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TRANSIENT SENSITIVITY REDUCTION AND BIPHASIC PHOTORESPONSES OBSERVED WHEN FROG RETINAL RODS ARE OXIDIZED

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Abstract—1. Rod photoresponses and the effects of oxidation have been studied by recording either the transretinal voltage in aspartate-treated retinas or the outer segment current of single rods.

2. Oxidizing conditions transiently decreased, reducing conditions increased sensitivity.

3. Biphasic photoresponses were seen when the level of oxidation was rising and also in some other sensitivity-depressing conditions.

4. A model is proposed which explains the biphasic responses in terms of sensitivity differences between the tip and the base of the rod outer segment.

INTRODUCTION

Earlier reports indicate that the oxidation level affects sodium channel functioning in retinal rods. Bownds and Brodie (1975) observed that isolated rod outer segments (ROSs) deteriorated in oxygen-rich solutions. Further, a reduction in oxygen concentration decreased the leakage resistance of the ROS. Wormington and Cone (1978) concluded that oxygen somehow inactivates sodium channels. Deoxygenation of the perfusion fluid, or the addition of EGTA (an antioxidant in lipids), preserved a large sodium conductance.

The impetus for the present work was the discovery of peculiar biphasic photoresponses in the aspartate-isolated mass receptor potential from the frog retina. Normally the photoresponse (the decrease in sodium current) is seen as a simple vitreous-negative deflection, but in some retinas a small positive deflection preceded the negative one in responses to low stimulus intensities. As the biphasic responses occurred only for a certain time after the retina had been put under perfusion, it was thought that they might be connected with a rising level of oxidation.

In the experiments reported here the state of oxidation of the rods was varied and the effects on the sensitivity, maximum amplitude and waveform of aspartate-isolated mass receptor photoresponses were recorded. Two main transient effects of oxidation were found: a depression of sensitivity and the appearance of biphasic photoresponses. For the latter, subsequent suction pipette recordings from single rods lead to the suggestion of a more general explanation in terms of differences between the distal and proximal parts of the ROS. The implication is that any deleterious treatment acting inhomogeneously on the ROS can bring out biphasic photoresponses. Our results emphasize that although a rod remains functional over a wide range of oxygen levels, these may importantly affect the specific electrophysiological outcome.

MATERIALS AND METHODS

Common frogs (*Rana temporaria*) had been stored at 4°C. Dark-adapted cooled retinas were dissected in cooled Ringer under dim red light and placed receptors upwards in a specimen holder similar to that of Bastian and Fain (1979). The receptor side was perfused at 12°C with a Ringer solution containing 95 mM NaCl, 3 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 12 mM phosphate buffer (pH 7.5), 10 mM glucose and 2 mM sodium aspartate. (Because of the low temperature, our responses are usually long and slow.) 'Deoxygenated' Ringer was obtained by driving nitrogen bubbles through the normal Ringer for about 10 min. The oxygen content of the aerated Ringer was 8.8 mg/l and that of the deoxygenated Ringer 2.5 mg/l as determined with a calibrated oxygen electrode. The gravitation-controlled perfusion system involved two tubes joining at the inlet to the specimen chamber. The flow could be switched from one to the other by valves outside the light-tight box with a delay of about 30 sec.

Stimuli were 0.1-sec, 493-nm flashes. The intensity was controlled with neutral density filters and wedges. The calibration of intensity was based on a spectrophotometrical measurement of the rate of rhodopsin bleaching.

The mass receptor potential was recorded with two Ag/AgCl electrodes, one connected to the Ringer space beneath the retina and the other in chloride solution connected to the perfusion fluid through a porous plug. The photoresponses were recorded on a fast recorder.

The use of the mass receptor potential for studying receptor function is inherently problematic, because it is determined not only by longitudinal receptor currents, but also by other radial currents of non-receptor origin [for a discussion, see Hemilä (1983)]. Thus the time courses of large responses are strongly distorted and so is, to some extent, the decay phase even of smaller responses (see Donner and Hemilä, 1985). Non-receptor currents also boost the maximal response amplitude. Still, the mass receptor potential served the present purpose well. On the one hand, the amplitudes and times-to-peak of small responses are practically free from non-receptor contributions. On the other hand, the automatic averaging of signals from many receptors (which, in effect, is what the mass receptor potential achieves) was essential, for example, in recording response waveforms during fast transients,

where there was no time to obtain several comparable responses in succession for the purpose of signal averaging. Finally, the maximal response was left practically unchanged by the treatments used, so there was no need to assess the exact proportions of receptor and non-receptor currents.

