

Light-induced Oxygen Consumption in *Limulus* Ventral Photoreceptors Does Not Result from a Rise in the Intracellular Sodium Concentration

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ABSTRACT Illumination of *Limulus* ventral photoreceptors leads to an increase in the intracellular concentration of sodium, $[Na^+]_i$, and to an increase in the consumption of O_2 (ΔQ_{O_2}). After a 1-s light flash, it takes ~ 480 s for $[Na^+]_i$ to return to within 10% of its preillumination level, whereas ΔQ_{O_2} takes ~ 90 s. Thus, the ΔQ_{O_2} is complete long before $[Na^+]_i$ has returned to its resting level. Pressure injection of Na^+ into the cell in order to elevate $[Na^+]_i$ to the same levels as attained by illumination causes a rise in $[Na^+]_i$ that returns to baseline with the same time course as the light-induced rise in $[Na^+]_i$. However, the injection of Na^+ does not lead to an increase of the consumption of O_2 . We conclude that activation of the Na pump by a rise in $[Na^+]_i$ is not a factor involved in the light-induced activation of O_2 consumption in these cells.

INTRODUCTION

A fundamental problem of neurobiology is the question of how neurons control their metabolism to provide metabolic energy for cellular work. Typically, when neurons are active, there is an influx of Na^+ into the cell and an efflux of K^+ into the extracellular space. The entry of Na^+ elevates the intracellular concentration of Na^+ , $[Na^+]_i$, which activates the Na pump to extrude Na^+ at the expense of ATP, which is hydrolyzed to ADP and P_i (for reviews, see Ritchie, 1973; De Weer, 1975). From biochemical studies on isolated mitochondria, it was predicted that the increase of cytoplasmic ADP would stimulate ATP production by mitochondrial respiration and thereby increase the rate of neuronal O_2 consumption (Chance and Connelly, 1957). This prediction was confirmed when it was found that ouabain, an inhibitor of the Na pump, reduced or abolished the steady state increase in O_2 consumption associated with repetitive nerve stimulation (Baker and Connelly, 1966; Ritchie, 1973). Recently, the prediction was directly tested in a sensory neuron, the bee photoreceptor, where

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the time course of O_2 consumption (ΔQ_{O_2}) (Tsacopoulos and Poitry, 1982) was compared with the time course of the increase in $[Na^+]_i$ ($\Delta[Na^+]_i$) after a brief flash (Tsacopoulos et al., 1983). It was found that the decay of ΔQ_{O_2} preceded the decline of $\Delta[Na^+]_i$ to its preillumination level. Tsacopoulos et al. (1983) hypothesized that mitochondrial respiration in bee photoreceptors is stimulated by a signal that precedes the rise in ADP resulting from the increased activity of the Na pump.

These experiments, which appeared to exclude activation of the Na pump as the cause of increased mitochondrial respiration in bee photoreceptors, had a weakness. The measured ΔQ_{O_2} was the response of thousands of photoreceptors, while $[Na^+]_i$ was measured in a single cell that might have been damaged by impalement and consequently could have given an artificially slow time course for $\Delta[Na^+]_i$. Therefore, we decided to extend these studies to individual *Limulus* ventral photoreceptors, where we could record both ΔQ_{O_2} and $\Delta[Na^+]_i$ from the same cell and where we could artificially elevate $[Na^+]_i$ by pressure-injecting Na^+ into the cell in the dark. We found that the decay of ΔQ_{O_2} precedes the return of $\Delta[Na^+]_i$ to its preillumination level, confirming the findings in bee photoreceptors. We also found that pressure injection of Na^+ so as to elevate $[Na^+]_i$ to the same levels induced by light does not activate O_2 consumption. We conclude that activation of the Na pump by an elevation of $[Na^+]_i$ does not activate oxidative metabolism in *Limulus* ventral photoreceptors. Some preliminary results have been published in abstract form (Tsacopoulos et al., 1986).

METHODS

Ventral nerves were removed from the animal, desheathed, treated with 1% Pronase (Calbiochem-Behring Corp., La Jolla, CA) for 45 s, and pinned to the bottom of a transparent chamber coated with Sylgard (Dow Corning Corp., Midland, MI) (Millecchia and Mauro, 1969). The cells were bathed in an artificial seawater (ASW) solution consisting of (millimolar): 435 NaCl, 10 KCl, 20 $MgCl_2$, 25 $MgSO_4$, 10 $CaCl_2$, 10 HEPES, pH 7.0. Miniature recessed-tip platinum O_2 electrodes were fabricated as previously described (Tsacopoulos et al., 1981). Double-barreled quartz Na^+ -selective electrodes were fabricated as described previously (Munoz et al., 1983; Coles and Orkand, 1985; Munoz and Coles, 1987). Intracellular electrodes for injection of Na^+ were pulled on a BBCH microelectrode puller (Mecanex, Geneva, Switzerland) of thin-walled capillary tubing (Frederick Haer & Co., Brunswick, ME) and were filled with 500 mM Na-aspartate and 10 mM HEPES, pH 7.0. Throughout the experiment, during pressure injection, cells were observed on a video monitor under infrared illumination as described by Corson and Fein (1983).

