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# THE RECOVERY FROM A TRANSIENT INHIBITION OF THE OXIDATIVE METABOLISM OF THE PHOTORECEPTORS OF THE DRONE (*APIS MELLIFERA* ♂)

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SUMMARY

1. In the honey-bee drone retina (*Apis mellifera*) oxidative metabolism is carried out largely by the photoreceptors. We have studied the effects of a transient inhibition of this metabolism, by recording receptor potential simultaneously with measurements of local $P_{O_2}$ and extracellular $[K^+]$ ($[K^+]_o$), using $O_2$- and $K^+$-sensitive microelectrodes.

2. When the retina was subjected to anoxia or exposed to Na-amobarbital (amytal), the photoreceptors depolarized by about 30 mV, in parallel with an increase of $[K^+]_o$ of up to 30 mmol $l^{-1}$, and the receptor potential was abolished in 2–3 min.

3. The reduction of the receptor potential followed the beginning of anoxia with a delay, which we define as a 'resistance' of the photoreceptors to anoxia.

4. The resistance to anoxia was greater if the photoreceptors were stimulated with only low intensity light flashes, suggesting that the effects of anoxia are due to the progressive consumption of a substance stored in the photoreceptors. This substance is probably ATP.

5. When $O_2$ was reintroduced, or amytal washed out, oxidative metabolism rapidly resumed. $[K^+]_o$ quickly decreased and, after a large undershoot, returned to the baseline in less than 5 min. There was in parallel a repolarization of the photoreceptors, followed by recovery of the receptor potential.

6. About 5 min after reintroduction of $O_2$, when the $[K^+]_o$, the membrane potential and the amplitude and kinetics of the receptor potential had completely recovered, exposure of the retina to a second anoxia suppressed the receptor potential faster than had the first anoxia.

7. Full recovery of the resistance to anoxia thus takes longer (by about 10 min) than recovery of the electrical properties of the photoreceptor cells. The amplitude of the extra oxygen consumption measured after a flash of light recovered in parallel with the resistance to anoxia.

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Key words: Drone, photoreceptor, receptor potential, oxidative metabolism, oxygen consumption, anoxia, amytal, potassium, pH.

## INTRODUCTION

The histology of the honey-bee drone retina suggests a remarkable metabolic compartmentation: the photoreceptors contain numerous mitochondria ranged at the periphery of the cell close to the cytoplasmic membrane, facing fin-like processes of the adjacent glial cells, which contain few mitochondria, and large quantities of glycogen beta particles (Perrelet, 1970). This suggests that the oxidative metabolism of the drone retina is largely confined to the photoreceptors (see Fig. 1).

Oxidative metabolism of the drone retina, as measured by $O_2$ consumption ($Q_{O_2}$), increases when the retina is exposed to flashes of light (Tsacopoulos, Poitry & Borsellino, 1981; Tsacopoulos & Poitry, 1982). The drone retina cannot function without this $Q_{O_2}$: anoxia depolarizes the photoreceptors and suppresses the receptor potential within 2 min (Baumann & Mauro, 1973). These effects of anoxia have also been observed in the photoreceptors of other arthropods (Wong, Wu, Mauro & Pak, 1976; Lantz & Mauro, 1978; Payne, 1981) but the molecular mechanism by which anoxia blocks the receptor potential is not clear. It is reasonable to suppose that suppression of $Q_{O_2}$ by anoxia leads to a decrease of the ATP concentration in the photoreceptor, and thus to an inhibition of ATP-dependent processes, such as the working of the sodium pump. The present study examines the extent to which inhibition of the pump is involved in suppression of the receptor potential, and the role of secondary factors, such as an accumulation of $K^+$ in the extracellular space.

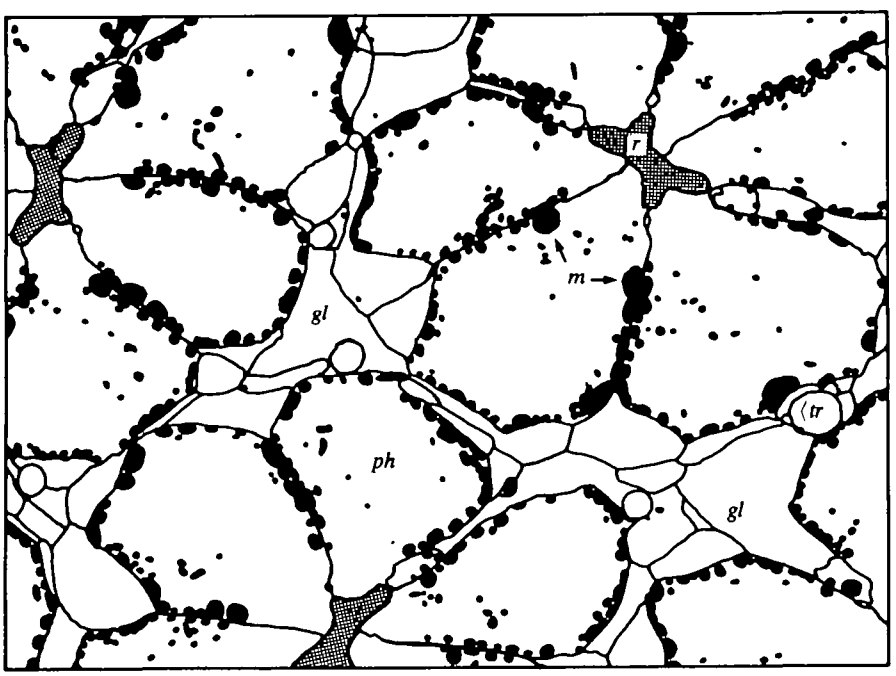


Fig. 1. Outline drawing obtained from an electronmicrograph of the drone retina. The section was at the middle of and transversal to the long axes of the ommatidia. Mitochondria (*m*) are marked solid in black, and are found almost completely within photoreceptor cells (*ph*), which are of fused rhabdom type (*r*); *gl*, glial cells; *tr*, tracheoles. Traced by J. A. Coles from an electron micrograph by A. Perrelet.

## METHODS

For some experiments, the cut head preparation described by Fulpius & Baumann (1969) and slightly modified by Tsacopoulos *et al.* (1981) was used. Briefly, the drone was decapitated, the antennae were cut and the head was fixed with the posterior surface up in a stainless steel microcuvette previously filled with melted wax (at 37–38 °C) (Eicosan, Fluka, Buchs, Switzerland). A cut was made close to the posterior surface and parallel to the axes of the ommatidia with a new razor blade vibrating at 300 Hz. This exposed a layer of ommatidia which were then placed flush with the floor of a chamber (see Tsacopoulos *et al.* 1981) and which was superfused with oxygenated Ringer's solution (composition in mmol $l^{-1}$: NaCl,280; KCl,10; $CaCl_2$, 1·6; $MgCl_2$,10; Tris,10; pH 7·4). Under these conditions only a retinal layer of 400 $\mu$m is oxygenated in darkness and therefore functioning (Tsacopoulos *et al.* 1981). When the retina is repetitively stimulated by light flashes this oxygenated layer is reduced to about 200 $\mu$m (Tsacopoulos & Poitry, 1982). In some other experiments, more particularly those in which $\Delta Q_{O_2}$ was measured, slices 300 $\mu$m thick were made, as described by Tsacopoulos & Poitry (1982). The effect of anoxia on the receptor potential was identical in the two preparations. The Ringer's solution was pumped to the chamber by a peristaltic pump with a flow rate of about 8 ml $min^{-1}$. Before reaching the chamber, the flow passed through a Teflon membrane thin layer gas exchanger (Dunnschicht Dialysator, L. Eschweiler and Co., Kiel, West Germany). This system was effective in quickly replacing $O_2$ by $N_2$ (anoxia) in the Ringer's solution (see Tsacopoulos *et al.* 1981).

