LUMINESCENCE OF ISOLATED PHOTOCYTES FROM *PORICHTHYS* PHOTOPHORES: ADRENERGIC STIMULATION

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SUMMARY

1. A fast transient peak of light, followed by a slow luminescence, was produced by isolated photophores of *Porichthys notatus* when exposed to 1 mM-epinephrine, norepinephrine or phenylephrine, an α-adrenergic agonist. Only a slow luminescence was induced by 1 mM-isoproterenol, the β-adrenergic agonist.

2. After removal of the reflector and surrounding tissues, the isolated photogenic cells, i.e. the photocytes, responded to norepinephrine and phenylephrine by producing a fast transient peak of light, and to isoproterenol by a slow luminescence. Epinephrine evoked both types of luminescence. The emission latency time, the amplitude and the total duration of the light responses were significantly less than those of the photophore.

3. The results suggest that both epinephrine and norepinephrine combine with sympathetic α or β receptors upon the photocytes to trigger light production.

INTRODUCTION

The catecholamines norepinephrine and epinephrine induce a light emission both *in situ* and in isolated photophores of the midshipman fish *Porichthys notatus* (Nicol, 1957; Baguet, 1975). Though there is strong evidence for the presence of a catecholaminergic innervation in the photophore (Anctil & Case, 1976; Anctil, Brunel & Descarriers, 1981), the mechanism of nervous control of production by the photocytes is not yet understood.

The aim of the present work was to investigate how the putative neurotransmitters, epinephrine and norepinephrine, could directly activate the photocytes without interfering with nervous elements present in the different tissues surrounding the photogenic cells (Strum, 1969). After the overlying tissue had been removed, photocytes were exposed to epinephrine, norepinephrine, isoproterenol (β-agonist) or phenylephrine (α-agonist), and the light responses were compared.

Key words: Luminescence, *Porichthys*, photocytes.
METHODS

Dissection of the photophores and the photocytes

Specimens of Porichthys notatus, air-shipped by the Pacific Bio-Marine Laboratories (Venice, California), were kept in aquaria (751) provided with aerated running sea water (15–17 °C) from the North Sea. The fish used (five males and three females) were 17-7–21-5 cm in length.

Fish were anaesthetized for 5–10 min by total immersion in a tray of sea water containing 1-5 ml/l of a mixture of 25% quinaldine and 75% acetone. Following anaesthesia, a strip of skin with six photophores from the mandibular, branchial, gastric, gular or ventral regions (using Greene’s terminology, 1899) was excised and maintained in a small vessel filled with air-saturated saline.

To make the ‘isolated photophore’ preparation, a photophore was isolated from the strip and mounted in the light recording chamber. To make the ‘isolated photocytes’ preparation, a photophore was dissected under the binocular microscope. The layers of epidermal and dermal cells were dissected away using fine scissors and forceps (Dumont no. 5). The layer of reflector cells was then easily torn away from the connective tissue which surrounds the photocytes that cling to the underlying lens (Fig. 1). The photocytes of such a preparation observed with the interference microscope do not show any sign of membrane rupture: the photocytes never coloured after application of vital dye and they showed the typical green fluorescence when exposed to u.v. light excitation (365 nm) (Baguet & Zietz-Nicolas, 1979). Isolated preparations (photophore or photocytes) used in the experiments did not luminesce spontaneously during a period of 120 min following their manipulation.

Solutions

The preparation was immersed in a saline of the following composition: NaCl, 188 mM; KCl, 7-4 mM; CaCl₂, 3-5 mM; MgCl₂, 2-4 mM; adjusted to pH 7-3 with Tris-HCl buffer (20 mM). Epinephrine (S.C. Federa), norepinephrine (Fluka), isoproterenol (Wintrops S.A.) and phenylephrine (Sigma) hydrochlorides were dissolved at a concentration of 1 mM in saline (20 °C) immediately before each experimental series.