While this study was in progress, the construction of equipment was completed for recording the current from isolated rods with suction pipettes (see e.g. Hodgkin *et al.*, 1984). With this, a few control recordings were performed with a view to comparing the photocurrent of isolated ROSs with the mass receptor responses. Isolated rods (including the inner segment but without synaptic pedicles) were obtained by gently tearing the retina in the 2 mm × 10 mm well of a portable specimen holder. Micrometer mechanisms enabled us to position the tip of the pipette anywhere at the bottom of the well. The inner segment of a rod was sucked into the fire-polished micropipette (inner diameter about 6 μm) under a microscope and the holder was moved to the light-tight box. The perfusion tubes were fastened to the inlet and the outlet of the well and the electrodes connected (the virtual electrode coupling was similar to that used by Hodgkin *et al.*, 1984). Photoresponses were recorded using a conventional current-to-voltage transducer. Stimulation, perfusion and cooling were as in the mass receptor potential recordings. In these first suction pipette recordings, a deep red light was used when isolating and sucking the rods. Because of the large magnification needed this exposure caused considerable light-adaptation of the rods.

If a weak stimulus, intensity I (in the linear range), elicits a response U and the maximal response amplitude is U_{max}, the relative sensitivity is defined as $S = (U/U_{max})/I$. When determining sensitivity it was necessary to take into account the small nonlinearity apparent even in 'small responses' (10–15% of U_{max}), therefore, suitable stimulus–response functions were used (z-functions, one type of generalized Michaelis functions; see Bäckström and Hemilä, 1979) to extrapolate to the linear range.

RESULTS

Changing the state of oxidation affects sensitivity

The most straightforward way of varying the state of oxidation is switching back and forth between well-aerated and deoxygenated Ringer solutions. It was found that such changes did not affect the maximal response amplitude U_{max}, as would have been the case if the state of oxidation affected the sodium conductance in the dark. However, there were marked changes in sensitivity, as seen in Fig. 1. Introducing deoxygenated Ringer produced a transient sensitivity rise followed by a slow decay, while the subsequent introduction of aerated Ringer caused a transient fall in sensitivity. In five retinas the average rise caused by lowering the oxidation level was 0.24 log units (range 0.10–0.42), peaking 4 min after the switch, while the average sensitivity decrease caused by oxidation was 0.34 log units (range 0.10–0.70) peaking at 5 min. It is noteworthy that the desensitized responses tended to be somewhat slower and the sensitized ones somewhat faster than normal, as opposed to the situation when desensitization is achieved by normal light-adaptation.

An attempt was also made to manipulate the state of oxidation with various chemicals. Hydrogen peroxide in 0.1 mM concentration had an effect similar to that of dissolved oxygen. It produced a small but clear transient reduction of sensitivity. Higher concentrations reduced sensitivity much more, but left it

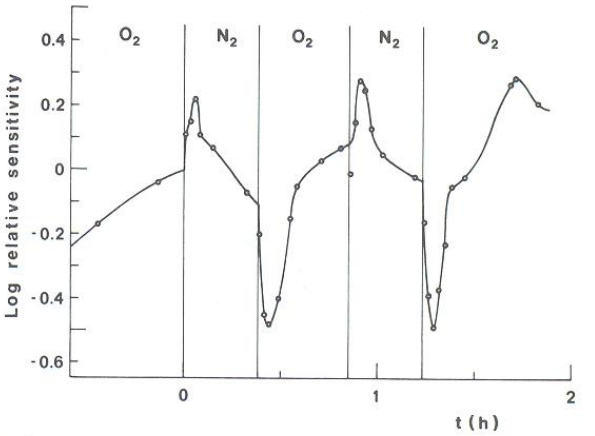

Fig. 1. Changes in the relative sensitivity of the rods (log scale) recorded when switching between aerated and deoxygenated Ringer solution. Abscissa: Time scale in hours, with zero set at the moment of the first switch to the deoxygenated solution.

permanently depressed, suggesting some form of non-specific deterioration.

The oxidizing form of vitamin C, dehydroascorbate (DHA), put down sensitivity very effectively (but very transiently) even in low concentrations (20 μM), as shown in Fig. 2A. Again, there was no effect on U_{max}. Ascorbic acid (0.1 mM) acted similarly to DHA, but with a slower and more variable time-course (Fig. 2B). Although often thought of as an anti-oxidant, here it appeared to act as an oxidizing agent. Its action was faster and stronger the longer the period before the experiment that the drug had been dissolved in Ringer. It was presumed that ascorbic acid was spontaneously oxidized in the aerated Ringer and entered the rods as DHA, as it is known to do in other types of cells (e.g. Bigley and Stankova, 1974). The idea was tested by spectrophotometrically determining the time-course of the conversion of ascorbic acid, starting when the solution had just been prepared. Indeed, the 260-nm absorbance peak corresponding to ascorbic acid fell nearly exponentially with a half-time of about 6 hr. The slower action of ascorbic acid became useful to us when we studied response waveforms (see below): it gave us time to record a few responses during the transients.

Biphasic photoresponses in the mass receptor potential

As mentioned in the Introduction, the spontaneous appearance of photoresponses having a positive "hump" in front of the normal negative-going response was initially puzzling; this response type usually persisted only for a rather short time after dissection. In Fig. 3, examples from two retinas are seen, showing some of the large variation in the size and shape of these dissection-related humps. Often they were very small, making signal averaging necessary for reliable hump/noise discrimination, but sometimes they were quite prominent in responses to low stimulus intensities (Fig. 3B). As exemplified by Fig. 3B, the gradual disappearance of the dissection-related humps was often accompanied by a conspicuous decrease in low-frequency noise.

