Light-Evoked Oxygen Responses in the Isolated Toad Retina

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Transient changes in retinal oxygen in response to light stimuli were studied to further understand the light-evoked change in oxygen consumption. Double-barreled microelectrodes, which measured oxygen and local voltage simultaneously, were positioned near the photoreceptor inner segments of the toad neural retina–retinal pigment epithelium–choroid preparation. Light-evoked oxygen responses were measured in a normal $[Na^+]$ solution, and in a test solution with lowered extracellular $[Na^+]$ to inhibit Na^+/K^+ pumping. Under the normal $[Na^+]$ condition, retinal oxygen tension increased in response to light indicating that oxygen utilization had decreased. When the Na^+ concentration was lowered in the retina, the oxygen tension decreased in response to light, indicating an increase in oxygen utilization which was smaller than the Na^+/K^+ pump effect and therefore masked under normal conditions. The increase in oxygen utilization in lowered $[Na^+]$ was suppressed by adding 0·7 mM 3-isobutyl-1-methyl-xanthine, a phosphodiesterase inhibitor, suggesting that the response was largely due to hydrolysis and subsequent resynthesis of cyclic GMP. Results of fitting the light-evoked responses to exponential functions suggested that the decrease in oxygen consumption caused by slowing of the photoreceptor Na^+/K^+ ATPase had a time constant between 130 and 180 sec and that the increase in oxygen utilization from increased cyclic GMP synthesis was faster. © 1995 Academic Press Limited

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1. Introduction

In the retina of both frog and toad, inhibition of the photoreceptor Na^+/K^+ ATPase decreases photoreceptor oxygen consumption (QO_2) by at least a factor of two (Kimble, Svoboda and Ostroy, 1980; Zuckerman and Weiter, 1980; Haugh-Scheidt, Linsenmeier and Griff, 1995), indicating that this pump requires a large part of the energy used by the photoreceptors. Furthermore, the light-evoked decrease in photoreceptor QO_2 is believed to reflect a slowing of the rate of sodium pumping by their Na^+/K^+ ATPase. In light, the Na^+ influx is reduced (e.g. Woodruff, Fain and Bastian, 1982), thereby decreasing the need for Na^+ pumping. Under conditions which inhibited Na^+/K^+ pumping, however, the light-evoked change in photoreceptor QO_2 was not abolished, but reversed (Kimble et al., 1980). This increase in QO_2 probably originated from the photoreceptors, since it persisted during aspartate treatment. A light-induced increase in cGMP synthesis has been found in the toad retina (Dawis et al., 1988), and this could be responsible for a light-evoked increase in QO_2 in the photoreceptors. Some direct evidence for this was reported in rabbit retina by

Ames et al. (1992) and by a preliminary report of the present work (Haugh, Griff and Linsenmeier, 1991).

The present work was undertaken to characterize the light-evoked increase in QO_2 and to determine whether there are two processes (or possibly more) whose metabolic needs change substantially with illumination. The pharmacology of these PO_2 changes was examined to better identify the light-dependent processes which affect photoreceptor QO_2, and to investigate whether the light-evoked increase in QO_2 reflects an increase in cGMP synthesis. In addition, the amplitudes, sensitivities, and time courses of the processes were compared. These questions were addressed by examining transient, light-evoked changes in retinal PO_2 (PO_2 responses) that were measured from the in vitro toad retina during normal function and when Na^+/K^+ pumping was inhibited.

2. Materials and Methods

A description of the toad retina preparation and recording of electrical signals is found in the companion paper (Haugh-Scheidt et al., 1995). Briefly, intraretinal PO_2 and local electroretinogram (ERG) were recorded with double-barreled microelectrodes in normal $[Na^+]$ and in low $[Na^+]$ solutions. In low $[Na^+]$ solutions, $[Ca^{2+}]$ was buffered at a markedly reduced level to prevent a large influx of Ca^{2+} into the photoreceptors (Haugh-Scheidt et al., 1995). Retinas were dark-adapted for at least 2 hr after dissection.

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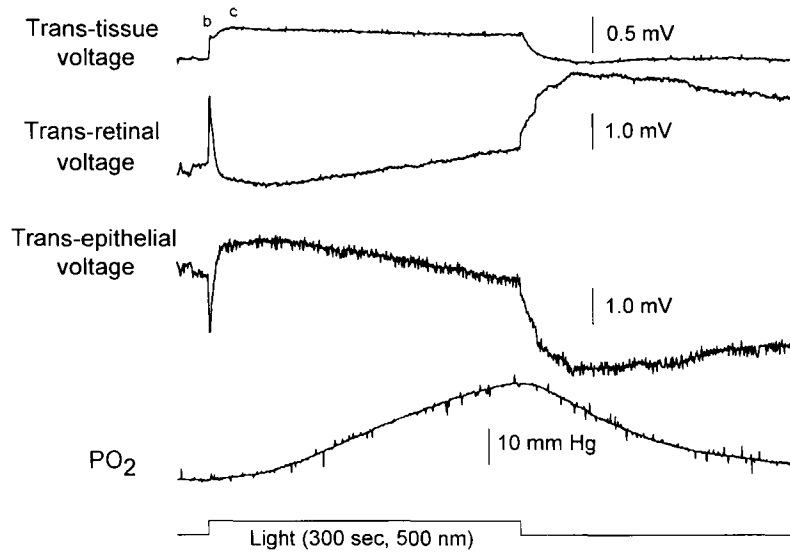
FIG. 1. Measurement of the light-evoked retinal responses. A microelectrode positioned in the outer retina recorded the trans-retinal and trans-epithelial voltages along with retinal PO_2. The trans-tissue voltage was recorded simultaneously. The stimulus level (at 500 nm for 300 sec) was about 2 log units below saturation of the c-wave. The b- and c-waves of the trans-tissue response are indicated on the trace. The trans-tissue voltage is given at twice the gain of the microelectrode voltages. Retinal PO_2 increased during illumination and returned to its baseline while the retina was in darkness (not completely shown). (Data = 44LLR18.)