The kinetics of O_2 consumption (ΔQ_{O_2}) were computed from the P_{O_2} recording by considering the photoreceptor as a sphere that consumes O_2 uniformly throughout (Tsacopoulos and Poitry, 1982). The radius of the photoreceptors was measured on the screen of the video monitor and the value, in the range of 40–50 μm , was included in the calculation. The diffusion (D) and solubility (α) coefficients of O_2 in the cell were assumed to be the same as in the bath ($D = 2 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$, $\alpha = 31 \mu l \text{ O}_2 \text{ STP cm}^{-3} \cdot \text{atm}^{-1}$): tests of the method have shown that plausible errors in D , α (Tsacopoulos et al., 1983), and the radius (Poitry, S., and H. Widmer, personal communication) can affect the amplitude of ΔQ_{O_2} , but affect only negligibly the kinetics of ΔQ_{O_2} . Samples of the P_{O_2} record were taken by an analog-to-digital converter every 400 ms or by hand every 2 s. The Fourier transform of the P_{O_2} was computed using a fast Fourier transform (FFT) routine; the result was mul-

multiplied by the transfer function obtained, as previously described (Tsacopoulos and Poitry, 1982), by solving the equation of diffusion for the case of a sphere consuming a substance uniformly; the inverse Fourier transform of the product was then computed with the FFT routine to yield the ΔQ_{O_2} . For convenience, the values are connected with a continuous line. The voltage difference between the two barrels of the Na⁺ microelectrode was transformed by the computer to an Na⁺ signal (Dimitracos and Tsacopoulos, 1985) on the basis of the calibration curve of each microelectrode (see details in Coles and Orkand, 1985).

The data shown in Figs. 1 and 7 were obtained at the Marine Biological Laboratory, Woods Hole, MA. In Woods Hole, an on-line computer was not available and the record of Fig. 1 was digitized by hand. The data in all the other figures were obtained at the Experimental Ophthalmology Laboratory, Geneva, Switzerland, where a computer was available. The optical bench in Woods Hole used a quartz iodide lamp and the one in Geneva used a xenon arc. The stimuli in Figs. 1 and 7 had a duration of 5 s and were attenuated 0.5 log units from the maximum intensity available from the quartz iodide lamp. The stimuli from the brighter xenon arc were 1 s in duration and were attenuated by 2.1 log units unless otherwise specified in the figure legends.

RESULTS

Fig. 1 shows a simultaneous recording of the transmembrane potential (*D*) and the local P_{O_2} (*C*) in the region immediately outside the cell shown in *A*. The miniature O_2 electrode used to record the P_{O_2} outside the cell can be seen in *A*; the intracellular microelectrode used to record the transmembrane potential is not in focus. In the dark-adapted photoreceptor, a flash of light induces a decrease in P_{O_2} (ΔP_{O_2}) that begins after the peak depolarization of the light response. Comparing the time courses of the recorded ΔP_{O_2} (*C*) with the calculated ΔQ_{O_2} (*B*), it is clear that there is little difference. This is because any region of the cell that consumes O_2 is $<50 \mu\text{m}$ away from the bath, which supplies O_2 , so that diffusion of O_2 does not introduce as much of a delay between ΔQ_{O_2} and the resulting ΔP_{O_2} as in other preparations (Tsacopoulos et al., 1981; Tsacopoulos and Poitry, 1982). The ΔQ_{O_2} returns to baseline long before the transmembrane afterhyperpolarization has returned to baseline. We have previously shown (Fein and Tsacopoulos, 1988) that the light-induced ΔP_{O_2} originates at the photoreceptor cell body and that it is mitochondrial in origin since it is blocked by rotenone, a specific mitochondrial inhibitor.

