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# EFFECT OF LOW EXTRACELLULAR CALCIUM CONCENTRATION ON PHOTOSENSITIVITY OF ISOLATED RODS FROM FROG RETINA

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## Summary

The effects of extracellular calcium concentration ($[Ca^{2+}]_o$) on the light sensitivity of isolated single rods from the retina of the frog (*Rana catesbeiana*) were investigated by sucking the rod inner segments into tightly fitting pipettes.

Light flashes (500 nm, 1 s duration) evoked transient outward changes of membrane current (photoresponses). The peak amplitude of maximal photoresponses in normal physiological solution varied between 6 and 12 pA. Reducing $[Ca^{2+}]_o$ from 0·9 mmol $l^{-1}$ (normal $Ca^{2+}$) to 90 $\mu$mol $l^{-1}$ (low $Ca^{2+}$) increased the peak amplitude of photoresponses and shortened the recovery phase of the responses.

The effects of larger changes in light intensity were also investigated. After light-on there was a steady outward change of membrane current, and after light-off the current recovered to the initial dark level. With a low external $Ca^{2+}$ concentration, light-off induced a large inward change of membrane current which transiently overshot the initial dark level. During the overshoot stage, light flashes evoked photoresponses which were larger than those in the initial dark period.

## Introduction

In the frog retina, superfused with low-$Ca^{2+}$ solution, the response of the transretinal potential to a light flash shows an increase in amplitude when it is elicited soon after the end of a period of illumination at a steady intensity. This phenomenon has been termed hypersensitivity on the basis that the sensitivity is the reciprocal number of photons needed to elicit a certain amplitude of response (Azuma & Azuma, 1979). The results of experiments using light of different wavelengths have suggested that hypersensitivity is mainly due to red rods (Azuma & Azuma, 1982).

In the present study, we have further investigated hypersensitivity by measuring the photocurrents of single rods, using a suction electrode method.

Key words: rod, $Ca^{2+}$, photosensitivity, suction electrodes.

## Materials and methods

Adult bullfrogs (*Rana catesbeiana*) were dark-adapted overnight. After the animal had been pithed, the eyeballs were enucleated. The isolated retina was placed in normal physiological saline in a Petri dish and shaken to detach the rods. The suspension of rods in saline was put into a small chamber. The bottom of the chamber was made from a glass microscope slide and the top consisted of a cover-glass. The front and back sides were made of plastic. The lateral sides were open to allow flow of superfusion solution and to permit entry of the suction electrode pipette. The superfusate (pH 7·6) contained (in mmol $l^{-1}$) NaCl, 85; KCl, 2·5; $NaHCO_3$, 25; $MgCl_2$, 1·2; glucose, 25; Hepes, 3, and an appropriate concentration of $CaCl_2$. The superfusate was run off at the rate of 2 ml $min^{-1}$. The temperature of the superfusate was maintained at 20 ± 1 °C.

The suction electrodes were constructed from Pyrex capillary glass (1·5 mm outer diameter, 1 mm inner diameter; Narishige). The glass was broken off squarely at a position where the outer diameter was about 50 $\mu$m, and the electrodes were then made with a tip of about 6 $\mu$m inner diameter over a length of 10 $\mu$m, as described by Baylor *et al.* (1979).

The chamber was set on the stage of an inverted microscope (Nikon). A single rod which had outer and inner segments was sucked at the inner segment following the methods described by Hodgkin *et al.* (1984). All these procedures were carried out under infrared light using an infrared image converter (NEC).

The apparatus for optical stimulation was essentially the same as that described by Baylor *et al.* (1979). The unattenuated light intensity for light flashes and for longer-term exposures (adaptation) was $9\times10^4$ photons $\mu m^{-2} s^{-1}$ at 500 nm.

