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Ca^{2+}-DEPENDENT PHOTORECEPTOR POTENTIAL IN *PARAMECIUM BURSARIA*

BY YASUO NAKAOKA, KOHSAKU KINUGAWA AND TOHRU KUROTANI

Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka 560, Japan

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SUMMARY

Photostimulation of deciliated specimens of chlorella-free *P. bursaria* elicited a transient depolarization of the membrane potential. The amplitude of this receptor potential became larger as light intensity was increased and the relationship showed a Michaelis equation. The action spectrum of the receptor potential showed two peaks at around 420 and 560 nm. When constant current was injected to depolarize the membrane gradually, the receptor potential showed a decrease in amplitude. The potential at which the response disappeared depended on the external concentration of Ca^{2+} (27 mV/log $[Ca^{2+}]_o$), and the amplitude of the receptor potential was also dependent upon external Ca^{2+} concentration. Therefore, the receptor potential is primarily caused by a transient increase in the membrane conductance to Ca^{2+}.

INTRODUCTION

Paramecium bursaria is a ciliated protozoan which contains symbiotic green algae, chlorella (Muscatine, Karakashian & Karakashian, 1967; Brown & Nielsen, 1974). It shows an avoiding reaction when it comes to a shaded area (Engelmann, 1882) or when exposed to a step-decrease in light intensity (Saji & Oosawa, 1974). Such a photoresponse is mediated by the presence of the chlorella. In chlorella-free *P. bursaria*, an avoiding reaction is generally given to lighted areas or in response to a step-increase in light intensity (Iwatsuki & Naitoh, 1981; Niess, Reisser & Wiessner, 1981).

In *P. caudatum* and *P. aurelia*, swimming behaviour has been shown to be closely related to the membrane potential (Naitoh & Eckert, 1974; Eckert & Brehm, 1979) and a transient change in this potential, termed a receptor potential, is given in response to a mechanical or thermal stimulus (Naitoh & Eckert, 1969; Ogura & Machemer, 1980; Toyotama, 1981; Hennessey, Saimi & Kung, 1983; Nakaoka, Kurotani & Itoh, 1987). *P. bursaria* has not been studied electrophysiologically.

In this investigation, we show that a step-increase in illumination induces a receptor potential in chlorella-free *P. bursaria*. The receptor potential is caused by a transient increase in Ca^{2+} conductance of the membrane.

Key words: *Paramecium bursaria*, photoreceptor, membrane potential, action spectrum, Ca^{2+}.

MATERIALS AND METHODS

Cells

A chlorella-free type of *Paramecium bursaria* (Mit-C^{w}, supplied by Dr I. Miwa of Ibaraki University) was cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. The cultures were maintained on a fixed illumination cycle of 12 h dark and 12 h light (a fluorescent lamp of about 10^3 lux) at 22°C. Stationary phase *Paramecium* were collected by low-speed centrifugation and suspended in a control solution containing 0·25 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} KCl and 2 mmol l^{-1} Tris-HCl (pH 7·2).

Deciliation

To carry out the experiments in the absence of action potentials, the cells were deciliated by incubation in control solution containing 5 % ethanol for 2 min at 22°C, and returned to control solution (Ogura & Machemer, 1980). Intracellular recordings were started about 30 min after deciliation and continued for 2–3 h. Data were obtained only from cells which showed no action potentials.

Intracellular recording

Methods of intracellular recording were similar to those described by Naitoh & Eckert (1972). The electrodes were filled with 0·1 mol l^{-1} KCl, or 0·1 mol l^{-1} KCl plus 0·1 mol l^{-1} EGTA neutralized with KOH, and their resistances were 100–130 MΩ. The cells were placed in a glass vessel mounted on an inverted microscope, and electrodes were inserted from the upper side (Nakaoka *et al.* 1987).

The membrane potential was set at various levels by injection of constant current of the order of 10^{-10} A. Twenty to thirty seconds after the potential had been set, and when it had become relatively stable, photostimulation was commenced.