Brown and Lisman (1972) have shown for ventral photoreceptors that the afterhyperpolarization following the depolarizing light response results from activation of an electrogenic Na pump. If we assume that the duration of the hyperpolarization reflects the duration of the electrogenic Na pumping, then the data in Fig. 1 suggest that the light-induced ΔQ_{O_2} is complete long before cessation of Na pumping. This might suggest that consumption of ATP by the Na pump does not lead to activation of O_2 consumption. In order to rigorously test this suggestion, we quantitatively compared, in one cell, the time course of ΔQ_{O_2} with the time course of the change in $[\text{Na}^+]_i$ following a flash of light, as described below.

Fig. 2 shows the calculated ΔQ_{O_2} for five intensities of a 1-s light flash. For light intensities greater than the brightest shown in Fig. 2 ($\log I/I_0 = -2.1$),

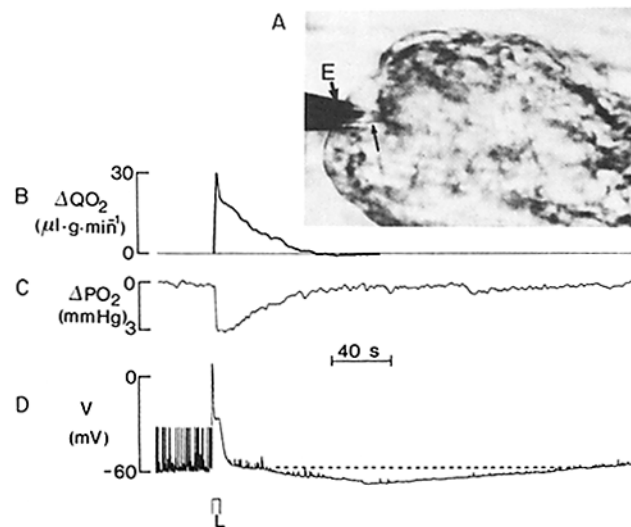


FIGURE 1. Light-induced increase in O₂ consumption. (A) This photograph of the cell and O₂ electrode (E), taken from the video monitor used to observe the cell with infrared illumination during the experiment, shows the size of the electrode relative to the cell. The arrow below the electrode points to the recess of the platinum wire inside the open electrode. (B) ΔQ_{O_2} computed from ΔP_{O_2} . (C) Recording of P_{O_2} made by the O₂ electrode. (D) Recording of the cell's transmembrane potential made by conventional intracellular microelectrode, which is out of focus in A.

there was often a photoartifact associated with the light flash (see Fig. 1 in Tsapopoulos and Poitry, 1982). Thus, the data in Fig. 2 span the working range over which we could reliably measure ΔQ_{O_2} in these experiments. In order to obtain the best signal-to-noise ratio in the calculated ΔQ_{O_2} , we used the highest-intensity flash possible ($\log I/I_o = -2.1$) in the experiments that follow.

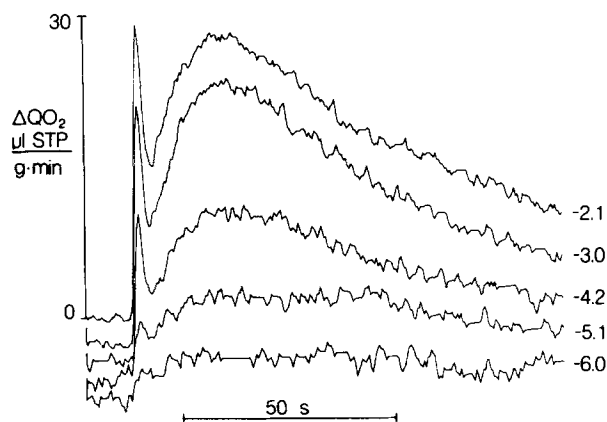


FIGURE 2. Time course of light-induced O₂ consumption (ΔQ_{O_2}) for different light intensities. The ΔQ_{O_2} was calculated from recordings of P_{O_2} as described in the Methods. The light intensities (I) of the 1-s light flash are given as the \log_{10} attenuation relative to the unattenuated intensity (I_o) of the source.

Measurements of [Na⁺]_i

For an individual photoreceptor, measurement of changes in [Na⁺]_i as a function of time after a light flash is probably the most reliable indicator of the activity of the Na pump. We used double-barreled quartz Na⁺-selective electrodes to record [Na⁺]_i and transmembrane potential from these cells. Fig. 3 shows a typical recording of the transmembrane potential and Δ[Na⁺]_i elicited by a 1-s light flash of intensity $\log I/I_0 = -0.9$. During the light response, [Na⁺]_i rose from 7 mM in the dark to ~15 mM. In the dark after the light flash, [Na⁺]_i slowly returned to the baseline as the Na⁺ was pumped out of the cell.

