The Increase of Oxygen Consumption after a Flash of Light Is Tightly Coupled to Sodium Pumping in the Lateral Ocellus of Barnacle

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ABSTRACT In the lateral ocellus of the barnacle, we have tested the hypothesis that the transient increase of oxygen consumption (ΔQO2) induced by light results from an increase in the rate of Na+ pumping. With a Na+-sensitive microelectrode, we measured the intracellular concentration of Na+ (Na) in the photoreceptor cells. Na was 17.6 ± 1.2 mM (SE; n = 18) in darkness and it increased transiently by 10–20 mM after an 80-ms flash of intense light. The increase of Na recovered in about the same time as the ΔQO2, and the Na+/O2 ratio was 19.2 ± 3.8 (SE; n = 6). Removing Na from the bath caused the ΔQO2 to decrease by 79 ± 3% (SE; n = 5). Exposure to 25 μM ouabain inhibited Na+ pumping and abolished the ΔQO2. Removal of K+ from the bathing solution inhibited Na+ pumping in darkness, but mostly shortened the duration of the ΔQO2; with a K+-sensitive microelectrode, we measured pericellular [K+] and found that it increased after the flash for about the same time as the ΔQO2. Increasing Na+ pumping in darkness by reintroducing K+ in the bath or by injecting Na+ into one of the photoreceptor cells induced a ΔQO2. Finally, intracellular injection of adenosine diphosphate and inorganic phosphate (ADP + P), the metabolic products of ATP splitting by the Na+ pump, also induced a ΔQO2 in darkness. We conclude that all the results obtained are consistent with the formulated hypothesis.

INTRODUCTION

In photoreceptor cells of invertebrates, a brief flash of intense light induces a transient increase of O2 consumption (ΔQO2) which reflects presumably an increase of mitochondrial respiration (Tsacopoulos and Poitry, 1982; Fein and Tsacopoulos, 1988a; Poitry and Widmer, 1988). Since a brief flash causes also a large entry of Na+ in the cells, and since the extra amount of Na+ is subsequently extruded from the cell by the Na+ pump at the expense of adenosine triphosphate (ATP), it was reasonable to postulate that the light-induced ΔQO2 replenished the ATP consumed for pumping Na+ out of the cell. Surprisingly, this postulate was not confirmed by...
the experimental results obtained in studies on the kinetics of the \( \Delta QO_2 \) in the honeybee drone and in *Limulus* (Tsacopoulos et al., 1983; Fein and Tsacopoulos, 1988b): the \( \Delta QO_2 \) recovered faster than \( Na^+ \) pumping. In the lateral ocellus of the barnacle however, the time course of the postillumination hyperpolarization (PIH), which is attributed to \( Na^+ \) pumping (Koike et al., 1971), is similar to the time course of the \( \Delta QO_2 \) (Poitry and Widmer, 1986, 1988). Since this difference was intriguing and since the PIH may be a poor index of \( Na^+ \) pumping (see, e.g., Edgington and Stuart, 1981), we decided to study further and in more detail the relation between the light-induced \( \Delta QO_2 \) and \( Na^+ \) pumping in that preparation.

With a \( Na^+ \)-sensitive microelectrode, we have measured the transient increase of \([Na^+](\Delta Na)\) elicited in the photoreceptor cells by a brief flash of light, and we have compared the time course of its recovery to that of the light-induced \( \Delta QO_2 \). We have also observed the effects on the \( \Delta QO_2 \) of altering \([Na^+],[Ca^{2+}],\) or \([K^+]\) in the bath, or of exposing the cells to 25 \( \mu \)M ouabain. Finally, we have injected \( Na^+ \) or adenosine diphosphate and inorganic phosphate (ADP + P\(_i\)) into one of the three photoreceptor cells and recorded the effects on the \( QO_2 \). All the results obtained are consistent with the hypothesis that the \( \Delta QO_2 \) results from an increase in the rate of \( Na^+ \) pumping.

A summary of these results (Poitry et al., 1989) has already been published.

**MATERIALS AND METHODS**

Barnacles (*Balanus eburneus*) were obtained from the Marine Biological Laboratory, Woods Hole, MA. The lateral ocellus was dissected from the animal and the tapetum was removed with fine forceps. For intracellular injections and for measurements with ion-selective microelectrodes, the preparation was softened with collagenase (17449, Serva Feinbiochemica, Heidelberg, Federal Republic of Germany), 1 mg/ml in artificial seawater (ASW) for about 10 min, after dissection. It was then placed corneal side down on a small cotton net in a perfusion chamber. During the experiment and unless otherwise specified, it was stimulated about every 5 min with an 80-ms flash of white light. The light came from below, through the transparent floor of the chamber (see Fig. 2 in Poitry and Widmer, 1988). All the experiments were performed at room temperature (22°C).

**Chambers for the Measurement of \( \Delta QO_2 \)**

To measure the time course of the \( \Delta QO_2 \), the solution bathing the preparation had to be almost stagnant (Poitry and Widmer, 1988). Therefore, to change the composition of the bathing solution rapidly, we designed two mobile perfusion chambers: the first one was used when the density of the test solution was close to that of the ASW, and the second one when it was different (which was the case for the 0 \( Na^+ \), arginine solution).

**Chamber 1.** This chamber, ~7 mm deep, was divided along most of its length into two compartments (Fig. 1 A). The solutions, which flowed continuously at a low rate (1 ml/min), were channeled along the partition between the compartments and met near the downstream end of the chamber. The measurements were made in the bulk of the solutions, which was out of the main stream and virtually stagnant. The chamber was mounted on an X-Y movement that was controlled pneumatically. To expose the preparation to the solution flowing in the other compartment, the chamber was moved so that the position of the preparation relative to the chamber followed the path indicated by the broken arrow.

**Chamber 2.** This chamber was about 1 cm deep (Fig. 1 B). It had two entries, one above the other: the lower entry received the denser solution (i.e., the 0 \( Na^+ \) solution), which flowed...
thus through the lower half of the chamber, whereas the lighter solution (i.e., the ASW) flowed through the upper half. The flow rate at each entry was the same as in chamber 1. The chamber was mounted on the coarse movement of a micromanipulator and was manually moved up or down to expose the preparation to one solution or the other.

To ensure that the solutions did not mix during the experiment, we added traces of Na-fluoresceinate (Laboratoire H. Faure, Annonay, France; final concentration < 0.5 mM) in one of the solutions which appeared thus lightly green under the light. In chamber 2, we always marked in this way the denser solution. In such low concentrations, Na-fluoresceinate had no detectable effect on the responses.