Light stimuli

The light source for stimulation was a 100-W halogen lamp with a variable-supply voltage. An i.r. filter and an interference filter with a half-bandwidth of 9–11 nm were placed in front of the lamp to obtain monochromatic light. Short pulses of light were obtained by using a camera shutter between the lamp and the microscope stage. A photocell was placed between the shutter and the stage to record the light pulse.

Light intensity was measured with a calibrated silicon photodiode. Light stimuli were usually of white light, with an intensity of about 1 mW cm^{-2}, in the plane of the specimen which elicited a maximum amplitude of receptor potential. To determine the dependence of the receptor potential upon light intensity or wavelength, stimulations were made using monochromatic light.

The light source for observing the cell was a 15-W tungsten lamp with a 700-nm long-pass filter.

RESULTS

EGTA in the microelectrode

A step-increase in illumination produced a transient change in membrane potential (Fig. 1A). In initial experiments, using electrodes filled with 0·1 mol l^{-1} KCl, it was

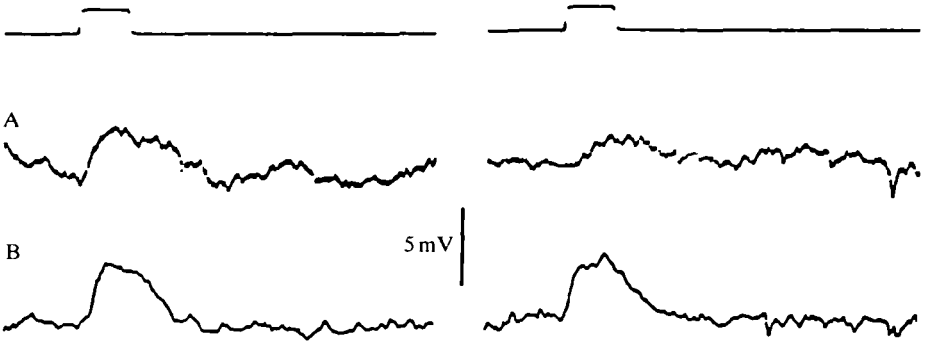

Fig. 1. Recording of potential change elicited by photostimulation. Upper trace shows light pulses of 0·5 s duration. (A) Potential change recorded when the electrode was filled with 0·1 mol l^{-1} KCl. Resting potential was −27 mV. Left; record of the third stimulation. Right; record of the fifteenth stimulation. (B) Potential change recorded when the electrode was filled with 0·1 mol l^{-1} KCl plus 0·1 mol l^{-1} EGTA. Resting potential was −25 mV. Left; record of the third stimulation. Right; record of the sixtieth stimulation. External medium contained 4 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} KCl and 2 mmol l^{-1} Tris-HCl (pH 7·2).

found that the amplitude of the potential change was gradually reduced by repetition of the stimulus (Fig. 1A). If electrodes were filled with 0·1 mol l^{-1} KCl plus 0·1 mol l^{-1} EGTA, then the potential change was not decreased by repetitive stimuli (Fig. 1B), so these electrodes were adopted for the following experiments.

Duration of the light stimulus

When the duration of the light pulse was 10^{-3} s, no potential change could be detected amongst the potential fluctuations (Fig. 2). A small potential change was elicited by a light pulse of 2×10^{-3} s. As the duration of the pulse was increased, the potential change grew larger in amplitude and duration, until at pulse lengths greater than 30^{-1} s, no further increase was observed (Fig. 2). The potential change was transient and the membrane potential recovered to almost the original level within 2 s, even when the light was continuously on.

Usually, a step-down in light intensity induced little change in the potential, but in a few cases a step-down induced a transient hyperpolarization.

Effect of light intensity

The effect of light intensity was examined using a standard pulse duration of 0·5 s. The response gradually increased with the increase in light intensity (Fig. 3A), and the relationship was fitted by the Michaelis equation,

$$R/R_{max} = I/(I + I_o)\,, \qquad (1)$$

where R represents the amplitude with maximum R_{max}, and I represents the light intensity at half-saturation intensity I_o (Fig. 3B).