### *Electrodes*

Receptor potentials were recorded intracellularly with micropipettes using conventional techniques (Bader, Baumann & Bertrand, 1976). The local $P_{O_2}$ was recorded with double-barrelled platinum microelectrodes as described previously (Tsacopoulos & Lehmenkuhler, 1977; Tsacopoulos *et al.* 1981).

The extracellular $K^+$ concentration ($[K^+]_o$) was measured with double-barrelled $K^+$-sensitive microelectrodes as described by Coles & Tsacopoulos (1977, 1979) and Munoz, Deyhimi & Coles (1983).

### *Stimulation*

Light from a 150 W xenon arc was focused on a diaphragm which could be occluded by an electromechanical shutter. The image of the diaphragm was focused on the retina with a microscope objective (Bader *et al.* 1976). The intensity at the retina was about 1·1 W $cm^{-2}$ and the standard flashes were 20–40 ms long.

### *Data storage and calculation*

Recordings were digitized at 10 Hz, and were then displayed on a graphics terminal (Tektronix 4006) and stored on magnetic disc by use of a microcomputer (IMSAI 8080 microcomputer system, San Leonard, CA). The kinetics of the extra $O_2$ consumption induced by a single flash of light ($\Delta Q_{O_2}$) were calculated using a Fast

Fourier Transform routine as described by Tsacopoulos & Poitry (1982). The voltage signal from the $K^+$ microelectrodes was transformed by the computer to a potassium signal on the basis of the calibration curve of each microelectrode.

## RESULTS

### *The effects of anoxia*

Recordings were made from photoreceptors which were exposed to light flashes every 4 s to induce depolarizing receptor potentials. When $O_2$ was replaced by $N_2$, a $P_{O_2}$ level of near zero was achieved in about 15 s. About 15 s later there was a depolarization of the resting potential, and the amplitude of the receptor potential diminished (Fig. 2). Depolarization was completed, and receptor potential was abolished in about 2 min, as found previously (Baumann & Mauro, 1973). When $O_2$ was restored, the membrane potential quickly recovered to the pre-anoxia level followed by a progressive recovery of the amplitude of the receptor potential. These effects of anoxia were qualitatively the same in all cells studied.

Hypoxia with local $P_{O_2}$ around 10 mmHg only slightly depolarized the photoreceptor without any appreciable effect on the receptor potential. This is in accord with the observation that $Q_{O_2}$ in the superfused drone retina is independent of the local $P_{O_2}$, down to a $P_{O_2}$ as low as 10 mmHg (Tsacopoulos *et al.* 1981).

The delay between the onset of anoxia and the effects upon the photoreceptor (Fig. 2), which we term the resistance of the photoreceptor to anoxia, would indicate that the metabolic needs of the photoreceptor during this period were met by anaerobic metabolism, as in the brain (McIlwain & Bachelard, 1971), or by an energy store. Glycolysis does not appear to be involved because extracellular pH measurements (using pH-sensitive microelectrodes) and biochemical analysis of the superfusate have shown that anoxia does not cause either acidification of the extracellular space or accumulation of lactate in the drone retina (Coles & Tsacopoulos, 1985).

### *Resistance of the photoreceptor to anoxia*

To investigate whether an ATP store was depleted during anoxia, the effect of light intensity upon the time required for anoxia to block the receptor potential was examined. It has previously been shown that the amplitude of $\Delta Q_{O_2}$ and $\Delta[Na^+]_i$

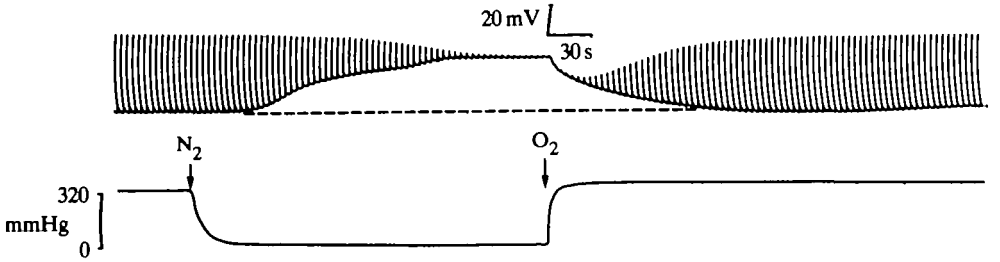


Fig. 2. The effect of anoxia on the intracellularly recorded receptor potential. In the upper trace are receptor potentials elicited by strong light flashes. The lower trace is the local $P_{O_2}$ recorded at the exposed surface of the superfused retina. Calibration of the $O_2$ microelectrode was done as described in Tsacopoulos, Poitry & Borsellino (1981).

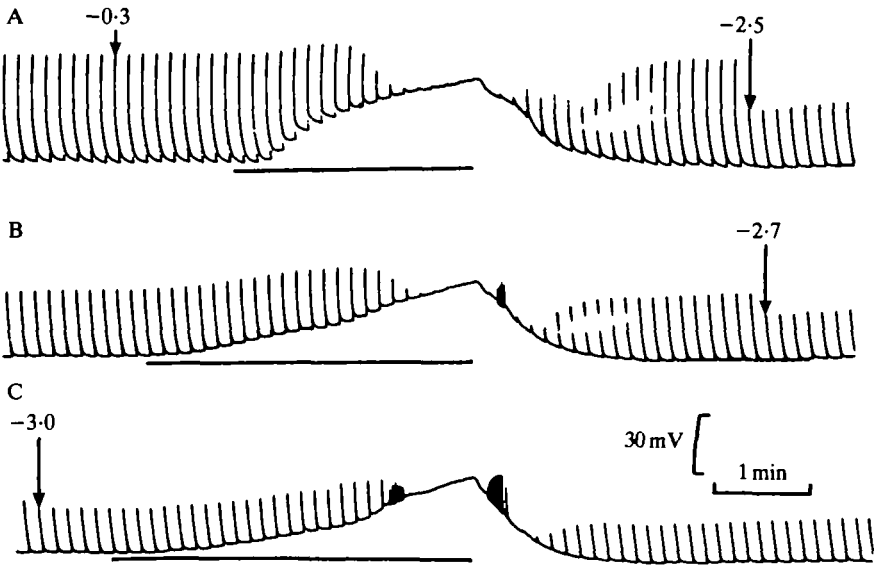


Fig. 3. The effect of anoxia on the intracellular receptor potential during stimulation with three different light intensities. The relative absorbance of the neutral density filters used for the attenuation of the light beam is indicated. The record was continuous from the same cell, but it was separated in three portions A, B and C, for better presentation. The horizontal bars indicate the period of tissue anoxia (measured with an $O_2$ microelectrode). The black marks on the records are fused trains of action potentials occurring in the dark, similar to those described by Baumann (1968). These are present also in other following figures.

elicited by a single flash of light are linearly related to the logarithm of the intensity of the light (Tsacopoulos & Poitry, 1982; Coles & Orkand, 1982).

