

# Alteration of Sensitivity and Time Scale in Invertebrate Photoreceptors Exposed to Anoxia, Dinitrophenol, and Carbon Dioxide

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**ABSTRACT** The effects of anoxia, 2,4-dinitrophenol (DNP), and carbon dioxide (CO<sub>2</sub>) on the late receptor potential of *Balanus* lateral ocelli, *Limulus* ventral eyes, and the reticular cells of *Limulus* lateral eyes have been studied. Either anoxia, DNP, or exposure to 100% CO<sub>2</sub> causes a depolarization of 5–30 mV and a gradual reduction and eventually abolition of the late receptor potential and an increase in the latency and time to peak of the response. This lengthening of the time scale is in contrast to the response obtained in photoreceptors that have been light-adapted or injected with calcium. In that case a loss in sensitivity is associated with a decrease in latency and time to peak. Because of these observed differences, the effects of metabolic inhibition cannot be attributed merely to a loss in regulation of intracellular free calcium. Rather, because alteration of intracellular pH (pH<sub>i</sub>) by using either (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or CO<sub>2</sub> produced changes in the photoresponse similar to those caused by metabolic inhibition, it is suggested that changes in pH<sub>i</sub> during metabolic inhibition can account in part for the lengthening of the time scale. In addition to the changes in pH<sub>i</sub> and internal Ca<sup>++</sup> concentration due to metabolic inhibition, the possible role of other consequences of metabolism in the transduction mechanism is also discussed.

## INTRODUCTION

The initial step in the transduction process is generally agreed to be the isomerization of the photopigment, rhodopsin, by light, but the mechanisms by which this isomerization eventually gives rise to conductance changes of the plasma membrane remain unknown. One model, which has been proposed to describe the response to light of *Limulus* lateral eye reticular cells, states that the transduction process can be described by a sequence of first-order chemical reactions (Fuortes and Hodgkin, 1964; Borsellino et al., 1965; Fuortes, 1965; and Borsellino and Fuortes, 1968 *a*). One line of experimental evidence which suggested that chemical reactions may be involved is that the amplitude and time to peak of the response evoked by a dim flash are logarithmically related to the temperature.

The hypothesis that sequential chemical reactions may be involved in the transduction mechanism is also suggested by experiments with agents or procedures which interfere with the metabolism of the photoreceptor. For

example, Borsellino and Fuortes (1968 *b*) found that exposure of *Limulus* lateral eye reticular cells to 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, reversibly abolished the late receptor potential and increased the latency and time to peak of the light response. This occurred without appreciably affecting the membrane potential or the resting membrane conductance. More recently, Baumann and Mauro (1973) have shown that exposure to anoxia can cause similar effects in the reticular cells of the lateral eye of *Limulus* and of the honeybee drone, *Apis mellifera*. In addition, Brown and Meech (1976) have reported that exposure of the photoreceptors in *Balanus* lateral ocelli to 100% CO<sub>2</sub> suppressed the late receptor potential and decreased the internal pH as measured by a pH microelectrode (see also Brown et al., 1976). A similar effect by CO<sub>2</sub> on the light response was seen subsequently in *Drosophila* by Wong et al. (1976).

In this study we have examined the effects of anoxia, 2,4-dinitrophenol, and carbon dioxide on the late receptor potential recorded intracellularly from the photoreceptors in the lateral ocelli of *Balanus* and the lateral and ventral eyes of *Limulus*. All of the procedures produced qualitatively similar results in each of the photoreceptors. Application of either anoxia, DNP, or 100% CO<sub>2</sub> caused a depolarization of 5–30 mV and a gradual reduction and eventually abolition of the late receptor potential with an increase in its latency and time to peak. This relatively unexplored feature in the response to light, namely, retardation in the kinetics of the late photoreceptor potential, is the main finding reported here. We also discuss the possible modifications of the transduction mechanism by changes in the internal concentration of Ca<sup>++</sup> and H<sup>+</sup> that might arise from the action of anoxia, 2,4-dinitrophenol, and carbon dioxide. Part of this work has been communicated in preliminary form (Lantz and Mauro, 1977).

#### MATERIALS AND METHODS

Lateral ocelli from *Balanus eberneus* (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 recordings were made directly through the connective tissue.