## Results

The response to a light flash at the normal external calcium concentration of 0·9 mmol $l^{-1}$ (Fig. 1A) had a smaller peak amplitude than the response at a 10-fold lower $Ca^{2+}$ concentration (Fig. 1B). In both cases, peak amplitude increased as the stimulus intensity was raised, until a saturation level was reached. A further increase in intensity prolonged the duration of the saturation level (Fig. 1). The falling phase of the response showed a different time course at the two calcium concentrations (Fig. 1). The falling phases of responses obtained at the same stimulus intensity were compared after normalization to the peak amplitude of the response at normal $[Ca^{2+}]_o$, showing that the time course was shortened by reducing the $Ca^{2+}$ level (Fig. 2). The rate of the falling phase can be expressed as the time between the middle points of the rising and falling phases, since the two responses almost coincide in the rising phase. The duration was 4·8 s at normal $Ca^{2+}$ and 3·7 s at low $Ca^{2+}$ concentrations.

From experiments such as those shown in Fig. 1, response–intensity curves were obtained (Fig. 3). The peak amplitudes of responses at normal and low $Ca^{2+}$ concentrations are plotted against the logarithm of the relative intensity of the stimulus. The peak amplitude of each response is normalized to that of a maximum

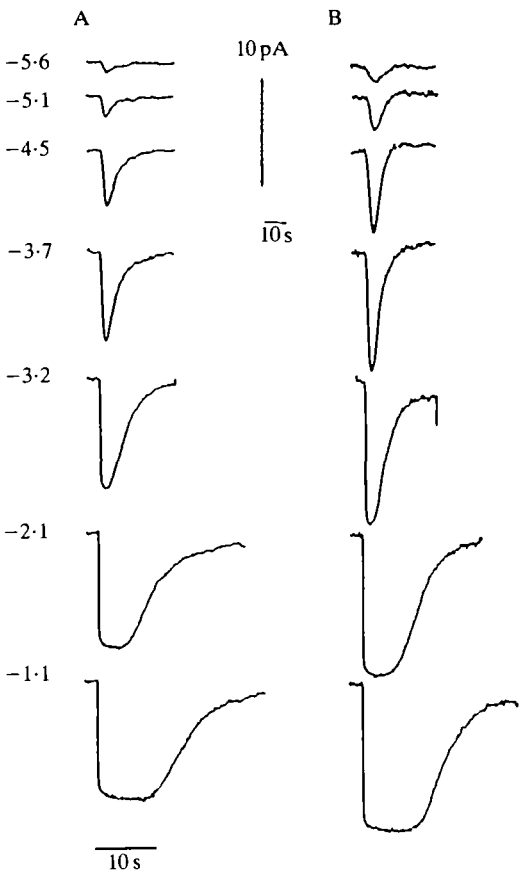


Fig. 1. Measurements of photoresponses from a detached rod. The inner segment of the rod was sucked into a pipette filled with the normal-$Ca^{2+}$ solution (0·9 mmol $l^{-1}$). Downward deflection indicates a decrease in dark current of the rod. The responses in A were evoked by 1-s stimuli of seven different intensities at 2-min intervals after 10 min of superfusion with the normal-$Ca^{2+}$ solution (0·9 mmol $l^{-1}$). The numeral on the left of each response indicates the stimulus intensity expressed by the negative logarithm of the (neutral density) filter. After recording these responses, the superfusate was changed to the low-$Ca^{2+}$ solution (90 $\mu$mol $l^{-1}$). The responses in B were obtained, after 10 min of superfusion, by stimulation at the intensities indicated.

response at normal $Ca^{2+}$ concentration. These are fitted by the equation of Naka & Rushton (1966)

$$r/r_{max} = i^n/(i^n + i_o{}^n). \quad (1)$$

Here r represents the peak amplitude of a response (pA), with maximum $r_{max}$, and i represents stimulus intensity with half-saturating intensity $i_o$. The value of $r_{max}$ at low $[Ca^{2+}]_o$ was about 1·2 times larger than that at normal $[Ca^{2+}]_o$. When the value of n was 1·0, $i_o$ was −4·6 at low $[Ca^{2+}]_o$ and −4·4 at normal $[Ca^{2+}]_o$.