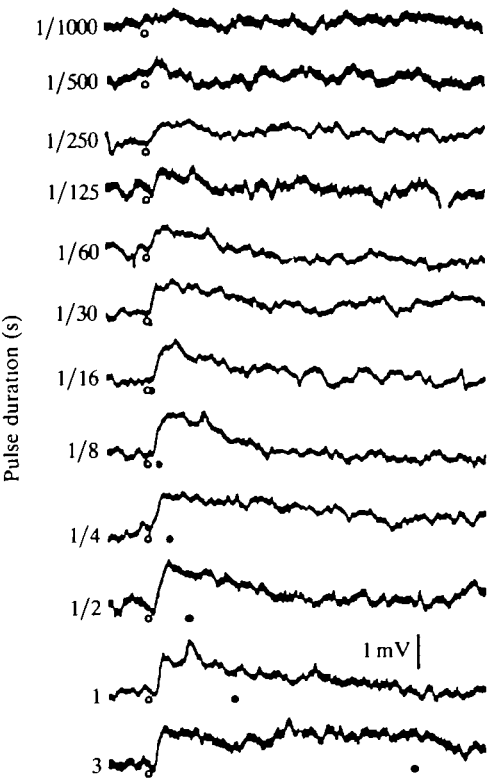

Fig. 2. Dependence of receptor potential upon light pulse duration. Figures on the left indicate pulse duration (in s). Open and closed circles show light-on and light-off, respectively. Resting potential was −29 mV. External medium contained 0·1 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} KCl and 2 mmol l^{-1} Tris-HCl (pH 7·2).

Action spectrum of the receptor potential

To obtain an action spectrum, the wavelength of the stimulating light was changed and the amplitude of the elicited potential change was measured (Fig. 4). Large depolarizations were induced at two wavelengths: approx. 420 nm and approx. 560 nm.

Voltage-dependence of the receptor potential

The resting potential was shifted by injection of a constant current, and then a light stimulus was applied (Fig. 5A). The elicited depolarization became larger when the membrane potential was made more negative than the resting potential. When the membrane potential was made more positive, the response to the light stimulus became smaller. Further positive shift caused the response to disappear and increased the fluctuation of the membrane potential. The polarity of the receptor potential was not reversed by the positive shift. A negative shift increased the amplitude of the response.

The positively shifted potential at which the response disappeared was largely dependent on the Ca^{2+} concentration in the external medium (Fig. 5B). This

