

Lability of the Prolonged Depolarizing Afterpotential in *Balanus* Photoreceptors

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ABSTRACT A large cell to cell variability of the prolonged depolarizing afterpotential (PDA) decay time constant (τ) has been measured in *Balanus eberneus* lateral ocelli. While 25% of the cells had PDAs of long duration, $\tau > 10$ min, 45% of the cells tested showed either weak ($\tau < 60$ s) PDA or none at all. The variability was not reflected in the late receptor potential. All the cells showed normal light-coincident responses. The variability was not due to some alteration of the thermal stability of the pigment states, since after monochromatic adaptation the amplitude of the early receptor potential remained unchanged for at least 30 min. In addition, in some cells that initially showed PDAs of long duration, the decay time was either shortened or abolished after exposure to anoxia. Again, the late receptor potential and the stability of the pigments remained unaffected. These results indicate that the mechanisms which give rise to the PDA are not always tightly coupled to the direct chain of events that lead to the light-coincident response.

INTRODUCTION

It has been found in several arthropod photoreceptors that stimulation with an intense monochromatic light that converts a large portion of the photopigment, rhodopsin, to metarhodopsin induces a conductance increase that can far outlast the period of stimulation (Hochstein et al., 1973; Nolte and Brown 1972*a, b*; Muijser et al., 1975; Cosen and Briscoe, 1972). This phenomenon has been termed the prolonged depolarizing afterpotential (PDA) or "latchup." If the photoreceptors remain in the dark the PDA will slowly decay away, but photo-conversion of the metarhodopsin back to rhodopsin will immediately terminate the PDA.

The ionic mechanisms in *Limulus* median ocellus and *Balanus* lateral ocellus that produce the PDA appear to be the same as those underlying the light-coincident response (Nolte and Brown, 1972*a*; Brown and Cornwall, 1975*b*). Also, Minke et al. (1975*b*), from experiments on *Drosophila* photoreceptors, suggest that the PDA, like the light-coincident response, is produced by the summation of discrete potential fluctuations. Because of these similarities it may be possible to utilize the PDA to study how the isomerization of the photopigment can lead to membrane conductance changes.

One suggestion that has been advanced is that metabolism may play a role in the phototransduction process (Borsellino and Fuortes, 1968; Baumann and

Mauro, 1973). A number of metabolic agents have been shown to abolish the light-coincident response (Lantz and Mauro, 1977; Brown and Meech, 1975). Wong et al. (1976) demonstrated that the light-induced conductance increase and the conductance increase produced during a PDA in *Drosophila* can be blocked with anoxia or carbon dioxide. Also, although no observable electrical response was evident when a PDA-inducing light was presented during anoxia, the effects of the stimulation could be seen after recovery. Therefore, whatever is producing the prolonged depolarization can be produced during anoxia and will persist until it is able to alter the membrane conductance.

Our original intentions had been to reproduce and extend the findings of Wong et al. (1976) with *Balanus* lateral ocelli. One advantage of using *Balanus* rather than *Drosophila* photoreceptors is the larger cell size which allows more stable impalements for longer periods of time. We also planned to study the effects on the PDA of 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, and sodium azide, in addition to those of anoxia and carbon dioxide.

Unexpectedly, we found these experiments difficult to perform on *Balanus* since in a large number of cells, the PDA was either of short duration or entirely absent. In addition, exposure of other cells to anoxia irreversibly reduced or abolished PDA responses that were initially of long duration. The following is a report of these findings.

MATERIALS AND METHODS

Lateral ocelli from *Balanus eberneus* (obtained from the Marine Biological Laboratory, Woods Hole, Mass.) were dissected free from the rest of the animal. The tapetum and a small square of connective tissue were left intact surrounding the photoreceptors and the tissue was pinned down in the recording chamber, corneal side up. Penetrations for intracellular recording were made directly through the connective tissue. Standard intracellular recording techniques were used.