Male *Limulus polyphemus*, approximately 8–10 inches across the carapace, were obtained from either Marine Biological Laboratory, Woods Hole, Mass. or Gulf Specimen Co., Panacea, Fla. Lateral eyes were cut free from the rest of the animal and a slice, parallel to the long axis of the eye, was made with a razor blade thus exposing a layer of ommatidia. After the slice had been mounted in the recording chamber, reticular cells were impaled by placing the microelectrode on the surface of an ommatidium and "buzzing" i.e., increasing the capacitance neutralization feedback thus throwing the pipette amplifier into oscillation. The ventral photoreceptors (Clark et al., 1969) were dissected as described by Millecchia and Mauro (1969). In order to soften the connective tissue for impalement, 0.5% (wt/vol) Pronase (Calbiochem, San Diego, Calif.) in buffered sea water was applied to the preparation for 3 min.

Micropipettes were made from glass capillary tubing, which contained a small glass fiber (Hilgenberg Glass, Malsfield, West Germany). The micropipettes (DC resistance 10–30 M) were filled with 3 M KCl by injection through a 30-gauge needle, and were connected to an Ag-AgCl electrode via a 3 M KCl bridge (micropipette holder EH-2R,

W-P Instruments, Inc., New Haven, Conn.). The bath was connected to a calomel electrode with an artificial sea water bridge. The output of the electrode pair was fed into a unity gain high input-impedance amplifier. The output of the amplifier was displayed simultaneously on an oscilloscope, which could be photographed with a Grass Kymograph camera (Grass Instrument Co., Quincy, Mass.) for measurement of the latency and time to peak, and on a Gould Brush 220 pen recorder (Gould Inc., Instrument Systems Div., Cleveland, Ohio) for measurement of the membrane potential. Figs. 2-5, which show the latency and time to peak, are composite smoothed tracings made from this film. The noise level in the film recordings was 50-100 microvolts. Latency measurements were made in a total of 31 cells. Recordings in Fig. 1 were obtained with the pen recorder.

The recording chamber (1 ml) could be continuously perfused with artificial sea water (435 mM NaCl, 10 mM KCl, 10 mM CaCl<sub>2</sub>, 20 mM MgCl<sub>2</sub>, and 25 mM MgSO<sub>4</sub>, pH obtained with 5 mM Tris) by use of a peristaltic pump. The flow rate was maintained at approximately 10 ml/min which allowed drugs to be washed out quickly or kept the preparation in a steady state of N<sub>2</sub> (anoxia) or CO<sub>2</sub> (pH 5.5) exposure. The gas exchange system, oxygen electrode for maintaining oxygen tension, and protocol for dinitrophenol application have been described previously (Lantz et al., 1977). (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions were made immediately before use to minimize loss of ammonia to the atmosphere. The ammonium-sea water solution was adjusted to pH 7.4 with NaOH.

The light source was a 150-W xenon arc lamp (Osram, Munich, West Germany) powered by a regulated supply manufactured by Oriel Corp. of America, Stamford, Conn. The light was brought to the preparation by a light pipe which was positioned ~ 3-5 mm above the level of the solution in the recording chamber. The beam could be interrupted by an electromagnetic shutter which was usually driven at 0.1 Hz. The photoreceptors were allowed to adapt to the given intensity and stimulation before being exposed to any of the treatments. When unsaturating light intensities were used the intensity was attenuated, using calibrated neutral density filters (Eastman Kodak Co., Rochester, N. Y.), until the adapted late receptor potential amplitude began to decrease.

The intensity of the unattenuated light source was calibrated as described previously (Lantz et al., 1977).

## RESULTS

Fig. 1 shows the effect of anoxia on the lateral ocellus of *Balanus*. The control response shows a typical intracellular recording from an arthropod photoreceptor. The resting membrane potential is ~ -60 mV. When light is presented to the receptor it undergoes at first a transient depolarization which may overshoot zero membrane potential and then returns to some steady state depolarization which is maintained throughout the stimulation. It is known from voltage clamp studies in *Balanus* ocellus (Brown et al., 1970) and *Limulus* ventral eye (Millecchia and Mauro, 1969) that the receptor potential is due to a voltage dependent, time variant, conductance increase, the major current carrier being sodium.

The initial downward deflection in each of the receptor potentials of Fig. 1 is the early receptor potential (ERP) of Brown and Murakami (1964) which is believed to be caused by changes in the pigment states. That this signal was indeed the ERP was determined by the fact that it appeared with almost no latency after the start of stimulation, its amplitude was proportional to light intensity, and it survived all treatments which affected the late receptor potential. This included exposure to 500 mM KCl solution. The small upward

deflection at the termination of the light stimulus also seems to be associated with the pigment states inasmuch as it also survived all treatments which affected the late receptor potential.