Measurement of Retinal PO_2 Responses to Light

To measure light-induced PO_2 changes, the O_2 microelectrode was positioned in the outer retina by first advancing through the retinal pigment epithelium (RPE) and then withdrawing about 70 μm into the neural retina, close to the minimum PO_2 in this preparation and at the level of the inner segments. An example of the microelectrode recordings in response to 300 sec of illumination is shown in Fig. 1. The ERG measured across the tissue is the sum of the trans-retinal and trans-epithelial voltages recorded by the microelectrode. Retinal PO_2 increased in response to light when the retina was superfused with a normal [Na^+] solution. Light-evoked PO_2 responses were recorded for 300 sec of illumination at different stimulus levels. For longer responses that were recorded, the PO_2 reached a steady level by 600 sec. The unattenuated illumination (500 nm) was about 8×10^6 quanta (μm^2 sec)$^{-1}$, and neutral density filters were added for dimmer illumination. The retina was allowed to dark adapt for at least 15 min between responses, or until the b-wave returned to its dark-adapted amplitude.

Modelling Light-evoked PO_2 Responses

The light-evoked PO_2 response was modelled as an exponential function, or as the sum of two exponential functions, as a means of describing the time course of the processes that affect retinal QO_2. The purpose of the modelling was to describe mathematically the PO_2 changes and then, hopefully, relate the quantitative results to cellular processes. For the single exponential

model, the time dependent PO_2 change was described by:

$$P = P_0 \qquad\qquad 0 \leqslant t \leqslant td \qquad (1)$$

$$P = P_0 + (P_\infty - P_0)\left\{1 - \exp\left(\frac{-(t-td)}{\tau}\right)\right\}$$
$$t \geqslant td \qquad (2)$$

In this model, P_0 is the initial PO_2 and P_∞ is the steady state PO_2 during illumination. The time delay, td, is the time between light onset and the initial change in PO_2. The response has an amplitude of $P_\infty - P_0$ and a time constant of τ.

The second model that was used to describe the light-evoked PO_2 change was the sum of two exponential functions with delay times, td_1 and td_2, and time constants, τ_1 and τ_2. For $td_1 < td_2$, the time dependent PO_2 change was described by:

$$P = P_0 \qquad\qquad 0 \leqslant t \leqslant td_1 \qquad (3)$$

$$P = P_0 + P_1\left\{1 - \exp\left(\frac{-(t-td_1)}{\tau_1}\right)\right\}$$
$$td_1 \leqslant t \leqslant td_2 \qquad (4)$$

$$P = P_0 + P_1\left\{1 - \exp\left(\frac{-(t-td_1)}{\tau_1}\right)\right\}$$
$$+ P_2\left\{1 - \exp\left(\frac{-(t-td_2)}{\tau_2}\right)\right\} \quad t \geqslant td_2 \qquad (5)$$

The parameters P_1 and P_2 are the magnitudes of the two exponential functions. The steady state PO_2 during illumination, P_∞, is equal to $P_0 + P_1 + P_2$. The amplitude of the response is $P_1 + P_2$. The optimal parameter values were found by fitting the models to responses using a nonlinear least-squares routine that employed Marquardt's method (Press et al., 1989) to minimize

the sum of the squared error between the model and the data. Other similar models were tested, for example using only one delay time or using the same delay time for both exponential functions. These were found to give larger confidence intervals for the parameters and in some cases the fits did not even converge on a set of parameters.

The 95% confidence interval for P_0 was about ± 0.5 mmHg, and for td, td_1 and td_2 was within ± 4 sec. For the single exponential model, the parameters P_∞ and τ were closely correlated within a fit (coefficient of correlation > 0.9). As τ increased P_∞ became smaller ($P_0 - P_\infty$ grew larger), with little reduction in error. This probably occurred because not all responses reached a clear plateau during 300 sec of illumination. The values of τ reported are the smallest ones yielding good fits. For the two exponential model, the parameters P_1, P_2, τ_1 and τ_2, were highly correlated (coefficients of correlation > 0.9). For τ_1 and τ_2, there was a negative correlation, i.e. as τ_1 grew τ_2 diminished. For fits in which the confidence intervals were large, the reported parameter values were found by fixing τ_1 about 10 sec above its lower limit and allowing the other parameters to adjust. This adjustment did not change the error of the fit. Thus, the reported τ_1 may be an underestimate, but it is not likely to be an overestimate of the actual value. Likewise, the reported τ_2 is probably an upper limit.