In a total of 12 cells, the value of [Na⁺]_i measured in the dark after the cell had recovered from any previous illumination was 9.7 ± 3.9 mM (mean \pm SD). The value of [Na⁺]_i ranged from a low of 6.0 mM to a high of 21 mM for these cells. Excluding the one cell in which we measured a value of 21 mM, [Na⁺]_i ranged from 6.0 to 11.8 mM. Since impalement of the cell by a microelectrode will cause Na⁺ to leak into the cell and thereby artificially elevate [Na⁺]_i around the tip of the electrode, we suggest that the true value of [Na⁺]_i in the dark-adapted cell is probably closer to 6 mM.

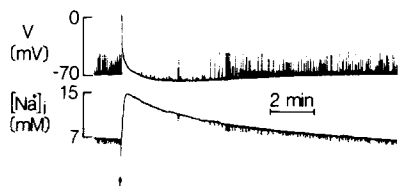


FIGURE 3. Light-induced increase in [Na⁺]_i in a ventral photoreceptor. The upper trace is the transmembrane potential and the lower trace is [Na⁺]_i. The time of occurrence of the 1-s light flash of intensity $\log(I/I_0) = -0.9$ is marked by the arrow below the lower trace.

In Fig. 4 we compare the light-induced Δ[Na⁺]_i for four different intensities of a 1-s light flash. It can be seen that Δ[Na⁺]_i is graded with the intensity of the flash (A) and returns to baseline with a similar time course for different intensities of the flash (B). This is consistent with the finding in voltage-clamped Purkinje fibers at constant extracellular K⁺, where the decline of [Na⁺]_i appeared to follow first-order kinetics with a rate constant independent of [Na⁺]_i (Gadsby and Cranefield, 1979).

In Fig. 5 we plot the peak amplitude of Δ[Na⁺]_i over a 6-log-unit range of light intensities. The Δ[Na⁺]_i shows no evidence of saturating even up to the highest-intensity light flashes available from our light source. Similar findings were reported in the drone retina (Coles and Orkand, 1982).

The Na⁺ sensor we used in this study is not perfectly selective for Na⁺ over Ca⁺⁺ (Steiner et al., 1979). Inasmuch as there is a large light-induced rise in [Ca⁺⁺]_i (Δ[Ca⁺⁺]_i), it is a concern whether the recordings of Δ[Na⁺]_i are contaminated by cross-talk from Δ[Ca⁺⁺]_i. Quantitative measurements of Δ[Ca⁺⁺]_i in ventral photoreceptors using ion-selective electrodes have shown that after a light flash, [Ca⁺⁺]_i rises and then returns to baseline with an initial time constant

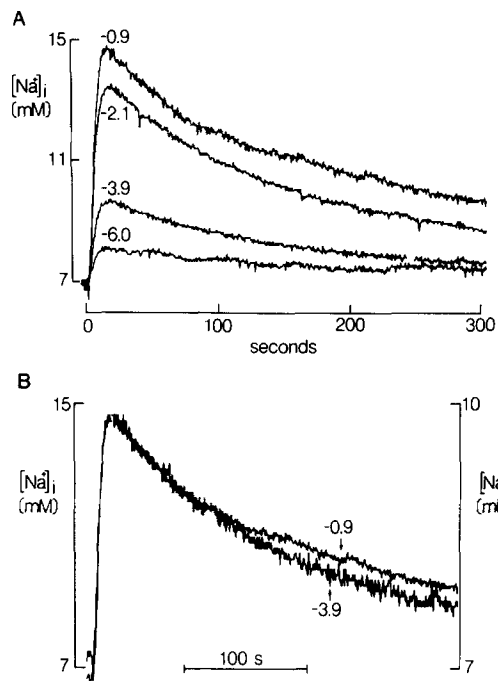


FIGURE 4. Light-induced increase in $[Na^+]_i$ for different light intensities. (A) The numbers next to the four superimposed records give the log relative intensity of the 1-s light flash that evoked the increase in $[Na^+]_i$. (B) Comparison of the time courses of the change in $[Na^+]_i$ evoked by two light intensities differing in intensity by 3 log units. The left-hand scale corresponds to the log $I/I_0 = -3.9$ intensity flash and the right-hand scale to the log $I/I_0 = -0.9$ intensity flash.

of tens of seconds (Levy and Fein, 1985). It is clear that there is no component with that time constant in the return of $[Na^+]_i$ to baseline after the light flash. Rather, $[Na^+]_i$ returns to baseline with a time constant of several minutes (~ 3 min). Thus, we conclude that our recordings of $[Na^+]_i$ are basically uncontaminated by changes in $[Ca^{++}]_i$.