When the light intensity was reduced by 3 log units from the maximum (Fig. 3C) the duration of the anoxia necessary to block the photoresponses was increased by about 1·5 times. Also, in the case of stimulation with low intensity flashes (Fig. 3C), the membrane depolarization and the reduction of the amplitude of the receptor potential had clearly different time courses.

The time required for anoxia to block the photoresponse depended also on how recently the photoreceptors had been exposed to a previous anoxia. In the experiment shown in Fig. 4, the first exposure to anoxia produced 90 % inhibition of the receptor potential in 90 s (Fig. 4A). The retina was then reoxygenated for different periods before further anoxic exposures. After 5 min of reoxygenation, anoxia produced 90 % inhibition in 45 s (Fig. 4B), whereas after 16 min recovery, an anoxic period of 94 s was required (Fig. 4C), similar to that for the first exposure (Fig. 4A).

Resting potential and membrane potential returned to similar values after all three exposures, indicating that these parameters do not determine the resistance to anoxia.

### *Extracellular $[K^+]$ during anoxia*

To examine whether ionic perturbations are induced in determining the resistance of the photoreceptors to anoxia, we measured changes of the extracellular $K^+$ concentration ($[K^+]_o$) as described by Coles & Tsacopoulos (1979). In the typical experiment shown in Fig. 5, the retina was stimulated with strong flashes of light presented

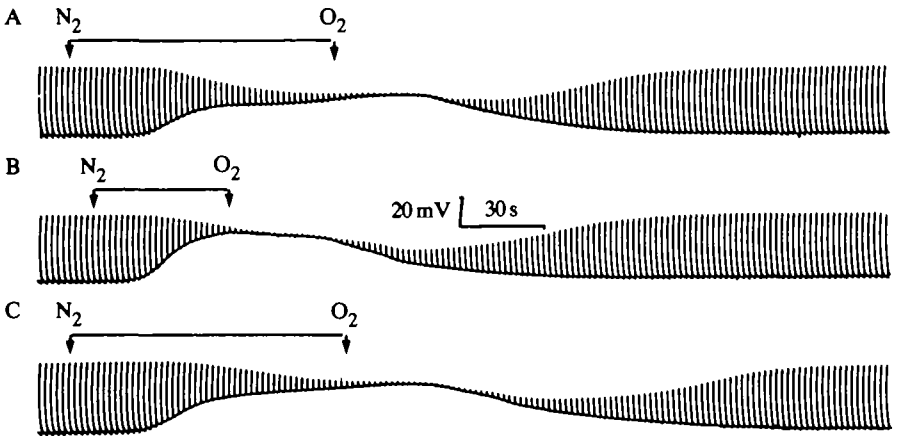


Fig. 4. Repetitive exposure of a photoreceptor to anoxia. Stimulation with flashes presented to the retina every 2 s. The record lasted for about 2 h and the retina was exposed to anoxia several times. Here, three portions of the record are shown. (A) The effect of the first anoxia. After about 5 min of recovery a second anoxia was applied (B) followed by others, alternated with recoveries every time. Before the exposure to anoxia shown in C, the photoreceptor recovered in normoxia for about 16 min.

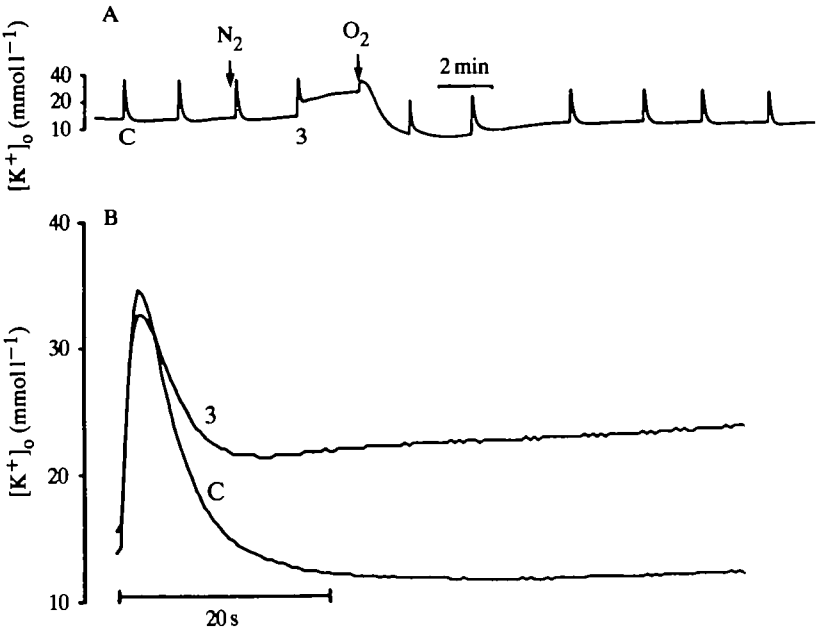


Fig. 5. Extracellular $K^+$ changes during anoxia. (A) A typical record of the extracellular $K^+$ concentration ($[K^+]_o$) obtained with a double-barrelled $K^+$-sensitive microelectrode. The position was identified as extracellular when the apparent resting potential was close to zero and the electrical response to a light flash recorded by the reference barrel was a negative-going potential with an amplitude of about 10 mV. Each flash of light elicited a transient increase of $[K^+]_o$ of characteristic shape and amplitude (see also Tsacopoulos *et al.* 1983). Arrows indicate when the $O_2$ was replaced by $N_2$ and *vice versa*. (B) Two selected traces are superimposed by the computer on the same, expanded, time scale for better examination of the effect of anoxia on the kinetics of $\Delta[K^+]_o$.

every 2 min. Each flash induced a transient increase of $[K^+]_o$ (Fig. 5A) (see Tsacopoulos *et al.* 1983). In physiological conditions $[K^+]_o$ reached a peak of about 40 mmol $l^{-1}$ 1–2 s after the flash (Fig. 5B). Then $[K^+]_o$ rapidly declined, presumably

because $K^+$ enters glial cells (Coles & Tsacopoulos, 1979, 1981). It then undershot the baseline. This undershoot is abolished by strophanthidin (M. Tsacopoulos & J. A. Coles, unpublished) and so is probably due, in part at least, to the pumping of $K^+$ into the photoreceptors (see also Heinemann & Lux, 1975). Anoxia was verified by measurements of the local $P_{O_2}$ (not shown) and occurred just before the response labelled 3. It was manifested by a slow increase of $[K^+]_o$ in the dark, presumably because of inhibition of the $Na^+$ pump. The flash labelled 3 elicited a $\Delta[K^+]_o$ response of almost normal amplitude but with a time course completely distorted when compared to that recorded in normoxia: the recovery to the baseline is incomplete, $[K^+]_o$ remained at a higher concentration in the dark than before the flash and even continued to increase slowly.