**Figure 1.** Schematic view of the two chambers: (A) chamber 1; (B) chamber 2.
**QO₂ Measurements**

The method for measuring the time course of the ΔQO₂ has been described in detail (Poitry and Widmer, 1988). In brief, we recorded the partial pressure of O₂ (PO₂) near the surface of the cluster of photoreceptor and glial cells with an O₂-sensitive microelectrode (Tsacopoulos et al., 1981). The ΔQO₂ of the cluster was then calculated from the transient decrease of PO₂ (∆PO₂) elicited by a flash of light. The formula used in the calculation was based on a model of diffusion with spherical symmetry (see Poitry and Widmer, 1988).

**Ion-selective Microelectrodes**

The ion-selective microelectrodes were made as described by Munoz and Coles (1987). Micropipettes were pulled from double-barreled quartz capillaries. The active barrel was silanized and filled with the appropriate sensor at the tip; the rest of the barrel was filled with ASW. The reference barrel was filled with either 3 M KCl or 4 M K-acetate for Na⁺-sensitive electrodes, and with 3 M NaCl for K⁺-sensitive electrodes. The tip of the microelectrode was then beveled to the desired outer diameter: 0.5–1.0 μm for intracellular measurements and 1–2 μm for extracellular measurements. Both barrels were connected to a high-input impedance amplifier. The ionic potential was obtained as the difference between active and reference barrels.

| Composition of ASW and of calibrating solutions for Na⁺-sensitive microelectrodes |
|---------------------------------|-------|------|------|------|------|
| **Calibrating solution**        | NaCl  | KCl  | CaCl₂ | MgCl₂ | Tris-HCl | pH  |
| ASW                             | 462   | 8    | 10    | 22    | 10      | 7.4 |
| 40 mM Na⁺                       | 40    | 322  | —     | 32    | 100     | 7.5 |
| 15 mM Na⁺                       | 15    | 347  | —     | 32    | 100     | 7.5 |
| 5 mM Na⁺                        | 5     | 357  | —     | 32    | 100     | 7.5 |

Na⁺-calibrating solutions with 10⁻⁶ M [Ca²⁺] were made by adding the appropriate amount of CaCl₂ to the calibrating solutions given in the table.

We used two Na⁺ sensors, one based on ligand ETH 227 (Fluka, Buchs, Switzerland; see Steiner et al., 1979), and the other on ligand bis(12-crown-4-methyl) dodecylmethylmalonate (Dojinido laboratories, Kumamoto, Japan; see “derivative 10” of Kimura and Shono, 1984). Microelectrodes with the sensor based on ETH 227 also respond to changes in [Ca²⁺] (see Coles and Orkand, 1985): in our calibration solutions (Table I), raising [Ca²⁺] from < 10⁻⁶ to 10⁻⁴ M produced a deflection of ~12 mV in 5 mM [Na⁺] and of ~2 mV in 40 mM [Na⁺]. In contrast, as shown in Fig. 2, microelectrodes with the bis(12-crown-4) sensor did not respond to such changes of [Ca²⁺].

For K⁺ measurements, we used either of the two sensors described by Coles and Orkand (1983); one was an ion exchanger, and the other was based on valinomycin, a neutral ligand. Valinomycin microelectrodes were much more sensitive to changes of [K⁺] at concentrations below 1 mM.

The electrodes were usually calibrated before and after the experiment in a small calibrating chamber at three or more concentrations of the ion. The time constant of the electrode response was measured in the calibrating chamber by switching solutions rapidly from a low to a high concentration. The Na⁺-sensitive electrodes that we used for the measurement of ΔNa⁺ had time constants in the range 0.5–3.0 s.
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FIGURE 2. Recordings made with Na$^+$-sensitive microelectrodes. The Na$^+$ sensor was either (A) ETH 227, or (B) bis(12-crown-4). $V_{m}$, potential measured by the reference barrel; $V_{Na}$, ionic potential of Na$^+$; LM, light monitor (80-ms flashes appear as vertical bars). Calibration bars are 20 mV and 2 min throughout. (a) Recordings in a photoreceptor cell; numbers indicate either the optical density of the gray filter attenuating the light stimulus, or the wavelength of the interference filter used; a light stimulus with the 410-nm interference filter (Balzers) was approximately as effective as with a gray filter of 3 logarithmic units. (b) Changes in $V_{m}$ and $V_{Na}$ when the microelectrode was withdrawn from the cell (note the change of scale for $V_{Na}$ between a and b in A. The dotted line indicates the level of identical voltage). (c) Calibration of the microelectrode; arrows indicate when the solution was changed. The solutions (see Table I) were successively (A) ASW, 40 mM Na$^+$, and 15 mM Na$^+$; or (B) ASW, 40 mM Na$^+$, 15 mM Na$^+$, 5 mM Na$^+$, 5 mM Na$^+$/10$^{-4}$ M Ca$^{2+}$, and 40 mM Na$^+$.

The changes of ionic concentration corresponding to the ionic responses were calculated with a computer. The results of the calibration of the ion-selective microelectrode were fitted with a modified version of Nikolsky's equation: $V(x) = R \cdot \ln(A \cdot x + B)$, where $x$ is the concentration of the ion and $V(x)$ is the corresponding ionic potential. The values of $R$, $A$, and $B$ for which the fit was the best were then used by the computer to display the responses in concentration units (millimolar). Usually, the value found for $R$ differed by <20% from the theoretical value (25.5 mV) for a monovalent ion at room temperature.

Solutions

The composition of the ASW and of the calibrating solutions for the Na$^+$-sensitive microelectrodes are given in Table I. The calibrating solutions for the K$^+$-sensitive microelectrodes were
made by replacing, in ASW, KCl by equimolar amounts of NaCl or NaCl by equimolar amounts of KCl; the concentrations of K⁺ chosen were 0, 1, 20, and 50 mM.

For bathing solutions in which the concentration of one ion was changed, we replaced this ion by equimolar amounts of another ion: Ca²⁺ was replaced by Mg²⁺, K⁺ by Na⁺, and Na⁺ by arginine. The density of the 0 Na⁺ solution was ~1% larger than that of ASW. This difference of density was large enough to cause the denser solution to flow under the lighter one in chamber 1 (see above); therefore, we used chamber 2 for 0 Na⁺ experiments.

Ouabain-containing solutions were made by first dissolving ouabain (g-strophanthin, 7897, E. Merck, Darmstadt, FRG) in ethanol; the final concentration of ethanol was <0.5% and had in itself no effect on the responses.

Storage of the Data

The PO₂, the membrane potential and the ionic potential were monitored continuously on a multichannel pen-recorder (Watabane Instruments Corp., Tokyo, Japan); the responses to light were also digitized and stored on magnetic disks by a computer (QT Computer Systems Inc., Lawndale, CA; see Poitry and Widmer, 1988). ΔQO₂ and ion concentration changes were calculated by the computer.

Intracellular Injections

For the intracellular injection experiments, the preparation was pinned to the Silastic (Dow Corning, Senneffe, Belgium) floor of a large unperfused chamber. The method for pressure injecting solutions was the one used by Fein and Tsacopoulos (1988a, b) and it was similar to that described by Corson and Fein (1983). Throughout the experiment, the preparation was observed on a video screen under infrared illumination. This allowed us to visualize the disturbance caused by the injection in the cell (Corson and Fein, 1983).

