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Properties of the On-Transient of the Intracellular Response in the Barnacle Photoreceptor*

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Abstract. We have studied the on-transient of the receptor potential of the barnacle photoreceptor. Its amplitude has previously been shown to depend on light intensity and state of light-dark adaptation. We have examined its dependence on 1) the presence of a prolonged depolarizing afterpotential (PDA), 2) a background light, 3) added alcohol, or 4) decreased K^+ concentration in the bath. We find that the relative on-transient amplitude tends to increase initially with increasing depolarization arising from 1)–4) and then to decrease again at higher depolarization. This behavior is qualitatively explainable by the cell's current-voltage characteristics and by the adapting effect of the stimulus on the conductances arising from the PDA, the background light and the alcohol.

Key words: Photoreceptor – Late receptor potential.

Introduction

The invertebrate photoreceptor's response to light (the late receptor potential or LRP) is a depolarization which may exhibit at least six phases. In time order they are:

1. An on-transient – a dynamic phase with rapid rise time.
2. A dip – a brief and generally partial repolarization.
3. A steady state or plateau.
4. An off-transient.
5. A prolonged depolarizing afterpotential or PDA. The PDA is normally induced by a net transfer of visual pigment from the rhodopsin to the metarhodopsin state.
6. An after-hyperpolarization which presumably arises from an electrogenic pump.

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We have noticed in the barnacle photoreceptor a great variability in the appearance and amplitude of these phases. Therefore we have examined some factors which can influence the shape of the LRP.

In this article we deal with the initial phase of the LRP – the on-transient. The on-transient is known to depend upon light intensity and state of light/dark adaptation (Millecchia and Mauro, 1969; Brown et al., 1970). We have examined the dependence of the on-transient upon membrane potential changes induced in various ways. These results form the background for a later report on the effect of pigment transfer on the shape of the LRP. The existence and nature of such an effect should throw new light on the transduction process.

Method

The lateral ocelli of *Balanus amphitrite* were excised and placed in a chamber perfused with seawater. The cornea was removed after bathing the preparation for 4 min with 1.5% collagenase and 1.5% protease.

The techniques of intracellular recordings and the optical apparatus have been described previously (Hillman et al., 1973; Minke et al., 1973). "Blue" and "red" stimuli were obtained by filtering the light from the quartz-iodide lamp through K3 (λ_{max} = 480 nm) and K5 (600 nm) Balzers broad band interference filters. Calibrated neutral density filters (Ditric Optics) provided the required intensity. We used long trains of stimuli composed of 1–2 s duration pulses with $\frac{1}{2}$–2 s intervals so as to monitor the development of the various phases of the LRP with stimulus duration.

Results

On-transient Dependence upon Various Treatments Modifying Membrane Potential

a) PDA. After blue adaptation (putting most of the pigment into the rhodopsin state) and 3 min of dark adaptation, a train of 1-s red stimuli was given. For stimuli of low intensity (Fig. 1a) the PDA developed gradually, and the on-transient amplitude correlates roughly, but definitely not uniquely, with the dark depolarization (PDA). For a higher stimulus intensity, with the PDA reaching its maximal depolarization in 2 s, the maximal on-transients appeared later on the PDA (Fig. 1b).

b) Background Light. On a train of bright neutral stimuli (blue or red), the on-transient is normally lower than the steady-state. A background blue light resulting in a steady depolarization raised the on-transient above the steady-state level (Fig. 2).

c) The Effect of Butanol 0.9%. Perfusing the cell with alcohol in seawater causes membrane depolarization (Shaw et al., 1978). When perfused with 0.9% butanol in