The simplest explanation of this phenomenon is that the $Na^+$ pump is inhibited by the anoxia (due to lack of ATP) and thus the $K^+$ released by the photoreceptor after the flash accumulates in the extracellular space. This is despite some influx into the glial cells due to the spatial buffering mechanism, which is not dependent on metabolic energy (Gardner-Medwin, Coles & Tsacopoulos, 1981; Coles & Orkand, 1983). Interestingly, after about 2 min, the $[K^+]_o$ increase reached a plateau, at about 30 mmol $l^{-1}$. At this level, a flash elicited only a small $\Delta[K^+]_o$, presumably because there was only a tiny light-induced $Na^+$ influx into the photoreceptors. Tissue reoxygenation led to a rapid decrease of $[K^+]_o$ in the dark, towards the normal baseline existing before the exposure to anoxia. It then undershot the baseline since, following anoxia, the photoreceptors are loaded with $Na^+$, which in the renewed presence of ATP greatly activates electrogenic sodium pumping (Thomas, 1972). A similar pattern for $[K^+]_o$ has been found to occur after anoxia in the mammalian cortex (Morris, 1974; Ullrich, Steinberg, Baierl & ten Bruggencate, 1982). Apparently, during the recovery from anoxia, the physiological redistribution of ions in the different tissue compartments is completed by the end of the undershoot of $[K^+]_o$. Indeed, at this point, a flash elicited a $\Delta[K^+]_o$ response very similar to that recorded before the anoxia. (Fig. 5A). Inhibition of the sodium pump by anoxia and the related ionic perturbations recovered on average within 5 min (five experiments) after the onset of reoxygenation, i.e. faster than the resistance to anoxia. Similar results were obtained when the oxidative metabolism was inhibited by amytal (see below).

### *Role of calcium during anoxia*

Conceivably anoxia could cause an increase of the intracellular ionized calcium (Lo, Wong & Pak, 1980) which does not necessarily recover with the same time course as the activity of the $Na^+$ pump. This is important because it is known that an increase of $[Ca^{2+}]_i$ causes a decrease in sensitivity of invertebrate photoreceptors to light (Lisman & Brown, 1972; Bader *et al.* 1976). To examine whether there was a rise in $[Ca^{2+}]_i$, the effects of anoxia upon the kinetics of the receptor potentials were examined (Figs 6, 7). Fig. 6A shows that during anoxia, the latency and the time-to-peak of the photoresponse progressively increased as the amplitude decreased. The decrease of amplitude could be due to a rise in $[Ca^{2+}]_i$, but the first two effects are opposite to those expected for a rise in $[Ca^{2+}]_i$, and are similar to those found in another invertebrate photoreceptor (Lantz & Mauro, 1978). Following reoxygenation, recovery of the latency and the time-to-peak occurred rapidly (Fig. 6B). Then,

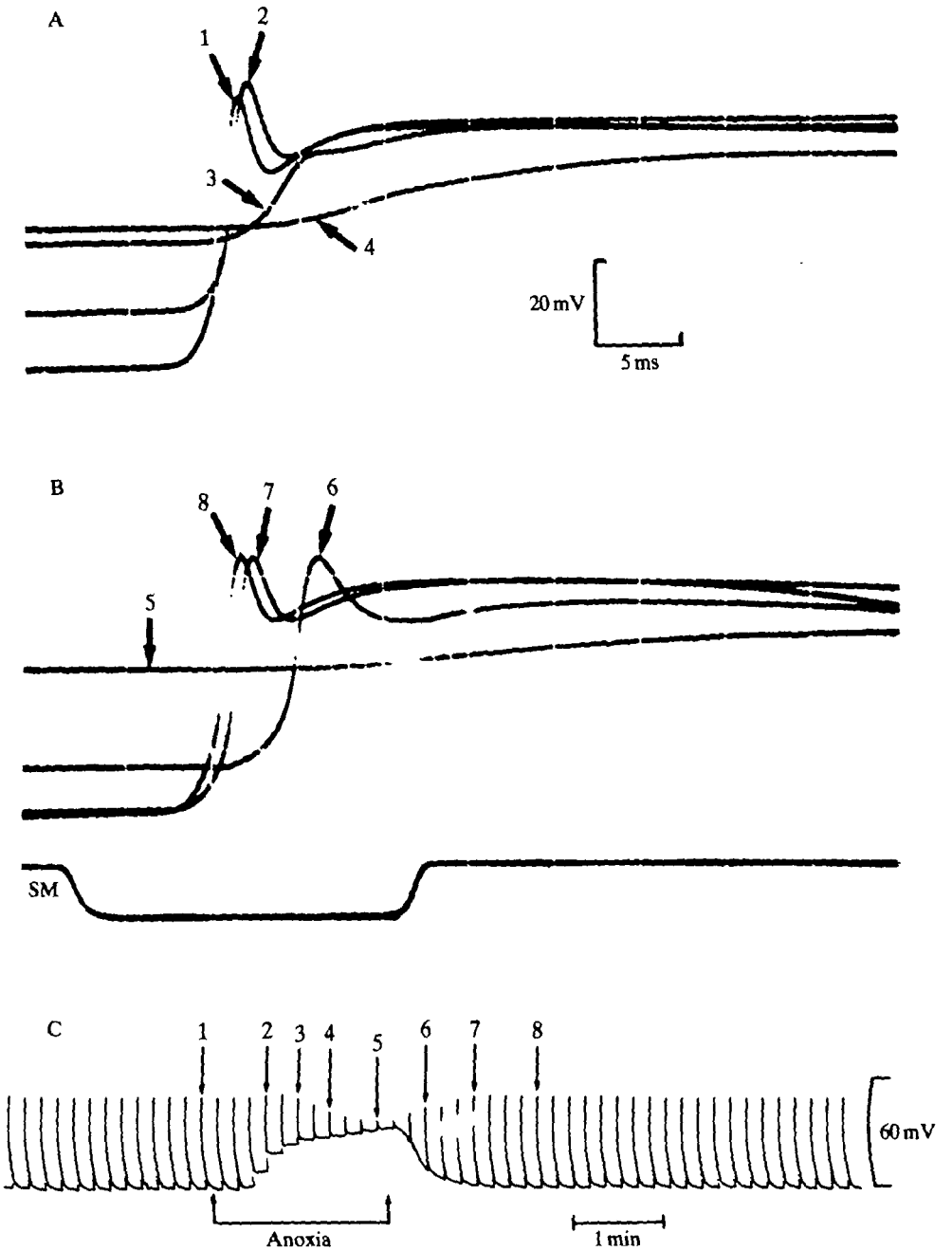


Fig. 6. The effect of anoxia on the latency and the time-to-peak of the receptor potential. Fast oscilloscope recordings of the receptor potential during the effect of anoxia (A) and during recovery from anoxia (B). In (C), the chart record, the individual reaponses are numbered so as to make it easier to identify them. The stimulus monitor (SM) is indicated.