The remaining traces in Fig. 1 demonstrate the effect of anoxia on *Balanus* lateral ocelli. The anoxia was produced by equilibration of continuously perfused sea water with 100% N<sub>2</sub>. Time  $t = 0$  would correspond to the time the oxygen tension of the sea water began to drop. As can be seen, the amplitude of the late receptor potential decreased as a function of time in the anoxic state until after 30 min the response was almost completely abolished. Along with the decrease in amplitude of the late receptor potential there was also a depolarization of the membrane potential. The amplitude of the membrane potential decrease varied from cell to cell ranging from almost no change to up to 30 mV

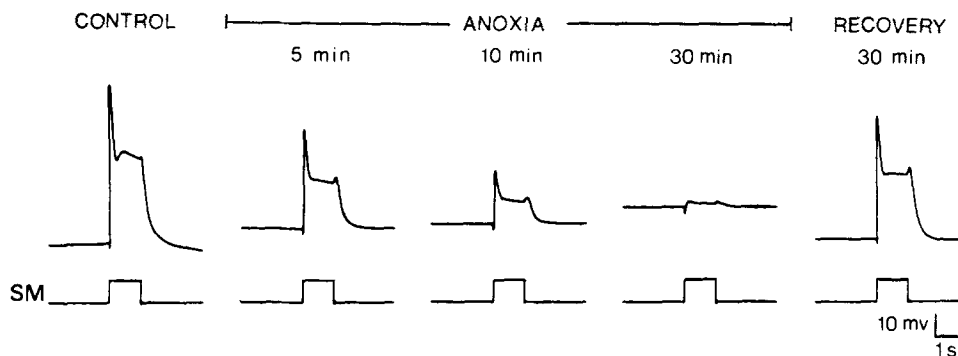


FIGURE 1. Effect of anoxia on the late receptor potential of *Balanus* lateral ocelli. Time  $t = 0$  corresponds to the time when the oxygen tension of the sea water began to fall. After 30 min in anoxia the membrane potential is depolarized by  $\sim 15$  mV, and the late receptor potential is almost completely abolished. Subsequent introduction of air recovered the response to near control values after 30 min. Note the early receptor potential (initial negative deflection) was not affected by anoxia. SM = stimulus monitor. Full intensity ( $I_{\max}$ ) 1-s light pulse presented every 10 s. Recorded with pen recorder.

depolarization. Although this membrane depolarization may contribute to the decrease in amplitude of the late receptor potential, it is not the primary cause of the receptor potential decrease. Rather, it is the light induced conductance increase that is blocked during anoxia (Baumann and Mauro, 1973; Wong et al., 1976). As can be seen, anoxia had no effect on the ERP. The last trace of Fig. 1 shows that the effect of the anoxia on the late receptor potential is reversible, in this case recovery taking 30 min from the time air was reintroduced.

The effects of anoxia are qualitatively similar for each of the photoreceptors studied. Also there does not appear to be any qualitative differences between the effects caused by anoxia, 2,4-dinitrophenol (DNP), or 100% CO<sub>2</sub> on any particular photoreceptor. Quantitative differences between different arthropod receptors are seen in the time in anoxia or in the effective concentration of DNP necessary to reduce or abolish the late receptor potential. Although large cell to cell variations are seen, in general the reticular cells of *Limulus* lateral eyes were

the most sensitive to anoxia and DNP. The late receptor potential could usually be abolished in these cells with a 15–30-min exposure to anoxia or with 0.05 mM DNP. Slightly more resistant were *Balanus* lateral ocelli which required, on the average, 0.1–0.2 mM DNP or 30–60 min in anoxia to abolish the response. *Limulus* ventral cells were usually the least sensitive (0.5–1.0 mM DNP or several hours in anoxia) although some cells were found with a sensitivity comparable to the *Balanus* lateral ocelli. All the arthropod photoreceptors responded very rapidly to 100% CO<sub>2</sub> (< 5 min). The abolition caused by the CO<sub>2</sub> was not simply a result of anoxia, inasmuch as cells in which the late receptor potential had been abolished by 100% CO<sub>2</sub> could be recovered in 100% N<sub>2</sub> (anoxia).