The recording chamber (1 ml vol) could be continuously perfused with artificial seawater (435 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 20 mM MgCl₂, and 25 mM MgSO₄, pH 7.2, with Tris) by use of a peristaltic pump. The flow rate was adjustable but was normally maintained at approximately 10 ml/min. This flow rate allowed drugs to be washed out quickly or the preparation to be maintained in a state of anoxia. The oxygen tension of the seawater was lowered by equilibration with 100% N₂ by using a Teflon membrane gas exchanger (L. Eschweiler and Co., Kiel, West Germany). The oxygen tension of the seawater was measured with a Yellow Springs Instrument Co. (Yellow Springs, Ohio) O₂ electrode placed in the recording chamber. The electrode was calibrated for zero oxygen tension by addition of NaHSO₃, an oxygen scavenger. During anoxia experiments the oxygen tension of the seawater fell to within 5 mM Hg of the calibrated zero.

Dinitrophenol was not continuously perfused but was applied through a local perfusion system. When DNP was applied, 10 ml of the inhibitor in a seawater solution were initially washed into the recording chamber. 5 ml were introduced every 10–15 min thereafter to keep the external bathing medium constant.

Stimulation was from a 150-W xenon lamp (Osram) powered by a regulated supply manufactured by Oriol Corp. of America, Stamford, Conn. The light was focused on to one end of a light pipe and the other end was positioned approximately 3 mm above the photoreceptor. The maximum light intensity at the photoreceptor, measured with a

calibrated photodiode and interference filters, was equivalent to 2×10^{17} photons/s/cm² at 594 nm. The power measured by the photodiode was not from 594-nm photons exclusively, since the filter passes other wavelengths (half-maximal bandpass of 17 nm). However, the average energy of the photons passed by the filter is close to the energy of a 594-nm photon. Thus for convenience the calculations were performed as if all the photons were 594-nm photons. The beam could be interrupted by an electromagnetic shutter. Narrow bandpass interference filters (IF) (Spectrocoat Monopass Filters, Optics Technology Inc., Palo Alto, Calif.) and wide bandpass Wratten filters (Eastman Kodak Co., Rochester, N. Y.) were used for monochromatic stimulations. The spectral characteristics of the filters used are given in Table I. A glass IR absorbing filter was positioned between the light source and the light pipe to reduce any effects of heating.

TABLE I
SPECTRAL CHARACTERISTICS OF FILTERS USED FOR
MONOCHROMATIC STIMULATIONS

| | λ_{\max} | Transmission at λ_{\max} | Half-maximum bandwidth |
|-------------------------------|------------------|----------------------------------|---------------------------|
| | nm | % | nm |
| Wide bandpass Wratten filters | | | |
| 49B | 455 | 17.6 | 40 |
| 74 | 540 | 14.8 | 30 |
| 72B | 605 | 6.1 | 30 |
| Narrow bandpass IF | | | |
| 466 | 463 | 34 | 15 |
| 533 | 527 | 41 | 17 |
| 600 | 594 | 41 | 17 |
| 633 | 626 | 31 | 14 |

The values for the Wratten filters are from the spectral data given in the handbook of Chemistry and Physics, 56th edition. The values for the interference filters were obtained from the spectral data supplied by Optics Technology. Since the measured λ_{\max} values for the interference filters do not correspond precisely to those given on the filter labels, the wavelengths given in the text are the λ_{\max} wavelengths of the filters.

The early receptor potential (ERP) (Brown and Murakami, 1964) was examined in several cells by exposing the cells to an isotonic potassium chloride solution (500 mM KCl, 10 mM CaCl₂). This procedure abolishes the late receptor potential but leaves unaffected the ERP, which is not dependent on the ionic medium.

RESULTS

Fig. 1*a* shows a typical receptor potential from *Balanus* lateral ocelli produced by stimulation with white light. The response shows both a transient and a steady phase and returns to the resting potential very quickly after the light is turned off.

Fig. 1*b* shows the response to red light ($\lambda_{\max} = 605$ nm) of a cell that had been adapted previously with 455-nm light. Again, as with white light, the transient and steady phases of the receptor potential are evident but upon termination of the light the membrane potential remains depolarized. This is the prolonged depolarizing afterpotential (PDA). If, during the PDA, the photoreceptor is exposed to intense blue ($\lambda_{\max} = 455$ nm) or white light, the membrane potential

will return to its resting level after the stimulation. But when the cell is left in the dark after PDA induction, the membrane potential will slowly decay back to its resting level.