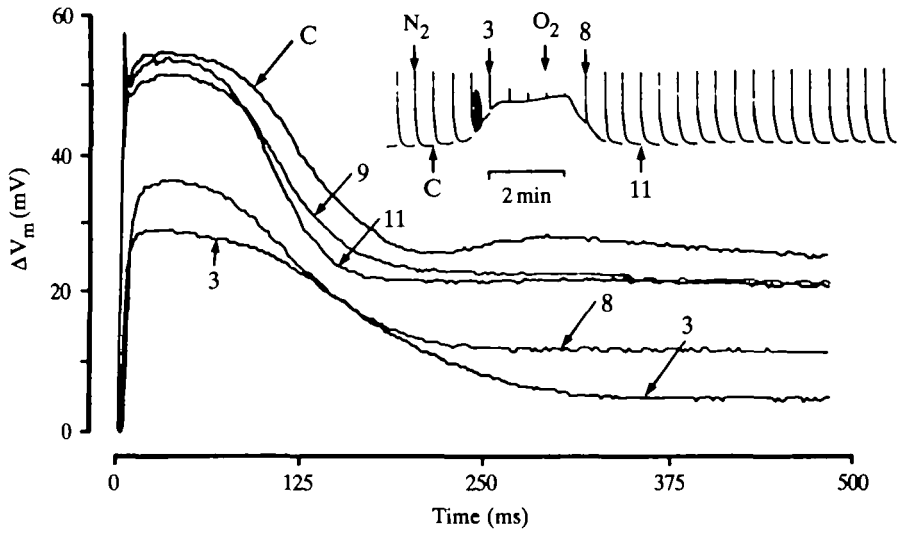


Fig. 7. The effect of anoxia on the time course of the receptor potential. The inset shows the slow chart recording. Individual responses from C (control) to 11 were digitized and stored on floppy disk. The responses are superimposed by the computer and displayed on the same time scale. They are numbered so as to make it easier to identify them on the chart record.

the fast repolarizing phase became progressively shorter by an amount comparable to that recorded by Bader *et al.* (1976) after intracellular Ca-EGTA injection, despite a return of the amplitude to the normal pre-anoxia level (Fig. 7, traces 9 and 11). The effects of Ca injection were fully reversible in 6 min (Bader *et al.* 1976, their Fig. 8); in our experiments the reversibility of the shortening was variable: in some cells there was a partial recovery in about 5 min after the complete recovery of the amplitude from anoxia, but in many other cells the shortening persisted for more than 60 min in normoxia. Careful comparison of the persistence of the shortening of the response with vulnerability to anoxia showed no correlation between the two.

In the following section of this paper, we present evidence suggesting that the resistance of the receptor potential to anoxia is related to the recovery of the oxidative metabolism.

### *The recovery of oxidative metabolism from anoxia or amytal*

As mentioned above, a flash of light induces a rapid increase of the oxygen consumption ($\Delta Q_{O_2}$) which in turn causes a transient drop of the local $P_{O_2}$ ($\Delta P_{O_2}$) (Tsacopoulos & Poitry, 1982).

In the experiment shown in Fig. 8, a photoreceptor located about 150 $\mu$m from the exposed surface of a retinal slice was impaled and the membrane potential was recorded. The $P_{O_2}$ was also recorded at two sites: one at the exposed surface and another located deep in the tissue at about the same depth as the impaled photoreceptor. The replacement of $O_2$ by $N_2$ caused a drop of the local $P_{O_2}$. It reached a value close to zero faster deep in the tissue than at the surface because in addition to the reduction of the $O_2$ flux, the superficial layers of the tissue consumed $O_2$. The last light-induced $\Delta P_{O_2}$ is indicated by an arrow on record D. The tissue anoxia was followed by depolarization of the photoreceptor, and progressive diminution of the amplitude of the

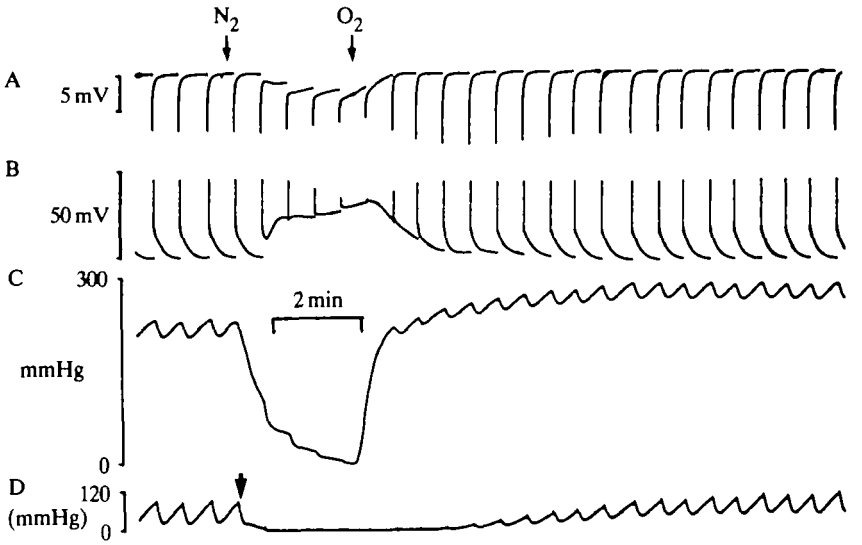


Fig. 8. Simultaneous recording of receptor potentials (A and B) and local $P_{O_2}$ (C and D). Three microelectrodes were used: one KCl-filled micropipette to impale a photoreceptor (trace B) and two $O_2$-sensitive, double-barrelled microelectrodes. One $O_2$ microelectrode was positioned at the surface of the 300 $\mu$m slice of retina (C) and the other was introduced in the retina at 150 $\mu$m from the surface (D). The reference barrel of the second $O_2$ microelectrode recorded the extracellular receptor potential shown in A.