For Na⁺ injections, the composition of the injected solution was: 500 mM Na-aspartate, 10 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES), pH 7.0. For ADP+Pᵢ injections, the injected solutions contained either 17 mM K₂-ADP (A5285, Sigma Chemical Co., Munich), 17 mM KH₂PO₄, 466 mM K-aspartate, 10 mM HEPES, at pH 7.0, or 100 mM K₂-ADP, 100 mM KH₂PO₄, 300 mM K-aspartate, 10 mM HEPES, at pH 7.0.

To check that the injection did not in itself elicit a ΔPo₂, we injected some photoreceptor cells with a solution containing 500 mM K-aspartate, 10 mM HEPES, pH 7.0 (see, e.g., Fein et al., 1984). In four preparations that had a sensitivity to light comparable to that of the cells injected with Na⁺ or ADP+Pᵢ, K⁺ injections visible on the video screen elicited no change in the ΔPO₂ or membrane potential. We also observed in these experiments that some large or repeated injections caused a rapid and large (>5 mV) depolarization that was accompanied by a ΔPO₂; therefore, when we injected other test solutions, we discarded those injections which produced a rapid and large depolarization.

RESULTS

In the lateral ocellus of the barnacle, an 80-ms flash of light of moderate or strong intensity induces a ΔQO₂ in the cluster of photoreceptor and glial cells (Poitry and Widmer, 1988). In the photoreceptor cells, this stimulus elicits a depolarizing receptor potential followed by a PIH. Even though the mechanisms of the receptor potential and of the PIH are not completely understood, it seems well established that the receptor potential is initiated by an increase of the permeability of the cell membrane mainly to Na⁺ (Brown et al., 1970) and that the PIH, or at least part of it, reflects the consequent extrusion of Na⁺ by an electrogenic Na⁺ pump (Koike et al.,
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1971; Edgington and Stuart, 1981). As we have shown already (Poitry and Widmer, 1988), the ΔQO₂ and the PIH share some common features: both become measurable at about the same light intensity and they take about the same time to recover after stimulation (see, e.g., Figs. 3 and 4). To investigate whether Na⁺ pumping might give rise to the ΔQO₂, we compared first the PIH and the ΔQO₂ under conditions that affect the light-induced entry of Na⁺.

Replacement of Na⁺ in the Bath

The light-induced entry of Na⁺ is greatly reduced by removing Na⁺ from the bath (Brown et al., 1970). The effects of this treatment on the ΔQO₂ of the cluster and on the electrical response of the photoreceptor cells are shown in Fig. 3. In that experiment, the test solution contained arginine instead of Na⁺ (see Tsacopoulos and Poitry, 1982). After the preparation had been exposed for 13 min to the 0 Na⁺ solution, both the ΔQO₂ and the PIH were greatly reduced. The effects on the receptor potential were less conspicuous, but its amplitude and its duration were also slightly reduced (as reported by Brown et al. [1970], the receptor potential decreases much less than the light-induced current in 0 Na⁺). The treatment did not significantly affect the resting potential of the cell.

In the five preparations tested, the results were similar. On average, after 7–15 min in 0 Na⁺, the amplitude of the receptor potential was reduced by 16 ± 4% (SE), whereas the PIH was reduced by 87 ± 3% (SE) and the ΔQO₂ by 79 ± 3% (SE).
These effects were reversed by returning the preparation to ASW, but the time needed for a complete restoration of the responses was variable: in two preparations, the responses were restored after 15 min in ASW, whereas in the other three preparations it took up to 45 min.

Replacement of Ca\(^{2+}\) in the Bath

As shown by Brown et al. (1970), the concentration of Ca\(^{2+}\) in the bath ([Ca\(^{2+}\)]\(_o\)) strongly influences the light-induced current: decreasing [Ca\(^{2+}\)]\(_o\) from 10 to 1 mM causes a large increase in the light-induced current and a prolongation of the transient phase of the receptor potential. We confirmed here that a decrease of [Ca\(^{2+}\)]\(_o\) down to ~100 μM causes a prolongation of the receptor potential; in addition, we observed that it caused an increase of the ΔQO\(_2\) (not shown). In contrast, replacing all the Ca\(^{2+}\) in the bath by Mg\(^{2+}\) caused a decrease of the ΔQO\(_2\), of the receptor potential and of the PIH (Fig. 4). In four preparations, an exposure to 0 Ca\(^{2+}\) for <15 min was sufficient to reduce dramatically the ΔQO\(_2\) and the PIH, and to a lesser extent the receptor potential. On average, the receptor potential was reduced by 33 ± 15% (SE), the PIH by 97 ± 2% (SE) and the ΔQO\(_2\) by 95 ± 3% (SE). After returning the preparation to ASW, the responses recovered completely within <15 min.
Measurement of $\text{Na}_i$

Since the hyperpolarization generated by electrogenic pumping depends on the electrical conductance of the cell membrane, and since changes of conductance or of the equilibrium potential of a permeant ion can also affect the membrane potential of the cell, the PIH does not necessarily reflect the rate of $\text{Na}^+$ pumping. Instead, once the $\text{Na}^+$ influx is back to its resting value, the rate of recovery of the intracellular $\text{Na}^+$ concentration should be a much better index of the rate of pumping.

Using $\text{Na}^+$-sensitive microelectrodes, we measured the intracellular $\text{Na}^+$ concentration ($\text{Na}_i$) in the photoreceptor cells. Usually, when the microelectrode entered a cell, the ionic potential decreased abruptly at first and then more slowly for at least 15 min, to reach finally a stable resting value that was maintained for up to 4 h. With no other treatment than occasional light flashes, the average resting value of $\text{Na}_i$ measured in 18 preparations was $17.6 \pm 1.2$ mM (SE). The microelectrodes with the larger tips tended to yield higher values of $\text{Na}_i$ (average closer to 20 mM). No statistical difference was found between the resting values of $\text{Na}_i$ measured with either of the $\text{Na}^+$ sensors (see Materials and Methods).

As already shown in Fig. 2, brief flashes of light that produced a receptor potential of maximal amplitude caused a transient increase of $\text{Na}_i$ (Δ$\text{Na}_i$). Whereas the amplitude of the Δ$\text{Na}_i$ increased almost linearly with the logarithm of the light intensity, ranging up to 35 mM in two cells, its time course did not vary appreciably over the range of intensities used here (Fig. 5).

