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METABOLIC CONTROL OF LUMINESCENCE IN ISOLATED PHOTOPHORES OF *PORICHTHYS*: EFFECTS OF GLUCOSE ON OXYGEN CONSUMPTION AND LUMINESCENCE

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Summary

1. Basal oxygen consumption of isolated photophores from *Porichthys* sp. at rest, i.e. without light emission, increased significantly from $0.101\pm0.021 nmol min^{-1}$ to $0.173\pm0.016 nmol min^{-1}$ in response to the addition of $5.5 mmol l^{-1}$ glucose.

2. $5.5 mmol l^{-1}$ glucose pretreatment modified the time course of the two phases of adrenaline-induced luminescence; an increase in oxygen consumption was observed during the fast phase of light production but a decrease occurred during the slow phase of luminescence.

3. Pretreatment of isolated photophores with $5.5 mmol l^{-1}$ glucose totally inhibited the light emission induced by $1 mmol l^{-1}$ potassium cyanide. With this treatment, the respiration rate decreased progressively and after 40min reached a value not significantly different from zero.

4. Even after blockage of cellular respiration by cyanide, an increase in the rate of oxygen consumption was observed during the fast adrenaline-induced luminescence.

5. Glucose utilisation by glycolysis or by oxidative metabolism may provide energy to an inhibitory mechanism that maintains the photophores in a non-luminescent state.

6. We suggest that the oxygen consumed during the fast phase of adrenaline luminescence could represent the activity of an extramitochondrial oxidative pathway involved in the light reaction.

Introduction

Luminous organs of the teleost fish *Porichthys* remain functional when isolated from the fish and immersed in Young's teleost saline (Baguet and Case, 1971). In these *in vitro* conditions, luminescence can be evoked by external electrical or chemical stimuli. Christophe and Baguet (1983, 1985) described a biphasic luminescence triggered by adrenaline or noradrenaline application: the fast phase (L_{max1}: maximum intensity within 2min of stimulus application) is under alpha-adrenergic receptor control while the slow phase (L_{max2}: maximum intensity after 25min of stimulation) shows some beta-adrenergic

Key words: *Porichthys notatus*, *Porichthys myriaster*, luminescence, oxygen consumption, metabolic control.

receptor characteristics. It has been observed that, after immersion in saline for several hours, isolated organs developed a spontaneous long-lasting luminescence (Baguet and Case, 1971). This slow spontaneous luminescence was abolished by the addition of glucose to the saline (Rees and Baguet, 1988). It has been proposed that the presence of an energy-requiring inhibitory mechanism maintains the light organ in a non-luminescent state (Baguet, 1975; Mallefet and Baguet, 1984). When iodoacetic acid (IAA), which specifically inhibits glycolysis, is applied to the photophores a luminescence similar to that seen during the spontaneous slow phase is seen, suggesting that the glycolytic pathway may provide energy to this inhibitory mechanism (Rees *et al.* 1989).

This hypothesis was supported by the observation that blockade of cellular respiration by potassium cyanide (KCN) induced isolated photophores to glow (Mallefet and Baguet, 1984). Since pyruvate inhibits spontaneous and IAA-induced luminescence, it has been suggested that glycolysis might inhibit luminescence by passing its end product on for oxidative metabolism (Rees *et al.* 1989).

The aim of the present work was to demonstrate that glucose can be metabolized by the isolated photophore and that this metabolism is involved in the control of photogenesis in isolated *Porichthys* photophores.

Our results show that glucose significantly increased the oxygen consumption of the non-luminescent photophore and inhibited the luminescence evoked by blockade of photophore respiration by KCN. Such poisoned and glucose-treated preparations still responded to the putative physiological mediator adrenaline with the typical biphasic luminescence associated with a large increase in oxygen consumption. The origin of the oxygen uptake after blockade of respiration and the nature of metabolic control in the isolated photophore are discussed.

Materials and methods

Specimens of *Porichthys notatus* and *Porichthys myriaster*, shipped by Pacific Bio-Marine Laboratories (Venice, California), were kept in separate aquaria (70l) provided with aerated running sea water (18°C).

After anaesthesia of the fish with quinaldine (Allen and Sills, 1973), pieces of skin containing photophores were excised from the pleural, ventral, gular and branchial regions (using the terminology of Greene, 1899) and were immersed in saline. Following dissection, one photophore was isolated under a binocular microscope and was placed in a specially designed recording chamber containing 0.95ml of saline. A description of the apparatus used in this work has already appeared (Mallefet and Baguet, 1989). Light calibration was performed using a tritium-irradiated phosphor emitting at the same wavelength as the light organ; calibration and control of stability of the polarographic oxygen sensor were checked before and after each experiment. Light units are calculated in quanta s^{-1} and oxygen consumption is expressed in nmol min^{-1}.