3. Results

PO_2 Responses to Light in Normal [Na^+]

Transient retinal PO_2 responses to illumination ($n = 144$; 30 toads) were measured in the outer retina during 300-sec periods of illumination at different light levels. The threshold for detecting the PO_2 response was about a log unit above the threshold for detecting a voltage response. Light-evoked PO_2 responses and the corresponding trans-tissue voltage responses are shown in Fig. 2 for stimuli of increasing illumination. The time course of the PO_2 change was much slower than any component of the ERG. In Fig. 2(A), the PO_2 did not change much during the first 30–40 sec of illumination, and then the PO_2 increased for the duration of the light stimulus. For an illumination that saturated the oxygen response, the PO_2 usually returned to baseline within 600 sec. Sometimes the light-evoked PO_2 response had a slightly different shape, decreasing initially and then increasing as shown in Fig. 2(B). The dip in the beginning of the response was smallest for the lowest light level and, with increasing illumination, its amplitude did not change as much as the subsequent PO_2 increase. This biphasic type of response was seen regularly, but less often than the monotonic type of response shown in Fig. 2(A). In any one tissue the response was always one type, either an initial increase or an initial decrease in PO_2. The shape of the response was not correlated with the sensitivity of the retina. The initial PO_2 decrease that was observed in some responses was not expected, but has also been observed from single rod photoreceptors of the salamander retina (Poitry et al., 1993).

The amplitudes of both the c-wave of the ERG and the PO_2 response increased as stimulus magnitude increased. The c-wave, rather than the b-wave, was used as a measure of the retinal electrical activity because it was a prominent component of these responses and because its integration time was more similar to the PO_2 change. Plots of the peak response to different illuminations in a single retina (intensity series) were examined in ten experiments. The responses were normalized to the maximal response within each retina, and are shown in Fig. 3(A) with points from the same retina connected. The PO_2 response increased in amplitude through 3 to 4 log units of illumination and then saturated. The shape of the intensity–response plots were quite similar across retinas, but the threshold for detecting a PO_2 response varied over more than 2 log units of illumination. The sensitivity of the retina was related to the length of time it had been in the dark after dissection, which varied from 2 to 10 hr. The illumination required to produce a half-maximal PO_2 response was highly correlated ($r = 0.96$) to the stimulus required for a half-maximal c-wave response (not shown).

PO_2 Responses to Light in Lowered [Na^+]

A substantial portion of the normal light-evoked PO_2 change in the retina is thought to be due to a decrease in the demand for Na^+ pumping in the photoreceptor inner segments. When a low [Na^+]–low [Ca^{2+}] solution was used in this preparation, a substantial amount of extracellular Na^+ was removed from the retina, as determined by the disappearance of the b-wave and c-wave (not shown). Although there was no Na^+ in the superfusate, it was unlikely that the [Na^+] was zero in the subretinal space because there were multiple layers of the retina to diffuse through. A combination of low [Na^+] and ouabain, however, did not produce a larger effect than low [Na^+] alone, suggesting that low [Na^+] completely inhibited the pump. After the flow of solution into the retinal bath was switched to the low [Na^+] solution, the PO_2 increased slowly and took about 30 min to reach a steady level. Sometimes PO_2 did not remain constant but continued to increase at a slow rate, about 0.8 mmHg min^{-1}. In these cases the steady drift was subtracted from the PO_2 response.

With the microelectrode positioned in the outer retina about 70 μm from the RPE, light-evoked PO_2 responses (93 responses from 16 toads) were measured during 300 sec of illumination while the retina was in low [Na^+]–low [Ca^{2+}]. The light-evoked PO_2 response in low [Na^+] was very different from the PO_2 response while the retina was in normal [Na^+] as shown in Fig.
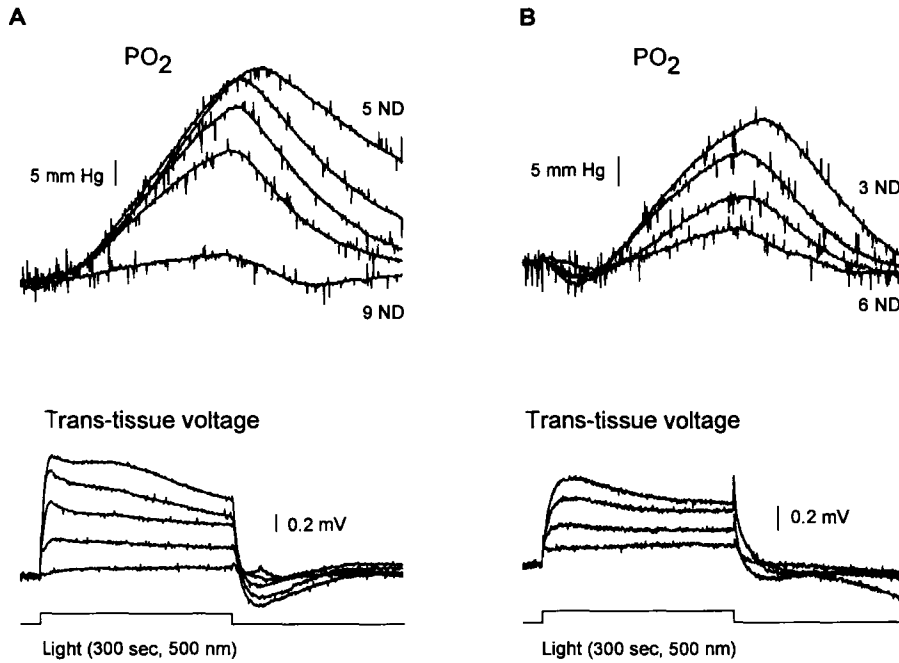
FIG. 2. Light-evoked PO_2 responses measured in the outer retina. Retinal PO_2 (top) and the corresponding trans-tissue voltage (bottom) were measured in response to illumination that was increased by a factor of 10 between responses as indicated by log attenuation provided by neutral density (ND) filters. The b-wave is obscured by overlying responses. Light evoked an increase in intraretinal PO_2 (A). In some retinas (B), light caused an initial decrease, followed by an increase in PO_2. (Data = 44LLR16-20 and 51LLR03-06.)
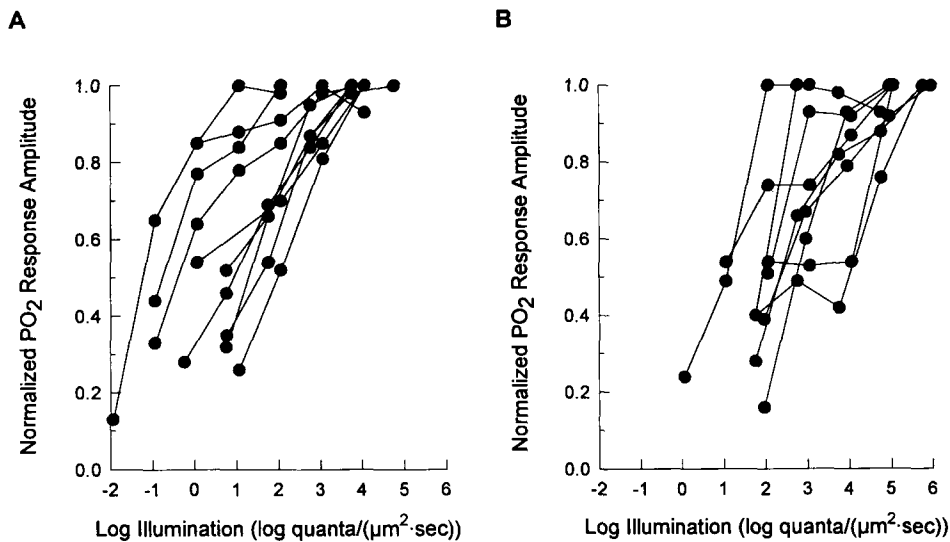