One possibility which might explain the observed suppression of the late receptor potential is that regulation of intracellular calcium has been decreased. It has been reported by Brown and Lisman (1975) and by Charlton and Fein (1977) that, in the ventral photoreceptor of *Limulus*, intracellular injection of calcium can mimic the effects of light adaptation. That is, increasing intracellular calcium decreases the sensitivity of the photoreceptor and also decreases the latency and time to peak of the response.<sup>1</sup> Thus, if increased intracellular calcium is the only cause for the observed suppression, there should be a decrease not only in the late receptor potential amplitude but also in the latency and time to peak of the response.

Fig. 2 shows that this is not the case. This is a composite tracing showing the effects of anoxia on the latency in a *Balanus* lateral ocellus. As has been demonstrated above, anoxia causes a depolarization of the membrane potential and a decrease of the amplitude of the late receptor potential. In addition to these changes, anoxia causes an increase in the latency of the light-coincident response. The latency in all our records is given as the time to reach 10% of the peak amplitude. This value was chosen rather than the absolute latency because in some instances it was difficult to decide the exact point where the depolarizing receptor potential began. In this particular cell, exposure to anoxia for 60 min caused an increase in the receptor potential latency ( $t_l$ ), from 29 ms during control to 51 ms during anoxia, and time to peak ( $t_p$ ), from 95 ms during control to 127 ms during anoxia. These values recovered toward control levels after 30 min in air ( $t_l = 41$  ms,  $t_p = 97$  ms). The initial downward deflection in Fig. 2 is the ERP.

Inasmuch as the latency measurements of Fig. 2 were made using maximum intensity, 1-s light stimulation, any decrease in latency may not be readily observable because the cell may already be responding with the smallest possible latency. This possibility was examined using short nonsaturating light intensities. Fig. 3 shows that the effect of anoxia on the ventral cell of *Limulus* is the same under these stimulation conditions. In this case exposure to anoxia for 90 min increased the latency from 43 ms during control to 73 ms during anoxia. The time to peak was also dramatically increased, control  $t_p = 94$  ms; anoxia  $t_p = 162$  ms.

The increase in latency and time to peak was found no matter which

<sup>1</sup> It should be noted that this experiment has not been reported for *Balanus*. In our investigations we have assumed that because *Limulus* and *Balanus* are both arthropods, their photoreceptors behave qualitatively in a similar manner with regard to change in (Ca<sup>++</sup>).

photoreceptor or metabolic agent was used. In some cases the latency during metabolic inhibition was three to four times greater than the control values. These findings indicate that, although a decrease in regulation of intracellular calcium may play a role in the suppression of the receptor potential amplitude during metabolic inhibition, it cannot account for the increase in latency which is observed. The fact that calcium does play a role in the suppression of the

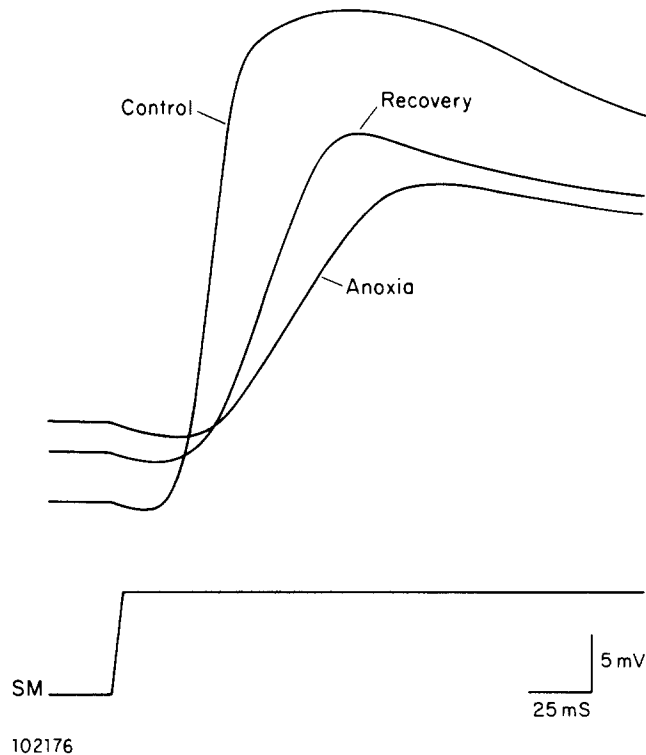


FIGURE 2. Effect of anoxia on the time scale of the response in *Balanus* lateral ocelli. The latency ( $t_l$ ) and time to peak ( $t_p$ ) of the response to a full intensity, 1-s stimulation were increased by exposure to anoxia for 60 min (control:  $t_l = 29$  ms,  $t_p = 95$  ms; anoxia:  $t_l = 51$  ms,  $t_p = 127$  ms). The values recovered toward control values after air was reintroduced (recovery:  $t_l = 41$  ms,  $t_p = 97$  ms). Curve shown was taken 30 min after recovery was begun. SM = stimulus monitor. Full intensity ( $I_{max}$ ) 1-s light pulse presented every 10 s. Figs. 2-5 are composite smoothed tracings made from oscillographic film.

