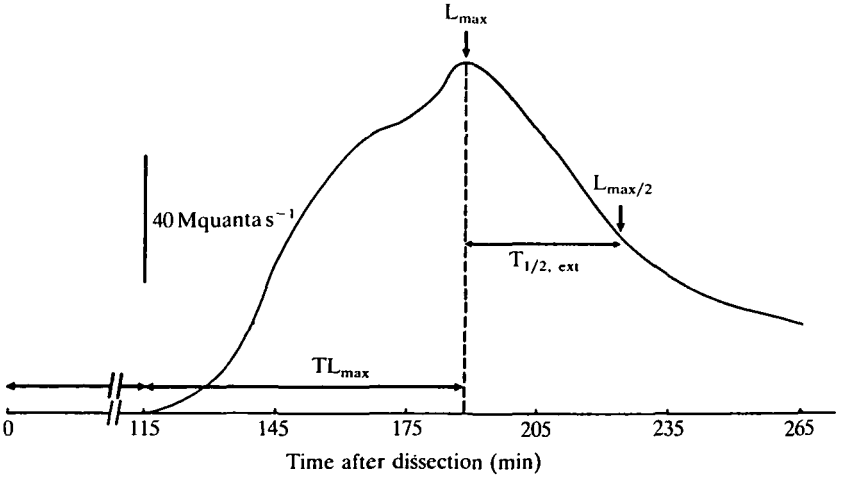

Fig. 1. Typical record of luminescence intensity of a spontaneously glowing photophore in glucose-free saline as a function of time after dissection. See text for definitions of the abbreviations.

terminals after dissection of the organ. Adrenaline and noradrenaline present in the nerve varicosities (Anctil, Descarries & Watkins, 1984) should bind to α- and β-adrenergic receptors present on the photocell membranes (Christophe & Baguet, 1985) and stimulate light production. However, the addition in saline of 10^{-5} mol l^{-1} phentolamine, an α-adrenergic antagonist, or 10^{-5} mol l^{-1} propranolol, a β-adrenergic antagonist, did not significantly change the parameters of the spontaneous luminescence (Table 1).

Effect of glucose on spontaneous glowing

An isolated photophore immersed in saline containing 5·5 mmol l^{-1} glucose did not show any spontaneous luminescence even after 20 h; after this long period, the photophore remained excitable by adrenaline and noradrenaline (Fig. 2A). Moreover, the addition of 5·5 mmol l^{-1} glucose to a spontaneously luminescent photophore at its maximal level of light production induced a rapid extinction. In the example shown in Fig. 2B, extinction began about 20 s after addition of glucose and was complete in about 2 min. In this case, the half-extinction rate was 0·92 % L_{max} s^{-1}. There was no relationship between the half-extinction rate and the light intensity when glucose was added. However, the half-extinction rate depended on the glucose concentration.

In Fig. 3, the half-extinction rate is expressed in % L_{max} s^{-1} as a function of the log glucose concentration. At 10^{-6} mol l^{-1} glucose, the half-extinction rate was similar to the value measured on control photophores injected with glucose-free saline; the lowest concentration at which extinction was significantly accelerated was 10^{-5} mol l^{-1} glucose. Above this concentration, the half-extinction rate increased with the glucose concentration. The maximal half-extinction rate occurred at $5{\cdot}5\times10^{-3}$ mol l^{-1} glucose and was 46 times higher than in the control. There was no further increase at 10^{-2} mol l^{-1} glucose. The ED_{50}, i.e. the concentration inducing 50 % of the maximal effect, was estimated to be $2{\cdot}4\times10^{-4}$ mol l^{-1} glucose. The 'extinction effect' of glucose was reversible; after washing out the preparation with

Table 1. *Effects of 10^{-5} mol l^{-1} phentolamine and 10^{-5} mol l^{-1} propranolol on the parameters of spontaneous glowing*

	TL (min)	TL_{max} (min)	L_{max} (Mquanta s^{-1})
Controls ($N = 8$)	76·7 ± 6·1	49·7 ± 3·7	98·1 ± 18·4
+ Phentolamine ($N = 8$)	72·6 ± 4·3	43·4 ± 3·1	122·9 ± 27·4
+ Propranolol ($N = 7$)	68·2 ± 8·1	58·3 ± 5·4	57·5 ± 11·9

Both antagonists were added immediately after the dissection.
Mean values ± S.E.M., N = number of experiments.
There were no significant differences between experimental and control values ($P < 0{\cdot}05$).
See text for definitions of abbreviations.