Table II is a summary of the PDA decay time constants found under a variety of conditions. The decay could be fit very closely by a single exponential and the time constants (τ) listed in the table are calculated from a least-squares fit of the data ($A_t = A_{\max} [\exp(-t/\tau)]$), where A_{\max} is the maximum amplitude of the late receptor potential and A_t is the amplitude at time t).

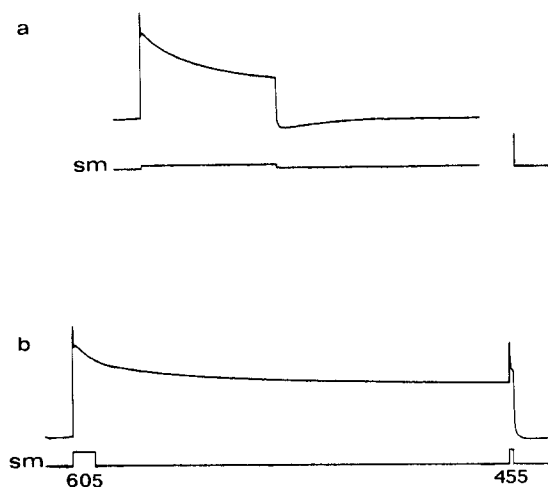


FIGURE 1. Intracellular recordings from a *B. eberneus* lateral ocellus. Trace (a), response of the photoreceptor when stimulated with white light. The cell responds with a transient and steady-state depolarization and returns to the resting level at light off. Trace (b) (same cell as a), effect of stimulation with monochromatic 605-nm light. The cell had been adapted previously with 455-nm light for 1 min and left in the dark for 5 min. In this case the membrane potential remains depolarized after the light is turned off. This is the prolonged depolarizing afterpotential (PDA). The depolarization was terminated with a 455-nm light. Voltage scale = 20 mV and time scale 10 s. *sm* = stimulus monitor.

Each cell was presented first with adapting light followed by a dark period before the PDA was induced. An adapting light at wavelengths below 550 nm was found by Hochstein et al. (1973) and Brown and Cornwall (1975a) to be essential for PDA induction. Conversely, if the cell is adapted to wavelengths above 580 nm no PDA will be produced by subsequent red stimulation. After appropriate color adaptation, a PDA will be produced by stimulation at wavelengths between 600 and 650 nm. We have followed this procedure in most of our experiments, using saturating light intensities during both adaptation and stimulation. By "saturating light intensities" it is to be understood that an increase in either the duration or the intensity of the light flash did not cause a change in the photoreceptor response. A PDA can also be induced after adaptation with white light, such as in the case of cells 1 and 2, Table II, because the

predominant absorption components are at wavelengths below 550 nm, favoring absorption by metarhodopsin.

In order to study the effects of inducing the PDA during anoxia or dinitrophenol application, it is necessary that the PDA persist longer than the time required for recovery from these treatments. Time for recovery from either anoxia or DNP varies from cell to cell as was evident from the range of 15–30 min observed in our experiments.

Since we found only a few cells in which the PDA persisted longer than the time for recovery from anoxia or DNP, several parameters were varied to ascertain if they had any influence on the decay time constant. If saturating light intensities are used, wide bandpass Wratten filters (455 nm and 605 nm) or narrow bandpass interference filters (463 nm and 626 nm) for adaptation and stimulation appear to induce an equally good PDA, as seen in cell 17. Because there is a possibility that the most effective adapting and stimulus wavelengths may have different values from cell to cell, we varied both parameters to test their effects on the PDA decay. Changing the adapting light from $\lambda_{\max} = 463$ nm to $\lambda_{\max} = 527$ nm or altering the stimulus light to 594 nm did not prolong the PDA decay on the two cells (8 and 12) which were tested.

Only one procedure that we tried lengthened the decay time constant, namely, increasing the time the cell remained in the dark between the 463 nm adapting and the 626 nm stimulus lights (cell 35). As the dark period increased from 1 min up to 30 min, the time constant increased by a factor of almost five (213 s to 1,010 s). This phenomenon has been reported by Hochstein et al. (1973). Since PDAs produced in this manner were sufficiently long to outlast the recovery from anoxia, this procedure was adopted for all subsequent recordings.