amplitude of the light-coincident response during DNP application was found by Wong et al.<sup>2</sup> In *Limulus* ventral cells, injection of ethylene glycol-bis ( $\beta$ -aminoethyl ether)- $N,N'$ -tetraacetic acid (EGTA), a calcium chelator, during DNP application recovered the receptor potential amplitude to its control level.

<sup>2</sup> Wong, F., R. C. Lantz, and A. Mauro. 1977. The role of calcium in the suppression of the photoresponse during anoxia and application of metabolic inhibitors. Submitted for publication.

It should be noted that the time-course of the response (i.e., latency and time to peak) did not recover to control values with EGTA injection. Therefore, the increase in latency that is seen with metabolic inhibition must counteract and eventually overcome the effects of increased intracellular calcium concentration.

If it is assumed that the transduction process is a consequence of a sequence of chemical reactions, then an increase in latency may be indicative of a decrease in some reaction rate. One parameter which controls reaction rates and has already been implicated in the latency of the photoresponse (Coles and Brown,

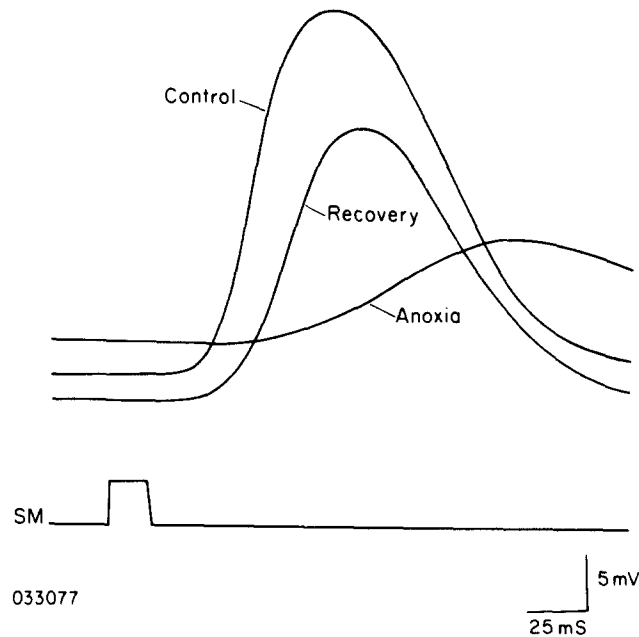


FIGURE 3. Effect of anoxia on the time scale of the response in *Limulus* ventral eyes. These particular records were taken with short (25 ms), unsaturating flashes so that any increase in latency could be seen. As shown in Fig. 2, the latency and time to peak increase with exposure to anoxia for 90 min (control:  $t_l = 43$  ms,  $t_p = 94$  ms; anoxia:  $t_l = 73$  ms,  $t_p = 162$  ms). 30 min after reintroduction of air the values had recovered toward control values (recovery:  $t_l = 49$  ms,  $t_p = 104$  ms). SM = stimulus monitor. 25-ms light pulse presented every 10 s;  $I_{\max}$  attenuated by 3 log units with neutral density filters (ND - 3.0).

1976) is the intracellular pH ( $\text{pH}_i$ ). If changes in intracellular pH cause the increased latencies observed during metabolic inhibition, then direct experimental manipulation of the intracellular pH should cause similar effects. In fact, as we have seen, carbon dioxide, whose mode of action is presumably through changes in intracellular pH, does increase the latency of the photoresponse.

Another method of altering intracellular pH is by addition of an ammonium salt to the external bathing solution (Thomas, 1974). While the ammonium is in the external solution, the intracellular pH should become more basic. Removal

of the ammonium from the bath will lead to a decrease of  $\text{pH}_i$  which may undershoot the normal pH level (cf. Thomas, 1974).