The response to light flashes was compared with the response to long-term

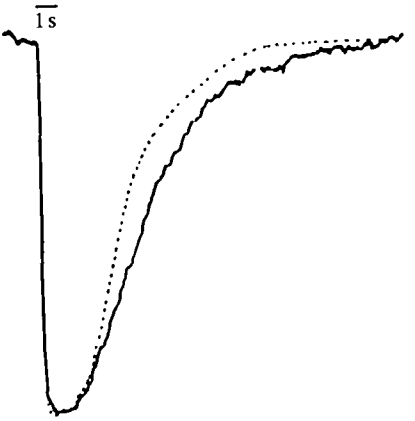


Fig. 2. Effect of lowering $[Ca^{2+}]_o$ on the time course of photoresponses. Responses in normal $[Ca^{2+}]_o$ (continuous line) and low $[Ca^{2+}]_o$ (dotted line) were evoked by stimuli of equal intensity (−3·7 log units) to the same rod. The amplitudes of the responses were normalized to the peak amplitude of the response in normal $[Ca^{2+}]_o$.

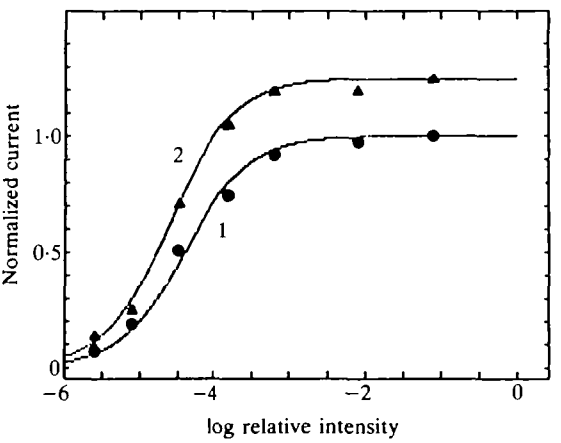


Fig. 3. Effect of lowering $[Ca^{2+}]_o$ on the response–intensity relationship. The measurements were carried out consecutively with the same rod as in Fig. 1. Curve 1: normal $[Ca^{2+}]_o$. Curve 2: low $[Ca^{2+}]_o$. The peak amplitude of the photoresponse evoked by light flashes of various intensities is plotted *versus* the logarithm of relative intensity, normalized to unity at the peak amplitude of maximal response in normal $[Ca^{2+}]_o$.

(adaptation) illumination. At the normal $Ca^{2+}$ level (Fig. 4A), the onset of the adaptation illumination produced a decrease in membrane current similar to that induced by a preceding light flash. During adaptation, current steadily increased and the response to light flashes showed a marked decrease in amplitude. After the adaptation illumination had been turned off there was a small transient increase in dark current followed by a recovery to the original level, and there was recovery