Recording luminescence

The photophore was placed on a glass slide with the light emitting area facing downward. The photocyte preparation was placed with the lens downward. A piece of 0-5 mm mesh tulle moistened with saline covered the preparation and was clamped on the glass slide by a stainless steel ring to prevent any movement. The chamber was placed on a photomultiplier (PM 270D, International light) in such a way that the preparation faced the photocathode at a distance of 2-0 cm. The apparatus was calibrated with a standard light source (betalight, peaking at 470 nm) held at the site of placement of the tissue (Baguet & Zietz-Nicolas, 1979). The light response was amplified and recorded simultaneously on an oscillographic recorder (HP 17400 A) and a 20 cm flat-bed recorder (Servogor, full scale response within 0-30 s).
Fig. 1. Longitudinal sections of 'isolated photophores' (A) and 'isolated photocytes' (B) of Porichthys notatus. l, lens; r, reflector; ph, photocytes; ct, connective tissue.
Luminescence of Porichthys photophores

Experimental procedure

Each of the four agonists was applied to 18 photophores and 18 photocytes. The chamber with the preparation immersed in 0.1 ml saline saturated with air, was placed on the light recording apparatus. In the dark, 1.1 ml saline containing a given agonist was applied with a syringe on a photophore preparation at a final concentration of 1 mM. The same volume of agonist was applied 5 min later on a photocyte preparation from the same fish. The light response was recorded from both with two separate light recording units.

Calculation of results

The amplitude of the light response of isolated preparations showed large variations from fish to fish, according to the season. For instance, the extreme values of the norepinephrine response recorded on the eight experimental fishes from February to October 1980, extended from 94–1721 Mq/s. To make a comparison of the absolute values of the light responses among the different fish used, we expressed the results for each fish relative to the response to norepinephrine, this agonist usually giving the largest luminescence.

Light organs isolated from a given fish showed a similar response throughout captivity, and a similar response from the different regions of a given fish.

The number of light organs used for the determination of the mean value of a parameter is indicated by \( n \) and each mean value is expressed with its standard error (mean ± s.e.m.). Significance of differences was calculated by \( t \)-test. The decrease in luminescence of photocytes is described by an exponential function, of which the parameters are estimated by the modified Prony method applied to inverted exponentials (Simon, 1972).

RESULTS

Time-course of luminescence

When the photophore was exposed to epinephrine, norepinephrine or phenylephrine, there was a fast, transient luminescence followed by a slowly decaying luminescence (Fig. 2A, B, D). Isoproterenol triggered only a slow luminescence (Fig. 2C). The luminescence pattern was very reproducible since on the 18 photophores stimulated by each drug, only two photophores did not emit a fast luminescence in response to phenylephrine and only one photophore did not produce a slow luminescence in response to norepinephrine.

In contrast, photocytes developed fast and slow luminescences only in response to epinephrine stimulation (Fig. 2F) (Christophe & Baguet, 1981). Norepinephrine and phenylephrine induced solely a fast light response (Fig. 2E, H), whereas isoproterenol triggered a slow luminescence (Fig. 2G). As with the photophores, these effects were very reproducible.

The fast luminescence of the photophore

Response time course

The latency of the fast luminescence for epinephrine was not statistically different
Fig. 2. Typical light responses of isolated photophores (A–D) and photocytes (E–H) induced by different adrenergic agonists. Horizontal bar, 120s. Vertical bar, A, 6·8 Mq/s; B, 7·81 Mq/s; C, 35·1 Mq/s; D, 15·6 Mq/s; E, 1·4 Mq/s; F, 3·4 Mq/s; G, 6·71 Mq/s; H, 1·67 Mq/s. The beginning of the photocyte response is shown in the upper right part of the figure (time scale, 1 s).
Luminescence of Porichthys photophores

1. Values (±S.E.) of different parameters of luminescence of isolated photophores in response to norepinephrine, epinephrine, isoproterenol and phenylephrine (10⁻³ M for each substance)