In order to compare the time courses of $\Delta[Na^+]_i$ and ΔQ_{O_2} , we measured both in the same cell. The upper record in Fig. 6 shows the ΔQ_{O_2} and the lower record shows the $\Delta[Na^+]_i$ when the cell was stimulated by a 1-s flash of intensity

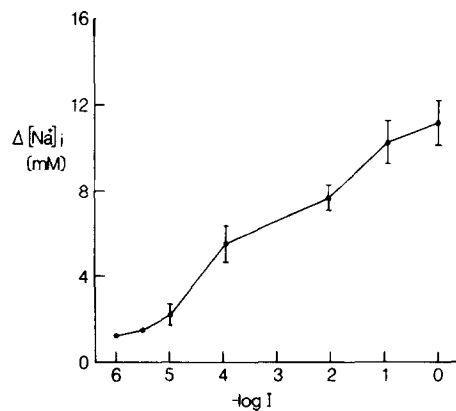


FIGURE 5. Peak amplitude of light-induced increase in $[Na^+]_i$ as a function of light intensity. The peak amplitude of the light-induced increase of $[Na^+]_i$ was determined from data such as those in Figs. 3 and 4. The intensity is that of the 1-s light flash used to stimulate the cell.

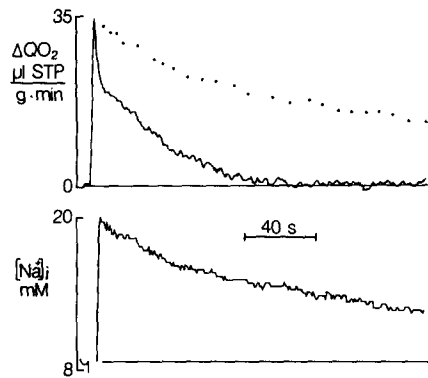


FIGURE 6. Comparison of the time courses of O₂ consumption and the change in [Na⁺]_i measured simultaneously for the same cell. The upper record is the ΔQ_{O_2} calculated from the P_{O_2} recording, such as the one in Fig. 1. The lower record is the recording of [Na⁺]_i. For the purpose of comparison, the dots in the upper record give the time course of [Na⁺]_i arbitrarily scaled to the same peak amplitude as the Q_{O_2} .

$\log I/I_0 = -2.1$. The dots in the upper record are for the purpose of comparison, and give the time course of $\Delta[Na^+]_i$ arbitrarily scaled to the same amplitude as the ΔQ_{O_2} . It is clear that ΔQ_{O_2} had returned to baseline at a time when the pump was still actively extruding Na⁺ from the cell.

Intracellular Injection of Na⁺

To further test the possibility that the light-induced $\Delta[Na^+]_i$ might somehow contribute in part toward the light-induced ΔQ_{O_2} , we tried to mimic the effect of light by artificially injecting Na⁺ into the cell. We recorded the P_{O_2} outside the cell while impaling the cell with an injection pipette containing Na⁺ (see Methods). Fig. 7 shows simultaneous recordings of P_{O_2} (A) and transmembrane

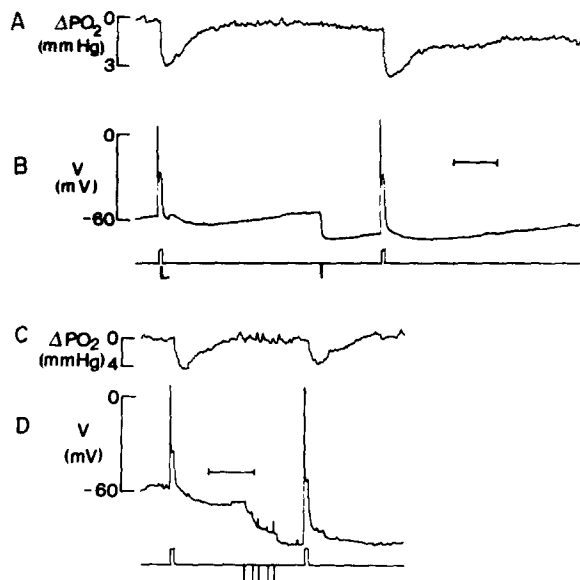


FIGURE 7. Injection of Na⁺ does not activate O₂ consumption (A and C). Recordings of P_{O_2} were made as in Fig. 1. (B and D) Transmembrane potential measured with an intracellular microelectrode containing 500 mM Na-aspartate and 10 mM HEPES, pH 7.0. The traces below B and D show the occurrence of the light flash as an upward deflection and the pressure pulse used to inject Na⁺ as a downward deflection.