One unexpected finding has been that in 45% of the cells examined (19 out of 41) we found either no PDA or a very weak PDA whose time constant of decay was less than 60 s. The cell to cell variations did not seem to be correlated in any way with the length of time that the animals had been kept in the aquarium or with the possibility that individual animals might have deteriorated. Table II includes animals from two shipments which were received in late summer and fall. All the animals were stored in an oxygenated constant-flow aquarium at 10°C until used. Both shipments showed considerable variation in PDA decay time constants. In addition, there was a large variability in cells that were impaled in the same ocellus or the same animal (for example cells 10 and 11, and 17 and 18). This was not due to damage caused by the first impalement because in some cases the second cell impaled gave a longer decay time (cells 33 and 34). None of the variations attempted above had any effect on lengthening the response. This demonstrates that even among animals which had been kept in similar environments, there was a wide variability of the PDA response. It should be noted that while the PDA varied from cell to cell, in all cases the cells showed normal receptor potentials.

The lability of the PDA does not arise from thermal decaying of the pigment states. An indication of the stability of the pigment states can be obtained by observing the amplitude of the early receptor potential (ERP). Minke et al.

TABLE II
 PDA DECAY TIME CONSTANT τ FOR CELLS THAT HAD
 BEEN COLOR ADAPTED, KEPT IN THE DARK, AND
 STIMULATED UNDER A VARIETY OF CONDITIONS

The table includes two shipments of animals received in late summer and fall. Cells 1-11 were from animals received in the first shipment while cells 12-41 were from the second shipment. In addition, cells 3, 4; 7, 8; 10, 11; 12, 13; 14-16; 17, 18; 20, 21; 23, 24; 26, 27; 28, 29; 31, 32; 33, 34; and 38, 39 were recorded from the same ocellus while cells 5, 6 were recorded from different ocelli in the same animal. Also cells 22, 25, and 30 were recorded from the second ocellus of the animals from which cells 23, 24; 26, 27; and 28, 29, respectively, were recorded.

| Adapting light | Dark period | Stimulus light | τ | Cell no. |
|----------------|-------------|----------------|----------|----------|
| <i>nm</i> | <i>s</i> | <i>nm</i> | <i>s</i> | |
| White | 10 | 605 Wratten | 130 | 1 |
| | 10 | | 59 | 2 |
| | 10 | | — | 3 |
| | 10 | | 59 | 5 |
| | 10 | | 30 | 6 |
| | 10 | | — | 10 |
| | 20 | | 110 | 13 |
| 455 Wratten | 120 | 605 Wratten | 112 | 12 |
| | 120 | | 122 | 14 |
| | 120 | | 120 | 16 |
| | 240 | | 1,040 | 9 |
| | 300 | | 1,810 | 17 |
| | 600 | | 19 | 8 |
| 540 Wratten | 120 | 605 Wratten | 25 | 7 |
| | 180 | | 520 | 11 |
| | 300 | | 59 | 15 |
| White | 10 | 626 IF | — | 20 |
| | 10 | | — | 22 |
| | 25 | | 403 | 29 |
| | 45 | | — | 24 |
| 463 IF | 25 | 626 IF | 13 | 21 |
| | 25 | | 26 | 26 |
| | 30 | | 92 | 27 |
| | 30 | | 58 | 33 |
| | 40 | | 49 | 23 |
| | 50 | | 170 | 28 |
| | 60 | | 203 | 34 |
| | 300 | | 2,290 | 17 |
| | 300 | | 1,930 | 19 |
| | 300 | | 126 | 25 |

TABLE II—*Concluded*

| Adapting light | Dark period | Stimulus light | τ | Cell no. |
|----------------|-------------|----------------|----------|----------|
| <i>nm</i> | <i>s</i> | <i>nm</i> | <i>s</i> | |
| | 60 | | 213 | 35 |
| | 300 | | 296 | 35 |
| | 900 | | 692 | 35 |
| | 1,800 | | 1,010 | 35 |
| | 1,800 | | 1,700 | 36 |
| | 1,800 | | 764 | 37 |
| | 1,800 | | 725 | 38 |
| | 1,800 | | 700 | 39 |
| | 1,800 | | 910 | 40 |
| | 1,800 | | 692 | 41 |
| 527 IF | 60 | 626 IF | 18 | 31 |
| | 60 | | — | 32 |
| | 300 | | 28 | 18 |
| | 600 | | 11 | 30 |