We measured Δ$\text{Na}_i$ in nine preparations: even though its general shape was always the same, consisting in a rising phase followed by an approximately exponential

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Simultaneous recordings of membrane potential (upper traces) and $\text{Na}_i$ (lower traces) for various intensities of light; the responses were superimposed for comparison. The intensity of the light was increased in steps of 0.6 log units. The $\text{Na}^+$ sensor was ETH 227. LM, light monitor.
decline, its precise time course was quite variable from one preparation to another. However, we found no definite correlation between this time course and the sensitivity of the cell or the sensor used (for six of the nine preparations, the sensor used was ETH 227). The time required for $\Delta N_a$ to reach its peak ranged from 8 s in one preparation to 21 s in another. As for the rate of recovery of the $\Delta N_a$, it had no apparent correlation with the time to peak. We selected two $\Delta N_a$ responses for each preparation tested and we measured the time they took to return from their peak to 10% of it: the values found ranged from 66 to 152 s, with an average of $103 \pm 6$ s (SE; $n = 18$).

**Comparison of $\Delta QO_2$ and $\Delta N_a$**

**Time course of the responses.** Since the $\Delta N_a$ recovered with an approximately exponential time course, the rate of its recovery ($-d\Delta N_a/dt$) was proportional at any time to its amplitude. Therefore, the time course of the recovery phase of $\Delta N_a$ should approximate the time course of the rate of $Na^+$ pumping.

In six preparations, we compared the $\Delta N_a$ measured in one of the photoreceptor cells with the $\Delta QO_2$ measured simultaneously in the whole cluster. Examples of such comparisons are given in Fig. 6. To make the comparisons easier, the $\Delta N_a$ and the $\Delta QO_2$ responses were scaled to the same height (in the second comparison of Fig. 6 A, we assumed that the fast deflexion upward on top of the $\Delta N_a$ response was an electrical artifact due to the difference in the electrical time constant of the reference and sensor barrels). The $\Delta N_a$ responses of Fig. 6 A were measured with the sensor ETH 227 and those of Fig. 6 B with the sensor bis(12-crown-4). In the three preparations in which we measured $Na_i$ with the sensor ETH 227, the responses had an almost identical time course (see Fig. 6 A). In the three preparations in which we measured $Na_i$ with the sensor bis(12-crown-4), there was a slight difference in their time to peak and their rate of recovery (see Fig. 6 B), but those differences were not systematic (i.e., the sign of the difference was not always the same for one pair of responses and for another). In addition, we did not find any significant difference in the time necessary for the $\Delta N_a$ and the $\Delta QO_2$ to return back to the baseline.

**Na$^+$/O$_2$ ratio.** If the metabolic energy produced by the $\Delta QO_2$ balances the energy used in pumping $Na^+$ out of the photoreceptor cells after the flash, the total extra amount of O$_2$ consumed by the cluster should be proportional to the extra amount of $Na^+$ that entered the cells. From our data, we can estimate the extra amount of O$_2$ consumed by the cluster as the time integral of $\Delta QO_2$ ($\int \Delta QO_2 dt$) times the volume of the cluster ($V_c$). Similarly, the extra amount of $Na^+$ that entered the cells can be estimated as the peak value of $\Delta N_a$ ($\Delta N_{a_{max}}$) times the volume of the photoreceptor cells ($V_{ph}$). Thus, the $Na^+$/O$_2$ ratio of the responses to light can be calculated as the ratio $\Delta N_{a_{max}}/\int \Delta QO_2 dt$ times the ratio $V_{ph}/V_c$, which is 0.48 (Krebs and Schaten, 1976).

We compared $\int \Delta QO_2 dt$ with $\Delta N_{a_{max}}$ for the six preparations for which we had compared the time course of $\Delta QO_2$ and $\Delta N_a$. While both $\int \Delta QO_2 dt$ and $\Delta N_{a_{max}}$ increased with light intensity, their relative increase was not always the same and, thus, the ratio $\Delta N_{a_{max}}/\int \Delta QO_2 dt$ varied with the intensity of the light. We attributed this lack of parallelism between $\int \Delta QO_2 dt$ and $\Delta N_{a_{max}}$ to differences in sensitivity.
FIGURE 6. Pairs of ΔO₂ and ΔNa responses obtained in four preparations. (A) The sensor used was ETH 227; the noisier trace is Na. (B) The sensor used was bis(12-crown-4); trace a is Na. LM, light monitor.
between the photoreceptor cells within a cluster. For this reason, and also because
the relative errors of our measurements were then smaller, we considered the
Na+/O₂ ratio in each preparation as the ratio calculated for the flashes of the highest
intensity tested. These values of Na+/O₂ ranged from 10 to 32, with an average of
19.2 ± 3.8 (SE; n = 6), which is not statistically different from the maximal value of
Na+/O₂ expected from theory (see Discussion).

**Effects of Ouabain**

To investigate further the relation between ΔQO₂ and Na⁺ pumping, we examined
whether treatments that inhibit Na⁺ pumping affect also the ΔQO₂. Initially, we
exposed a preparation to 25 μM ouabain, a specific inhibitor of the Na⁺ pump (see
Glynn and Karlish, 1975). This treatment caused both the ΔQO₂ and the PIH to
decrease and to disappear irreversibly within 15 min. However, the cell depolarized
slowly (10 mV after 10 min); in addition, the receptor potential was slightly
shortened and its peak value was decreased, indicating that the treatment may have
decreased the light-induced entry of Na⁺.

Brown and Lisman (1972) reported that, in the ventral eye of *Limulus*, the
decrease of the receptor potential observed with ouabain is prevented by lowering
[Ca²⁺] in the bath to 100 μM. So we tested the effect of exposing the preparation to
a 25 μM ouabain solution containing 100 μM [Ca²⁺]. (As mentioned above, a similar
decrease of [Ca²⁺] in the bath causes per se an increase of the ΔQO₂.) The results of
this experiment are presented in Fig. 7. After 30 min in the ouabain/low [Ca²⁺]
solution (Fig. 7 A), the effects on the ΔQO₂, the PIH and the resting potential were
similar to those observed in the ouabain/normal [Ca²⁺] solution: the ΔQO₂ and the
PIH were abolished, and the cell depolarized by ~8 mV. In contrast, the receptor
potential became slightly prolonged, this time, and its peak value did not decrease. In
addition, the simultaneous measurement of Naᵢ showed that the peak amplitude of
ΔNaᵢ did not diminish during the treatment, but that the recovery phase was
gradually slowed down (one cell; not shown here). This gradual inhibition of the
recovery phase of ΔNaᵢ, together with a sustained increase of Naᵢ in the dark,
resulted in the overall increase in Naᵢ shown in Fig. 7B. None of these effects was
reversed by returning the preparation to ASW. The effects of ouabain/low [Ca²⁺] on
the ΔQO₂ and on the PIH were reproduced in two other preparations.