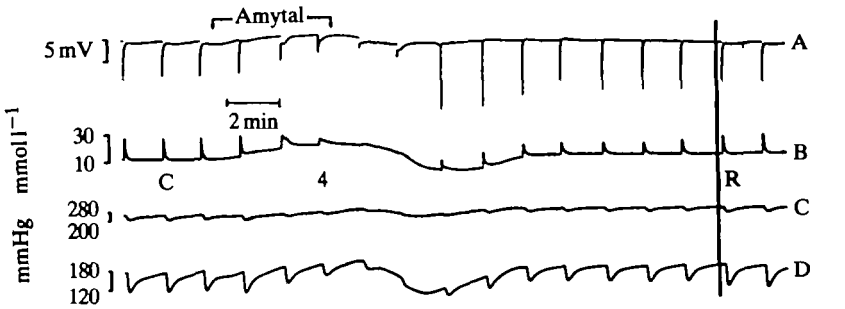


Fig. 9. The effect of amytal (3 mmol $l^{-1}$). Simultaneous recording of extracellular receptor potential (A), extracellular $[K^+]_o$ (B), $P_{O_2}$ at the exposed surface (C) and $P_{O_2}$ at a site located 150 $\mu$m deep in a 300-$\mu$m thick slice of retina (D). The extracellular receptor potential was recorded with the reference barrel of the $K^+$-sensitive microelectrode. The retina was stimulated regularly with intense light flashes presented about every 90 s. The sample records C (control), 4 and R (recovery) were digitized and the $\Delta Q_{O_2}(t)$ was calculated by the computer and presented as Fig. 10B. The vertical black line indicates that between record R and the preceding record there were nine responses that have not been included in order to avoid overloading of the figure. Thus R was recorded 23 min after washout of amytal.

receptor potential until it was completely blocked. This record clearly shows the tight coupling between oxidative metabolism and photoreceptor function. When the $O_2$ in the Ringer was reintroduced, $P_{O_2}$ increased rapidly at the surface and more slowly inside the tissue. This slowness in the recovery of $P_{O_2}$ deep in the tissue was due to a higher $Q_{O_2}$ in the dark following the anoxia. This became clearer when the oxidative metabolism was transitorily inhibited by Na-amobarbital (amytal) because this drug rapidly blocked the light-induced extra $O_2$ uptake, but for short times affected but little the resting $P_{O_2}$ (small steady increase).

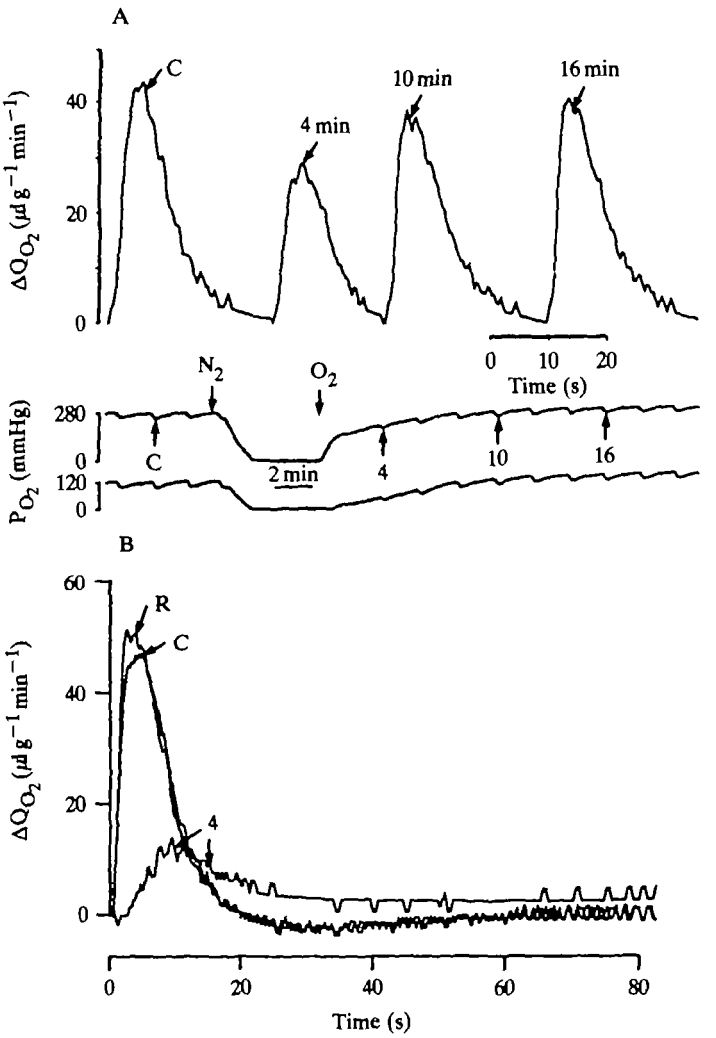


Fig. 10. Recovery of $Q_{O_2}$ from a brief exposure to anoxia (A), or amytal (B). In A, the top trace is the $\Delta Q_{O_2}$ calculated from the $P_{O_2}$ traces presented below: the upper $P_{O_2}$ trace was recorded at the exposed surface of a superfused slice (300 $\mu$m) and the lower at a site located 160 $\mu$m inside the tissue. Selected traces showing the control (C) and the recovery for 4, 10 and 16 min, are plotted by the computer at the same time scale, side by side, for a better appreciation of the progressive recovery of the amplitude. (B) Three selected $\Delta Q_{O_2}(t)$ responses from the experiment illustrated in Fig. 9C, D plotted on the same scale. The labels are explained in the legend of Fig. 9.

Amytal blocks the mitochondrial respiratory chain (see e.g. Nishiki, Erecinska & Wilson, 1979) by inhibiting the transfer of electrons at the level of flavoproteins (Chance & Hollunger, 1960), whereas anoxia blocks the transfer of electrons from cytochrome *a* to $O_2$. Fig. 9 shows that, like anoxia, 3 mmol $l^{-1}$ amytal inhibited the light-induced $\Delta Q_{O_2}$ and $\Delta[K^+]_o$ and suppressed the receptor potentials. As with anoxia, exposure to amytal caused $[K^+]_o$ to increase to a plateau of 30 mmol $l^{-1}$ (Fig. 9B). The small increase of the local $P_{O_2}$ in the dark (Fig. 9C,D) indicates that basal $Q_{O_2}$ was also partly inhibited by amytal (see Tsacopoulos *et al.* 1981). After removal of amytal, $P_{O_2}$ returned to the baseline in the dark, and transitorily undershot it. This

undershoot clearly indicates that $Q_{O_2}$ in the dark was transitorily higher than before inhibition of the oxidative metabolism.

Inspection of Figs 8 and 9 reveals that the recovery of the amplitude of $\Delta P_{O_2}$ is much slower than the recovery of the receptor potential or the activity of the Na-pump as expressed by the time course of the $[K^+]_o$ undershoot. This indicates that the light-induced $\Delta Q_{O_2}$ recovers, after a transient inhibition of the oxidative metabolism, more slowly than most of the electrophysiological parameters, including the return of the post-anoxic transient undershoot of $[K^+]_o$ to the baseline. The return of $\Delta Q_{O_2}$ to normal after a brief inhibition of the oxidative metabolism by anoxia or amytal was studied quantitatively in the experiments illustrated in Fig. 10. In Fig. 10A, $P_{O_2}$ was recorded at a depth of 160 $\mu$m in a 300-$\mu$m slice, and at the exposed surface. The retina was stimulated with strong light flashes, one every 2 min. During oxygenation, an intense flash induced a $\Delta Q_{O_2}$ which reached a peak of 45 $\mu l\, g^{-1} min^{-1}$ above the baseline in the dark (control: trace C). The retina was then exposed to anoxia for a period sufficient to block the receptor potential (not shown). When $O_2$ was reintroduced to the Ringer, $P_{O_2}$ rose but, as in the other experiments, the return towards the baseline was much slower than the drop during the exposure to $N_2$. The $\Delta Q_{O_2}$ response recorded 4 min after the onset of the increase of the local $P_{O_2}$ had an amplitude about 50 % of the control. Note that at this time both the receptor potential and the activity of the pump had recovered almost completely to the pre-anoxia values (Fig. 8A,B). It took 12 min more before the amplitude of $\Delta Q_{O_2}$ had recovered to about 90 % of the amplitude of the control response. Similar results were obtained in six slices and also, as shown in Fig. 10B, when the retina was exposed to 3 mmol $l^{-1}$