FIG. 3. Intensity series of PO_2 responses. The normalized PO_2 response amplitudes, measured in normal [Na$^+$] (A, positive amplitude) and in low [Na$^+$] (B, negative amplitude), are plotted as a function of illumination. Responses from the same retina are connected by a line. The response amplitude increased through 3–4 log units, and in general, PO_2 responses in low [Na$^+$] were less sensitive than responses in normal [Na$^+$].

4. In low [Na$^+$], the PO_2 increase was replaced with a retinal PO_2 decrease during illumination. The amplitude of the response increased as light intensity increased, but for the same light level the magnitude of the response in normal [Na$^+$] was larger than the corresponding PO_2 response in low [Na$^+$]. The PO_2 responses in low [Na$^+$] did not usually saturate at the same illumination as PO_2 responses in normal [Na$^+$]. The PO_2 decrease in low [Na$^+$] appeared to have a shorter latency following the onset of light than the

latency of the PO_2 increase in normal [Na$^+$]. This timing was consistent with the biphasic responses sometimes observed in normal [Na$^+$] (Fig. 3). In two experiments, [Ca^{2+}] was buffered at a low level by adding 2·0 mM EGTA to the normal [Na$^+$] solution to determine whether low [Ca^{2+}] alone caused the PO_2 to decrease in response to light. In fact, in normal [Na$^+$]–low [Ca^{2+}], there was a larger light-evoked PO_2 increase (not shown).
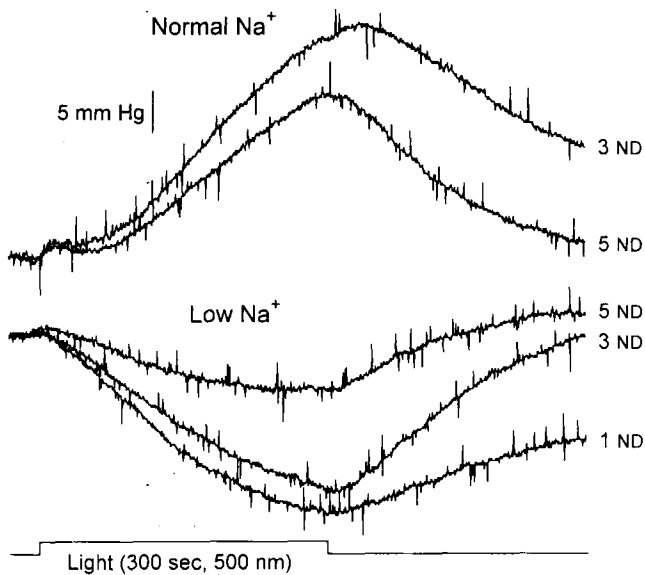
The intensity series from nine experiments were

FIG. 4. Effect of lowered [Na$^+$] on the light-evoked PO$_2$ response. The response of retinal PO$_2$ measured in normal [Na$^+$] at two light levels was a large increase in PO$_2$. When the superfusate was changed to a 0 Na$^+$ solution, the light-evoked PO$_2$ response changed to a decrease in PO$_2$. The un-attenuated illumination was 9·2 × 10^6 quanta (μm^2 sec)$^{-1}$. (Data = 41LLR09-11, 13, 15.)