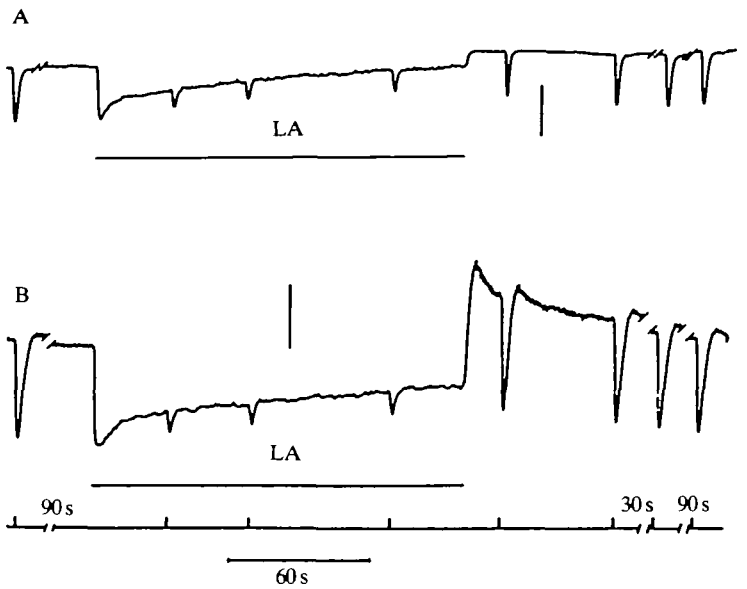


Fig. 4. Effects of low $[Ca^{2+}]_o$ and steady illumination (adaptation) on the photoresponses of a detached rod. The rod outer segment was superfused with normal $[Ca^{2+}]_o$ (A) and low $[Ca^{2+}]_o$ (B). Horizontal bars labelled LA indicate 150 s adaptation (−3·7 log units). Photoresponses evoked by 1-s stimuli were recorded 2 min before the onset of the adaptation illumination, at appropriate periods during the illumination, and after the light had been turned off (indicated by light flash monitors). Vertical lines correspond to 5 pA.

of the response to light flashes. At the low $Ca^{2+}$ level (Fig. 4B), some differences were observed: during adaptation, there was greater suppression of the response to light flashes; and after adaptation the transient increase in dark current was larger and was accompanied by an increase in the amplitude of the response to light flashes (about 1·2 times larger than before adaptation). The effect of adaptation illumination upon the stimulus–response curve for light flashes was studied at low $[Ca^{2+}]_o$ (Fig. 5). At 15 s after turning off a weak adaptation light (−4·0 log units), the curve (Fig. 5, curve 2) was similar to that obtained in the dark (Fig. 5, curve 1): $i_o(n)$ was −4·6(1·2) compared with −4·6(1·0); in the range of stimulus intensity stronger than −4·5 log units the responses after turning off the weaker adaptation light were about 1·1 times larger than those in the initial dark period. In contrast, under bright adaptation illumination (−2·0 log units), $i_o(n)$ was −2·9(4·0); in the range of stimulus intensity dimmer than −3·0 log units the responses 15 s after turning off the light were completely suppressed, but the maximum level (Fig. 5, curve 3) was about 1·3 times larger than that in the initial dark period (Fig. 5, curve 1).

## Discussion

This study has indicated that the dark current and the maximum light responses

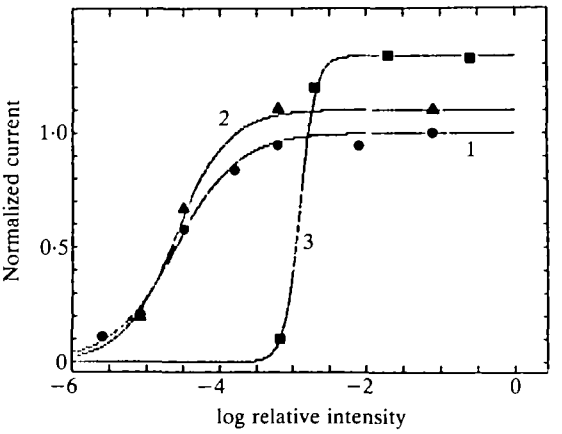


Fig. 5. Effects of illumination at steady intensity (adaptation) on the response–intensity relationship at low $[Ca^{2+}]_o$. Curve 1 was obtained in the dark. Curve 2 was obtained from the measurements 15 s after turning off the dim adaptation light (−4·0 log units, 150 s). Curve 3 was obtained after the bright adaptation light (−2·0 log units, 150 s). Curves 2 and 3 were obtained by repeating the same adaptation light stage several times and giving 1-s stimuli of different intensities 15 s after each light stage. The peak amplitudes of responses evoked by 1-s stimuli of various intensities were normalized to unity at the peak amplitude of the maximal response of the same rod during the initial dark period.

of a single rod photoreceptor in low-$Ca^{2+}$ solution are increased soon after the end of a period of steady illumination, and the $i_o$ value is raised (Fig. 5). Therefore, we will call the phenomenon 'enhancement', which indicates an increase in dark current and in the maximum light response.