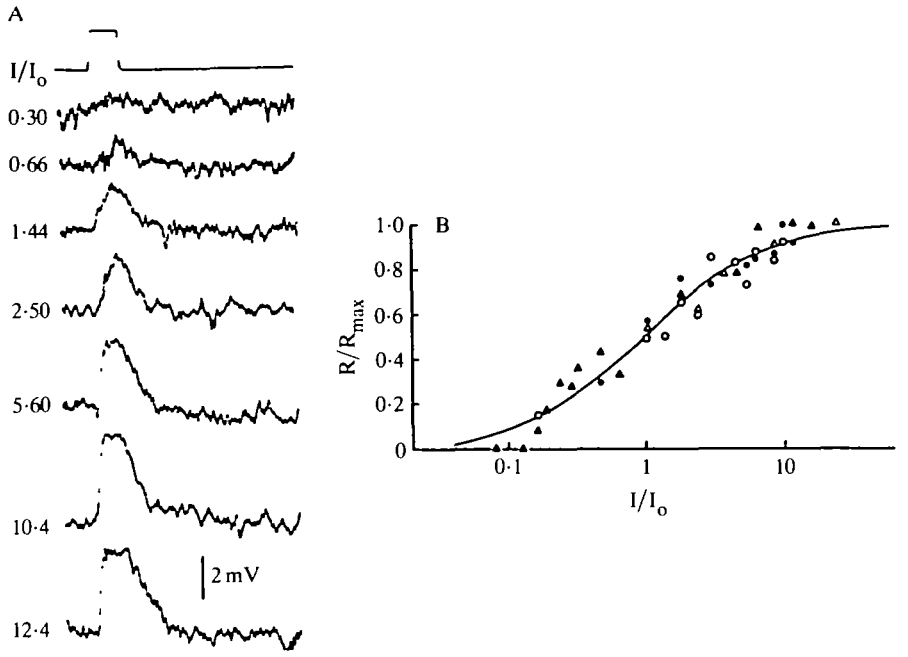

Fig. 3. Dependence of potential change upon light intensity. (A) Potential changes elicited by light pulses of graded intensity at 560 nm. Figures on the left indicate normalized light intensity (I/I_o). (B) Relationship between normalized amplitude of potential change (R/R_{max}) and normalized light intensity. Three cells are shown with a different symbol for each. The curve is equation 1. External medium was similar to that given in the legend to Fig. 1.

potential showed a slope of 27 mV for a 10-fold change in Ca^{2+} concentration. Addition of K^+ or Mg^{2+} to the external medium had little effect on the potential at which the response disappeared, but resulted in depolarization of the resting potentials.

DISCUSSION

The receptor potential obtained in response to successive step-increases in illumination showed a reproducibility when the electrodes contained EGTA, and showed a gradual decrease when the electrodes did not. It is therefore likely that some of the EGTA in the electrode diffuses into the cytoplasm and acts to maintain a low Ca^{2+} concentration, which is a necessary condition for generation of the receptor potential.

The action spectrum in the present study (Fig. 4) was obtained by measurements of receptor potentials. However, this spectrum differs from those of previous reports which were obtained by counting cells accumulating in response to light stimuli. Iwatsuki & Naitoh (1981) reported that chlorella-free specimens show photodispersal at 560 nm, but no dispersal at around 420 nm. Pado (1972) showed that chlorella-containing specimens accumulated at about 420 nm, but did not accumulate at

560 nm. We have observed that, at both 420 nm and 560 nm, ciliated and chlorella-free specimens show a photophobic swimming response (unpublished data). Such a difference in the action spectra may come from some differences in the specimens used for the experiments. Accurate determination of the action spectrum is needed for identification of the photoreceptor chromophore.

A positive shift of the membrane potential, produced by current injection, decreased the amplitude of the potential change induced by the light stimulus, and a negative shift increased the amplitude (Fig. 5A). This observation rules out the possibility that the membrane permeability for Na^+ or K^+ contributes to the potential change, because the concentrations of these ions inside the cell are higher than those outside (Yamaguchi, 1963; Oka, Nakaoka & Oosawa, 1986) and the equilibrium potentials of these ions are at negative levels below the resting potential in our experimental conditions. The positively shifted potential at which the response disappeared was Ca^{2+}-dependent (27 mV/log $[Ca^{2+}]_o$) and was almost independent of K^+ or Mg^{2+} concentration (Fig. 5B). Because the Ca^{2+} dependency approaches the theoretical slope of 29 mV for a Ca^{2+} diffusion potential, the transient depolarization elicited by step-up photostimulation is interpreted to be primarily caused by a transient increase in the membrane conductance to Ca^{2+}. This interpretation is in agreement with the observation that the polarity of the receptor potential is not reversed by a positive potential shift, because an extremely low