FIG. 5. Effect of aspartate. In a normal [Na$^+$] solution, retinal PO$_2$ increased upon illumination and that response persisted when aspartate was added to the superfusate and the stimulus repeated. The PO$_2$ response in lowered [Na$^+$] and aspartate was a PO$_2$ decrease just as it had been in the absence of aspartate (Fig. 4). The bottom trace shows recovery of the light-evoked PO$_2$ response when [Na$^+$] was reintroduced to the retina. All responses were recorded from the same retina in the order presented. (Data = 42LLR02, 4, 5, 9.)

examined by normalizing to the maximum response amplitude as shown in Fig. 3(B). The points from a single retina are connected by a line. The threshold for detecting a response varied over about 2 log units of illumination, and the response amplitudes increased through 3 to 4 log units of illumination. It required about 2 log units less light to detect a light-evoked PO$_2$ response in normal [Na$^+$] than in low [Na$^+$]-low [Ca^{2+}]. Thus, the average half-maximal response was at 2·3 log quanta (μm^2 sec)$^{-1}$ in low [Na$^+$] [Fig. 3(B)] as compared to 0·4 log quanta (μm^2 sec)$^{-1}$ in normal [Na$^+$] [Fig. 3(A)]. Retinas that had a high sensitivity in normal [Na$^+$] tended to also have higher sensitivity in low [Na$^+$].

Origin of the Light-evoked PO$_2$ Response in Low [Na$^+$]

The light-evoked O$_2$ utilization change could have originated in cells that were affected by photoreceptors or in the photoreceptors themselves, and to investigate this, 40 mM aspartate was added to the superfusate. Aspartate mimics the endogenous photoreceptor transmitter and saturates postsynaptic receptors so that second order retinal cells cannot detect a change of transmitter release during illumination. The effect of aspartate on the ERG was to abolish the b-wave, but the c-wave was still observed. As shown in Fig. 5, the normal [Na$^+$] PO$_2$ response and the low [Na$^+$] PO$_2$ response both persisted, indicating that the QO$_2$ change occurred in the photoreceptors ($n = 3$).

The light-evoked increase in O$_2$ utilization observed when Na$^+$ pumping was inhibited could be due to some component of phototransduction. For example, many molecules of cGMP are hydrolysed as a result of
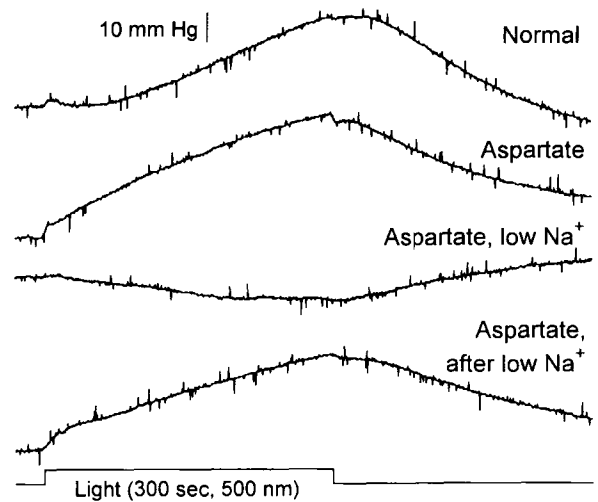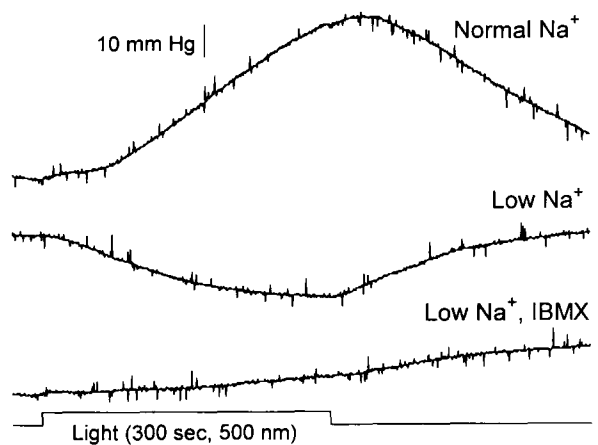

FIG. 6. Effect of IBMX. A light-evoked PO$_2$ response measured in normal [Na$^+$] exhibits a PO$_2$ increase. A PO$_2$ response measured in the same retina after lowering [Na$^+$] (and low [Ca^{2+}]), results in a PO$_2$ decrease (same stimulus). When IBMX (0·7 mM) was added to the superfusate, still in lowered [Na$^+$], the PO$_2$ decrease was inhibited. Similar results were obtained in eight of nine retinas examined. (Data = 45LLR21, 24, 27).

rhodopsin absorbing photons, and the resynthesis of cGMP requires a supply of ATP. To test this hypothesis, the rate of cGMP turnover was manipulated by adding IBMX (3-isobutyl-1-methyl-xanthine), which is known to block the action of phosphodiesterase (PDE), to the low [Na$^+$]-low [Ca^{2+}] superfusate. By inhibiting the hydrolysis of cGMP to GMP, it was expected that any light-dependent increase in cGMP turnover would be inhibited. Adding IBMX to the low [Na$^+$]-low [Ca^{2+}] superfusate produced little change in baseline PO$_2$ measured in the outer retina. The major effect of 0·7 mM IBMX, shown in Fig. 6, was to inhibit the

A   B   C

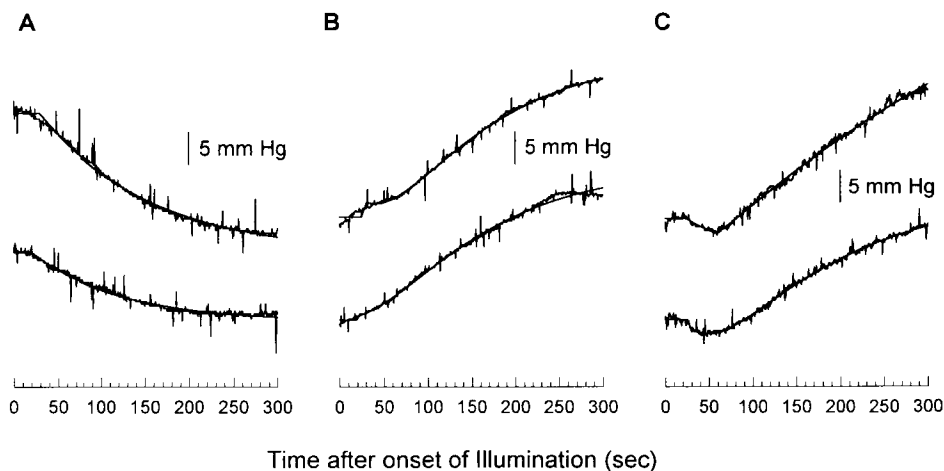Time after onset of Illumination (sec)