There are four points worth noting about the

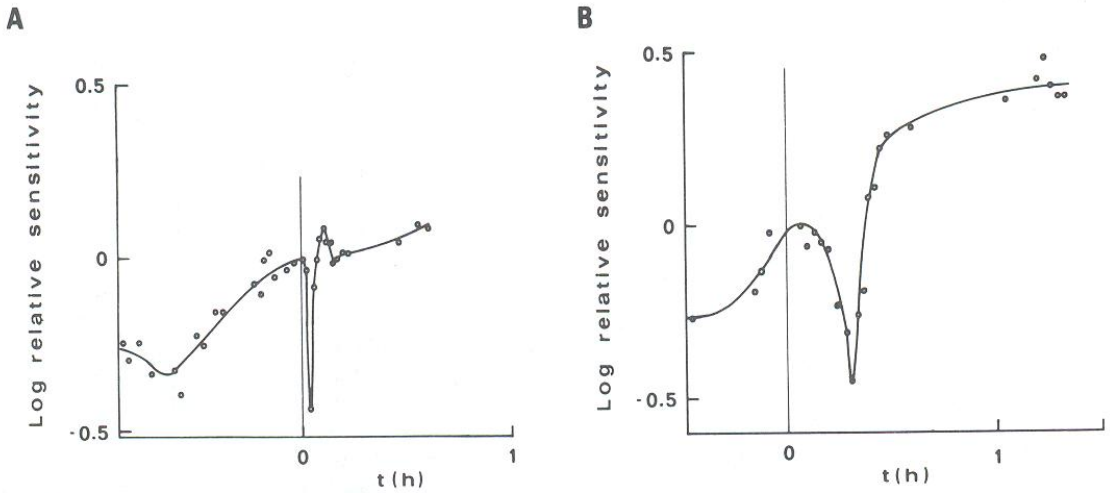

Fig. 2. Changes in sensitivity upon the introduction of oxidizing agents: A, a sensitivity transient in 20 μm dehydroascorbate, B, a slower transient following the introduction of 200 μm ascorbate. Coordinate axes as in Fig. 1.

positive going hump response. First, it constitutes the very first visible reaction to a light stimulus and must accordingly be of receptor origin. Secondly, its sensitivity is as high as that of the normal response and, as shown in Fig. 4, it reaches its highest amplitude at a stimulus intensity of only about one photoisomerization per rod. Thirdly, it is never large compared with the maximal amplitude of the negative response component: even the largest observed humps were only 7% of the latter (in the retina from which the slow and noisy responses of Fig. 3B were also recorded). Fourthly, humps can normally be

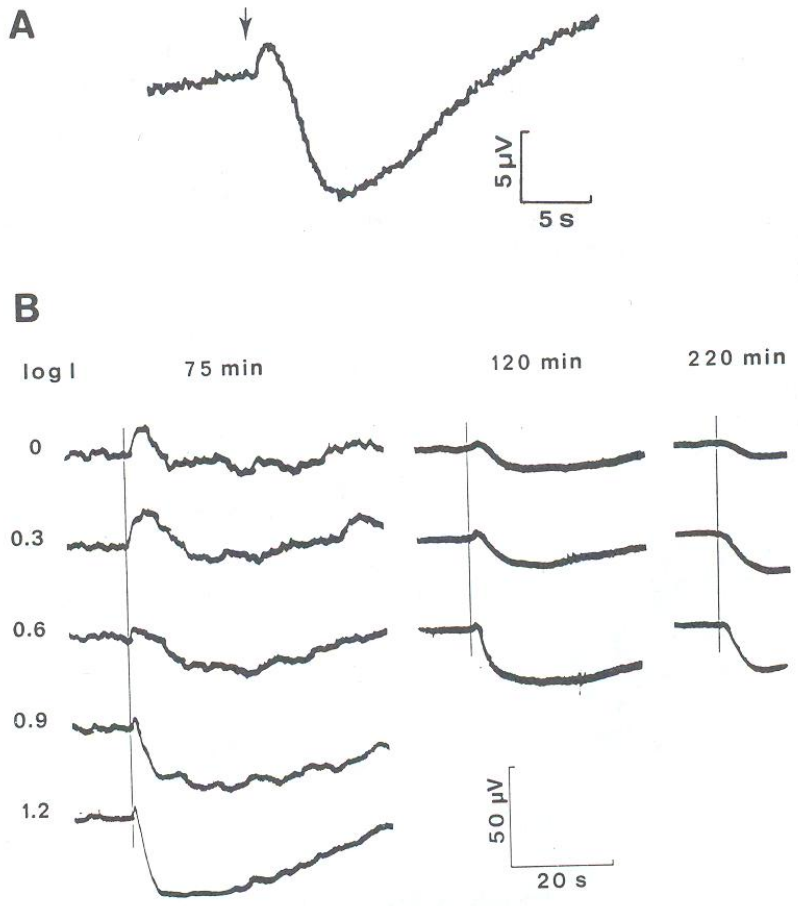

Fig. 3. Examples of dissection-related biphasic 'hump' responses: A, a response with a typical smallish hump (about 2% of the maximum amplitude U_{max} of the normal response) preceding a small normal photoresponse (about 10% of U_{max}), B, recordings from an exceptional retina with initially very slow and noisy photoresponses and unusually big and persistent humps. Series of responses to a few low stimulus intensities recorded at different times after the retina had been put into perfusion. Log $I = 0$ corresponds to 0.54 Rh^* per rod. Note how the low-frequency noise tends to decrease in parallel with the humps.