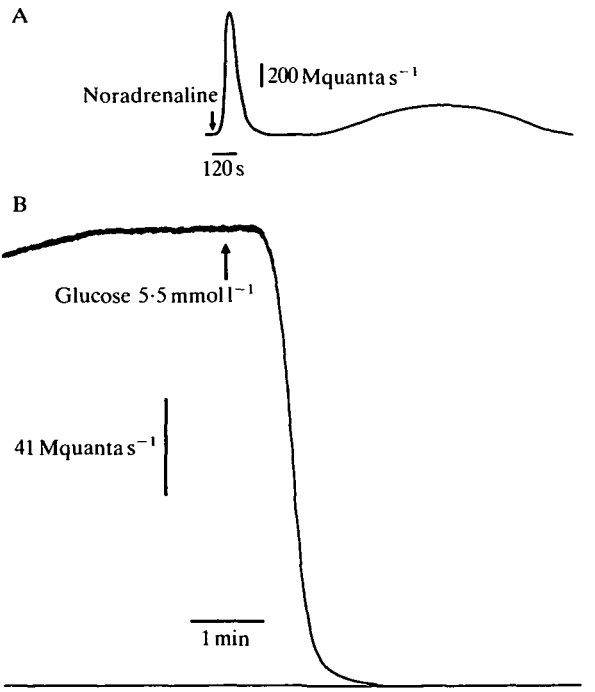

Fig. 2. (A) Record of the typical luminescence evoked by the application (arrow) of 10^{-4} mol l^{-1} noradrenaline to an isolated photophore immersed in saline containing 5·5 mmol l^{-1} glucose. (B) Record showing the rapid extinction of spontaneous luminescence following the addition of 5·5 mmol l^{-1} glucose to glucose-free saline. Glucose was added when the luminescence was maximal.

glucose-free saline, the preparation started to luminesce at the typical slow rate of the spontaneous luminescence.

Effects of various sugars on spontaneous luminescence

Mannose, fructose and galactose

In fish, the capacity of sugars to serve as cellular substrates is about 10-fold higher for glucose and mannose than for fructose (Hochachka, 1969). We compared the half-extinction rates of luminescent photophores after addition of glucose, mannose, fructose or galactose at concentrations varying from 10^{-6} to 10^{-2} mol l^{-1}. The value of ER_{50} for each sugar concentration is expressed as a percentage of the ER_{50} when $5{\cdot}5\times10^{-3}$ mol l^{-1} glucose was the substrate (Fig. 4).

Glucose and mannose had similar ED_{50} values of approximately $2{\cdot}5\times10^{-4}$ mol l^{-1}, although the threshold of the extinction was 10^{-4} mol l^{-1} for mannose and 10^{-5} mol l^{-1} for glucose. Even though fructose had a similar threshold at 10^{-4} mol l^{-1}, the value of the half-extinction rate remained very low, ranging from 2·38 to 6·63 % from 10^{-4} to $1{\cdot}8\times10^{-3}$ mol l^{-1}. At higher concentrations, the value was only $30{\cdot}53\pm4{\cdot}68$ % of that measured for glucose. ED_{50} for fructose was

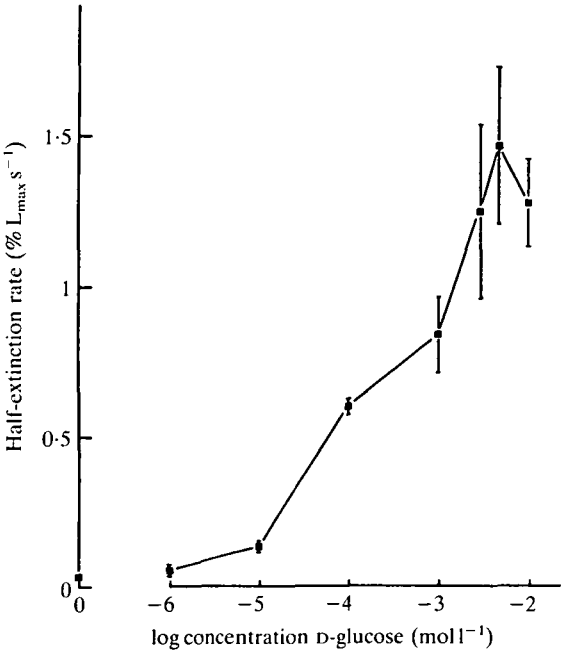

Fig. 3. Effect of glucose concentration on the half-extinction rate of luminescence in spontaneously glowing photophores. Each point (mean ± S.E.M.) represents four experiments.