**Replacement of K⁺ in the Bath**

Another way to inhibit Na⁺ pumping, yet reversibly, is to remove K⁺ from the bath
(see, e.g., Edgington and Stuart, 1981) and thereby to prevent the exchange of
intracellular Na⁺ for extracellular K⁺ by the Na⁺ pump. For reasons analogous to
those given above in the case of the ouabain treatment (see also Brown and Ottoson,
1976), we tested here the effects of exposing the preparation to 0 K⁺ solutions
containing either normal or low [Ca²⁺].

In both 0 K⁺ solutions, the ΔQO₂ and the PIH returned much faster to the
baseline than in ASW (Fig. 8 A). However, the effects of those solutions on the
amplitude of the responses were different. In 0 K⁺/normal [Ca²⁺], the amplitude of
the PIH increased slightly, whereas that of the ΔQO₂ diminished gradually (three
preparations). In contrast, in 0 K⁺/low [Ca²⁺], the amplitude of the PIH was
decreased and the amplitude of the $\Delta QO_2$ was maintained or even increased (the increase of the $\Delta QO_2$ and the decrease of the PIH shown in Fig. 8 A were the largest observed in the three preparations tested). In addition, there was a slight depolarization of the cell in 0 K$^+$/low [Ca$^{2+}$] (10 mV in 15 min), and $Na_i$ increased more and faster during this treatment, reaching ~100 mM within 15 min (two preparations; Fig. 8 B).
FIGURE 8. Effects of exposing the preparation to 0 K+/low [Ca^{2+}] (100 μM). (A) Recordings of membrane potential in a photoreceptor cell (upper traces), ΔQO₂ in the cluster (middle traces), and pericellular [K⁺] (lower traces). LM, light monitor. (B) Graph of Naᵢ in darkness during the treatment; the vertical arrows indicate the time when the membrane potential traces in A were recorded. ΔQO₂ₑ and K₀ were measured simultaneously in one preparation, whereas membrane potential and Naᵢ were recorded in another preparation.
Role of pericellular K⁺ (Kₒ). The increase in Naᵢ indicated that Na⁺ pumping was certainly inhibited when the cells were exposed to 0 K⁺. Therefore, the persistence of a large ΔQO₂ was at odds with the results obtained with ouabain, unless the inhibition of Na⁺ pumping by 0 K⁺ was transiently relieved after a flash of light. Since this could happen if the cellular response to light triggered a large efflux of K⁺, we measured the pericellular concentration of K⁺ (Kₒ) in the cluster with an ion-selective microelectrode.

For these measurements, we pushed the K⁺-sensitive microelectrode inside the preparation, as if we intended to impale a cell. When the preparation was bathed in ASW, a flash of light elicited a large increase of Kₒ (ΔKₒ) (see Fig. 8 A, left) that ranged from 10 to 35 mM (four preparations). In 0 K⁺, with either normal or low [Ca²⁺] (one and three preparations, respectively), Kₒ diminished rapidly, falling to 1 mM or less within 12 min. At that point, there was still a large ΔKₒ and its time course was similar to the time course of the ΔQO₂ measured in those conditions (Fig. 8 A, right). In addition, the amplitude of ΔKₒ decreased gradually in 0 K⁺/normal [Ca²⁺], and it was maintained in 0 K⁺/low [Ca²⁺], like that of the ΔQO₂.

Restoration of [K⁺] in the Bath

If the persistence of the ΔQO₂ in 0 K⁺ is due to the transient relief of the inhibition of Na⁺ pumping by the light-induced ΔKₒ, there should be an increase of Na⁺ pumping and of the QO₂ upon returning the preparation from 0 K⁺ to ASW.

In the 0 K⁺ experiments described above, we recorded a prolonged hyperpolarization of the cell membrane upon returning to ASW (not shown here, but see, e.g., Edgington and Stuart, 1981), indicating that Na⁺ pumping was activated. The hyperpolarization lasted from 3 to 6 min after the return to ASW. Unfortunately, we did not obtain a convincing record of an increase of QO₂ in these experiments because the displacement of the chamber, which was necessary for switching solutions, caused artifacts in the PO₂ signal. Therefore, in order to examine how the restoration of K⁺ in the bath affected the QO₂, we had to use another chamber which was constantly perfused at a high flow rate. Since the calculation of the QO₂ was based on the assumption that the bath was stagnant (see Materials and Methods), we could not calculate the QO₂ and we simply recorded the changes of the PO₂ measured at the surface of the cluster.

The PO₂ recorded in one of the three preparations tested is shown in Fig. 9. At the start of the recording, the preparation was bathed in ASW. When a flash of light was delivered, it elicited a transient decrease of the PO₂, reflecting an increase of the QO₂ of the cluster. The perfusion was then switched from ASW to 0 K⁺ (normal [Ca²⁺]) and the PO₂ increased slightly indicating a small decrease of the basal QO₂. At that time, a flash elicited a decrease of the PO₂ which then returned faster to the baseline than in ASW. After about 30 min in 0 K⁺, the preparation was exposed again to ASW which caused a decrease of the PO₂ that lasted ~7 min. The results obtained in the two other preparations were similar.

Intracellular Injections of Na⁺

The restoration of [K⁺] in the bath was one way to activate Na⁺ pumping without actually stimulating the cells with light. Another way to do so is to inject Na⁺ into
one of the three photoreceptor cells: if the ΔQO₂ is induced by an increase of Na⁺ pumping, an injection of Na⁺ should cause an increase of the QO₂.

The effects of pressure-injecting a solution containing 500 mM Na-aspartate into one of the cells are shown in Fig. 10 (since we did not know in which of the three cells the injection was made, we did not calculate the ΔQO₂ in these experiments and we simply present here the PO₂ recorded at the surface of the cluster). After the injection, the membrane hyperpolarized and this hyperpolarization had a time course similar to the PIH. At the same time, the PO₂ recorded at the surface of the cluster decreased transiently indicating an increase of the QO₂ of the cell.

We injected Na⁺ in six preparations and, in all of them, the effects of the injections were qualitatively the same: an injection that caused a hyperpolarization of the cell membrane caused also a transient decrease of the PO₂. The amplitude of the hyperpolarization varied from ~5 mV in one cell to 50 mV in another, and the decrement of PO₂ from ~1 mm Hg to 3 mm Hg. Except in one cell, the PO₂ fell somewhat more slowly after an injection than after a flash. Usually, a single injection was enough to produce those effects.

**Intracellular Injections of ADP+P₁**

To extrude Na⁺ from the cell and to exchange it for K⁺, the Na⁺ pump must split ATP to ADP+Pᵢ. Therefore, in an attempt to test whether an increase in the rate of ATP splitting by the Na⁺ pump causes the ΔQO₂, we pressure-injected ADP+Pᵢ into one of the photoreceptor cells.