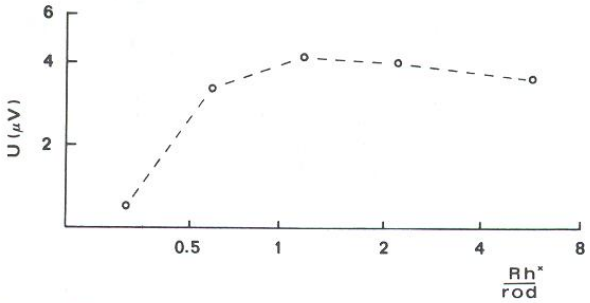

Fig. 4. The amplitude of the positive 'hump' as a function of flash intensity.

seen only at rather low stimulus intensities: they shorten rapidly with increasing intensity, as the onset of the main response covers the hump process.

Our original hypothesis that humps were connected with a rising level of oxidation required that they could be artificially produced by oxidizing treatments. This was borne out by the experiments. Figure 5 shows two such cases: one in response to the return to aerated Ringer after a period in nitrogen Ringer (Fig. 5A), the other in response to the introduction of ascorbic acid, whose slow action made it advantageous for this kind of study (cf. Fig. 2B). Humps could generally (but not always) be observed during the periods of reduced sensitivity connected with an oxidizing treatment. They were always quite small, however.

Biphasic responses in single ROS current recordings

While finishing the series of experiments reported above, the construction of suction pipette equipment was completed, for recording the current from isolated rods. This made it possible to study whether the biphasic photocurrent suggested by the mass receptor potential recordings could be found in single rods under some conditions. Part of the rod, usually the inner segment first, was sucked into the micropipette, so at least part of the outer segment was left exposed to the perfusing fluid.

What was found was quite striking. If a significant part of the outer segment was within the pipette, so that the constriction was between its base and tip, the photocurrent appeared biphasic: the expected decrease due to a flash was preceded by an increase in current, occasionally even in near-saturated responses (Fig. 6A). In one experiment where the tip of the outer segment had been drawn into the pipette, leaving the inner segment and the basal part of the outer segment outside, quite curious triphasic photoresponses were obtained (Fig. 6B). Here, the photoresponse proper is upwards (because the direction of the rod current was reversed), but in addition, a leading hump and a trailing overshoot are seen. Obviously, these deviating waveforms were not simply related to oxidation, but suggested a more general account of biphasic (and triphasic) responses in terms of base/tip differences in the ROS.

DISCUSSION

The site of the sensitivity changes

A change in the state of oxidation transiently affected the rod's sensitivity to weak stimuli without changing its maximal response amplitude (U_{max}). This gives two clues to the site of action. First, any change in the ROS's sodium conductance in the dark would change U_{max}. Thus, a direct effect on the sodium channels (e.g. on their kinetic parameters) is ruled out and so is any mechanism affecting the balance of internal transmitter in the dark. Secondly, the transient nature of the sensitivity changes makes a direct effect on rhodopsin (e.g. changing its quantum efficiency) unlikely, since a permanent change in oxidation level would then be expected to give a

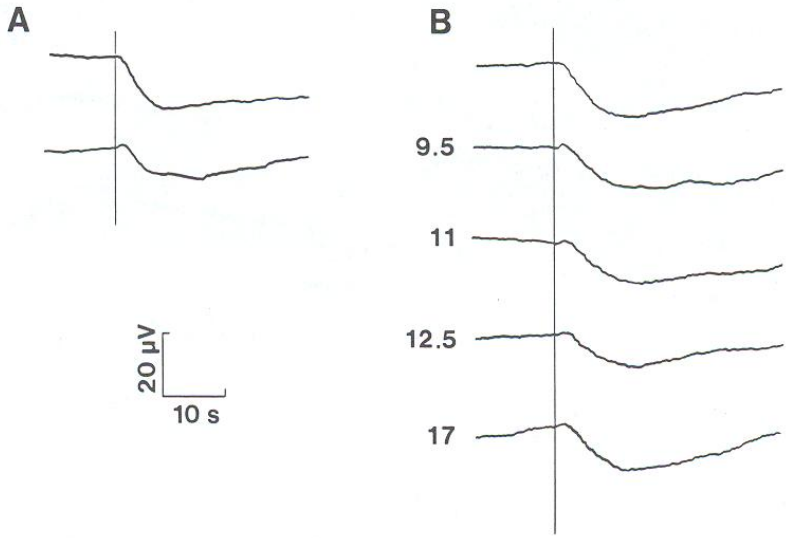

Fig. 5. Oxidation-induced 'hump' responses: A, two photoresponses to weak flashes, one (log $I = 0.5$, top) recorded just before, the other (to a slightly higher stimulus intensity: log $I = 0.6$, bottom) 3.5 min after the switch to oxygen-rich perfusion. Note decreased amplitude, increased noise and initial hump in the bottom response. B, A series of photoresponses to the same weak flash intensity (log $I = 0.3$) recorded before (top response) and at different times (in minutes as indicated along the curves) after the introduction of 100 μm ascorbate. The humps are barely perceptible but appear repeatedly. As in Fig. 3, log $I = 0$ corresponds to 0.54 Rh^* per rod.