potential (*B*). As in Fig. 1, a light flash causes a change in P_{O_2} and the receptor potential, followed by an afterhyperpolarization. After the cell had recovered from the light flash, sufficient Na^+ was injected into the cell to cause a membrane hyperpolarization greater than the hyperpolarization following the flash. The O_2 electrode recorded no change in P_{O_2} above the baseline noise. A subsequent light flash showed that the cell was not damaged by the injection and we could still record ΔP_{O_2} and receptor potential values similar to those before injection. Although we did not see an activation of O_2 consumption by Na^+ injection, we usually observed a temporary decrease in the amplitude of the light-induced ΔP_{O_2} after Na^+ injection, as illustrated in Fig. 7, *C* and *D*.

In the experiments illustrated in Fig. 7, we assumed that if the Na^+ injection produced a greater transmembrane hyperpolarization than light, then the injection elevated $[Na^+]_i$ to a higher level than that attained after the light flash. If this assumption is true, we could then conclude from Fig. 7 that elevation of $[Na^+]_i$, although it activates the Na pump, does not lead to an activation of oxidative metabolism.

In order to test whether this assumption was in fact correct, we injected Na^+

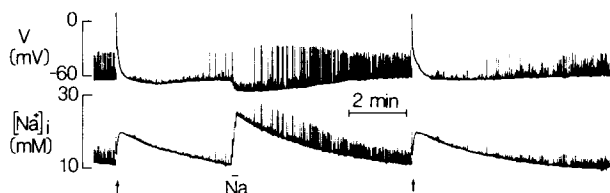


FIGURE 8. Measurement of $[Na^+]_i$ while Na^+ was injected into a ventral photoreceptor. Upper trace: membrane potential; lower trace: $[Na^+]_i$.

into a ventral photoreceptor while measuring $[Na^+]_i$ with a double-barreled Na^+ -selective electrode. The upper trace in Fig. 8 shows the transmembrane potential and the lower trace shows $[Na^+]_i$ after illumination and Na^+ injection. As already seen in Fig. 3, illumination caused a transmembrane depolarization, followed by an afterhyperpolarization and a transient rise in $[Na^+]_i$. Injection of sufficient Na^+ to cause a membrane hyperpolarization greater than that induced by light caused a larger rise in $[Na^+]_i$ than did light. A light flash after the Na^+ injection shows that the cell had fully recovered from the injection and was not damaged. One can see in Fig. 8 that the time courses of the return to baseline of $[Na^+]_i$ after light and Na^+ injection are essentially identical. This is expected from the data of Fig. 4.

DISCUSSION

Light-induced O_2 Consumption

The ventral photoreceptor is the first preparation in which it is possible to measure the kinetics of O_2 consumption (ΔQ_{O_2}) from a single cell after a flash of light. The ΔQ_{O_2} elicited by a 1-s light flash of intensity $\log I/I_0 = -2.1$ was

typically biphasic, consisting of an initial rapid phase followed by a later slow phase (see Figs. 2 and 6). The respective amplitudes of the two phases were variable (compare Figs. 2 and 6) and we have no explanation for this variability. For 20 cells, the peak amplitude of the rapid phase was $28.3 \pm 18.5 \mu\text{l STP/g} \cdot \text{min}$ (mean \pm SD) and the peak amplitude of the slow phase was 15.2 ± 5.5 . Because the light-induced ΔQ_{O_2} originates in the photoreceptor's cell body and both phases are blocked by rotenone (Fein and Tsacopoulos, 1988), we believe they are both mitochondrial in origin.

We have shown elsewhere (Fein and Tsacopoulos, 1988) that intracellular pressure injection of the Ca^{++} buffer EGTA completely suppresses the rapid component and partially inhibits the slow component of ΔQ_{O_2} . On the basis of this and other findings, we have suggested that a rise in $[\text{Ca}^{++}]_i$ plays a central role in activating mitochondrial metabolism (Fein and Tsacopoulos, 1988). However, at present it does not seem fruitful to pursue a quantitative description of the kinetics of ΔQ_{O_2} because important information is missing. The ventral photoreceptor cell body is segmented into two functionally specialized lobes, the light-sensitive rhabdomeral (R) lobe, and the arhabdomeral (A) lobe, which contains the nucleus (Calman and Chamberlain, 1982; Stern et al., 1982), and it seems likely that the light-induced ΔQ_{O_2} is initiated in the R-lobe, where the light-induced rise in $[\text{Ca}^{++}]_i$ is initiated (Levy and Fein, 1985). At present, we do not know how the mitochondria are quantitatively distributed throughout the cytoplasm of these two lobes.