Solutions

Based on electrolyte analysis of *Porichthys* blood samples (J. Mallefet, unpublished results), we used a saline modified from that of Young (1933) containing 150mmol l^{-1}

NaCl, 7.5mmol l^{-1} KCl, 3.5mmol l^{-1} $CaCl_2$, 2.5mmol l^{-1} $MgCl_2$, buffered to pH7.3 with 20mmol l^{-1} Tris–HCl. Stock solutions of D-glucose, potassium cyanide (Merck) and L-adrenaline (Sigma) were dissolved in fully aerated saline just prior to use.

Experimental procedure

After calibration and stability control of the polarographic oxygen sensor, the oxygen consumption and the luminescence of an isolated photophore were simultaneously measured for 10min prior to the addition in saline of 9.6 μl of different stock solutions. This measurement corresponds to the resting rate of oxygen consumption of the photophore provided it was non-luminescent (spontaneously luminescent photophores were discarded as they were considered to be in poor physiological condition). The mean time between dissection and recording of the resting rate of oxygen consumption was standardized at approximately 10min to avoid the decrease in light emission described by Christophe and Baguet (1985). In a first series of experiments, glucose was added to a final concentration of 5.5 mmol l^{-1} and oxygen consumption was recorded over a period of 20min. Statistical analysis was performed using a paired *t*-test.

In a second series of experiments, after the initial 20min recording time in glucose saline, KCN or adrenaline was added to give a final concentration of 1 or 0.1mmol l^{-1}, respectively. Oxygen consumption and luminescence were measured for 60min after the addition of cyanide and for 30min after the addition of adrenaline.

In a third series of experiments, after the initial 20min recording time in glucose saline, KCN was added to a final concentration of 1mmol l^{-1}. 40min later 0.1mmol l^{-1} adrenaline was applied. Oxygen consumption and luminescence were then measured over the next 60min.

In control experiments, photophores were maintained for the same period in glucose-free saline and then stimulated by the addition of either adrenaline or cyanide. Each mean value is expressed with its standard error (mean ± S.E.M.) and the number of preparations tested (*N*). The significance (*P*) of differences between means was based on analysis of variance (ANOVA) and on *t*-tests.

Results

Effects of glucose on the resting rate of oxygen consumption

Isolated non-luminescent photophores immersed in saline showed a very stable oxygen uptake (0.101±0.021nmol min^{-1}) that increased significantly in two steps on addition of 5.5mmol l^{-1} glucose (Fig. 1).

During the first minute, the mean respiration rate (N=6) increased to 0.128±0.03nmol O_2 min^{-1}; 9min later it increased further to 0.173±0.02nmol O_2 min^{-1}. This new value corresponded to a 89.5±23% increment above the previous resting level (P<0.001).

Although the resting rate of oxygen uptake was very stable, it showed large variations among the photophores from the same fish. The magnitude of the effect of glucose varied as a function of the intensity of the resting respiration rate measured before the treatment:

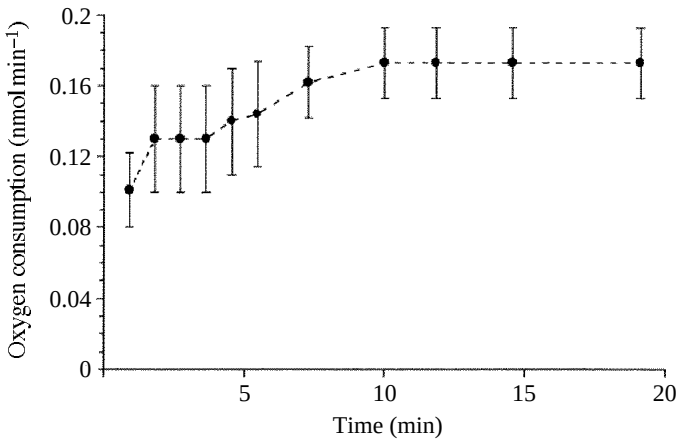

Fig. 1. Time course of oxygen consumption of isolated *Porichthys* photophores in response to addition of 5.5mmol l^{-1} glucose (applied at time zero). Mean values are shown (N=6); vertical bars represent one standard error.

the percentage increase was inversely related to the resting respiration rate. The relationship can be described by the equation:

$$\log y = -2.48 - 5.9x \ (r=-0.93;\ P<0.01;\ N=6);$$

where y corresponds to the percentage increase induced by glucose and x to the resting rate of oxygen uptake measured before the treatment, expressed in nmol min^{-1}.