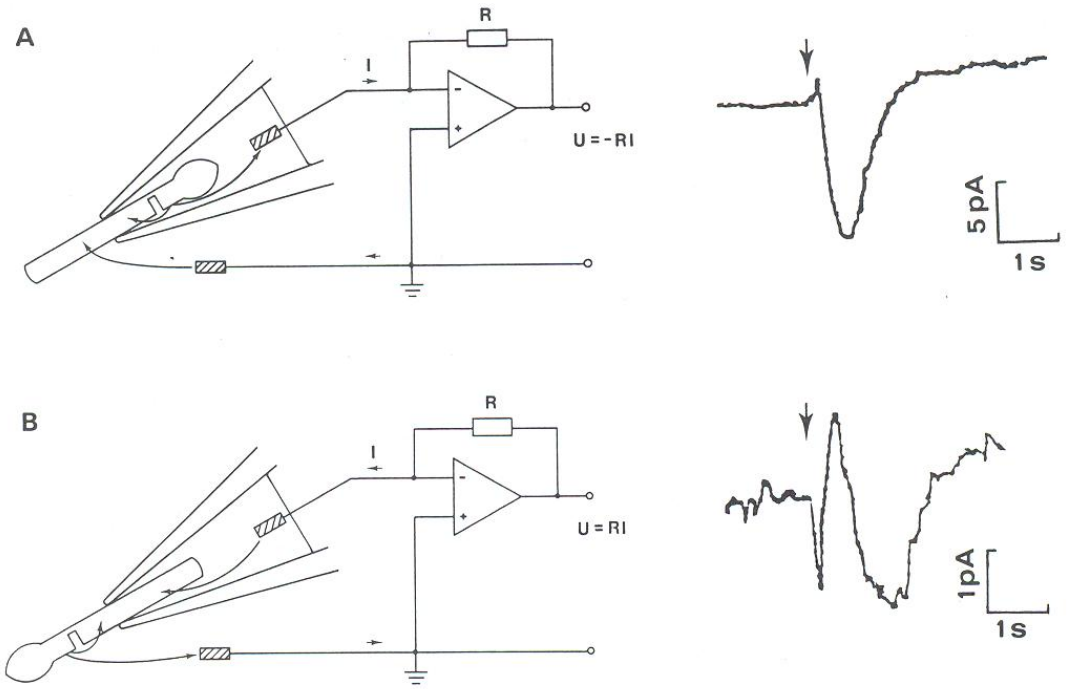

Fig. 6. Current recordings from single isolated rods: bi- and triphasic responses to comparatively strong flashes of light: A, the inner segment and the base part of the outer segment are inside the pipette, B, only the tip part of the outer segment inside the pipette, hence the main response is inverted. For an explanation of the waveforms, see Discussion.

permanent change in the properties of the visual pigment.

So this action of oxidation appears to lie somewhere in the excitatory chain, decreasing the gain of the cascade (photoisomerization) – (change in internal transmitter concentration). Therefore, it has an origin different from the deleterious effect of oxygen on the light-sensitive conductance of the ROS reported by Bownds and Brodie (1975) and Wormington and Cone (1978). The latter effect must be comparatively weak in the intact isolated retina, although the very slow reduction of U_{max} that is usually observed during the course of an experiment could be due to the oxidation of the plasma membrane. Arguably, a rod embedded in an intact retina is better shielded against the action of oxygen than an isolated rod.

The biphasic responses

Biphasic photoresponses have been observed before in current recordings from toad red rods by Baylor *et al.* (1979) and from green rods by Matthews (1984). Neither are clearly related to our responses. Baylor *et al.* (1979) give a plausible explanation of their responses in terms of the electrical coupling between rods in the piece of retina they were recording from. The opposite-going component recorded by Matthews is connected with the decay phase of the response, as opposed to ours.

A straightforward interpretation of the biphasic responses described in the present work would postulate a fast increase in ROS current preceding the closure of sodium channels following a flash. It is hard to think of a credible redox-modulated mechanism giving rise to such a complex reaction to light. Therefore, in this section, an explanation is proposed in terms of different properties of the outer segment base and tip. That notion receives direct support from the observation of Baylor *et al.* (1979) that base responses were always faster than tip responses. Also, for example, the susceptibility to desensitization by light is known to be greater at the tip than at the base (Hemilä and Reuter, 1981; Lamb *et al.*, 1981).