$2{\cdot}8\times10^{-3}$ mol l^{-1}. The extinction effect of galactose was negligible: it was detectable only at 8×10^{-3} mol l^{-1}, when there was only a two-fold increase compared with the control. These results show that the ability of these four sugars to inhibit the spontaneous luminescence of an isolated photophore seems to parallel their relative capacity to be metabolized by cells.

2-Deoxy-D-glucose (2-DG)

Although glucose and 2-DG penetrate the cell membrane by the same transport mechanism, 2-DG is not metabolized by the cell (Sols & Crane, 1954; Tower, 1958). We attempted to determine how glucose metabolism induces extinction by studying the effects of 2-DG on luminescent photophores. Fig. 5 shows the typical time course of the light level of a spontaneously luminescent photophore after addition of 10^{-2} mol l^{-1} 2-DG. After a slight increase in the light level, there was a temporary 30% decrease lasting about 1 min. Luminescence then increased again to reach a level after 4 min that was somewhat higher than that prior to the addition of 2-DG.

In a series of nine photophores we observed a significant increase of $10{\cdot}0 \pm 2{\cdot}2$% of the light level ($P < 0{\cdot}01$), 36 ± 4 s after addition of 2-DG; luminescence then decreased slowly and after $5{\cdot}2 \pm 0{\cdot}9$ min reached a level corresponding to $51{\cdot}4 \pm 6{\cdot}8$% of that measured prior to the addition of 2-DG. Luminescence then increased again and reached its initial level after another 15 min. The addition of

$5{\cdot}5\times10^{-3}$ mol l^{-1} glucose at this time always induced a typical rapid and complete extinction.

Extinction and glycolysis

These results suggest that extinction of a spontaneously luminescent photophore is associated with carbohydrate metabolism. Assuming that glycolysis could be a possible metabolic pathway, we investigated the capacity of pyruvate and phosphoenolpyruvate (PEP) to accelerate extinction (Fig. 6). The half-extinction rate was

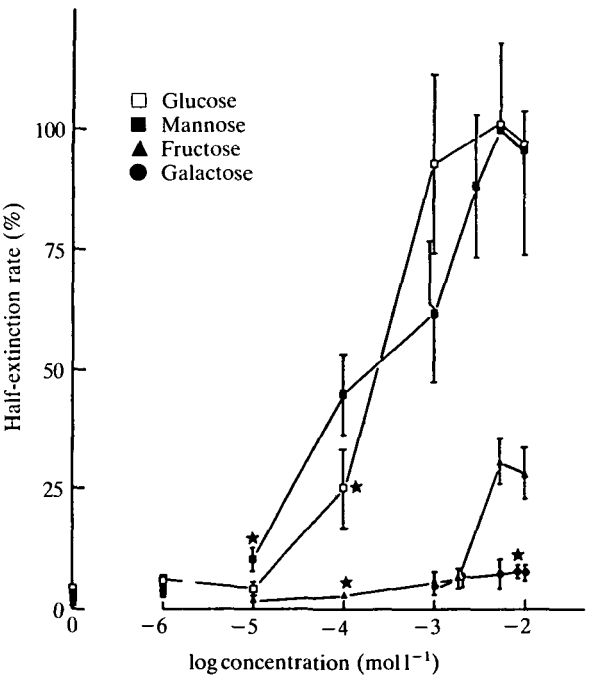

Fig. 4. Dose–response curves that illustrate the effects of different sugars on the half-extinction rate (ER_{50}) of spontaneous glowing. All values of ER_{50} are expressed as percentages of that measured for glucose at 5·5 mmol l^{-1}. Each point (mean ± S.E.M.) represents four experiments. * denotes the first value which significantly differs from controls for each sugar ($P<0{\cdot}05$).