Effects of glucose on the luminescent photophore

Rees and Baguet (1988) suggested that glycolysis and degradation of pyruvate through the Krebs cycle and oxidative phosphorylation could provide energy to an inhibitory mechanism required to maintain the photophore in a non-luminescent state. It is possible that the increased oxygen consumption of glucose-treated photophores corresponds to an enhanced activity of this inhibitory mechanism, resulting in a reduced capacity for luminescence. To test this hypothesis, we compared the adrenaline-induced luminescent responses of glucose-treated and untreated photophores.

Effect of treatment onset time on adrenaline-induced luminescence

The amplitude of the fast luminescence response to adrenaline decreases as a function of the time elapsing between the end of the photophore dissection and the beginning of stimulation (Christophe and Baguet, 1985). The decrease in amplitude becomes significant after the first 10min and can be described by a single exponential equation.

As reported in the experimental procedure, adrenaline stimulation of the glucose-treated photophore occurred 30min or more after dissection. In our work, it might be argued that the effect of glucose is time-dependent and could affect the light response to adrenaline in a different way. In the first series of experiments, we compared the effect of a 10min glucose treatment on photophores stimulated by adrenaline at five different times after dissection.

When photophores were excised from the fish, immediately immersed in glucose saline and then stimulated with adrenaline (Fig. 2, N=7) 10min later, L_{max1} was $167\times10^6\pm29\times10^6$ quanta s^{-1} and L_{max2} was $58.2\times10^6\pm9.0\times10^6$ quanta s^{-1}. When the stimulation occurred 30 and 60min after dissection, L_{max1} and L_{max2} were significantly reduced (P<0.05), although the values for L_{max1} at 30 ($75.8\times10^6\pm16.5\times10^6$ quanta s^{-1}) and 60min were not significantly different ($91.7\times10^6\pm26\times10^6$ quanta s^{-1}) from each other. The same phenomenon was observed for L_{max2} ($38.7\times10^6\pm6.4\times10^6$ quanta s^{-1} after 30min and $30.9\times10^6\pm4.7\times10^6$ quanta s^{-1} after 60min) (Fig. 2). After 90 and 120min, L_{max1} shows a further reduction of 50% while L_{max2} did not change.

Thus, in glucose-treated photophores, maximal intensities of adrenaline-induced luminescence showed the typical decrease described by Christophe and Baguet (1985), and the 10min glucose pretreatment did not seem to change the light response to adrenaline occurring 30–60min after dissection.

Effect of glucose on adrenaline-induced luminescence

Adrenaline (at 0.1mmol l^{-1}) applied to isolated photophores induced a typical biphasic response characterized by a series of variables summarized in Table 1. The time courses of luminescence and oxygen consumption are shown in Fig. 3A. The production of light started 16.6±1.4s (LT) after the addition of adrenaline and reached a first peak of $290\times10^6\pm102\times10^6$ quanta s^{-1} (L_{max1}) in 71s (TL_{max1}). A partial extinction brought the light level to about 64×10^6 quanta s^{-1} (L_{min}) 306s later; light emission then increased slowly to $77\times10^6\pm22\times10^6$ quanta s^{-1} over the next 5min and decreased over the next 30min to $51\times10^6\pm13\times10^6$ quanta s^{-1}.

The rate of oxygen consumption immediately increased on application of adrenaline and reached in 30s (i.e. prior to the peak of light emission) a rate equivalent to three times

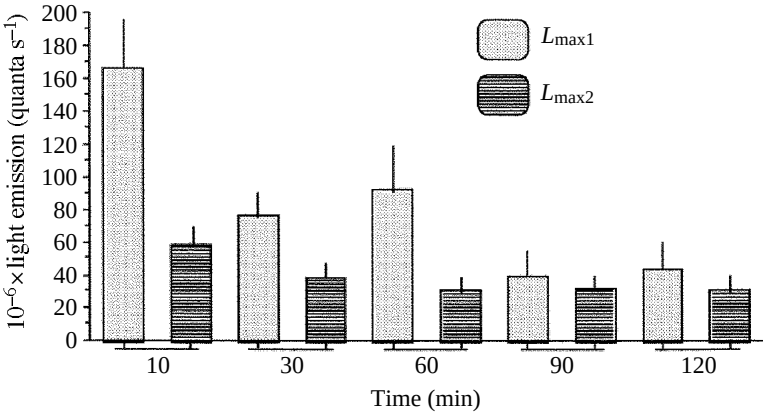

Fig. 2. Effect of time after dissection on adrenaline-stimulated bioluminescence of isolated *Porichthys* photophores. Photophores were pretreated for 10min with 5.5mmol l^{-1} glucose. Maximal amplitudes of the fast (L_{max1}, stippled bars) and slow (L_{max2}, striped bars) luminescence induced by 0.1mmol l^{-1} adrenaline applied at various times after dissection are shown. Mean values are given (N=7); vertical bars represent standard error.