The model predicts that biphasic responses occur (a) in current recordings from single ROSs if the electrode constriction divides the outer segment into 'tip' and 'base' and the former part responds to light more slowly and/or less sensitively than the latter part; (b) in mass receptor potential recordings, assuming the same difference between base and tip, and that the extracellular resistance is higher around the tips than around the bases.

The model

Figure 7A shows the equivalent circuit of the rod that is the basis of the simulated responses displayed in Fig. 7B–D. All driving forces are placed in the inner segment membrane. The light-sensitive sodium conductance is divided into tip and base portions, G_t and G_b, as is the extracellular resistance [R_{te} (tip) and R_{be} (base)]. The suction pipette (with the inner segment and outer segment base sucked in) is assumed to record only the current i through the conductance G_t (tip current). The mass receptor potential, on the other hand, is the extracellular voltage drop across the resistances R_{be} and R_{te} due to current flowing in the space between the outer segments. The photocurrent is assumed to follow the independent activation model of Baylor *et al.* (1974) with Michaelis-type saturation. The detailed equations are presented in the Appendix. Figure 7B shows 'normal' simulated photoresponses for comparison with the 'deviating' ones discussed below.

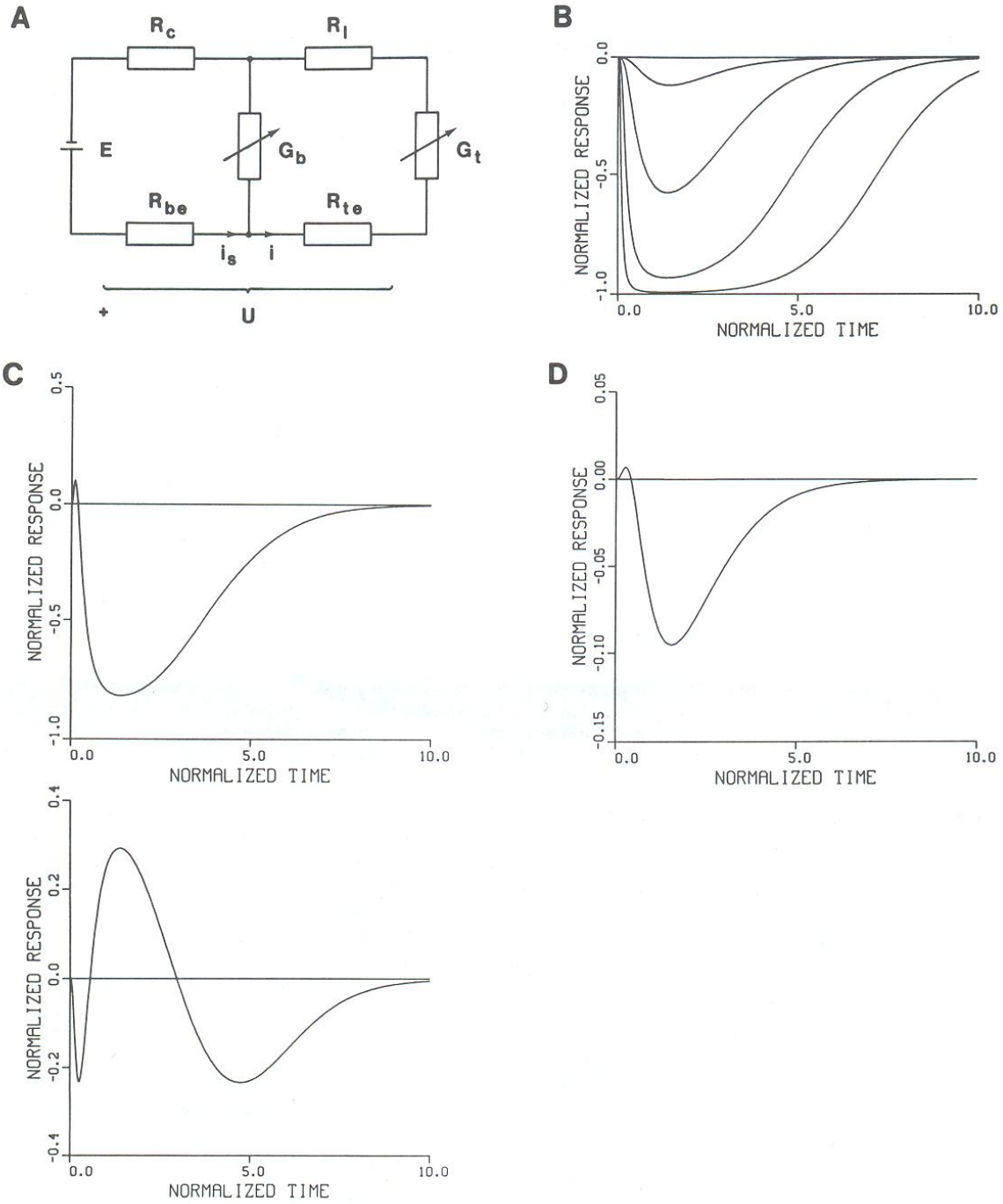

Fig. 7. A, An equivalent circuit of the rod. E is the driving force, placed in the inner segment membrane. R_c is the sum of the inner segment membrane resistance and the intracellular resistance along the cilium and a base part of the ROS. R_l is the tip part of the longitudinal intracellular resistance of the ROS. G_b and G_t are the base and tip parts of the ROS sodium conductance, respectively. R_{be} and R_{te} are the small extracellular longitudinal resistances around the ROS base and tip parts, respectively, i_s is the total current passing the outer segment conductance, while i is the current passing only G_t. The mass receptor potential is the light-induced change in the extracellular potential U; $\Delta U = U_{flash} - U_{dark}$, where the transretinal voltages U_{flash} and U_{dark} are due to the extracellular resistances: $U = R_{be} i_s + R_{te} i$. B–D show different photoresponses simulated by varying the values of the following parameters: c is the ratio of base/tip sensitivities, k is the ratio of base/tip response rates, X is the base fraction of the total extracellular resistance along the ROS, Y is the tip fraction of the total light-sensitive membrane conductance. B, Simulated 'normal' photoresponses to flashes $I = 3$, 30, 300 and 3000. ($c = 1$, $k = 1.2$, $X = 0.1$, $Y = 0.75$.) C, Simulated current responses ($X = 0$) of an isolated rod. Top, corresponding to a situation where the rod inner segment and one third of the outer segment are inside the pipette and the tip is slow and insensitive; the nearly saturated response to $I = 100$ is seen to be biphasic, starting with a sharp 'hump'. ($c = 10$, $k = 1.6$, $X = 0$, $Y = 0.67$.) Bottom, corresponding to a situation where one third of the outer segment (the tip) is inside the pipette and this part is rather insensitive, but not slower than the base. Response to a moderate flash $I = 15$. ($c = 25$, $k = 1$, $X = 0$, $Y = 0.33$.) D, Simulation of a biphasic 'mass receptor response' to a weak flash ($I = 3$; a small response: observe scaling). The base part, one fourth of the ROS, is assumed to be 0.6 log units more sensitive and 25% faster than in the 'normal' responses simulated in B. The extracellular resistance is assumed to be 2.5 times higher in the tip area. ($c = 4$, $k = 1.5$, $X = 0.1$, $Y = 0.75$.) The normalization of response amplitude is such that -1.0 corresponds to a saturated response.