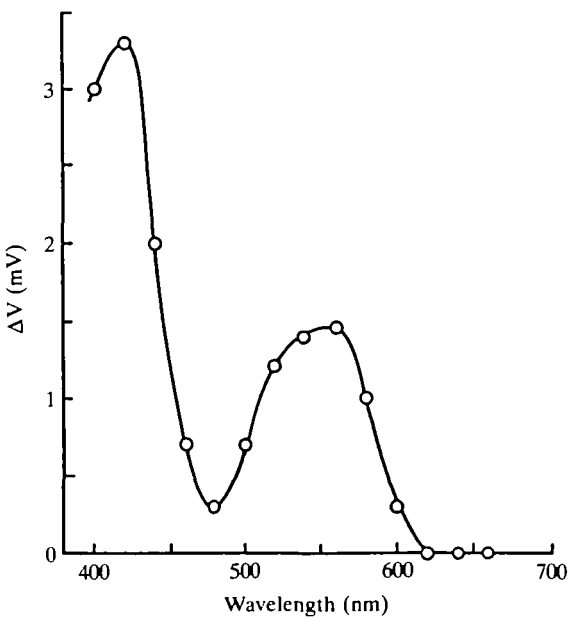

Fig. 4. Action spectrum of potential change. 0·5 s light pulses of various wavelengths were applied and the amplitudes of the potential changes were measured. Light intensity at the specimen was controlled at 0·7 mW cm^{-2}. External medium was control solution. Resting potential was -27 ± 3 mV. Potential changes are the mean of three different specimens.

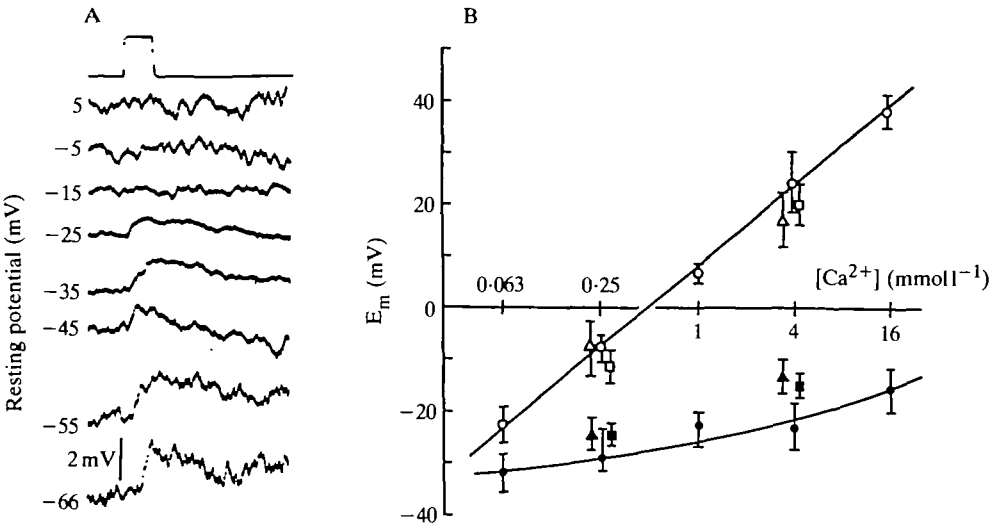

Fig. 5. Dependence of receptor potential upon potential shift. (A) Receptor potentials at various shifted potentials. Resting potential, −25 mV, was shifted to various levels indicated by the figures on the lefthand side, then a 0·5 s light pulse was applied. External medium was control solution. (B) Ca^{2+}-dependence of the potential at which the receptor potential disappears. When external media contained various concentrations of $CaCl_2$, 2 mmol l^{-1} KCl and 2 mmol l^{-1} Tris-maleate (pH 7·2), the resting potential (●) and the potential at which the response disappeared (○) were measured. To media containing 0·25 mmol l^{-1} and 4 mmol l^{-1} $CaCl_2$, 8 mmol l^{-1} KCl (▲, △) or 2 mmol l^{-1} $MgCl_2$ (■, □) was then added. Closed symbols show resting potentials and open symbols show the potential at which the response disappeared. $N = 3$–6. Vertical lines show range of measured values.

concentration of intracellular Ca^{2+} prevents outward flux of Ca^{2+}, even when the membrane conductance to Ca^{2+} increases.

In various vertebrate and invertebrate photoreceptors, the receptor potentials are primarily caused by a change in membrane conductance to Na^+ (Brown, Hagiwara, Koike & Meech, 1970; Yau, MacNaughton & Hodgkin, 1981). The receptor potential in scallop retina is, exceptionally, caused by an increase in membrane conductance to K^+ (Gorman & McReynolds, 1978).