Table 1. *Variables characterizing the luminescence of isolated* Porichthys *photophores*

Treatment	Luminescence response variable									
	LT (s)	TL_{max1} (s)	$10^{-6}\times L_{max1}$ (quanta s^{-1})	$T_{1/2ext1}$ (s)	TL_{min} (s)	$10^{-6}\times L_{min}$ (quanta s^{-1})	TL_{max2} (s)	$10^{-6}\times L_{max2}$ (quanta s^{-1})	$T_{1/2ext2}$ (s)	*N*
ADR	16.6±1.4	71±10	290±102	105±27	306±48	64±13	662±86	77±22	1032±307	12
ADR+GLUC	8.7±1.8	51±2.5	311±104	33.2±4.8	280±77	10.4±3.7	973±129	141±36	1117±232	7
KCN	118±28	358±15	1128±297	402±35	–	–	–	–	–	8
KCN+GLUC	0	0	0	0	0	0	0	0	0	8
KCN+GLUC+ADR	19.5±3.2	132±12.5	2806±1083	133±7.4	491±45	14.5±5.2	1659±109	423±219	1136±263	8

Each value represents the mean and standard error of *N* preparations. *LT*, latency time, corresponding to the time from the application of the drug to the beginning of light emission; TL_{max1}, time from the beginning of the response to the maximum emission of the first peak of luminescence; L_{max1}, maximum intensity of the first peak of light emission; $T_{1/2ext1}$, time to half-extinction of the first peak of light emission; TL_{min}, time from the beginning of the response to the minimum of light emission after the first peak; L_{min}, intensity of the minimum light emission; TL_{max2}, time from the beginning of the response to the maximum emission of the second peak of luminescence; L_{max2}, maximum intensity of the second peak of light emission; $T_{1/2ext2}$, time to half-extinction of the second peak of light emission; ADR, 0.1 mmol l^{-1} adrenaline; GLUC, 5 mmol l^{-1} glucose; KCN, 1 mmol l^{-1} potassium cyanide.

the resting value (from 0.188±0.02nmolmin^{-1} to 0.603±0.06nmolmin^{-1}). Oxygen consumption rapidly decreased to 0.196±0.03nmolmin^{-1} and returned 4min later to its resting value. The rate increased slightly to 0.24±0.03nmolmin^{-1} after 10min, a value that remained nearly constant to the end of the measurement period.

The pretreatment with 5.5mmol l^{-1} glucose enhanced the second peak of luminescence and clearly separated the two phases of luminescence (Fig. 3B).

Fast luminescence. A comparison of the light emission variables of control and glucose-treated photophores (Table 1) showed a significant acceleration of the fast luminescence: *LT* was reduced to 8.7±1.8s and TL_{max1} to 51.0±2.5s. However, L_{max1} was not affected by glucose. The kinetics of light extinction was also accelerated; $T_{1/2ext1}$

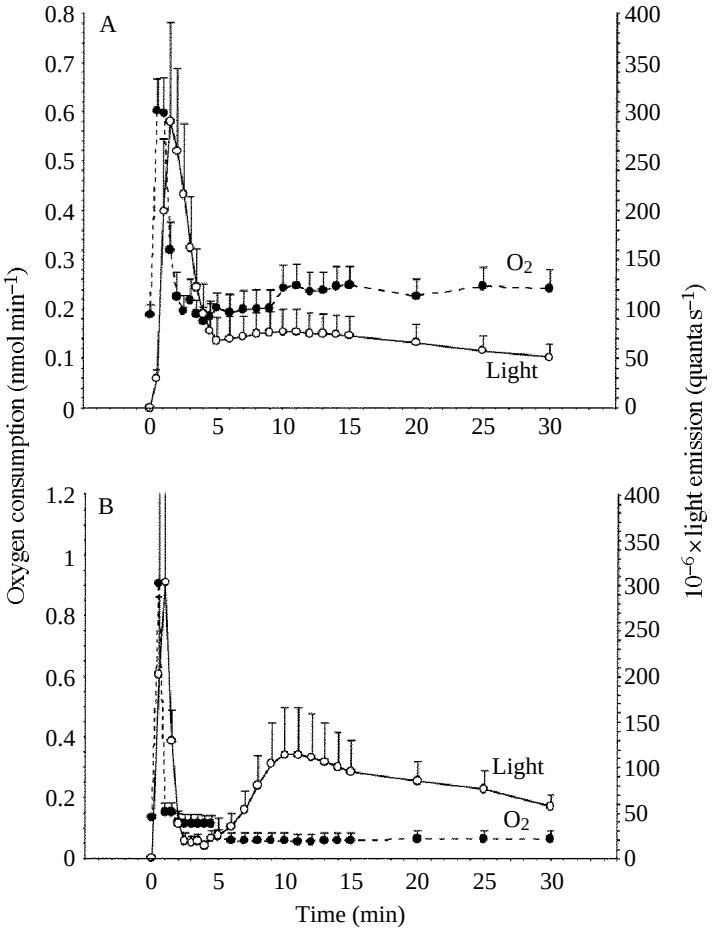

Fig. 3. Effect of glucose pretreatment on oxygen consumption and luminescence of isolated photophores following adrenaline application. Mean values of oxygen consumption (filled circles) and luminescence (open circles) are shown as a function of time after treatment with 0.1mmol l^{-1} adrenaline. Vertical bars represent one standard error. (A) No glucose pretreatment (*N*=12); (B) pretreatment with 5.5mmol l^{-1} glucose (*N*=8). Adrenaline was applied at time zero.