Fig. 4 shows the effect of ammonium sulfate on a *Balanus* lateral ocellus. After application of 5 mM  $(\text{NH}_4)_2\text{SO}_4$  for 5 min (trace A), the response to a 25-ms unsaturating flash was faster and larger than the control; control: latency = 47 ms,  $t_p$  = 107 ms; trace A: latency = 43 ms,  $t_p$  = 101 ms. The initial decrease in latency and increase in amplitude were seen in all cells exposed to  $(\text{NH}_4)_2\text{SO}_4$ . If

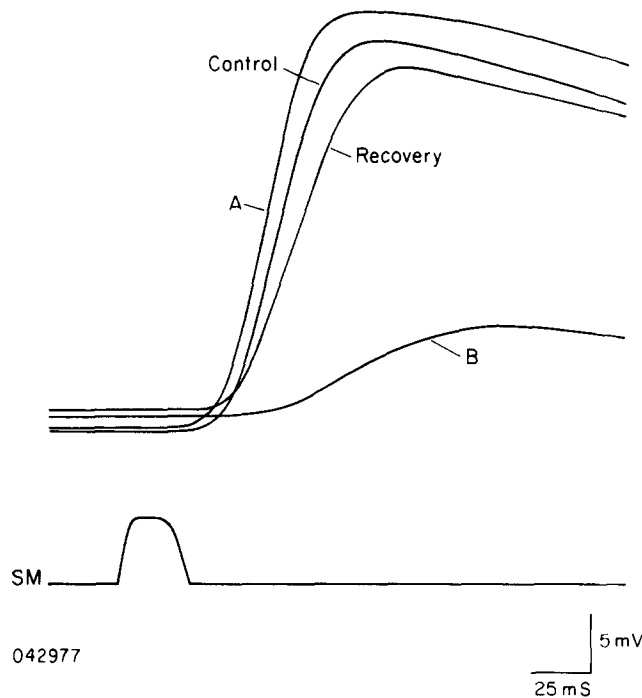


FIGURE 4. Effect of  $(\text{NH}_4)_2\text{SO}_4$  on the time scale of the response in *Balanus* lateral ocelli. 5 min after introduction of 5 mM  $(\text{NH}_4)_2\text{SO}_4$  (trace A) the latency and time to peak of the response have decreased (control:  $t_l$  = 47 ms,  $t_p$  = 107 ms; trace A:  $t_l$  = 43 ms,  $t_p$  = 101 ms). Upon washing out the  $(\text{NH}_4)_2\text{SO}_4$  the receptor potential amplitude quickly decreased and the time scale increased. Trace B was taken 3 min after the wash out was begun (trace B:  $t_l$  = 68 ms,  $t_p$  = 154 ms). After 10 min the response disappeared completely. With continuous perfusion (90 min) with artificial sea water, the response reappeared gradually, and the amplitude and time scale recovered toward control values (recovery:  $t_l$  = 51 ms,  $t_p$  = 113 ms). SM = stimulus monitor. 25-ms light pulse presented every 10 s. ND - 3.0.

the cell was exposed for longer times to  $(\text{NH}_4)_2\text{SO}_4$ , eventually the latency increased and the amplitude decreased with respect to the control values. When the  $(\text{NH}_4)_2\text{SO}_4$  was removed from the bath, thus lowering the intracellular pH below normal levels, the latency would increase and the response would eventually disappear. Trace B of Fig. 4 shows the response 3 min after normal artificial sea water was introduced (latency = 68 ms,  $t_p$  = 154 ms). The response



in this cell was completely abolished 10 min after the washing out of  $(\text{NH}_4)_2\text{SO}_4$  was begun. If the cell was continuously perfused, the response would eventually reappear and gradually recover toward the control level. The recovery trace of Fig. 4 was taken 50 min after the washing out of  $(\text{NH}_4)_2\text{SO}_4$  was begun. The latency (51 ms) and time to peak (113 ms) were still slightly greater than the control values. It should be noted that during the  $(\text{NH}_4)_2\text{SO}_4$  treatment the ERP, upon testing with a saturating flash, was not abolished indicating that the pigment states were not being affected.

The sensitivity to changes in  $\text{pH}_i$  of the three photoreceptors tested was the same as for the metabolic inhibitors. The *Limulus* lateral eye reticular cells were most sensitive, responding to the lowest concentrations and shortest exposure time of  $(\text{NH}_4)_2\text{SO}_4$ . *Balanus* lateral ocelli were slightly more resistant and the *Limulus* ventral eyes were the least sensitive, requiring high concentrations and long exposure to get results comparable with those seen in *Balanus*.