<table>
<thead>
<tr>
<th>Substance</th>
<th>n</th>
<th>LT (s)</th>
<th>TL_max (s)</th>
<th>T½_ext (s)</th>
<th>n</th>
<th>T½_ext (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>18</td>
<td>19.4 ± 2.6</td>
<td>46.2 ± 6.2</td>
<td>46.1 ± 3.6</td>
<td>17</td>
<td>174.6 ± 36.6</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>18</td>
<td>18.3 ± 3.4</td>
<td>76.8 ± 9.7</td>
<td>46.6 ± 4.1</td>
<td>18</td>
<td>273.5 ± 46.9</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>18</td>
<td>48.2 ± 8.0</td>
<td>90.3 ± 7.1</td>
<td>70.9 ± 12.9</td>
<td>18</td>
<td>329.1 ± 99.3</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>16</td>
<td>48.2 ± 8.0</td>
<td>188.6 ± 3.3</td>
<td>492.2 ± 84.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LT, latency time; TL_max, time elapsing from the latency time to the time to reach the peak of light; T½_ext, time to half extinction of the light response.

from that for norepinephrine (about 18.5 s) but was much less than that for phenylephrine (48 s) (Table 1). Rate of production of the fast luminescence was similar for epinephrine and phenylephrine stimulation, and less than that produced by norepinephrine (Table 1). It is not possible to calculate the time of complete extinction of the fast luminescence owing to the production of the slow luminescence. We estimated the half-extinction time which was very similar for the response to both epinephrine and norepinephrine (about 46 s), and somewhat longer for the response to phenylephrine (71 s).

Response intensity

Once dissected from the animal, the amplitude of the fast luminescence responses of the isolated photophore to the four adrenergic agonists decreased as a function of the time period elapsing from the end of the dissection, to the beginning of the stimulation, as shown for norepinephrine (Fig. 3). Over the length of our experimental period, i.e., 120 min, the decrease in amplitude of photophore slow response and the photocytes fast and slow responses, for all four agonists, can be described by a single exponential equation: \( L_t = L_0 e^{-\alpha t} \), where \( L_t \) is the amplitude of the fast response measured at a given time \( t \) after the dissection, \( L_0 \) the magnitude of the light response extrapolated to the end of the dissection, and \( \alpha \) the rate constant of the light response decay with a value of 0.56 min⁻¹ (±0.15; \( n = 207 \)). Thus the amplitude of any light emission \( (L_X) \) reported in the present paper, was corrected by using the following equation: \( \log L_X = \log L_t + 0.56t \), where \( L_t \) is the response amplitude measured at the time \( t \) after the dissection, added to a corrected term (0.56t) corresponding to the product of the rate constant and the time at which the response was recorded.

Applying these corrections to the isolated photophore preparation, the intensity of the fast luminescence produced by epinephrine (51.1 ± 5.1 arbitrary units) and phenylephrine (52.8 ± 9.2 units) was approximately half that induced by norepinephrine (94.8 ± 10.0 units) (\( n \) as for Table 1).
Fig. 3. Typical time course of decay of the fast response amplitude recorded on 19 isolated photophores stimulated by 1 mM norepinephrine at different times after the end of the dissection.

**The fast luminescence of the photocytes**

**Response time course**

The photocytes started to luminesce in less than 2 s after application of norepinephrine, epinephrine or phenylephrine, and maximal luminescence was reached 7 s later (Table 2).

**Response intensity**

The maximal amplitude of the fast response was similar for norepinephrine (12.0 ± 1.4 arbitrary units), epinephrine (9.5 ± 1.4 units) and phenylephrine (14.4 ± 4.6 units). No luminescence developed immediately after application of isoproterenol.

Thus the removal of the reflector and the surrounding connective tissue decreased the amplitude of the response of the photocytes but increased by more than 10 times the rapidity of this light production.

**Kinetics of light extinction**

The kinetics of extinction of the fast luminescence can be described by a single exponential equation of the type \( Y_t = Ae^{-\alpha t} \), where \( t \) is the time, \( Y \) the light level of the preparation at time \( t \), \( A \) the 100% light, and \( \alpha \) the rate constant of extinction. The rate constant for norepinephrine (3.50 ± 0.40%, \( n = 16 \)) was similar to that for phenylephrine (2.16 ± 0.43%, \( n = 6 \)).
Table 2. Mean values (± s.e.) of different parameters of luminescence of the photocyte preparations in response to norepinephrine, epinephrine, isoproterenol and phenylephrine (10^{-3} M for each substance)

<table>
<thead>
<tr>
<th></th>
<th>Fast response</th>
<th>Slow response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LT (s)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>18</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>18</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>14</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>16</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

Same parameters as in Table 1.