(1973) reported that with neutral, white light adaptation, the predominant component of the ERP is negative. If the cell is adapted with a saturating red light, the ERP produced by the first white flash is predominantly positive. Conversely, a saturating blue adapting light will produce an ERP which is more negative than that produced by neutral adaptation. Minke et al. (1973) found that the ERP amplitude produced by red or blue adaptation was not significantly altered even after 3 h in the dark, indicating no thermal decay of the pigments over this period. We examined the thermal stability of the pigment in several cells which showed weak or no PDA, by measuring the amplitude of the ERP after exposure to a saturating light, either $\lambda_{\max} = 626$ nm or $\lambda_{\max} = 463$ nm. No detectable change in the ERP amplitude was seen over a 30-min period. This indicates that the lability of the PDA arises at some point after the shift in pigment states.

In those few cells whose PDA could be expected to outlast recovery from anoxia or DNP, the effects of these metabolic agents on the PDA were examined. The procedure was as follows. Cells were first adapted with a saturating 463-nm light and then left in the dark for 30 min. A 626-nm stimulus light was then presented to the photoreceptor to induce the PDA and the time course of the decay was observed in the dark (Fig. 2*a*). If the depolarization remaining after 20–30 min was at least 5–10 mV above the dark-adapted resting potential, then the PDA in these cells could be expected to outlast the recovery from anoxia. The cells were then exposed to anoxia until the late receptor potential was abolished and then allowed to recover in normal oxygenated seawater in the dark (Fig. 2*b*). Cells which recovered in 20 min were regarded as being acceptable for the combined anoxia and PDA experiment. In this case the cell was again exposed to anoxia. When the late receptor potential produced by white light was abolished, the cell was adapted with 463 nm light and kept in anoxia in the dark for 30 min. The cell was then exposed to a 626-nm saturating light for PDA induction. This stimulation produced no discernible change in membrane po-

tential. The cell was allowed to recover in the dark for 20 min and then tested for a PDA with a 463-nm flash. If the PDA were present the 463-nm light should terminate it and the membrane potential should return to its more hyperpolarized resting potential upon termination of the light stimulus.