To estimate how much the Ca^{2+} conductance increases with photostimulation, the amplitude of the potential change which is elicited at -30 ± 2 mV in the experiments of Fig. 5 are summarized in Fig. 6. The amplitude increases linearly with the increase in Ca^{2+} concentration. The slope is 2·2 mV/log $[Ca^{2+}]_o$. For a conventional interpretation, the following assumptions are made. Resting potential, E_m, is described by the equations:

$$E_m = (g_K/G)E_K + (g_{Ca}/G)E_{Ca}\,, \tag{2}$$

$$G = g_K + g_{Ca}\,, \tag{3}$$

where g_K and g_{Ca} represent the membrane conductances to K^+ and Ca^{2+}, respectively, E_K and E_{Ca} represent the diffusion potentials of the respective ions, and

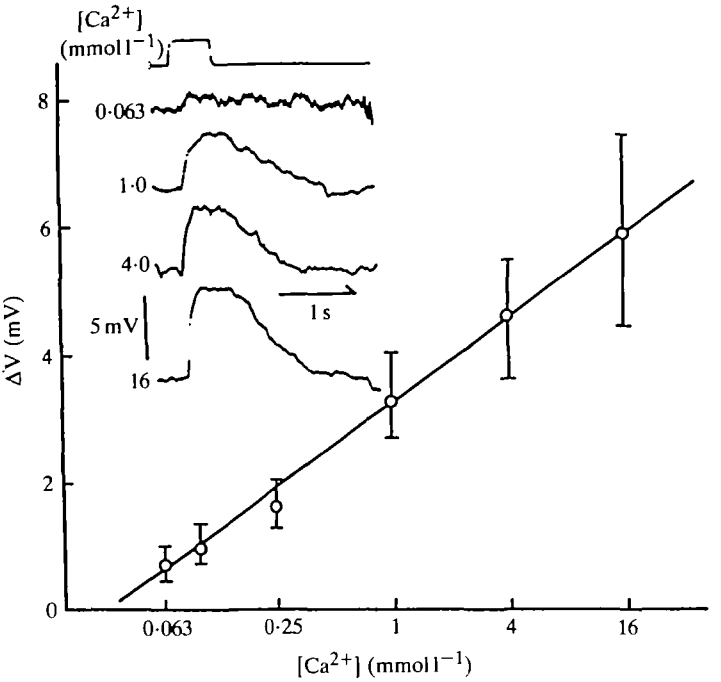

Fig. 6. Ca^{2+}-dependence of the amplitude of the receptor potential. The amplitudes of the receptor potentials elicited at -30 ± 2 mV in Fig. 5 are shown. Inset: typical recordings of receptor potentials in various concentrations of external Ca^{2+}. $N = 3$–7. Vertical lines show range of measured values.

application of photostimulation changes only g_{Ca} by Δg_{Ca}. When E_m is fixed at -30 mV, the slope of Ca^{2+} dependency roughly reflects $(\Delta g_{Ca}/G)E_{Ca}$. Ten-fold changes in external Ca^{2+} concentration change E_{Ca} by 29 mV and the response by 2·2 mV. Then, the ratio $\Delta g_{Ca}/G$ is 0·07. That is, 7% of the total conductance is increased by photostimulation.

In this study, measurements were started about 30 min after deciliation and continued for 2 h, and it is possible that the cilia had begun to regenerate during this period (Machemer & Ogura, 1979). Therefore, we cannot exclude the possibility that part of the Ca^{2+} conductance increased by photostimulation is located on the ciliary membrane. In a ciliated cell, the small depolarization elicited on the soma membrane can open voltage-sensitive Ca^{2+} channels on the ciliary membrane (Ogura & Takahashi, 1976; Dunlap, 1977). Opening of the voltage-sensitive channel could be accompanied by a large depolarization and an influx of Ca^{2+} into cilia, and this could induce photophobic behaviour.

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