Because the effects on the photoresponse of a decrease in  $\text{pH}_i$  are similar to the effects of the metabolic agents, this suggested that the increase in latency caused by the metabolic agents may be due to a decrease in the  $\text{pH}_i$ . If this hypothesis is true then it should be possible to recover a cell from the effects of a metabolic agent by increasing the  $\text{pH}_i$ .

Fig. 5 shows the result of an experiment of this type performed on a *Balanus* lateral ocellus. The control response had a latency of 52 ms and a time to peak of 127 ms. After being exposed to anoxia for 90 min (trace A), the latency had increased to 68 ms and the time to peak to 203 ms while the amplitude had decreased to 10 mV. At this point 10 mM  $(\text{NH}_4)_2\text{SO}_4$  was introduced into the perfusion solution while the cell was still in the anoxic state. The response quickly showed signs of recovery and after 10 min the latency had decreased back to 55 ms and the time to peak to 149 ms. Upon subsequently washing the cell in normal oxygenated sea water, the response disappeared just as it did when  $(\text{NH}_4)_2\text{SO}_4$  was removed in the previous experiment. But with continuous perfusion (90 min) the cell recovered to near the control values (latency = 49 ms,  $t_p$  = 126 ms). Similar results were obtained in *Limulus* ventral eyes.

Thus, increasing the  $\text{pH}_i$  during anoxia can recover the latency. This appears to be true only in the early stages of the anoxic effect. If the cell is kept in anoxia until the photoresponse is completely abolished, then exposure to  $(\text{NH}_4)_2\text{SO}_4$  has no noticeable effect on the response. In one cell, for instance, no recovery was seen during a 15-min exposure to 20 mM  $(\text{NH}_4)_2\text{SO}_4$  even though the cell recovered when placed back in normal oxygenated sea water.

#### DISCUSSION

Borsellino and Fuortes (1968 *b*) found that either DNP or lowering the temperature caused an increase in the time scale of the response of reticular cells in *Limulus*. Their interpretation, especially with regard to the effect of temperature, was that the rates of (hypothesized) sequential chemical reactions underlying the transduction process were being affected at more than one point in the chain of reactions, without dismissing the possibility that at some stages diffusion of "particles" might be involved.

By the use of either anoxia or DNP we have attempted to examine the

necessity, either direct or indirect, for cellular energy in the transduction process in several arthropods. Each of the procedures was found to cause qualitatively similar effects on the response. Anoxia or DNP, as well as the administration of  $\text{CO}_2$ , cause the photoreceptor to depolarize and the receptor potential to decrease in amplitude and eventually disappear. At the same time the latency and time to peak of the response increases.

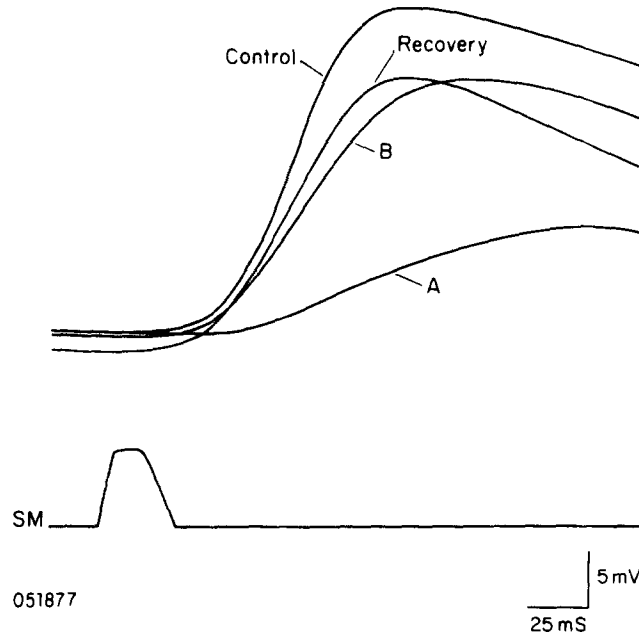


FIGURE 5. Effect of  $(\text{NH}_4)_2\text{SO}_4$  application during anoxia. A *Balanus* lateral ocellus was exposed to anoxia (trace A) for 90 min. This caused a lengthening of the time scale of the response (control:  $t_l = 52$  ms,  $t_p = 127$  ms; trace A:  $t_l = 68$  ms,  $t_p = 203$  ms). While the cell was still in the anoxic state, 10 mM  $(\text{NH}_4)_2\text{SO}_4$  was introduced into the bathing solution. The response quickly showed signs of recovery and after 10 min (trace B) the latency ( $t_l = 55$  ms) and time to peak ( $t_p = 149$  ms) had decreased toward control values. Upon washing out the  $(\text{NH}_4)_2\text{SO}_4$  solution, the response disappeared as described previously. With continuous perfusion (90 min) the cell recovered to near control values (recovery:  $t_l = 49$  ms,  $t_p = 126$  ms). SM = stimulus monitor. 25-ms light pulse presented every 10 s. ND - 3.0.