took only 33.2±4.8s instead of the 105±27s seen in control preparations. Moreover, extinction was nearly complete, L_{min} occurred 280s after adrenaline stimulation and corresponded to approximately 3% of the peak value in glucose-treated photophores while it still reached approximately 27% of the peak value in control photophores (Table 1).

Oxygen uptake showed a dramatic increase, reaching in 30s a rate almost 10 times the resting value (from 0.114±0.02nmolmin^{-1} to 0.906±0.252nmolmin^{-1}); after another 30s, i.e. when the production of light was maximal, it dropped back to near its resting value. After 2min, the rate of oxygen uptake was equivalent to that measured before stimulation.

The total amount of oxygen consumed during the first 5min was similar in glucose-treated and control organs, but the total amount of light produced in glucose-treated organs was half of that produced by control preparations (Table 2).

Slow luminescence. About 5min after the application of adrenaline, a second slow luminescence began to develop and reached a peak 6min later. The TL_{max2} value was not significantly different from the TL_{max2} value measured in control photophores (Table 1). In contrast, L_{max2} was approximately twice as high in the presence of glucose.

The oxygen consumption preceding and accompanying the light emission was particularly stable; but then, within 1min, the respiration rate dropped below the resting level to 0.06±0.03nmolmin^{-1} and remained nearly constant over the next 10min. During the last 15min of the extinction period, the level rose slowly, but it remained below the prestimulation resting level.

The total amount of oxygen consumed during the 5–30min period following glucose treatment was one-third of the amount consumed during the same period in control organs, but the total amount of light produced was not significantly different (Table 2).

It is concluded (i) that 5.5mmol l^{-1} glucose did not decrease the ability of the organ to luminesce but changed the time course of the production of light; and (ii) that glucose

Table 2. *Effects of glucose and cyanide treatments on the adrenergic responses of isolated* Porichthys *photophores*

		Time (min)			
Treatment	Variable	0–5	5–30	30–60	*N*
ADR	O_2 (nmol)	1.47±0.13	5.76±0.88		12
	Light (quanta×10^{-9})	48.83±9.39	99.16±26.39		
ADR+GLUC	O_2 (nmol)	1.06±0.05	1.70±0.03		7
	Light (quanta×10^{-9})	19.46±4.9	120.69± 31.6		
KCN+GLUC+ADR	O_2 (nmol)	0.79±0.11	0.34±0.03	0.23±0.01	8
	Light (quanta×10^{-9})	363.3±51.2	270.78±42.3	281.7±27.1	

Total oxygen consumption and luminescence was monitored for three time periods following adrenaline stimulation.

Values represent means ± standard errors; *N* is the number of preparations tested.

For further details, see Table 1.