The slow luminescence of the photocyte

Response time course

The latency of the slow response could not be measured accurately for epinephrine, norepinephrine and phenylephrine, but was 194.3 ± 37.8 s for isoproterenol. For all agonists, maximal amplitude occurred at a similar time (283 s for isoproterenol) and the time to decay to 50% of the maximum value was also similar (189 s for isoproterenol) (Table 1).

Response intensity

The maximal amplitude of the slow light response to norepinephrine (22.0 ± 5.3 arbitrary units) was not different from that for isoproterenol (29.1 ± 8.3 units) or phenylephrine (27.3 ± 4.1 units, n as in Table 1). The response to epinephrine was lower (8.4 ± 1.4 units).

The slow luminescence of the photocytes

Response time course

For isoproterenol, the latency was 42.3 ± 4.4 s and the maximum response was reached in 64.7 ± 4.9 s. For epinephrine, the response was slower: the latency was 90.6 ± 6.0 s and the maximum value was obtained in 91 s (Table 2).

Response intensity

Maximum amplitude for isoproterenol (8.2 ± 1.7 arbitrary units) was about twice that for epinephrine (4.4 ± 0.5 units, n as Table 2).

Kinetics of light extinction

The half-extinction time for isoproterenol (132 s) was much greater than that for epinephrine (92 s) (Table 2).

The extinction could be described by the same equation as for the extinction of the fast response. The rate constant was 0.66 ± 0.6 % of maximal light per minute for isoproterenol (n = 11) and 0.47 ± 0.04 % for epinephrine (n = 17).
DISCUSSION

Fast and slow luminescence of isolated photophores

The light produced by an isolated photophore of *Porichthys* in response to epinephrine or norepinephrine has previously been reported to last about 30 min and to be extremely variable in amplitude (Baguet, 1975; Anctil & Case, 1976). Our results show that the pattern of the luminescence response is extremely reproducible among the photophores from different fish if the stimulation is conducted at a fixed period after the excision from the animal; in this case the light response is characterized by a fast transient peak of light followed by a second slow luminescence with an amplitude about 20% that of the first peak. The amplitude of the light response decreases with time after dissection from the animal, probably because of a progressive decrease of the intracellular luminescent substrate. The exponential decrease of resting photophore fluorescence, which presumably corresponds to the oxidation of luciferin in a fresh photophore (Baguet & Zietz-Nicolas, 1979), proceeds at a rate which is similar to the loss of responsiveness observed whatever the adrenergic agonist used.

Luminescence of isolated photocytes

The adrenergic response of the isolated photocytes differed in three respects from that of the photophore. (i) The emission latency of the response was reduced by 20 to 30 times for norepinephrine, epinephrine and phenylephrine. It is suggested that the tissues of the photophore which surround the photocytes act as a barrier to the diffusion of the agonists. (ii) The duration of the light response of the photocytes preparation was shorter than that of the isolated photophores. The time course of the electrical light response of the isolated photophore (Baguet & Case, 1971) was very similar to that of the fast response of isolated photocytes. It is possible that electrical stimulation could liberate norepinephrine from nerve terminals present in the isolated photophore giving rise to the activation of photocytes. (iii) The magnitude of the fast peak corresponds to about 10% of that observed on the photophore for norepinephrine and to about 20% of that measured on the photophore for epinephrine. The amplitude of the long sustained luminescence induced by epinephrine corresponds to 50% of that measured on the photophore. This reduction of the light output from the photocytes after removal of the surrounding tissues might be ascribed to the absence of the multi-layer reflector. It should be noted that no photocytes or fluorescence could be observed on a removed reflector. While the reflective capacity of the reflector is not known for the *Porichthys* photophore, in the bacterial photophore of *Anomalops*, the reflector can reflect 70% of the incident light of the visible spectrum (Watson, Thurston & Nicol, 1978). However this could not explain why the responses to the different agonists are reduced by different amounts. Instead, it suggests that the surrounding tissues control the luminescence of the photocytes and that this control has different sensitivities to different agonists.