The similarity of the effects caused by anoxia and DNP supports the hypothesis that adenosine triphosphate (ATP) is necessary for the transduction process. This involvement of ATP is not mediated merely through the maintenance of the membrane potential, because in some cells very little change of membrane potential occurs during metabolic inhibition whereas the receptor potential is completely abolished. Nor is the primary effect of altering oxidative phosphorylation mediated through loss of volume regulation by the cell.

Borsellino and Fuortes (1968 *b*) showed that either doubling or halving the osmolarity of the bathing solution had no effect on the photoresponse.

It seems probable that the effects of metabolic inhibition are being mediated through several mechanisms, one of which may be an increase in intracellular calcium concentration. Increases in intracellular calcium are known to reduce the receptor potential (Brown and Lisman, 1975; Charlton and Fein, 1977), but this occurs with a decrease in the latency and time to peak of the response. In sharp contrast, metabolic inhibition always increases the latency and time to peak. Because it is likely that free intracellular calcium rises during metabolic inhibition, it follows that the effects of calcium, which shorten the latency, must be overridden by processes which slow down the response. Thus, it is reasonable to infer that at least two antagonistic processes are acting during metabolic inhibition.

A second probable condition which counteracts the effects of calcium on the time-course of the response during metabolic inhibition is a change in intracellular pH. The dependence of the time-course of the response on  $\text{pH}_i$  and intracellular buffering capacity has been shown by Coles and Brown (1976). Their results show that, in *Limulus* ventral eyes, injection of buffers with pK values below 7.7 caused a decrease in latency and time to peak. Our results with  $\text{CO}_2$  and  $(\text{NH}_4)_2\text{SO}_4$  indicate that lowering intracellular pH increases the latency and time to peak. (That  $\text{CO}_2$  causes a drop in  $\text{pH}_i$  has been established by Brown and Meech [1976] directly by means of a pH electrode in *Balanus* lateral ocelli: a 2-min exposure to 100%  $\text{CO}_2$  reduced  $\text{pH}_i$  by 0.7.)<sup>3</sup> The differences in these observations may be reconciled by considering the effects of additional buffering capacity on enzymatic reaction rates.

Although we have no direct measurement of the  $\text{pH}_i$ , we may infer from direct pH measurements in other preparations that metabolic inhibition can lead to a decrease in  $\text{pH}_i$ . Thomas (1974) demonstrated that, in *Helix* neurones, exposure to nitrogen for several minutes caused a gradual decrease in  $\text{pH}_i$ . In addition, Boron and De Weer (1976) showed that DNP will decrease  $\text{pH}_i$  of squid giant axons. Indirect evidence that anoxia causes a decrease in  $\text{pH}_i$  was found in our experiments where addition of  $(\text{NH}_4)_2\text{SO}_4$ , during anoxia, recovered the latency, time to peak, and amplitude of the receptor potential in *Balanus*.

Thus, we have discussed two factors resulting from metabolic inhibition which may affect indirectly whatever mechanisms underlie the photoresponse of the cell. However, the changes in the intracellular calcium concentration and  $\text{pH}_i$  are but two of the indirect effects which can be considered. There are undoubtedly more consequences of blocking oxidative phosphorylation. Their existence may be indicated by the observation, described above, that a cell in which the response has been completely abolished by anoxia cannot be recovered with ammonium sulfate. Whether or not these additional effects of

<sup>3</sup> It is relevant to note here that Brown and Meech (1976) have reported that the effects of exposure to 100%  $\text{CO}_2$  (pH 5.5), which caused a decrease in  $\text{pH}_i$  and the receptor potential amplitude, could not be reproduced by pH 5.5 phosphate buffered saline. This result indicates that changing the external pH to 5.5 is not the basis for the effects of the administration of 100%  $\text{CO}_2$ .

anoxia are mediated through direct involvement of metabolic energy in the transduction process remain to be established.

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