Since the stoichiometry of the Na⁺ pump implies that three Na⁺ are pumped out for each ATP split, we attempted to inject an amount of ADP+Pᵢ about three times less than we had injected for Na⁺ previously. In addition, to mimic the consequences of Na⁺ pumping, ADP+Pᵢ should not be injected briefly, but over tens of seconds, because this is the time during which Na⁺ pumping occurs normally. We injected a solution containing 17 mM ADP+Pᵢ. Thus, about 10 injections of that solution, with an interval of a few seconds in between, would introduce in the cell about the same amount of ADP+Pᵢ as produced by the pump after one of our Na⁺ injections. As
shown in Fig. 11A, a series of injections of that solution did elicit a drop of the $P_{O_2}$, with no measurable effect on the membrane potential of the cell. Such results were obtained in two preparations and the amplitude of the $P_{O_2}$ drops ranged from about 1 to 5 mmHg.

Because it was difficult to do a series of injections without impairing the quality of the impalement, we also injected some cells with a solution containing 100 mM ADP + Pi; with that solution, an effect on the $P_{O_2}$ should be seen with only one or two injections. The results presented in Fig. 11B show that this was actually the case. The solution was injected in five preparations and the injections caused drops of the $P_{O_2}$ that ranged in amplitude from $<1$ to $\sim5$ mm Hg. The time course of the $\Delta P_{O_2}$ was similar to that of a light-induced $\Delta P_{O_2}$, except in two preparations where it was slightly slower. In most cases, the injections also caused slow and maintained depolarizations up to 10 mV; these depolarizations usually appeared after a number of injections had been made.

**Figure 10.** Effects of injecting Na$^+$ into one of the photoreceptor cells. $P_{O_2}$ was recorded near the surface of the cluster. $V_m$, membrane potential; IM, injection monitor; LM, light monitor.

**DISCUSSION**

The results of a previous study (Poitry and Widmer, 1988) indicate that the light-induced $\Delta QO_2$ originates from mitochondria and that it is a consequence of the phototransformation of rhodopsin. In the present study, we have investigated the relation between the $\Delta QO_2$ and Na$^+$ pumping, and we found no evidence against the hypothesis that the $\Delta QO_2$ results from an increase in the rate of Na$^+$ pumping. In particular, we have shown that:

(a) flashes of light which elicit a $\Delta QO_2$ elicit also an increase of Na$_i$ ($\Delta$Na$_i$) of several millimolar in the photoreceptor cells;

(b) the ratio of this extra amount of Na$^+$ to the extra amount of O$_2$ consumed is close to the theoretical value of 18;

(c) the light-induced $\Delta$Na$_i$ and the light-induced $\Delta QO_2$ return to their baseline values with a similar time course;

(d) a large reduction of the light-induced entry of Na$^+$ causes a large reduction of the light-induced $\Delta QO_2$. 
(e) exposing the preparation to ouabain abolishes both Na\(^+\) pumping and light-induced ΔQO\(_2\);

(f) in 0 K\(^+\), there is still a light-induced ΔQO\(_2\), but its time course is then similar to that of the light-induced ΔK\(_w\);

(g) either intracellular injection of Na\(^+\) or restoration of [K\(^+\)] in the bath elicits a transient increase in the rate of Na\(^+\) pumping and a ΔQO\(_2\); and

(h) injection of ADP+P\(_i\), the metabolic products of Na\(^+\) pumping, elicits also a ΔQO\(_2\).

![Figure 11](image)

**Figure 11.** Effects of injecting ADP+P\(_i\) into one of the photoreceptor cells. The concentration of ADP+P\(_i\) in the pipette was (A) 17 mM or (B) 100 mM. PO\(_2\) was recorded near the surface of the cluster. V\(_m\), membrane potential; IM, injection monitor; LM, light monitor.

**Na\(^+\), in Darkness**

We have throughout expressed the measurements of Na\(^+\) in molarity units (see Materials and Methods). In doing so, we made the implicit assumption that the activity coefficient for Na\(^+\) in the cytosol was equal to that in the calibrating solutions. In drone retina, measurements of Na content by electron beam micro-probe analysis suggest that this assumption is valid (Coles and Rick, 1985), but the evidence is lacking in barnacle.

The resting value of Na\(_i\) that we measured in the photoreceptor cells was 17.6 ± 1.2 mM. This corresponds to an intracellular Na\(^+\) activity (a\(_{Na}\)) of about 12 mM, if we take the activity coefficient for Na\(^+\) in the calibrating solutions as 0.7 (see Taylor and Thomas, 1984). This value of a\(_{Na}\) is much lower than the 28 mM average reported by Brown (1976), but it comes close to the lowest values he measured (8–12 mM; see also Brown and Cornwall, 1975; Brown and Ottoson, 1976).

Our value for Na\(_i\) lies within the range of those obtained in other marine preparations, somewhat on the higher side (10–19 mM; Vaughan-Jones, 1977; Mullins et al., 1988; Taylor and Thomas, 1984; Fein and Tsacopoulos, 1988).
Taylor and Thomas (1984) concluded from their results that, in large cells, leakage around the site of penetration of the microelectrode causes often a substantial overestimation of $\Delta Na_i$. We made two observations consistent with this view: our microelectrodes with the largest tips gave on average a higher value for the resting $Na_i$, and they usually recorded a smaller PIH. However, we used them preferentially because they responded significantly faster to $Na^+$ changes and our aim was to measure the time course of the light-induced $\Delta Na_i$.

**Light-induced $\Delta Na_i$**

An 80-ms flash of moderate or intense light elicited a $\Delta Na_i$ in the photoreceptor cells. As observed in other photoreceptor cells of invertebrates (Coles and Orkand, 1982; Fein and Tsacopoulos, 1988b), the peak amplitude of $\Delta Na_i$ increased almost linearly with the logarithm of the light intensity, while the time course of the response remained the same.

**Time to peak.** $\Delta Na_i$ reached its peak between 8 and 21 s after the flash, i.e., a time when the cell membrane had repolarized. A delay of several seconds between the end of the stimulus and the peak of $\Delta Na_i$ is surprising. However, a similar delay is observed in other preparations: in the honeybee drone retina the delay is ~6 s (see Tsacopoulos et al., 1983), and it is ~15 s in the ventral eye of Limulus (see Fein and Tsacopoulos, 1988b). Tsacopoulos et al. (1983) attributed it both to the time constant of the $Na^+$-sensitive microelectrode and to a local damage of the cell membrane at the site of impalement; this is probably also true for our measurements.

**Peak amplitude.** At the lowest intensity eliciting a receptor potential of maximal amplitude, $\Delta Na_i$ was undetectable. For a light intensity ~100 times higher, its peak amplitude was in the range 10–20 mM, which is rather large when compared to the $\Delta Na_i$ measured previously in barnacle or in other preparations. In Limulus, for example, $\Delta Na_i$ reaches 11 mM for a 1-s flash of intense light (Fein and Tsacopoulos, 1988b), and in the drone, it reaches ~9 mM after an intense 20-ms flash (Coles and Orkand, 1982; note however that for the drone the $[Na^+]$ in the bath is less than half that in ASW). In the lateral ocellus, Brown and Cornwall (1975) measured a light-induced increase of $dNa_i$ from ~9 to 18 mM, corresponding to a $\Delta Na_i$ of 13 mM. Since this increase was obtained after 5-min illumination with intense light, whereas we used brief flashes, the results may seem conflicting. However, we don't think that they are: first, the amplitude at the peak of $\Delta Na_i$ does not increase much with the duration of the illumination (see Fig. 2); in addition, since our ASW contained only half as much $Ca^{2+}$ as theirs, the light-induced $Na^+$ entry was probably much larger in our experiments (see Brown et al., 1970).