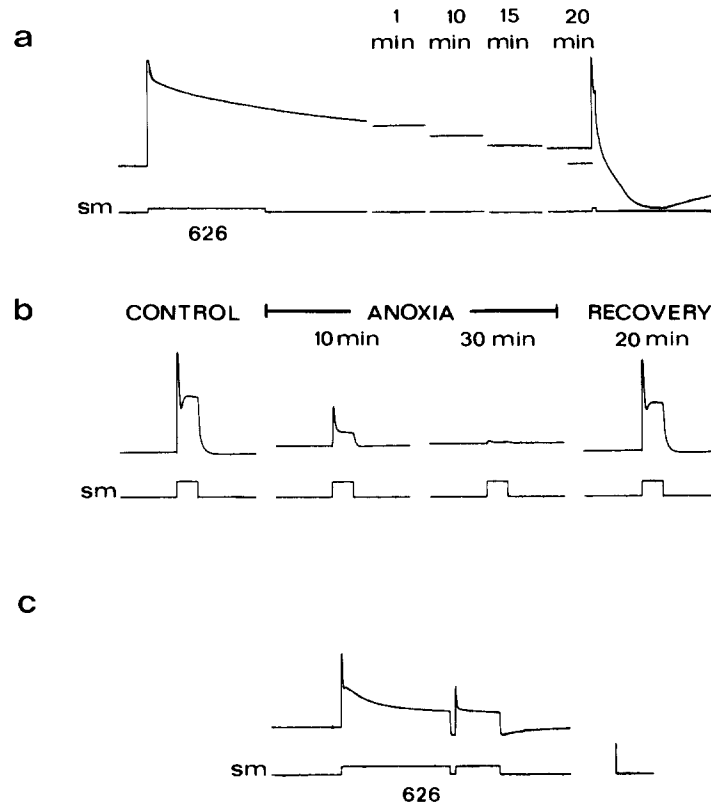


FIGURE 2. Lability of the PDA after exposure to anoxia. All records are from the same cell. Trace (a), PDA produced before exposure to anoxia by 626-nm stimulation. The cell had previously been adapted with 463 nm light and left in the dark for 30 min before stimulation. The times refer to the time after the stimulation light was turned off. As can be seen, at least 10 mV depolarization remains 20 min after PDA induction. The bar at 20 min is to allow comparison with the membrane potential before stimulation. In this case white light was used to terminate the PDA. Trace (b), Effect of anoxia on the light response. Stimulation was with white light. The times refer to the time after the oxygen tension of the seawater began to fall. At 30 min the response was almost completely abolished. At this point air was reintroduced and the cell was allowed to recover in the dark. The last record in (b) shows the light response 20 min after recovery was started. Trace (c), Response of the photoreceptor to 626-nm stimulation after recovery from anoxia. Both stimulations are 626 nm. The light was turned off briefly to test for PDA induction. No PDA is evident even though color adaptation wavelength and dark period are the same as in (a). This trace was not taken immediately after those in (b) but rather after recovery from a second exposure to anoxia. Voltage scale = 20 mV for each trace. Time scale = 10 s for (a) and (c) and 2 s for (b). *sm* = stimulus monitor.

In all the combined anoxia and PDA experiments that were attempted, we were surprised to find that after recovery from anoxia there was no evidence of a sustained depolarization, i.e. PDA, in any of the cells tested. When we tried to induce the PDA after recovery from anoxia we discovered that the PDA decay was either much more rapid than it had been before anoxia or that no PDA could be induced at all (Fig. 2c). Table III shows a summary of four cells in which this effect was seen. The values in the table are for cells which had been color adapted, kept in the dark, and stimulated in the same manner both before and after anoxia. In addition, longer periods in the dark, up to 1 h, were used to test for the PDA after exposure to anoxia. This was without effect. Although the PDA decay time course had been altered in these cells, there was no significant difference in the light-coincident responses seen before and after anoxia (Fig. 2b).

Some cells exposed to a different metabolic agent did not show the lability seen with anoxia. For example, Fig. 3 shows a cell which was exposed to 0.2 mM DNP

TABLE III
PDA TIME CONSTANT τ MEASURED IN CELLS BEFORE AND AFTER EXPOSURE TO ANOXIA

| Cell no. | τ | |
|----------|---------------|--------------|
| | Before anoxia | After anoxia |
| | s | |
| 19 | 1,930 | 307 |
| 38 | 725 | — |
| 39 | 700 | — |
| 41 | 692 | 75 |

The color adaptation wavelength, dark period, and stimulation wavelength used to measure τ after anoxia were identical to those used before anoxia.

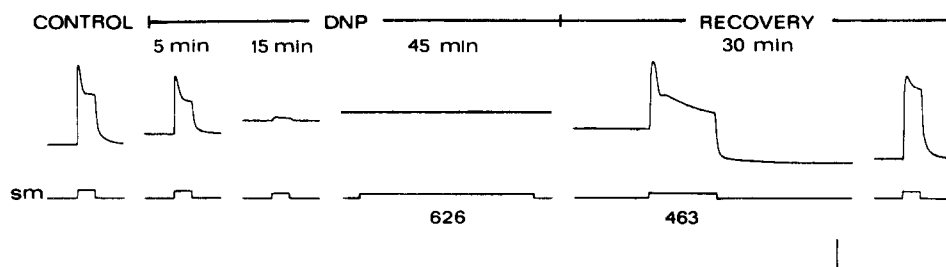


FIGURE 3. PDA induction during dinitrophenol application. *A. B. eberneus* lateral ocellus was exposed to 0.2 mM DNP until the response to white light was nearly abolished (15 min). The photoreceptor was adapted with 463-nm light and kept in DNP in the dark for 30 min. After the dark period, stimulation with 626-nm light for induction of the PDA produced no detectable response. The DNP was then washed out in the dark and after 30 min of recovery the cell was tested for a PDA. 463-nm light caused the membrane potential to return to a more negative value, indicating that stimulation with 626-nm light during DNP application had produced a PDA. The last record shows the response to white light after recovery. Voltage scale = 20 mV and time scale = 2 s. *sm* = stimulus monitor.