Fig. 7. Single exponential model (A) and two exponential model [(B) and (C)] fitted to light-evoked PO_2 responses. (A) Two PO_2 responses that were measured in lowered [Na^+] were fitted with the single exponential model. The parameter values were: $\tau = 127$, $td = 29$ (top trace), and $\tau = 102$, $td = 20$ (bottom trace). (B) Two PO_2 responses that were measured in normal [Na^+] were fitted with the two exponential model. The parameter values were: $\tau_1 = 60$, $td_1 = 40$, $\tau_2 = 158$, $td_2 = 38$ (top trace), and $\tau_1 = 60$, $td_1 = 21$, $\tau_2 = 104$, $td_2 = 20$ (bottom trace). (C) Another two PO_2 responses fitted with the two exponential model. The parameter values were: (A) $\tau_1 = 68$, $td_1 = 33$, $\tau_2 = 250$, $td_2 = 55$, and $\tau_1 = 40$, $td_1 = 25$, $\tau_2 = 142$, and $td_2 = 28$ (bottom trace). In each panel, the responses were from the same retina and the top trace is in response to a stimulus of 100-fold greater illumination. Values are given in seconds. The mean values from all retinas examined are given in Table I. (Data = 43LLR03, 4, 38LLR01, 3, 47LLR07, 8.)

TABLE I

Parameter values (mean ± s.D.) from the exponential models fitted to light-evoked PO_2 responses. For the two exponential model, the subscript 1 and 2 refer to the decreasing and the increasing exponentials, respectively. The units of τ and td are seconds. (n = number of responses)

	n	τ	td	τ_1	td_1	τ_2	td_2
Single exponential model							
Low [Na^+] (decreasing exponential)	93	156±66	38±22				
Normal [Na^+] (increasing exponential)	13	131±46	57±26				
Two exponential model							
Normal [Na^+]	131			68±21	42±18	180±64	41±20

light-evoked PO_2 decrease in low [Na^+]–low [Ca^{2+}]. The light-evoked PO_2 response recovered after the IBMX solution was replaced with control Ringer's solution. In fact, the effect of IBMX did not persist through a long exposure (about 60 min); however, a better inhibitor of PDE or guanylate cyclase was not available.

Modelling the Light-evoked PO_2 Response

Exponential functions were used to describe the time course of the light-evoked PO_2 change in the retina. In lowered [Na^+], the light-evoked PO_2 response was less complicated since a monotonic decrease in retinal PO_2 was always observed, and the response could always be fitted to the single exponential model. Figure 7(A) shows two responses fitted to the model. During the time delay the model did not fit the data precisely in the top trace, but the overall model fitted with 30% less sum-of-squares error when the delay

was included as compared to when the delay was omitted. The mean parameter values, given in Table I, were not highly correlated with the stimulus strength.

For light-evoked retinal PO_2 responses measured in normal [Na^+], a few responses were fitted well by the single exponential model (Table I). These responses were from stimuli at lower illumination, which did not usually generate a detectable PO_2 response in lowered [Na^+]. The remaining 131 responses, which could not be fitted to a single exponential function, were fitted reasonably well by the two exponential model, and required one decreasing exponential function (P_1, τ_1 and td_1) and one increasing exponential function (P_2, τ_2 and td_2), although this was not fixed in the model. Responses fitted to the two exponential model are shown in Fig. 7(B) and (C). When td_1 was less than td_2, the fitted curve decreased, and then increased, as was seen in some of the data. For PO_2 responses fitted by the two exponential model, the time constant of the decreasing exponential term, τ_1, was always smaller

than τ_2 (Table I). In addition, the absolute value of P_1 was always smaller than P_2, and the ratio of the amplitudes (absolute values), P_1/P_2, was 0.43 ± 0.15 (n = 131). If these functions approximated the PO_2 response of two photoreceptor processes, then the mechanism which lowered PO_2 in light had about 43% of the effect of the mechanism which raised PO_2.

4. Discussion

Light-evoked PO_2 Response

In the toad retina, light evoked a robust increase in PO_2, indicating a decrease in oxygen consumption as has been observed in other vertebrate retinas (e.g. Sickel, 1972; Linsenmeier, 1986). Light-induced PO_2 changes in the retina must have ultimately originated in the photoreceptors. Pharmacological isolation of the photoreceptors with aspartate did not affect the light-evoked PO_2 changes observed in normal or low $[Na^+]$, suggesting that these PO_2 changes must occur in the photoreceptors, Müller cells or RPE cells. A change in metabolism of the thin layer of RPE cells would not likely have much of an effect on retinal PO_2. A contribution from Müller cells can not be completely ruled out, but frog Müller cells have few mitochondria in their cytoplasm in the outer retina (Uga and Smelser, 1973), suggesting that they would contribute little to the changes in metabolism during illumination. Inner retinal oxygen consumption also changes with light (Haugh-Scheidt et al., 1995), but this change is smaller than the one in the outer retina. It can be shown, by using the steady-state diffusion model in that paper and average values of the parameters, that the change in inner retinal consumption accounts for less than 10% of the light-evoked PO_2 change recorded by an electrode located in the outer retina.