Current responses from isolated rods. In current recordings from isolated rods, that portion of the current which enters the rod through the tip part of the outer segment conductance is always recorded.

When the rod inner segment and part of the outer segment is drawn in, G_t is the tip membrane conductance outside the pipette, G_b the membrane conductance of the outer segment base which is inside the pipette. If a flash reduces G_b faster and more effectively than G_t, the tip current initially increases, although the total current decreases. Later the reduction in G_t becomes dominant and the result is the normal response—a negative deflection. The simulated response of Fig. 7C, top, is to be compared with the recorded one in Fig. 6A.

If only a small part of the outer segment has been drawn in and if that part loses much of its sensitivity, an inverted and triphasic photoresponse is expected. Figure 7C, bottom, shows a simulated response in such a situation. It is to be compared with the recorded one in Fig. 6B.

There is good reason to think that the tip part of the ROS really was less sensitive than the base part because of the light-adaptation caused during preparation (see Materials and Methods). As mentioned above, light-adaptation is known to desensitize the tip more strongly than the base.

The mass receptor recordings. In addition to the assumption that the ROS base is more sensitive to light than the tip (inherently or because of some treatment), it is also assumed that current flowing to the tip contributes more to the mass receptor potential than current entering the base. A trivial reason is that its path is longer, implying a higher resistance. But in addition it is specifically assumed that the extracellular resistance per unit length is higher distally than proximally, which is not unreasonable in view of the pigment epithelial protrusions which, in a dark-adapted retina, occupy the extra-receptor space primarily around the tips (see e.g. Bäck *et al.*, 1965). Under these conditions, a weak flash would reduce G_b mainly and although the total current would decrease, that through G_t would increase and so would the voltage drop across the extracellular resistances. Such a small (virtually monophasic) inverted response would usually be hard to discern from noise (see, however, the first response in Fig. 3B). With rising flash intensity, the response would become biphasic, involving a fast drop in G_b followed by a drop in G_t producing the normal (negative-going) response. Faster response kinetics at the base compared with the tip greatly enhances the initial hump.

Figure 7D shows a simulated small mass receptor response derived from the circuit of Fig. 7A. Obviously, any treatment causing the outer segment tip to be less sensitive and/or slower than the base would give the conditions where the above model can predict biphasic responses. Indeed it seems likely that the deleterious effects of oxygenation could transiently strike the tip more heavily than the base—either because the tip is more directly exposed to perfusion, or because of greater susceptibility. In Fig. 7D, the base part of the ROS, a quarter of the total light-sensitive conductance, is assumed to be 0.6 log units more sensitive and 25% faster than the tip. These changes are comparable in magnitude to the transient effects of oxidation. The ratio $R_{be}/(R_{be} + R_{te})$ is set to 0.1; a ratio 1:4 is accounted for by the relative lengths of the base and tip parts, the rest by the ratio of the cross sectional areas of the extracellular space around the base and the tip.