under conditions similar to those of the cells exposed to anoxia. In this particular cell a depolarization which could be terminated by a 463-nm light flash persisted through the recovery. This repolarization could be attributed to a PDA or, alternatively, to the light-activated hyperpolarizing pump that has been demonstrated in these cells (Koike et al., 1971). The magnitude of the pump hyperpolarization was determined by exposing the cell to a sequence of events similar to that represented in Fig. 3, excluding the 626-nm stimulation for PDA induction. In this case any hyperpolarization which is caused by the 463-nm light flash can be attributed to the pump. In the cell shown in Fig. 3 the pump hyperpolarization was found to be 8 mV. Thus, although the pump contributes to the hyperpolarization observed after the 463-nm light flash it cannot account for the entire change in membrane potential. The difference is most easily attributed to a PDA.

DISCUSSION

The prolonged depolarizing afterpotential is a very interesting and intriguing phenomenon. The fact that conductance increases, which are similar in nature to the light-coincident conductance changes, can be maintained after intense monochromatic stimulation may give us insight into the phototransduction mechanisms. In attempting to use the special properties of the PDA to examine the effects of metabolic agents on the phototransduction process we were surprised to find such a large variability from animal to animal. 25% of the cells had PDAs of long duration ($\tau > 10$ min) while 45% of the cells tested showed either weak ($\tau < 60$ s) PDA or none at all. These differences were found in animals that had been kept in the same environment and tested under similar conditions.

Although the PDA varied widely, the late receptor potential in all the cells tested was normal. The cells showed typical transient and steady-state potentials during illumination and the resting membrane potentials were at least -40 mV to -50 mV. In addition, the variability was not due to some alteration in the pigment states. The early receptor potential responded to monochromatic adaptation in the manner described by Minke et al. (1973) and the amplitude, tested over a 30-min period, was unchanged.

Besides the wide variability of the PDA from cell to cell, the duration of the PDA in some cells could be shortened or abolished after exposure to anoxia. Again, the late receptor potential and the stability of the pigment states remained unaffected.

Since we have demonstrated in *Balanus* lateral ocelli that the PDA is labile in some cells and that the PDA decay time can be irreversibly shortened after anoxia in other cells, it would be interesting to see if similar effects are seen in other photoreceptors where a PDA has been found, i.e. *Drosophila* retinular cells (Minke et al., 1975a), blowfly retinular cells (Muijser et al., 1975), and *Limulus* median ocellus (Nolte and Brown, 1972a, b). In their paper on the PDA in barnacle photoreceptors, Brown and Cornwall (1975a) commented briefly that they found the PDA in only 75% of the cells impaled. The percentage of cells in which a PDA could be induced has not been reported for *Drosophila*, *Limulus*, or blowfly photoreceptors. The effect of anoxia on the PDA decay was not seen by

Wong et al. (1976) in *Drosophila*. The reason may be that exposure to anoxia is much shorter in the *Drosophila* experiments because of a higher sensitivity to a drop in oxygen tension. In addition, recovery time from anoxia is very short, only a couple of minutes, compared to the time the PDA is maintained in *Drosophila*, i.e. several hours (Minke et al., 1975a), so that any decrease in PDA decay time might have gone unnoticed. The effects of anoxia on the PDA have not been studied in other photoreceptors.

It is interesting that in the few cells exposed to DNP, we were able to maintain the PDA until the cell recovered. This may be indicative of differences in the manner in which anoxia and DNP affect the PDA viability, but at this time we have no evidence which either supports or rejects this hypothesis.

Even though some variability of the PDA would be expected, the absence of the PDA in some cells and the irreversible abolition after anoxia in other cells were not. The lability of the PDA, in the absence of any alteration of other electrophysiologically measurable parameters (ERP, LRP), indicates that the mechanisms that give rise to the PDA are not always tightly coupled to the direct chain of events that leads to the light-coincident response. The site of the lability remains unknown but must be at some point after the change in pigment states. The steps in the transduction pathway that generate the PDA must be more labile than those leading to the light-coincident response.

At this time, we can do no more than discuss the lability in broad generalizations since the PDA phenomenon itself is not understood. The data are presented as another observable which must be considered when one is discussing possible mechanisms for explaining the prolonged depolarization afterpotential.

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