**Time course of the recovery.** On average, $\Delta Na_i$ returned from its peak to 10% of it within 103 s. Since the time course of the recovery was approximately exponential, this corresponds to an average time constant of 44 s. That $Na_i$ can recover with an exponential time course has been observed already in various preparations, either after physiological stimulation (Tsacopoulos et al., 1983; Fein and Tsacopoulos, 1988b) or after $Na^+$ loading (Thomas, 1969; Brown and Ottoson, 1976; Deitmer and Ellis, 1978; Eisner et al., 1981). However, even when they are measured at the same...
temperature, the values found for the time constant of the recovery vary greatly
from one tissue to another and from one species to another (see, e.g., Gadsby and
Cranefield, 1979): in photoreceptor cells for example, the value reported for the
honeybee drone is ~20 s (Tsacopoulos et al., 1983), whereas it is ~200 s for Limulus
(Fein and Tsacopoulos, 1988b). Since Na⁺ is extruded from the cytosol through the
plasma membrane, the time constant of the recovery depends on the surface-to-
volume ratio of the cell, and on the density of active sites and their turnover rate.
Therefore, the variability of the time constant reflects probably interspecies varia-
tions of some of those parameters, as suggested by Gadsby and Cranefield (1979). In
the photoreceptor cells indeed, part of the difference in the time constant of the
recovery may be due to a difference in the surface-to-volume ratio since this ratio is
~6 μm⁻¹ in barnacle (see Krebs and Schaten, 1976), 1.8 μm⁻¹ in the honeybee drone
(see Tsacopoulos and Poitry, 1982) and about 1 μm⁻¹ in Limulus (see Fein and
Tsacopoulos, 1988b).

Koike et al. (1971), who studied the PIH in the lateral ocellus, concluded that it is
due to electrogenic Na⁺ pumping and they estimated the time constant of its
recovery as 39 s, which is close to the 44 s measured here for the recovery of ΔNa⁺.
This suggests that the recovery of ΔNa⁺ is due to an increased rate of Na⁺ pumping,
which is confirmed by our observation that this recovery is blocked by ouabain, a
specific inhibitor of the Na⁺ pump (see, e.g., Glynn and Karlish, 1975).

Comparison of ΔNa⁺ and ΔQO₂

There are obvious similarities between the ΔNa⁺ and the ΔQO₂ in the lateral ocellus.
First, both responses are detectable only after flashes that elicit a receptor potential
of maximal amplitude. In addition, their peak amplitude increases linearly with the
logarithm of the light intensity, while their time course remains the same, except for
strong intensities where the ΔQO₂ becomes prolonged (see Poitry and Widmer,
1988). Finally, they return from their peak with a similar time course, reaching 10%
of the peak at about the same time on average, i.e., after 105 s for the ΔQO₂ (Poitry
and Widmer, 1988) and after 103 s for the ΔNa⁺, as shown in this study. This
similarity in the time course of ΔNa⁺ and ΔQO₂ contrasts with the results obtained in
drone (Tsacopoulos et al., 1983) and in Limulus (Fein and Tsacopoulos, 1988b)
where the ΔQO₂ recovers three to five times sooner than the ΔNa⁺ (see below for a
more detailed discussion of those differences).

If the ATP used in pumping out the extra Na⁺ after the flash is replenished via the
ΔQO₂, two conditions at least have to be fulfilled: (a) the increase in the rate of Na⁺
pumping must not last longer than the ΔQO₂, and (b) there must be at least as much
ATP produced via the ΔQO₂ as consumed for the increased pumping.

Except for a few seconds after reaching its peak, the ΔNa⁺ returned to the baseline
with an exponential time course. This observation suggests that, provided the Na⁺
influx was back to its preillumination level during the recovery of ΔNa⁺: (a) the
increase in the rate of Na⁺ pumping was equal to the rate of decrease of ΔNa⁺; and
(b) the rate of decrease of ΔNa⁺ was proportional to the amplitude of ΔNa⁺ at any
time during the recovery. Therefore, since the ΔNa⁺ did not recover later than the
ΔQO₂, condition a was fulfilled.

Assuming that six ATP molecules are produced per molecule of O₂ consumed
(Harris et al., 1980; Zweier and Jacobus, 1987) and that the Na⁺ pump extrudes three Na⁺ per ATP molecule hydrolyzed (see, e.g., Glynn and Karlish, 1975), we conclude that one molecule of O₂ can replenish the ATP consumed for the extrusion of 18 Na⁺. Therefore, condition b implies that the ratio (moles of extra Na⁺ entering after a flash)/(moles of extra O₂ consumed) be no more than 18. From the peak amplitude of ΔNa⁺ measured for intense flashes and from the time integral of the associated ΔQO₂, we estimated this ratio as 19.2 ± 3.8 (SE), which is not significantly larger than the limiting value prescribed by condition b. However, as indicated by the large scatter of the results, this value is certainly a poor estimate of the true Na⁺/O₂ ratio. In addition, the results may have been biased by the fact that we used the peak of ΔNa⁺ to estimate the extra amount of Na⁺ entering the cell: this implies that Na⁺ increases uniformly throughout the cells and that Na⁺ pumping did not increase until Na⁺ reached its peak.

**Light-induced Na⁺ Entry and ΔQO₂**

When either Na or Ca ions were removed from the bath, the receptor potential was decreased and the PIH almost abolished, suggesting that the light-induced entry of Na⁺ and the consecutive increase in Na⁺ pumping were both greatly reduced. In those conditions, the ΔQO₂ was also greatly reduced, as we expect it to be if it reflects the increased rate of pumping.

The persistence of the receptor potential in 0 Na⁺ has been already observed by Brown et al. (1970) who explained it by the fact that the light-induced increase of the membrane permeability is not very selective, although Na⁺ carries most of the light-induced current.

The reduction of the electrical response of the cells in 0 Ca²⁺ was unexpected: Brown and Blinks (1974) reported an abolition of the aequorin response with this treatment, but they did not mention any decrease of the receptor potential. In Limulus, Bolsover and Brown (1985) have shown that a prolonged exposure to 0 Ca²⁺ or EGTA, together with frequent stimulation with light flashes, causes a decrease of the receptor potential, and they concluded that Ca²⁺ participates in the excitation of the photoreceptor cells (see also Payne et al., 1986). Our results indicate that this may also be true for barnacle.