The 'dissection-related' biphasic photoresponses appearing at the beginning of many experiments could have additional causes, all expressed as different base/tip capacities of recovery from dissection (which inevitably involves both mechanical, thermal and chemical changes other than changing oxidation levels). The large variation in the size and persistence of the positive hump responses is well understandable by the special requirements. Certainly, for example, the distribution of pigment epithelium processes varies greatly, depending on temperature and light history (Arey, 1916).

It is remarkable that the biphasic responses have gone unreported although the mass receptor potential has been widely used. The only mention of a possibly related effect we have found in the literature is by Greenblatt (1983), who in a low Ca^{2+}, $CH_3SO_3^-$ solution observed inverted photovoltages to very dim flashes, but makes no further comments on this.

A possible reason why these deviating responses are rarely observed is that, if the dissection is done at room temperature, the pigment epithelium is usually easily and completely detached from the retina. On the other hand, although lower temperatures have been used in earlier work as well, the retina has usually been allowed to 'stabilize' and dark-adapt in perfusion for about an hour before starting the experiment. This stabilization is connected not only with a drastic decrease in the low-frequency noise, but most often also with the disappearance of the hump responses and a significant decrease in the maximal response amplitude. Assuming that the initial low-frequency noise reflects random conductance fluctuations in the injured tip region, a conceivable common cause of all these changes would be a gradually proceeding permanent inactivation of some of the membrane conductance, preferentially at the ROS tips.

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APPENDIX

The equations used for the simulation of photoresponses

Let G_{to} and G_{bo} be the values of G_t and G_b in the dark. At low intensities (I) the decrease in sodium conductance, $G_{to} - G_t$, is proportional to I, but at high intensities Michaelis-type saturation is assumed. Thus, if A_t is the time-dependent sensitivity factor at the tip, the relative decrease in conductance is

$$\frac{G_{to} - G_t}{G_{to}} = \frac{A_t I}{A_t I + 1}.$$

Taking the inverse, $R_t = 1/G_t$ and $R_{to} = 1/G_{to}$, one obtains

$$R_t = R_{to}(1 + A_t I).$$

The sensitivity factor of the tip, A_t, is calculated according to the 'independent activation' kinetics of Baylor *et al.* (1974) with four delay stages in the reaction cascade:

$$A_t = (1 - e^{-t/\tau})^3 e^{-t/\tau}.$$

The base sodium conductance is assumed to follow similar equations, but the light sensitivity factor and the time constant may be different (by factors c and $1/k$). Denoting $R_b = 1/G_b$ one obtains

$$R_b = R_{bo}(1 + A_b I)$$
$$A_b = c(1 - e^{-kt/\tau})^3 e^{-kt/\tau}.$$

Let us denote

$$G = \frac{1}{R_t + R_I + R_{te}}$$

$$G_o = \frac{1}{R_{to} + R_I + R_{te}}$$

$$R' = R_c + R_{be} \approx R_c$$

$$X = \frac{R_{be}}{R_{be} + R_{te}}.$$

The currents are then

$$i_s = \frac{E(G + G_b)}{1 + R'(G + G_b)}$$

$$i = \frac{EG}{1 + R'(G + G_b)}.$$

The transretinal voltage is $U = R_{be} i_s + R_{te} i$. In the dark, when $G = G_o$ and $G_b = G_{bo}$, the transretinal voltage is denoted U_o. Thus the mass receptor potential is $\Delta U = U - U_o$. Substituting the expressions of i and i_s in light and dark we obtain the relative photoresponse:

$$\frac{\Delta U}{U_o} = \frac{G + XG_b}{G_o + XG_{bo}} \cdot \frac{1 + R'(G_o + G_{bo})}{1 + R'(G + G_b)} - 1.$$

When the model is used for simulation of the ROS current responses, the relative signal is the relative change in current i. The above equation is valid when $\Delta i/i_o$ substitutes for $\Delta U/U_o$ and X is set to zero.

When calculating the curves of Fig. 7, scaling was used in order to reduce the number of parameters. The dimensionless time is t/τ and the dimensionless resistances are $r = R/R'$. Thus there are six parameters which may affect the shape of the response in this model, namely:

$$c, k, X = \frac{R_{be}}{R_{be} + R_{te}},$$

$$r_{to} = \frac{R_{to}}{R'}, \quad r_{bo} = \frac{R_{bo}}{R'},$$

$$r_i = \frac{R_I + R_{te}}{R'}.$$

A prerequisite for humps is that c and/or k is larger than 1 and X is small, or 0. The ratio r_i is determined by the resistive properties of the rod. Here it is always set to 0.3. The responses are not sensitive to this ratio. The total outer segment conductance is of the same order of magnitude as the inner segment membrane conductance, wherefore $1/r_{to} + 1/r_{bo} = 1$. Thus $1/r_{to}$ is the tip fraction of the total light-sensitive conductance (denoted Y in Fig. 7 and Discussion).