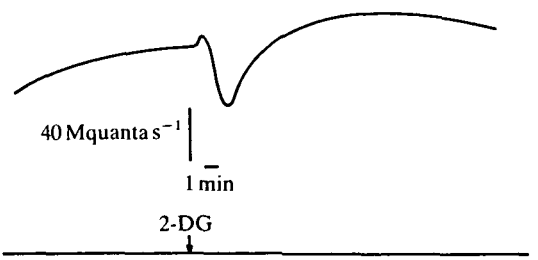

Fig. 5. Typical record showing the effects of the addition of 10 mmol l^{-1} 2-deoxy-D-glucose (2-DG) in saline at the maximal level of spontaneous glowing.

increased more by pyruvate than by phosphoenolpyruvate. The values of ED_{50} were estimated, respectively, as $7{\cdot}6\times10^{-4}$ mol l^{-1} and $4{\cdot}3\times10^{-4}$ mol l^{-1}. At higher concentrations, pyruvate was more effective and PEP less effective than glucose. This is particularly evident at $5{\cdot}5\times10^{-3}$ mol l^{-1}: the maximal ER_{50}, relative to that in 5·5 mmol l^{-1} glucose, was 135·2 ± 2·5 % in pyruvate and only 69·5 ± 17·0 % in PEP. The addition of pyruvate or PEP in saline also prevented the spontaneous luminescence of all isolated photophores. As glucose and these glycolytic metabolites prevent the development or induce the extinction of any spontaneous luminescence, we must assume that glycolysis is an essential pathway for maintaining an isolated photophore in a non-luminescent state. As a consequence, a blockade of glycolysis should induce spontaneous luminescence in an isolated photophore. Monoiodoacetate (IAA) at 5×10^{-4} mol l^{-1}, a classical blocking agent of glycolysis (Webb, 1966), added after extinction of spontaneous glowing following the addition of $5{\cdot}5\times10^{-3}$ mol l^{-1} glucose, induced luminescence (Fig. 7). However, when pyruvate instead of glucose was used to inhibit glowing, no preparations ($N = 7$) became luminescent following treatment with IAA.

Amino acids (Ala, Arg, Glu, Gly, His, Leu, Ser), tested at 5·5 mmol l^{-1}, had no inhibitory effect either on spontaneous or on IAA-evoked glowing.

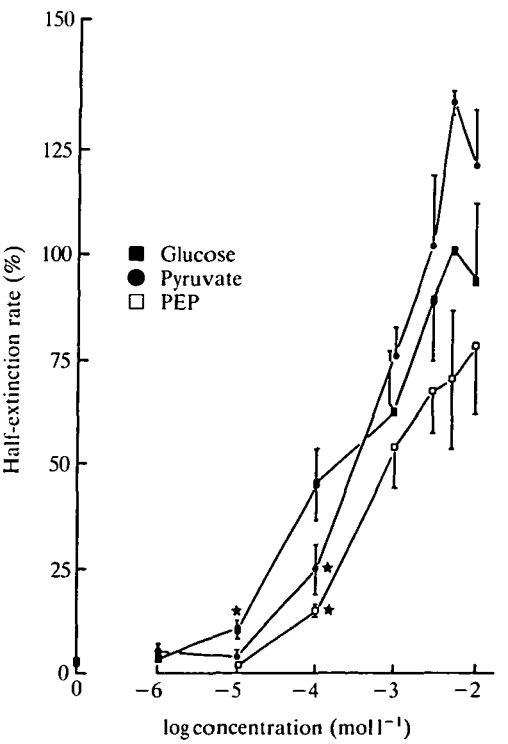

Fig. 6. Effects of the concentration of pyruvate, phosphoenolpyruvate (PEP) and glucose on the ER_{50} of spontaneously glowing photophores. Conventions as in Fig. 4. Each point represents four experiments.

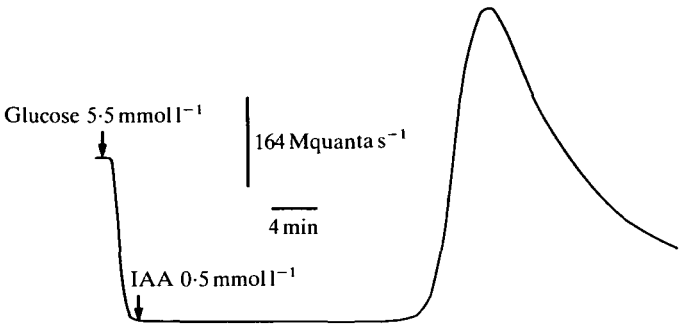

Fig. 7. Record showing the reversal by 0·5 mmol l^{-1} iodoacetate (IAA) of the inhibitory effect of 5·5 mmol l^{-1} glucose on spontaneous glowing.