The light-evoked PO_2 changes, therefore, are believed to reflect primarily changes in photoreceptor metabolism, and are expected to be rod dominated since the sensitivity was similar to that measured from toad rods (Fain, 1976). The illumination required for a half-maximal response from single toad rods, measured by Fain (1976), was in the upper end of the range of illuminations which produced half-maximal PO_2 (and c-wave) responses in this study. Amphibian retinas also contain cones (Liebman and Entine, 1968) which absorb maximally at 575 nm, and the cone population in the toad retina is substantial (Zhang and Straznicky, 1991), but their size and their percentage of total photopigment is small compared to the rods. Furthermore, a cone contribution to the b-wave could not be detected when a comparison was made between responses to 500 and 600 nm illumination at light intensities used in this study.

Inhibition of the light-evoked PO_2 increase in low $[Na^+]$–low $[Ca^{2+}]$ solution was not caused by the lowered $[Ca^{2+}]$ since lowering $[Ca^{2+}]$ alone produced a

larger light-evoked PO_2 increase. A possible explanation for that PO_2 response is that lowering $[Ca^{2+}]$ resulted in increased cGMP synthesis, more light-dependent channels open in the dark (e.g. Pepe et al., 1986; Koch and Stryer, 1988), and larger Na^+ conductance. Thus, the light-induced reduction in QO_2 when all the channels were closed would be larger because there had been more Na^+ pumping in the dark.

Model of the Light-evoked PO_2 Response

Modelling the light-evoked PO_2 responses as an exponential change in PO_2 was a means of describing and quantifying the time course of the two processes that effect QO_2. Diffusion of oxygen is rapid relative to the changes in PO_2 observed, so the time course of PO_2 changes is a direct measurement of the time course of consumption changes. Light-evoked PO_2 responses in low $[Na^+]$–low $[Ca^{2+}]$ were closely fitted by a decreasing exponential function. Light-evoked PO_2 responses in normal $[Na^+]$ could be detected at dimmer illumination than the responses in low $[Na^+]$–low $[Ca^{2+}]$. When the retina was in normal $[Na^+]$, responses at weaker light levels could be fitted by a single increasing exponential function, whereas responses at stronger light levels required a model that was the sum of increasing and decreasing exponentials. The amplitude and the time constant of the decreasing exponential were always smaller.

The time constant of the increasing exponential, attributed to the slowing of the Na^+ pump, and subsequent slowing of oxidative phosphorylation was about 180 ± 64 sec. These events are probably the same ones that are described by the responses at dim illumination which were fit by a single exponential. For those the time constant was 131 ± 46 sec. These values are close to estimates of the time constant of the light-induced decrease in intracellular $[Na^+]$, which ranged from 110 to 170 sec (Shimazaki and Oakley, 1984). The similarity suggests that the rate dependent process for the metabolic change, as well as for the slowing of the rate of pumping, is the change in intracellular Na^+ concentration.

If the PO_2 decrease is indeed a reflection of a change in cGMP turnover, then the time constant of the change in PO_2 is likely to reflect the synthesis of cGMP. From the two-exponential model, the time constant of the decreasing exponential, attributed to cGMP resynthesis, was 68 ± 24 sec, but this is a lower limit (see Results). From the responses in low $[Na^+]$–low $[Ca^{2+}]$, the time constant of this process was 156 ± 66 sec. A possible explanation for this difference is that lowering $[Na^+]$ or simultaneously lowering $[Ca^{2+}]$ had other effects besides inhibiting the Na^+/K^+ ATPase and that these affected the time course of cGMP resynthesis. In the physiological condition, light leads to a lowering of intracellular $[Ca^{2+}]$ and activation of guanylate cyclase, but when the Ca^{2+}-

activation of guanylate cyclase was manipulated, the time course of the photoresponse was changed (Gorczyca et al., 1994). Similarly, if the low [Na⁺]–low [Ca²⁺] solution blocked the light-evoked lowering of intracellular [Ca²⁺], then the time constant of cGMP synthesis in low [Na⁺]–low [Ca²⁺] would be different than under physiological conditions.

Two Competing Processes

The light-induced decrease in retinal QO_2 has been attributed to a slowing of the photoreceptor Na⁺/K⁺ ATPase. Lowering extracellular [Na⁺] inhibited the light-evoked PO_2 increase and revealed a PO_2 decrease, indicating that light also initiated events that increased oxygen consumption. The light-induced increase in oxygen consumption presumably occurs under normal [Na⁺] conditions, but is masked by the decrease in consumption due to the reduction of Na⁺/K⁺ ATPase activity. The fact that both an increasing exponential and a decreasing exponential were usually needed to model the normal [Na⁺] response supports this conclusion. Under conditions which inhibit Na⁺/K⁺ ATPase, the light-evoked PO_2 decrease has also been observed in frog (Kimble et al., 1980) and in rabbit (Ames et al., 1992).