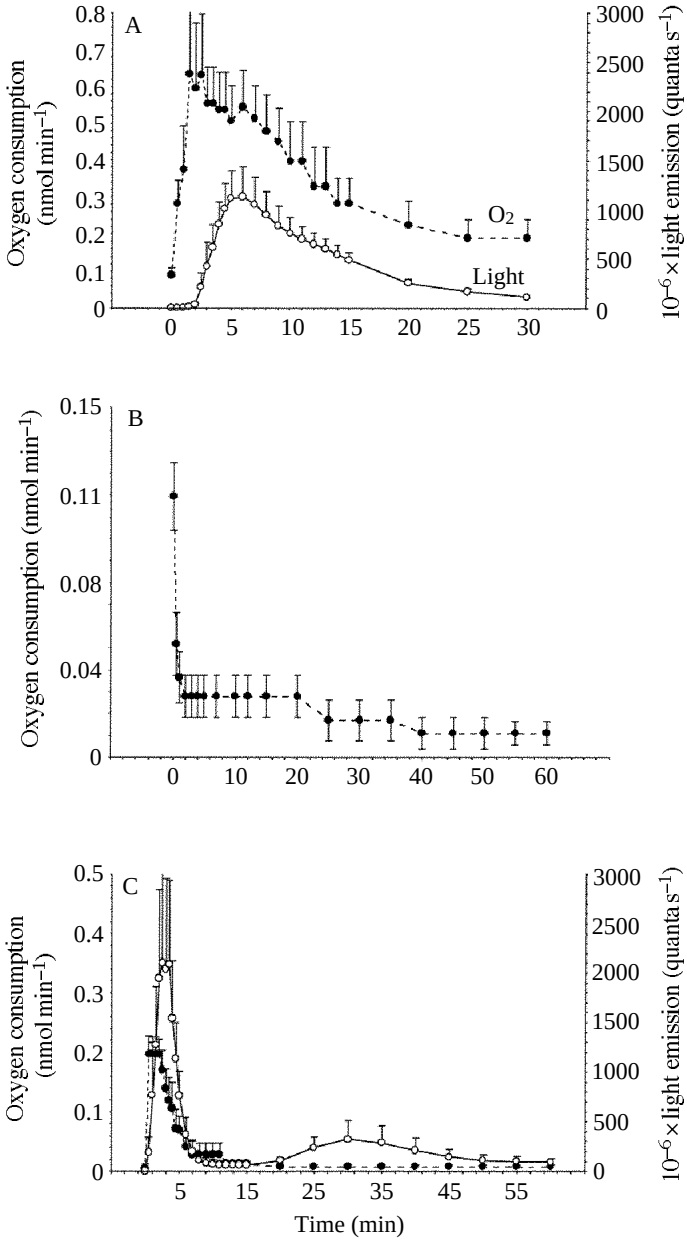

Fig. 4. Rate of oxygen consumption and luminescence of isolated photophores as a function of time after application of 1 mmol l^{-1} KCN. Values for oxygen consumption (filled circles) and luminescence (open circles) are given as means and are shown as a function of time after treatment. Vertical bars represent one standard error (N=8). (A) Control photophores (KCN applied at time zero); (B) response following pretreatment with 5.5 mmol l^{-1} glucose (KCN applied at time zero); (C) response to 0.1 mmol l^{-1} adrenaline following pretreatment with 5.5 mmol l^{-1} glucose and cyanide (see Materials and methods for detailed procedure).

stimulated the increase in the rate of oxygen uptake during the fast luminescence or inhibited oxidative metabolism during the slow luminescence.

This means that cellular respiration should not be essential for the slow luminescence while glycolysis is stimulated by the presence of external glucose. To test this hypothesis, we investigated the effect of glucose on the light response to adrenaline after blockade of cellular respiration by potassium cyanide (KCN).

Effects of glucose on KCN-stimulated photophores

In the presence of 1mmol l^{-1} KCN, the *TL* of control photophores was 118±28 s (*N*=8), a large light emission that reached a maximal value ($1128\times10^6\pm297\times10^6$ quanta s^{-1}) in 358±15s (Fig. 4A and Table 1). Light emission was nearly completed in 30min.

Prior to the production of light, oxygen uptake increased rapidly from 0.08±0.02 to 0.634±0.186nmol min^{-1} and typically reached a maximum during the second minute of incubation, i.e. when the photophore began to luminesce. During the phase of light production, the oxygen uptake remained nearly constant; it began to decrease in parallel with the light level. It is noteworthy that at the end of the measuring period (30min), when light emission was still occurring ($108\times10^6\pm19\times10^6$ quanta s^{-1}), the oxygen uptake (0.189±0.05nmol min^{-1}) was significantly higher ($P<0.05$; $N=8$) than the resting level (0.08±0.02nmol min^{-1}).

The glucose-treated photophores showed a rapid drop in the oxygen consumption on addition of 1mmol l^{-1} KCN (Fig. 4B): in 5min the level decreased to about 30% of the resting value and after 40min the inhibition of oxygen uptake was complete: the value corresponded to 10.5±4.2% of the resting level and was not significantly different from zero ($P<0.05$; $N=8$). None of the eight preparations tested showed a detectable production of light even 60min after addition of KCN.

When 0.1mmol l^{-1} adrenaline was added to glucose-pretreated preparations after 40min in KCN (Fig. 4C), there was a large light response with an *LT* of 19.5±3.2s (*N*=8); the peak of light emission ($2806\times10^6\pm1083\times10^6$ quanta s^{-1}) occurred after 133.0±7.4 s and remained at this level for 60s. Extinction was almost complete 4min later at a minimal constant value of $14.5\times10^6\pm5.2\times10^6$ quanta s^{-1} that was maintained for approximately 5min. After this, a slow second phase of luminescence developed with a maximal value of $423\times10^6\pm219\times10^6$ quanta s^{-1} occurring 1659±109s after the addition of adrenaline. The time course of extinction was also very slow, with a $T_{1/2ext}$ of 1136±263s (Table 1).