DISCUSSION

The spontaneous luminescence that isolated photophores slowly develop after immersion in glucose-free saline is not affected by adrenergic antagonists. This observation rules out the possibility that glowing could be induced by a release of neuromediators from damaged nerve endings. However, D-glucose is very effective in preventing development or inducing the rapid extinction of any spontaneous luminescence. As the photogenic cells remain excitable by natural neuromediators after extinction by glucose, glowing is probably not associated with irreversible cellular damage. The presence of glucose in saline is essential to maintain the functional properties of effector tissues such as mammalian smooth or cardiac muscles. The *Porichthys* photophore shows an exceptionally high resting respiration rate, about five times that of smooth muscle (Mallefet & Baguet, 1984). As metabolic inhibitors such as DNP or KCN induce luminescence in the photophore, Baguet (1975) proposed the presence of an energy-requiring inhibitory mechanism. In this context, we suggest that spontaneous glowing is induced by a progressive metabolic deficiency; glucose should reverse the situation by reactivating the metabolism of the photogenic cells.

Two arguments suggest that glucose effectively acts *via* the cell metabolism to maintain the photophore in a non-luminous state. (1) The relative extinction efficiency of the tested sugars (glucose = mannose > fructose > galactose = 0) seems to parallel their relative ease of metabolization in fish (Hochachka, 1969) and in other vertebrates. (2) 2-Deoxy-D-glucose, which competes with glucose for the phosphorylating enzyme hexokinase, is not metabolized and remains as 2-deoxy-D-glucose-6-phosphate in the cells (Sols & Crane, 1954). It does not induce the typical extinction effect of glucose. The reason why 2-DG induces a partial and temporary extinction is not yet understood.

Glycolysis seems to be the major pathway for glucose degradation; all the enzymes of this pathway have been detected in various fish tissues (for a review see Walton & Cowey, 1982). As pyruvate and phosphoenolpyruvate, two end-products of glycolysis, exhibit the same effect as glucose, it is suggested that glucose acts through the

glycolytic pathway to exert its inhibitory action. This assumption is supported by the results obtained with monoiodoacetic acid (IAA). IAA specifically inhibits glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase. Our results suggest that IAA could act similarly in *Porichthys* photophores; pyruvate, which bypasses the assumed site of inhibition by IAA, but not glucose, suppresses the IAA-induced luminescence. This observation excludes the possibility that IAA could trigger the luminescence by a direct activation of the luciferin–luciferase system, as showed in aequorin luminescence (Shimomura, Johnson & Morise, 1974). Because light production is evoked by IAA treatment, glycolysis seems to be implicated in maintaining the photophore at a resting non-luminous level. However, the efficiency of pyruvate as an inhibitor of spontaneous glowing suggests that the Krebs cycle and oxidative phosphorylation should be implicated in the glucose action. This assumption is supported by the observation that glucose stimulates the resting oxygen consumption (J. Mallefet, unpublished result) and that KCN, which inhibits photophore respiration, simultaneously elicits light production. The absence of any effect of amino acids indicates that they are probably poorly metabolized in the photophore.

In *Porichthys* photophores, glycogen stores appear to be very scarce. Strum (1969) did not observe typical images of glycogen rosettes or small chains, as in all fish muscle; only dense clusters of dark particles were evident in a few areas of the photogenic cells and are believed to represent glycogen.

However, blood vessels are very numerous in the connective tissue: a vast capillary network is intimately associated with the photogenic and supportive cells. Glucose is probably carried by the blood to the photogenic tissue. We have no information about the concentration of plasma glucose in *Porichthys*, but in other fish species it is present at 1–8 mmol l^{-1} (Cowey, de la Higuera & Adron, 1977; Cornish & Moon, 1985; Zammit & Newsholme, 1979), the same concentration range that induces the maximal extinction effect.

All these results confirm the previous hypothesis (Baguet, 1975; Mallefet & Baguet, 1984) that the luminescent system in *Porichthys* photophores is controlled by an inhibitory mechanism. They further show that this mechanism is under metabolic control: glucose breakdown by glycolysis, the Krebs cycle and oxidative phosphorylation should produce one or several metabolites mediating the inhibition of the luminescent system. After a time in glucose-free saline, a photophore becomes spontaneously luminescent as metabolism slows following dissection, or is rapidly lowered by treatment with metabolic inhibitors. Experiments are in progress to specify the nature of the metabolic control of photogenesis.

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