The light-induced increase in QO_2 was probably the result of some process of phototransduction; moreover, the synthesis of cGMP that has been hydrolyzed during phototransduction is likely to be the largest consumer of ATP (Dawis et al., 1989). When the hydrolysis of cGMP was inhibited indirectly by adding IBMX (in low [Na⁺]–low [Ca²⁺]), the light-evoked PO_2 decrease was blocked. In a normal extracellular environment, the effect of IBMX on cGMP turnover could occur via two mechanisms. An IBMX-evoked increase in [cGMP] could: (1) open light-dependent channnels, increase Ca²⁺ influx and inhibit activation of guanylate cyclase mediated by low [Ca²⁺]ᵢ, or (2) slow guanylate cyclase activity by cGMP-mediated product inhibition. In these low [Na⁺]–low [Ca²⁺] experiments, the normal light-induced decrease in [Ca²⁺]ᵢ is presumably inhibited by blocking Na⁺/Ca²⁺ exchange, and therefore the effect of IBMX on cGMP turnover is probably by product inhibition. Regardless of its mechanism of action, the inhibition by IBMX provides the first direct evidence that there is a light-evoked QO_2 increase due to cGMP turnover. Furthermore, since the IBMX/low [Na⁺] combination eliminated all light-evoked PO_2 changes, there are unlikely to be any further light-dependent processes that are energetically important.

A calculation of the absolute values of the energy utilization of each process would depend on knowing the contributions of glycolysis and of light-independent processes to the total metabolism. However, under the assumptions that cGMP synthesis is not seriously altered in low [Na⁺]–low [Ca²⁺], that glycolysis is a minor component, and that the processes add linearly,
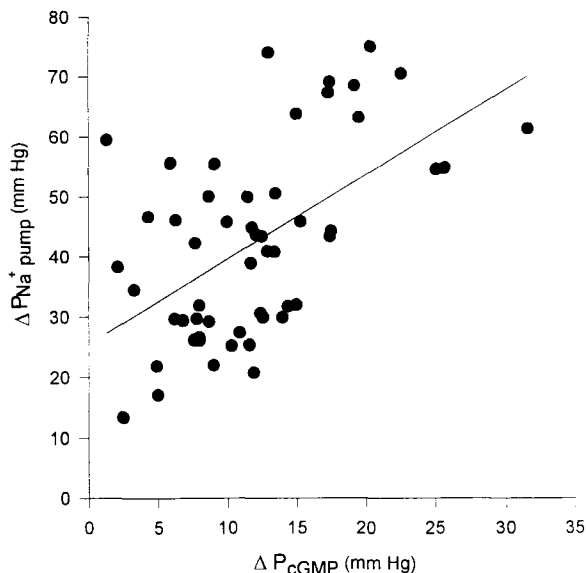

Fig. 8. Na⁺ pump dependent and cGMP dependent PO_2 changes. The light-evoked PO_2 changes are plotted for Na⁺-pump-dependent activity (calculated value) versus cGMP-dependent activity (measured value) in the same retina. The PO_2 change attributed to cGMP dependent activity was always smaller than the PO_2 change attributed to Na⁺ pump activity. The slope of the linear regression is 1·4 ($r = 0·55$).

then an estimate can be made of the relative changes in the metabolic demand of the two processes that occur during illumination. If one considers the light-evoked change in consumption under normal conditions to be ΔQ_{net}, and the Na⁺ pump dependent and cGMP dependent changes to be ΔQ_{Napump} and ΔQ_{cGMP}, then, at a particular illumination,

$$\Delta Q_{net} = \Delta Q_{Napump} + \Delta Q_{cGMP} \qquad (6)$$

Values have been obtained for ΔQ_{net}, but in low [Na⁺] it was not possible to estimate the light-evoked difference in QO_2 of the photoreceptors (Haugh-Scheidt et al., 1995). Therefore, a reliable estimate of ΔQ_{cGMP} could not be obtained from that data. Another approach is to use the values of ΔPO_2 measured near the photoreceptors as a reflection of ΔQ. From the steady-state diffusion model presented in the accompanying paper, it can be shown that observed changes in ΔP at the inner segment are linear with the underlying change in QO_2 of the photoreceptors, i.e. $\Delta P = k\Delta Q$, so that eqn (6) becomes

$$\frac{\Delta P_{net}}{k} = \frac{\Delta P_{Napump}}{k} + \frac{\Delta P_{cGMP}}{k} \qquad (7)$$

or $\qquad \Delta P_{Napump} = \Delta P_{net} - \Delta P_{cGMP} \qquad (8)$

To examine the relative magnitudes of ΔP_{Napump} and ΔP_{cGMP}, the derived values of ΔP_{Napump}, obtained from the measured values of ΔP_{net} and ΔP_{cGMP} were plotted as a function of ΔP_{cGMP} as shown in Fig. 8. The ratio of ΔP_{Napump} to ΔP_{cGMP} should give an indication of the relative sizes of the pump dependent and cGMP dependent changes in oxidative metabolism during illumination. This ratio is not really constant, but has

an average value of 3.58 ± 1.39 ($n = 48$) (omitting points where $\Delta P_{cGMP} < 5$ mmHg). This result, which depends on both normal and low [Na$^+$] responses, can be compared to another approximation of the same quantity, the inverse of the P_1/P_2 ratio given in the Results. That ratio, which depends only on fitting the time course of the normal [Na$^+$] responses, is 2.3. Taken together, these suggest that the change in metabolic requirement for Na$^+$ pumping during illumination is in the range of 2.3 to 3.6 times as large as (and, of course, in the opposite direction to) the change in the metabolic requirement for cGMP synthesis during illumination. This pump/synthesis ratio is similar to the ratio of 3.2 (41%/13%) that was observed in rabbit (Ames et al., 1992). It should be noted that this only provides information about changes with light. Further quantitative analysis of the total energy requirement of each process relies on knowing the absolute value of consumption for each process in the dark or light, which has not yet been obtained.

Acknowledgements

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