The extent of the enhancement is given by the ratio $r_{15}/r_d$ (where $r_{15}$ equals the peak amplitude of the response at 15 s after light adaptation; and $r_d$ equals the peak amplitude of the response in the initial dark period), with a value of 1·1–1·3 in these experiments. In contrast, the ratio obtained by measuring fast PIII in the retina is greater than 1·3 (Azuma, 1988). This discrepancy could be explained as follows. The mechanically detached rods are different from rods *in situ* in the following ways. The detachment procedure eliminates interactions between rods and neighbouring rods or cones and cuts off part of the inner segment. It should be noted that the $Na^+/K^+$ pump is localized in the rod inner segment membrane (Berman *et al.* 1977) and that the pump is essential to cause the enhancement as discussed in a previous paper (Azuma, 1988). Thus, a partial loss of the inner segment membrane may cause a decrease in pump activity and reduce the extent of the enhancement. However, it is not excluded that the interaction between photoreceptors has an influence on the enhancement.

Low $[Ca^{2+}]_o$ usually induces an increase in amplitude of a rod photoresponse (Fig. 1). In some cases low $[Ca^{2+}]_o$ also causes a decrease in the amplitude (data not shown). Hodgkin *et al.* (1984) have reported that the increase in dark current in low $[Ca^{2+}]_o$ is smaller in rods lacking a nucleus than in more intact cells. In the

recordings of fast PIII, low $[Ca^{2+}]_o$ was not observed to reduce response amplitude (Azuma, 1988). The decrease in amplitude could have the following cause. If detached rods lose a considerable amount of $Na^+/K^+$ pump activity (as discussed above) a reduction in $[Ca^{2+}]_o$ would cause a decrease in the $Na^+$ gradient, because low $[Ca^{2+}]_o$ leads to an increase in $[Na^+]_i$ in rods (Somlyo & Walz, 1985). The resultant decrease in the $Na^+$-dependent component of the membrane potential could explain the decrease in response amplitude.

Intense stimuli prolong the saturated level of photoresponses, i.e. the long cessation of the $Na^+$ dark current (Fig. 1). This result is consistent with that of Hodgkin *et al.* (1984) and suggests that strong light activates PDE (phosphodiesterase) for a long time and delays the increase of [cyclic GMP]$_i$ which controls the $Na^+$ dark current (Pugh & Cobbs, 1986).

A reduction in $[Ca^{2+}]_o$ shortens the recovery phase of photoresponses (Fig. 2). This indicates that $Ca^{2+}$ affects the kinetics of the photoresponse, which can be explained as follows. A reduction in $[Ca^{2+}]_o$ would decrease $Ca^{2+}$ influx through the rod outer segment membrane, and then induce a decrease in $[Ca^{2+}]_i$ *via* the $Na^+/Ca^{2+}$ exchange pump (Hodgkin *et al.* 1984). When a rod in low $[Ca^{2+}]_o$ receives light stimuli, $[Ca^{2+}]_i$ in the rod would decrease further, because light stimuli decrease $Ca^{2+}$ influx through the photosensitive channel but do not suppress the $Na^+/Ca^{2+}$ exchange pump (Yau & Nakatani, 1985). The decrease in $[Ca^{2+}]_i$ would activate guanylate cyclase (Koch & Stryer, 1988) and then induce an increase of [cyclic GMP]$_i$ which would cause the increase of $g_{Na}$, i.e. the increase of the $Na^+$ dark current (Pugh & Cobbs, 1986).

In summary, low $[Ca^{2+}]_o$ has dual effects of rod photoresponses. The first effect is an increase in response amplitude, which could be caused by an increase in [cyclic GMP]$_i$ in the dark. The second effect is the rapid recovery of photoresponses, which could be caused by an increase in [cyclic GMP]$_i$ in the light.

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