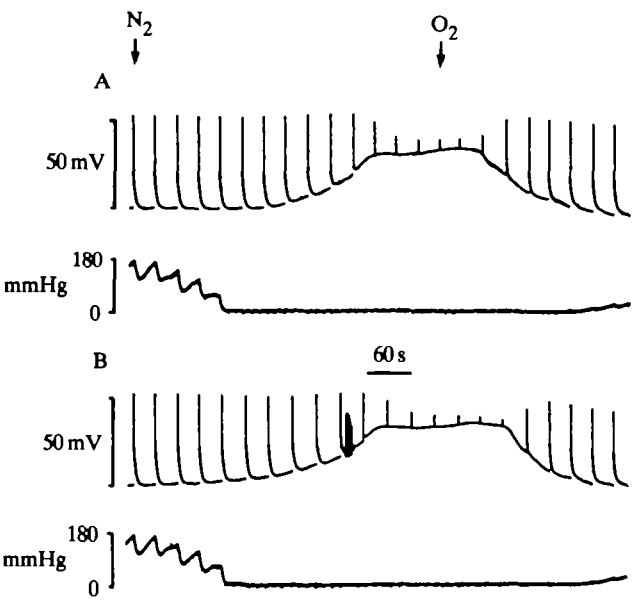


Fig. 11. Simultaneous recording of the intracellular receptor potential and of local $P_{O_2}$ at the same depth in a slice. The recording was continuous but here only two segments are shown. In the first, (A), the effect of the first exposure to anoxia is shown. The recording was continued until $P_{O_2}$ and $\Delta P_{O_2}$ had recovered completely. Then, the retina was exposed a second time to anoxia (second segment, B).

amytal: 6 min after the removal of amytal, the amplitude and the time course (Fig. 9A) of the receptor potential as well as of $[K^+]_o$ had completely recovered, but it took about 23 min for $\Delta Q_{O_2}$ to recover.

It appears, therefore, that the physiological resistance of the photoreceptors to anoxia is related to the recovery of the oxidative metabolism. This is clearly shown in Fig. 11. In this experiment, the receptor potential was recorded simultaneously with the local $P_{O_2}$ at the same depth (150 $\mu$m). The retina was exposed to anoxia until 90 % of the receptor potential was suppressed. Then the retina was allowed to recover for 22 min, until $P_{O_2}$ and the light-induced $\Delta P_{O_2}$ had recovered exactly to the same level as before the first anoxia. Thus, the first and the second anoxias were applied under the same conditions of oxidative metabolism. It then took the same time for the second anoxia to suppress 90 % of the receptor potential.

## DISCUSSION

### *The effect of anoxia*

The results presented in this paper unequivocally confirm earlier findings (Baumann & Mauro, 1973; Wong *et al.* 1976; Lantz & Mauro, 1978; Payne, 1981) showing that anoxia depolarizes the membrane of arthropod photoreceptors and abolishes the receptor potential. The observed accumulation of $K^+$ in the extracellular space during anoxia or exposure to amytal indicates that the depolarization produced by anoxia is probably due to inhibition of the $Na^+$ pump. This accumulation occurred slowly in the dark, but stimulation with light accelerated it (Figs 5,9), presumably because light opens $Na^+$ channels (Fulpius & Baumann, 1969; Coles & Orkand, 1982; Bacigalupo & Lisman, 1983) and much more $Na^+$ enters the photoreceptor (exchanged with $K^+$) than during the 'leakage' current in the dark. However, after about 2 min of anoxia or longer exposure to amytal, both the accumulation of $K^+$ and the depolarization of the membrane greatly slowed down, presumably because less $Na^+$ entered the photoreceptors, since anoxia blocks the opening of the $Na^+$ channels by light (see Baumann & Mauro, 1973). The depolarization and the suppression of the receptor potential, as well as their recovery, did not occur in parallel. This is more evident in *Limulus* or barnacle photoreceptors (Fuortes, 1965; Lantz & Mauro, 1978).

Measurements of local $P_O$, allowed a precise determination of the occurrence of complete anoxia and therefore of the arrest of the oxidative metabolism. Thus it was shown that depolarization starts after a certain delay following the anoxia and the abolition of the receptor potential also occurs progressively. In fresh preparations, it took about 3–4 min for anoxia to suppress completely the receptor potential (Fig. 2). We define this as a resistance of the photoreceptors to anoxia. As shown in a previous article (Coles & Tsacopoulos, 1985), this resistance is unlikely to be related to activation of anaerobic glycolysis leading to the accumulation of lactate in the tissue as is the case in the mammalian brain (McIlwain & Bachelard, 1971) or retina (Tsacopoulos, 1979). We hypothesize that the suppression of the receptor potential rather is related to the progressive consumption of a substance from an intracellular store which is not renewable during anoxia. This substance may be ATP or another high energy phosphate. Consequently, the resistance of the photoreceptor to anoxia would depend on the quantity of this energy substance and the rate of its use during complete

inhibition of oxidative metabolism. This hypothesis is supported by the observations that the suppression of the receptor potential was progressive and that the time required for complete abolition was dependent on the intensity of the light stimulation (Fig. 3) and on the interval between successive exposures to anoxia (Fig. 4) (see below). In addition, recent findings have shown that exposure of retinal slices for 3–4 min to anoxia causes about a 50 % decrease of the total retinal ATP concentration (Tsacopoulos & Coles, 1984). A single flash of light induces a transient increase of $Q_{O_2}$ in the dark ($\Delta Q_{O_2}$) which reaches a peak of about 50 $\mu l\, g^{-1}\, min^{-1}$ (Tsacopoulos & Poitry, 1982). This $\Delta Q_{O_2}$ is accompanied by a transient increase in the ATP concentration (Coles, Tsacopoulos & Dunant, 1984). Also, a single flash induces a transient increase of the intracellular $Na^+$ concentration ($\Delta[Na^+]_i$) of 3–4 mmol $l^{-1}$ (Coles & Orkand, 1982; Tsacopoulos *et al.* 1983). Most of the extra ATP produced after a flash appears to be used by the $Na^+$ pump (Tsacopoulos *et al.* 1983). Consequently, during stimulation of the retina with light flashes there is an increase in both production and consumption of ATP by the photoreceptors. Tsacopoulos & Coles (1984) have found that repetitive stimulation of the retina at 0·5 Hz did not cause any significant change of the [ATP] in the steady state.