The origin of the fast and the slow luminescence

The anatomical studies of the nervous system of *Porichthys* support the view that
Luminescence of Porichthys photophores

Photophores are innervated by catecholaminergic neurones of sympathetic origin (Nicol, 1957). Radioenzymatic assays and light microscope radioautographic studies have demonstrated uptake and storage of $^3$H-norepinephrine in the nerve fibres innervating Porichthys photophores (Anctil et al. 1981).

The isolated photocytes preparation, however, does not appear to have any functional nervous elements, for the following reasons. (i) Reserpine, which is known to deplete catecholamine storage, evokes a large light emission when applied to an isolated photophore, but when applied to the isolated photocytes preparation, it does not induce any luminescence. (ii) Electrical stimulation sufficient to trigger light production in the isolated photophore by indirect activation of nerve elements (Baguet, 1975) is ineffective on the photocytes preparation.

Thus light production by the photocytes is apparently stimulated by catecholamine agonists by a direct effect on the photocyte membrane. This is substantiated by the fact that the detergent Triton X-100 (10% concentration) applied to the isolated photocytes suppresses any response to epinephrine without impairing the light response to KCN which should act primarily on intracellular sites (Christophe & Baguet, 1982).

Our present pharmacological studies on the photocytes preparation presents evidence that both $\alpha$ and $\beta$ sympathetic receptors should be present on the photocyte membranes. Norepinephrine and phenylephrine, an $\alpha$-agonist, evoke only a fast transient peak of light, while isoproterenol, a $\beta$-agonist, induces only a delayed long lasting luminescence. Both responses are successively elicited by epinephrine which should stimulate $\alpha$ and $\beta$ receptors.

The high specificity of catecholamines on the pattern of light response is only observed for isolated photocytes. For photophores, even norepinephrine and phenylephrine, which presumably only stimulate the $\alpha$-receptors of photocyte membranes, also evoke the delayed long lasting luminescence. It might be argued that by removing the reflector, we discarded some structures excitable by $\alpha$-agonists which should control the lightening of the photocytes. Though nervous structures have been observed in the reflector by electron microscopy, (G. Zurstrassen & F. Baguet, in preparation) we need much more experimental evidence to define the nature of this delayed luminescence. While norepinephrine could be solely involved in the control of a fast luminescence of short duration, epinephrine could induce both a fast and slow luminescence of the photocytes.

The control mechanism of the fast and slow responses

Analysis of the kinetics of the light response of the isolated photocytes is a new approach to studying some aspects of the intracellular control mechanism of the light reactions. Assuming that in a given photophore the same photocytes produce successively the fast and the slow light responses, it is possible that the rate of light production by the photocytes is controlled by the rate of delivery of some activating agent, an ion perhaps, triggering the intracellular light reacting sites. In this case, our results would suggest that the light reacting sites are under the control of two different mechanisms or a single mechanism working at two different rates. Norepinephrine and phenylephrine would trigger the fast delivery of the activating agent, evoking the fast light response. Epinephrine and isoproterenol would deliver the activator at a low rate producing a slow delayed light emission.
A number of other possibilities exist. One such possibility is that the tissue contains two cell types. Norepinephrine could act directly on the photocytes while epinephrine could act indirectly through another cell.

Assuming that the extinction rate can be taken as an estimation of the spontaneous deactivation rate of the intracellular light reacting sites, our results also suggest the presence of an extinction control mechanism, quenching the light reactions at a different rate after a fast or a slow light response. The rate of withdrawal of the activating agent from the light reacting sites should determine the rate of light extinction.

Alternatively, one might suppose the presence of two types of photocytes. Fast photocytes may deliver and withdraw the intracellular activating agent at a high rate giving rise to a fast light response and slow photocytes would act in the opposite fashion.

Further experiments should be devoted to the study of substances that could specifically change the activation or the deactivation rate of isolated photocytes, in order to clarify the nature of the control mechanisms of photogenesis.

REFERENCES