Although the resting oxygen consumption was totally inhibited, oxygen uptake immediately increased on application of adrenaline and reached in 30s (i.e. prior to the peak of light emission) a value of 0.207±0.025nmol min^{-1} that remained constant for 2 min (Fig. 4C); it then decreased with a time course similar to that of the decrease in light production. During the first 5min, the total amount of oxygen consumed was 0.790nmol and the total light produced 363.3×10^9 quanta (Table 2).

About 5min after adrenaline stimulation, i.e. before the almost complete extinction of the fast peak of luminescence, oxygen consumption was slightly above the initial value. The slow luminescence phase was associated with a very low oxygen uptake. From 5 to

60min after adrenaline treatment, the total amount of oxygen consumed corresponded to 0.573nmol, which is less than the value measured over the first 5min, even though twice as much light was produced during this time (Table 2).

Discussion

Effect of glucose on resting respiration

After immersion in saline, isolated *Porichthys* photophores show large individual variations in the rate of oxygen uptake. The presence of 5.5mmol l^{-1} glucose abolishes these differences and reduces the range of oxygen consumptions by specifically increasing the individual values.

Isolated photophores from deep-sea fish are also sensitive to glucose but show different effects depending upon the fish species studied. For example, *Maurolicus muelleri* photophores show a constant 37% increase in their oxygen consumption irrespective of the amplitude of the resting level (Mallefet *et al.* 1990), while *Argyropelecus hemigymnus* photophores show a 40% decrease in their respiration rate (Mallefet and Baguet, 1991).

Our results support the hypothesis that, in isolated *Porichthys* photophores, external glucose acts through the glycolytic pathway to stimulate the Krebs cycle and oxidative metabolism. Both metabolic pathways should be tightly coupled to provide energy to an inhibitory mechanism maintaining the resting photophore in a non-luminescent state. Differences in the glucose content of individual photophores might explain the large variation in their respiration rates and the tendency sooner or later to become spontaneously luminescent (Rees and Baguet, 1988). However, glycolysis can supply energy directly for inhibitory control because external glucose inhibits the KCN luminescence.

Effect of glucose on the adrenaline-induced luminescence

The fast luminescence

The fast luminescent peak amplitude was similar in control and glucose-treated photophores. However, in the latter case the luminescent curve appeared as a brief flash characterized by a rapid extinction. In both cases, oxygen consumption increased prior to the production of light with a time course similar to the luminescent curve. As a consequence, we can assume that the acceleration of the oxygen uptake and its rapid fall in glucose-treated organs were responsible for the time course of the fast luminescence.

Mallefet and Baguet (1989) have suggested that this initial oxygen consumption could be due to cellular activity associated with the triggering mechanism of photogenesis. Assuming that the extra oxygen consumed above the resting level represents this activity, we can calculate that, to produce 10^9 quanta of light, the supplementary oxygen consumed is 26×10^{-3} nmol in glucose-treated organs and only 19.8×10^{-3} nmol in control organs.

Although the oxygen uptake was enhanced in the presence of glucose, our results clearly showed it was a transient effect. Although initially stimulating the response, glucose rapidly inhibited the oxidative metabolism of the photophore in response to

adrenaline. The stimulation of the glycolytic pathway by external glucose enhanced the degradation of pyruvate through the Krebs cycle and oxidative phosphorylation. However, such glycolytic activity can inhibit cellular respiration, since increased competition between glycolysis and oxidative phosphorylation for available inorganic phosphate or adenosine triphosphate can induce a rapid decrease in oxygen consumption.

This repression of cellular respiration under aerobic conditions by activated glycolysis known as the Crabtree effect (Crabtree, 1929), is observed in isolated guinea pig myocytes in the presence of glucose as an increase in the calcium concentration (Bailey *et al.* 1987). The activation of alpha-adrenoceptors by adrenaline is associated with an increase in intracellular free calcium level in all effector tissues studied. The fast luminescence in *Porichthys* photophores is mediated through alpha-adrenoceptor activation (Anctil and Case, 1976; Gariepy and Anctil, 1983; Christophe and Baguet, 1983, 1985) and could show a similar Crabtree effect.

Our results strongly support the view that glycolysis and oxidative pathways play a specific role in controlling photogenesis, with glycolysis being involved in triggering the production of light. The strong inhibition of luminescence when glycolysis is blocked (Rees *et al.* 1989) supports this hypothesis. However, cellular respiration is probably necessary for light production. When cellular respiration is reduced, light extinction is accelerated and can be complete, as in glucose-treated photophores. The successive stimulation of glycolysis and oxidative metabolism could produce the typical time course of the fast response to adrenaline.

The slow luminescence

After the extinction of fast luminescence in control preparations, the light level slowly began to increase and after a few minutes reached a maximal value corresponding to about 20% of the fast peak value. During this so-called ‘slow luminescence’ (Christophe and Baguet, 1983), the oxygen consumption level remained significantly higher than in the resting state. Glucose-treated preparations showed a slow luminescence separate from the fast luminescence, with a time course similar to that measured in control preparations, but with an amplitude twice as high.