We can compare the time course of ΔQ_{O_2} recorded from *Limulus* with those recorded from slices of bee retina and the lateral eye of barnacle (Tsacopoulos and Poitry, 1982; Tsacopoulos et al., 1983; Poitry and Widmer, 1986) because the measurements have been made using the same method. There is a striking difference in the duration of the ΔQ_{O_2} between *Limulus* and drone. At room temperature, it takes ~ 90 s for ΔQ_{O_2} to return to within 10% of its preillumination level in *Limulus*, while it only takes ~ 11 s in the bee, which is about the time course of the rapid phase of ΔQ_{O_2} in *Limulus*. In the barnacle, ΔQ_{O_2} is as slow as in *Limulus* but the biphasic nature of the shape is less striking; the ΔQ_{O_2} in barnacle appears to be composed primarily of a slow component.

In the present study, we were able to measure the light-induced ΔQ_{O_2} reliably from a single ventral photoreceptor over an ~ 4 -log-unit range of light intensities (Fig. 2). We were limited at the highest intensity by a photoeffect of the light stimulus on the O_2 electrode that often distorted the response (not shown). At the lower intensity, we were limited by noise in our measuring system (see Fig. 1, for example) and the difficulty in measuring polarographic currents of a fraction of a picoampere. Improvements in the O_2 electrode and the electronics would be useful in increasing the limited range of intensities over which changes in P_{O_2} can be measured. Nevertheless, the present measuring system was sufficient for the experiments reported here.

Light-induced Rise in $[\text{Na}^+]_i$

The average value of $[\text{Na}^+]_i$ in the dark-adapted cell was ~ 10 mM, a value comparable to what was found in other cells (Vaughan-Jones, 1977; Sheu and Foz-

zard, 1982; Taylor and Thomas, 1984; Coles and Orkand, 1985), but about double the level found in snail neurons (Thomas, 1972). Probably because the cell membrane does not fully seal around the Na^+ -selective electrode, the resulting leak may artificially increase the $[\text{Na}^+]_i$ near the electrode tip (Taylor and Thomas, 1984). Thus, we suggest that the true value of $[\text{Na}^+]_i$ in the dark-adapted cell is probably closer to 6 mM (the lowest concentration we have measured), which is fairly close to the 5 mM found in crab muscle by Vaughan-Jones (1977) when the leak was blocked (see Taylor and Thomas, 1984). The peak amplitude of the light-induced increase in $[\text{Na}^+]_i$ was monotonically graded with light intensity over a 6-log-unit range of intensities (Figs. 4 A and 5). The range of light intensities over which light-induced changes in $[\text{Na}^+]_i$ could be measured (Fig. 5) spanned the range over which ΔQ_{O_2} could be measured (Fig. 2). For the brightest flash, we measured a peak increase of $[\text{Na}^+]_i$ of ~ 11 mM. This increase is significantly larger than the 3.6-mM peak increase measured in bee (Tsacopoulos et al., 1983) or the 8-mM increase measured in barnacle photoreceptors after 5 min of continuous intense illumination (Brown and Cornwall, 1975).

Comparison of the Time Courses of $\Delta[\text{Na}^+]_i$ and ΔQ_{O_2}

In most recordings, the decaying phase of the light-induced $\Delta[\text{Na}^+]_i$ could be fitted with a single exponential with a time constant (τ) having an average value of 217 ± 41.8 s (mean \pm SD, $N = 7$ cells). Consistent with previous findings by Thomas (1969) in snail neurons, the time constants for the decaying phase of $\Delta[\text{Na}^+]_i$ for different light intensities for the same photoreceptor were independent of the amplitude of $\Delta[\text{Na}^+]_i$. Taking a mean value of 8 mM for $\Delta[\text{Na}^+]_i$ after a -2.1 -log flash, the average calculated Na^+ flux ~ 100 s after the flash is

$$\left. \frac{d[\text{Na}^+]_i}{dt} \right|_{t=100} = \frac{-[\text{Na}^+]_{i,t=0}}{\tau} e^{-t/\tau} \Big|_{t=100} = \frac{-8}{200} e^{-0.5} = -0.024 \text{ mM/s.}$$