It is of interest that hypoxia blocks synaptic transmission in hippocampal slices from guinea pig, largely as a result of a decline in [ATP], and that a resistance to this effect can be induced by raising phosphocreatine levels (Lipton & Whittingham, 1982). Such resistance is unlikely to be caused by creatine phosphate in the drone, since none can be detected in the retina (Tsacopoulos & Poitry, 1982), but might be induced by some other high energy phosphate.

The diminution of ATP during anoxia should inhibit the recently discovered light-induced phosphorylation reactions that probably lead to the opening of $Na^+$ channels on stimulation of invertebrate photoreceptors (Fein *et al.* 1984; Brown *et al.* 1984). Also, an increase in intracellular free $Ca^{2+}$ occurring during metabolic inhibition could reduce the sensitivity of the photoreceptors to light (see Brown & Lisman, 1975). Wong, Lantz & Mauro (1979) suggested such a mechanism since they observed that the intracellular injection of the $Ca^{2+}$ chelator EGTA in the *Limulus* ventral photoreceptor transitorily restored some of the receptor potential which was abolished by the metabolic inhibitor DNP. Pressure injection of calcium into drone photoreceptors reduces the amplitude of the receptor potential and shortens the fast repolarizing phase of the response (Bader *et al.* 1976) (see Baumann, 1974, for terminology); in contrast, injection of EGTA makes the response much larger. Also, Brown & Lisman (1975) have shown that injection of calcium reduces the latency and time-to-peak of the photoresponse. The effects of anoxia on the time course of the receptor potential (Figs 6,7) suggest that if a $Ca^{2+}$ increase in the drone photoreceptor is the cause of changes due to anoxia, this occurs mainly during the recovery phases.

Thus, it appears difficult to suggest a causality between an increase of $[Ca^{2+}]_i$ during anoxia and the progressive inhibition of the receptor potential. More probably, anoxia or amytal blocks one step in phototransduction in which the phosphorylation by a protein kinase is involved (Fein *et al.* 1984; Brown *et al.* 1984).

*Recovery from anoxia or amytal*

After the abolition of the receptor potential, when $O_2$ was reintroduced in the

Ringer's solution or amytal was washed out, oxidative metabolism resumed. It is reasonable to expect that the immediate consequence of this should be the progressive restoration of all ATP-dependent functions of the photoreceptor which were suppressed or diminished during the inhibition of the oxidative metabolism. Apparently, the first function that recovered was $Na^+$ pumping, since the photoreceptors quickly repolarized and even hyperpolarized, presumably because this pump is electrogenic (Thomas, 1972). Consistent with this finding was the parallel decrease of $[K^+]_o$, which, after a large undershoot, returned to the baseline in about 5 min (Fig. 5). The amplitude and the kinetics of the receptor potential and the light-induced membrane conductance (Baumann & Mauro, 1973) recovered after the repolarization. It has been observed that after a brief exposure of the retina to 100 mmol $l^{-1}$ $K^+$ the repolarization of the membrane and the recovery of the amplitude and the kinetics of the receptor potential are simultaneous (Dimitracos, 1983; see also Fulpius & Baumann, 1969). Consequently, the recovery from the effect of anoxia is not that of a simple ionic perturbation, but more complex, determined essentially by the time course of the recovery of metabolic energy-dependent processes that were inhibited during the anoxia. Among these processes the Na pump recovers first, maybe because it can use the first new molecules of ATP phosphorylated by the mitochondria immediately following the onset of oxidative metabolism, and this simply for anatomical reasons. In the drone photoreceptors, the mitochondria are located at the periphery of the cell very close to the cytoplasmic membrane facing the glial cells (Fig. 1), with which the photoreceptors display ionic exchanges (Coles & Tsacopoulos, 1981). Thus, it appears plausible that the pumping sites are located on the cytoplasmic membrane close to the mitochondria. When oxidative metabolism is resumed the new molecules of ATP may well be preferentially used for $Na^+$ pumping. As $Na^+$ is extruded from the cell (as seen in the recovery of the $[K^+]_o$ undershoot, Figs 5,9), more and more molecules of ATP produced by the mitochondria may be able to diffuse towards more distant sites of the cells, namely the subrhabdomeric cisternae (Perrelet, 1970) and the microvilli of the rhabdom, and allow ATP-dependent processes of the phototransduction process to recover from anoxia. Thus, cellular compartmentation and intracellular diffusion may explain the sequence of recovery of the various functions of the photoreceptors.

The effect of the duration of oxygenation upon the resistance of the photoreceptor to subsequent anoxia can be explained in terms of an ATP store. The effect could be produced if the duration of oxygenation was insufficient to allow replenishment of the store. This might be due to an increase in the basal use of ATP, as observed experimentally. The light-induced $\Delta Q_{O_2}$ which normally produces a net increase of ATP (Coles *et al.* 1984) also does not recover completely during this period (Fig. 10).

During recovery from anoxia or exposure to amytal, the resting $Q_{O_2}$ of the drone retina was transitorily higher than before the inhibition of the oxidative metabolism, and $\Delta Q_{O_2}$ of smaller amplitude (Figs 8, 9, 10, 11). Similarly, the oxidative metabolism of mammalian nervous tissue is transitorily higher after brief anoxia, and it has been suggested that this is due to an accumulation of ADP and phosphate during anoxia (Kreisman, Sick, Lamanna & Rosenthal, 1981; Chance & Schoener, 1962). An alternative explanation, at least in the drone retina, could be the following: during anoxia or exposure to amytal, the photoreceptor becomes loaded with both $Na^+$ and

metabolic substrate (a product of glycogen from the glial cells; Evêquoz, Stadelmann & Tsacopoulos, 1983) which is not consumed because of the lack of $O_2$ or the blockage of the respiratory chain by amytal. When $O_2$ is reintroduced, or amytal removed, $Q_{O_2}$ is strongly activated because the photoreceptors are loaded with this substrate and because of the large increase in activity of the sodium pump. However, it is not yet understood why the light-induced $Q_{O_2}$ recovers so slowly after anoxia or amytal, because the mechanism for the regulation of $\Delta Q_{O_2}$ is still not explained (see Tsacopoulos *et al.* 1983).

In conclusion, inhibition of the oxidative metabolism by anoxia or amytal depolarizes the photoreceptor and suppresses the receptor potential. The photoreceptor exhibits a certain resistance to anoxia which is probably due to an ATP store which is progressively consumed during the anoxia. The faster this store (or part of it) is consumed, the faster occurs the suppression of the receptor potential. A second exposure to anoxia, if applied shortly after the first, suppresses the receptor potential faster, even though most of the electrophysiological parameters are restored. A longer time interval between periods of anoxia is necessary for full recovery and this time interval is similar to that necessary for the recovery of light-activated oxidative metabolism. The reason for this link between full recovery from anoxia and recovery of light-induced $\Delta Q_{O_2}$ remains to be elucidated.

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