Unlike the fast luminescence, the slow luminescence was not associated with a large increase in oxygen uptake. Nevertheless, a slight increase in oxygen consumption may have occurred prior to the production of light, but was balanced by a simultaneous decrease visible after the event. This might explain why the oxygen level remained constant during the period preceding the slow luminescence.

The large drop in oxygen uptake to below the resting level, once the production of light had begun, suggested a specific inhibition of aerobic metabolism, probably induced by further stimulation of the glycolysis initiated during the fast luminescence.

In control preparations, the excess oxygen consumed during the slow luminescence can be interpreted as corresponding to an oxidative recovery process restoring the pool of luciferin, the substrate depleted in the light reaction (Mallefet and Baguet, 1989). In glucose-treated photophores, the depression of oxygen consumption may correspond to an enhancement of aerobic glycolysis resulting in a Crabtree effect. A similar

phenomenon has been described in ventral photophores isolated from the deep-sea fish *Argyropelecus hemigymnus* (Mallefet and Baguet, 1991).

The origin of the oxygen consumption of luminescing photophores

The glucose- and KCN-treated photophores showed a total inhibition of their cellular respiration and responded to adrenaline with the emission of a typical biphasic adrenergic luminescence: the amplitude of the fast and the slow components were, respectively, nine and four times higher than in glucose- and adrenaline-treated organs. This result clearly shows that the luminescent system of *Porichthys* photogenic cells involves more than control through oxidative metabolism. Nevertheless, oxidative metabolism still exerts some inhibition on the photogenic capability of the photophore because, when it is blocked, luminescence increases. Since oxygen uptake increased immediately after application of adrenaline to KCN-treated photophores, oxygen uptake associated with the adrenergic luminescence of non-poisoned organs may not be a respiratory response.

The non-respiratory oxygen consumption measured in KCN- and glucose-treated photophores (1.4×10^{-3} nmol per 10^9 quanta) represented 5.5% of the total oxygen consumption measured in adrenaline-stimulated photophores (26.7×10^{-3} nmol per 10^9 quanta). This means that the excess oxygen consumed above the resting level in the adrenergic luminescent response can be divided into two parts, one independent and the other dependent on cellular respiration. The non-respiratory oxygen consumption occurring prior to the fast luminescence represents only a small fraction of the total amount of oxygen consumed. Nevertheless, this oxidative phenomenon is sufficient to activate the luminescent reaction in the absence of functional cellular respiration.

Since the biochemical mechanism for luminescence in *Porichthys* photophores consists of a luciferase-catalysed oxidation of luciferin by oxygen (Tsuji *et al.* 1971), the results reported for KCN-and glucose-treated photophores suggest that the increase in non-respiratory oxygen uptake in the presence of light originated exclusively from the oxidation of luciferin by molecular oxygen.

The number of oxygen molecules consumed, calculated for the production of 1 quantum of light, is 898; this value is 299 times higher than the theoretical value (three molecules) calculated for the emission of 1quantum of light during the oxidation of luciferin *in vitro* (Stone, 1968; Shimomura and Johnson, 1970). It is possible that the oxidation of luciferin *in vivo* does not proceed through a simple direct reaction with molecular oxygen as it does *in vitro*. A peroxide mechanism has been proposed for the luminescent reaction of the *Vargula tsuji* system (Tsuji *et al.* 1977), which is similar to the *Porichthys* photophore system (Cormier *et al.* 1967). Moreover, extramitochondrial NAD/NADH activity, unaffected by KCN or NaN_3, has been measured in *Porichthys* photophores (J. Mallefet, in preparation).

We suggest that it is possible that the production of a significant quantity of H_2O_2, using extramitochondrial single-electron transfer reactions, could explain the low non-respiratory oxygen uptake described in adrenaline-stimulated photophores. To spare the cell from the toxic effects of free radical generation, this activity would be localized in specific areas, such as the numerous membranous vesicles that presumably contain the substrate for the light reaction (Strum, 1969; Baguet and Zietz-Nicolas, 1979).

In the absence of glucose, the KCN-induced luminescence is associated with an increase in oxygen uptake corresponding to about 50% of the total oxygen consumption measured in the adrenaline-stimulated photophore. The numerous ultrastructural deteriorations seen in KCN-treated photocytes (Anctil, 1979) are very similar to those described in free-radical-treated cells (Freeman and Crapo, 1982).

It is concluded that adrenaline-stimulated luminescence of isolated *Porichthys* photophores is independent of cellular respiration, as long as external glucose is provided, unlike the luminescence of the ventral photophores of the deep-sea fish *Argyropelecus hemigymnus* (Mallefet and Baguet, 1991).

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