The average volume of the ventral photoreceptor is 0.4×10^{-9} liter (Fein and Szuts, 1982). Thus, there was an efflux of $0.0096 \times 10^{-12} \times 6.2 \times 10^{23} = 0.06 \times 10^{11}$ ions/s. The maximum number of cations pumped per pumping site at 20°C is $\sim 22 \text{ s}^{-1}$ (Ritchie, 1973); hence, there should be $\sim 3 \times 10^8$ Na pumps per photoreceptor. Assuming the R- and A-lobes have the same density of pump sites, and taking the surface area of the ventral photoreceptor as $4 \times 10^{-3} \text{ cm}^2$ (Fain and Lisman, 1981; Calman and Chamberlain, 1982), we arrive at a density of Na-pumping sites of $\sim 700 \mu\text{m}^{-2}$. This is about the same density calculated for a variety of cells (Baker and Willis, 1972). If the rise in $[\text{Na}^+]_i$ caused the ΔQ_{O_2} through the consumption of ATP by the Na pump, one would have expected the cell to consume extra O_2 as long as the pump was actively extruding Na^+ and therefore consuming ATP. It took ~ 90 s (87.7 ± 15.2 , mean \pm SD, $N = 20$ cells) for ΔQ_{O_2} to recover to within 10% of its preillumination value, while $\Delta[\text{Na}^+]_i$ took ~ 480 s to return to within 10% of its preillumination value (see, for example, Fig. 6). Consequently, the time course of $\Delta[\text{Na}^+]_i$ is too slow for it to cause the change in Q_{O_2} .

Similarly, for the bee photoreceptor, ΔQ_{O_2} recovers 90% in ~ 11 s, while $\Delta[Na^+]_i$ took >32 s to recover 90% (Tsacopoulos et al., 1983). The findings in *Limulus* and bee photoreceptors are in sharp contrast to recent findings in barnacle photoreceptors, where the time courses of recovery of $\Delta[Na^+]_i$ and ΔQ_{O_2} were found to be similar (Poitry and Widmer, 1986).

Injection of Na⁺ Does Not Activate O₂ Consumption

Previous experiments in snail neurons (Thomas, 1969), barnacle photoreceptors (Koike et al., 1971), and *Limulus* ventral photoreceptors (Brown and Lisman, 1972) have shown that ionophoretic intracellular injection of Na⁺ causes a hyperpolarization of the cell membrane, probably because Na⁺ activates the working of an electrogenic Na pump. For example, in voltage-clamped snail neurons, an injection causing $[Na^+]_i$ to increase from ~ 7 to 15 mM induced an outward current of 1–2 nA (Thomas, 1969). In the experiments reported here, we pressure-injected Na⁺ into ventral photoreceptors. This elevated $[Na^+]_i$ by ~ 10 mM or more in the dark. The time constant of recovery of $\Delta[Na^+]_i$ after such an injection was the same as after a light flash (Fig. 8). Assuming that the ventral photoreceptor Na pump is similar to that of other neurons, then three Na ions are exchanged for two K ions. Multiplying the charge carried by each Na ion (1.6×10^{-19} A·s) by one-third of the Na⁺ efflux at 100 s after the Na⁺ injection (0.07×10^{11} ions/s) gives an electrogenic current of ~ 0.4 nA, a figure reasonably close to that measured by Thomas (1969). Using stoichiometries proposed by Harris et al. (1980), three Na ions are extruded for each ATP split; then ~ 3 mM of ATP is hydrolyzed to ADP and P_i in order to pump out this Na⁺. If ADP was a stimulus of mitochondrial respiration, as generally proposed from in vitro studies (Saktor, 1975), this pumping should induce an O₂ consumption of 10 μ l O₂/g of photoreceptor, i.e., about one-third of the O₂ used after a -2.1 -log flash of light (a signal clearly measurable; see Fig. 2). It is striking that we did not detect any increased O₂ consumption when we pressure-injected Na⁺ into the cell (Fig. 7).

One possibility suggested by the present and previous (Tsacopoulos et al., 1983) experiments is that production of ATP by oxidative phosphorylation precedes its utilization by the Na pump. If the cell has a powerful mechanism for buffering the level of ATP, then the ATP hydrolyzed by the pump would not result in a significant drop in the level of ATP. This possibility could be tested by measuring the level of ATP in ventral photoreceptors. Brown and Coles (1987) have published a preliminary report in which they injected ventral photoreceptors with luciferin luciferase in an attempt to monitor $[ATP]_i$. They observed a transient increase of 2–25% in the level of luminescence in 70 out of 118 responses to light stimulation, but a decrease was never observed. The luminescence increase peaked 10–60 s after the light flash, i.e., at a time after the peak of the ΔQ_{O_2} . Assuming that the luciferin luciferase luminescence measured from a living cell directly reflects $[ATP]_i$ in that cell, their results would support the hypothesis that production of ATP by oxidative phosphorylation precedes its utilization by the Na pump.

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