**Inhibition of Na⁺ Pumping**

We exposed the preparation to 25 μM ouabain or to 0 K⁺ solutions, with either normal or low [Ca²⁺] (100 μM). From their effects on the PIH or on Na⁺, we concluded that, as expected, all the treatments had an inhibitory effect on the rate of Na⁺ pumping. However, the most striking observation was that, whereas the exposure to ouabain abolished both the PIH and the ΔQO₂, the exposure to 0 K⁺ reduced the duration of the responses, but did not abolish them; in addition, in 0 K⁺, the amplitude of the PIH became larger with normal [Ca²⁺] than with low [Ca²⁺], whereas the converse happened for the ΔQO₂.

This apparent contradiction between the results obtained with ouabain and those obtained with 0 K⁺ was seemingly resolved by our measurements of the pericellular [K⁺] (Kₒ): we show here that Kₒ increases transiently after stimulation, and that the duration of this increase is similar to the duration of the ΔQO₂. Interestingly, in 0
K+, the steady value of K_o in darkness was between 0.5 and 1 mM; these concentrations, which are two to three times lower than the estimated K_m of the Na+ pump for K_o (see Gadsby, 1984), were apparently low enough to inhibit seriously the pumping of Na+ in the photoreceptor cells.

Together, the measurements of K_o and those of Na_i may provide an answer as to why, in 0 K+, the amplitude of the ΔQO_2 is larger with low [Ca^{2+}] than with normal [Ca^{2+}]: since Na_i increased faster and the light-induced rise of K_o was maintained longer with low [Ca^{2+}], the rate of Na+ pumping was probably much more activated by the rise of K_o (the K_m of the Na+ pump for Na_i is controversial, but it seems that the rate of pumping can still increase for values of Na_i higher than 50 mM in excitable cells; see, e.g., Gadsby, 1984; Brink, 1983). Besides, the light-induced rise in K_o suggests that, like in the median eye of the giant barnacle (Edgington and Stuart, 1981), there is a transient activation of a K+ conductance after the flash in the lateral ocellus. If such is the case, the PIH observed in 0 K+ probably reflects the time course of the conductance change and not the rate of Na+ pumping as it does otherwise; in addition, the smaller amplitude of the PIH measured in 0 K+/low [Ca^{2+}] may be due to the fact that, as the results of Brown and Ottoson (1976) have suggested, the equilibrium potential for K+ is less negative with low than with normal [Ca^{2+}].

The effects of ouabain or 0 K+ seen here are qualitatively similar to those reported by Rang and Ritchie (1968a, b) for the vagus nerve of rabbit: in that preparation, the posttetanic hyperpolarization and the associated ΔQO_2 are both abolished in ouabain, but not in 0 K+ where they recover slightly faster than in normal solution and are followed by an additional slow component. As we have, Rang and Ritchie explained their results by assuming that, in the absence of K+ in the bath, K_o increased after the stimulation enough to reactivate the pumping of Na+.

Activation of the QO_2 in Darkness

If the light-induced ΔQO_2 reflects a light-induced increase in the rate of Na+ pumping, it should be possible to elicit a ΔQO_2 in darkness either by causing a direct activation of Na+ pumping or by reproducing some of the effects of an increased rate of pumping.

We used two procedures aimed at increasing the rate of Na+ pumping, either in all three photoreceptor cells or in only one of them: the restoration of bath [K+] and the intracellular injection of Na-aspartate. With both procedures, we recorded a hyperpolarization of the cell membrane, indicating that Na+ pumping was activated. In addition, the PO_2 measured near the cells decreased, indicating that the QO_2 was increased.

To mimic the effects of the splitting of ATP by the Na+ pump, we injected ADP+P_i. Since these injections caused also an increase of the QO_2, all the results were consistent with our starting hypothesis. A detailed comparison of the light-induced ΔQO_2 with the ΔQO_2 elicited by injections is difficult: the preparation contains three photoreceptor cells and we were unable to determine precisely the location of the injected cell relative to the O_2-sensitive microelectrode, which is essential for the calculation of the ΔQO_2 (see Materials and Methods).

It is worth noting that, while an activation of the QO_2 by the restoration of [K+] in
the bath has already been reported by Rang and Ritchie (1968a), there is to our knowledge no previous report of such activation by intracellular injection of Na⁺ or of ADP+P_i (cf. Fein and Tsacopoulos, 1988b, and see below).

Role of Ca²⁺?

The results obtained in barnacle photoreceptor cells are in more than one respect at odds with those obtained in drone or in Limulus photoreceptor cells. To summarize: while the ΔQO₂ recovers at least three times faster than the PIH and the ΔNaᵢ in drone and in Limulus (Tsacopoulos et al., 1983; Fein and Tsacopoulos, 1988b), there is no such difference in barnacle; in addition, in either 0 Na⁺ or ouabain solutions, the ΔQO₂ does not decrease by >80% in drone, even after a 40-min exposure (Tsacopoulos and Poitry, 1982), whereas it is abolished in <20 min in barnacle; finally, an intracellular injection of Na⁺ in sufficient amount to hyperpolarize the cell membrane elicits a ΔQO₂ in barnacle, but not in Limulus (Fein and Tsacopoulos, 1988b).

In Limulus, Fein and Tsacopoulos (1988a) have shown that the intracellular injection of Ca²⁺ elicits a ΔQO₂ and they suggested that the light-induced ΔQO₂ is controlled by a light-induced rise of the intracellular [Ca²⁺] (Caᵢ). Since light induces a rise of Caᵢ in the photoreceptor cells of barnacle as well (Brown and Blinks, 1974), we have to consider whether our results are consistent with the idea that the QO₂ is controlled by Caᵢ. Even though we did not make intracellular injections of Ca²⁺ in this study, we think that, as a whole, our results are difficult to reconcile with this idea. There may have been a rise in Caᵢ when we injected Na⁺ or ADP+P_i (see Lisman and Brown, 1972; O'Day and Gray-Keller, 1989; Smith et al., 1985), but this seems unlikely in the case of the restoration of bath [K⁺] (see Brown and Lisman, 1972; Brown and Ottoson, 1976). In addition, while the effects of ouabain and of 0 K⁺ are certainly consistent with the "pump hypothesis," they are difficult to explain using the "Caᵢ hypothesis." Therefore, we conclude that the QO₂ of stimulated nervous tissue can be controlled by at least two different mechanisms: one in which the rate of ATP splitting by the Na⁺ pump plays a central role, and the other in which Caᵢ plays a central role. In the lateral ocellus of barnacle, like in mammalian nerve (see Ritchie, 1973), the QO₂ seems to be controlled primarily via the first mechanism, whereas in the ventral eye of Limulus, and maybe also in the retina of the honeybee drone, the control seems to occur via the second mechanism.

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