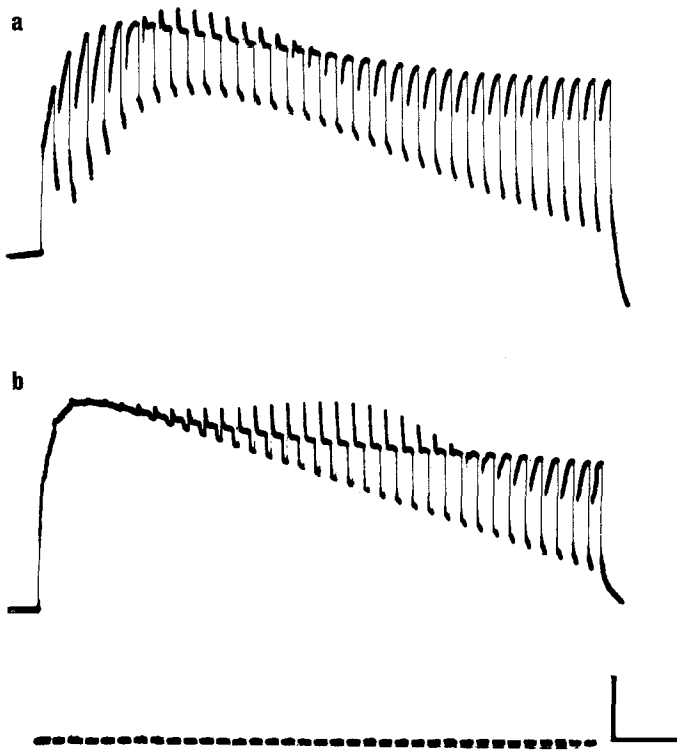

Fig. 1a and b. The on-transient during a PDA. All traces in all figures are intracellular recordings from *Balanus amphitrite* photoreceptors at 22° C. In all cases, a train of stimuli of 1–2 s duration is presented, with $^1/_2$–2 s dark intervals to make it possible to follow the time course of all the phases of the response. The "on-transient" in all figures is the initial rapid rise in the response each time the light is switched on and whose peak is indicated by the transition from a weak (or invisible) rising trace to a strong black trace. Calibration for all figures: 20 mV, 10 s. A few parts of the traces have been strengthened by hand for clarity. **a** A blue-adapted cell is exposed to relatively weak red light which transfers most of the pigment from the rhodopsin to the metarhodopsin state in about 20 s. The absolute amplitude of the on-transient increases with the PDA (lower envelope of the trace) but decreases initially more rapidly and then more slowly than the PDA. **b** A stronger red light, transferring the pigment in about 2 s. The time-course of the on-transient is now quite different from that of the PDA

seawater, the cell of Figure 3 depolarized slowly to a steady level. At the same time the on-transients increased gradually to their maximal size and then began to decrease despite continuing depolarization. During washing, the absolute amplitudes of the on-transients decreased as the depolarization decreased.

d) The Effects of Increasing External K^+ Concentration $c_K{}^+$. The amplitudes of the on-transients on the responses to PDA-inducing stimuli were examined: First, as a control, in reduced-Na^+ Ringer ($c_{Na}{}^+ = 362$ mM, $c_{Choline} = 100$ mM; normal $c_{Na}{}^+ = 462$ mM); then in high-K^+ Ringer ($c_K{}^+ = 108$ mM, $c_{Na}{}^+ = 362$ mM; normal $c_K{}^+ = 8$ mM). Figure 4b shows that in reduced Na^+, a small hyperpolarization developed

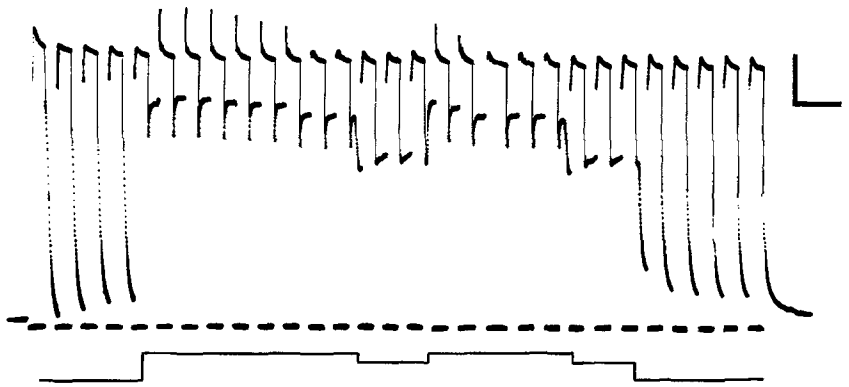

Fig. 2. The on-transient during background lights. The blue stimuli are presented in the dark and during two different intensities of blue background lights. The on-transient amplitude correlates with the light-induced depolarization, but not linearly nor uniquely

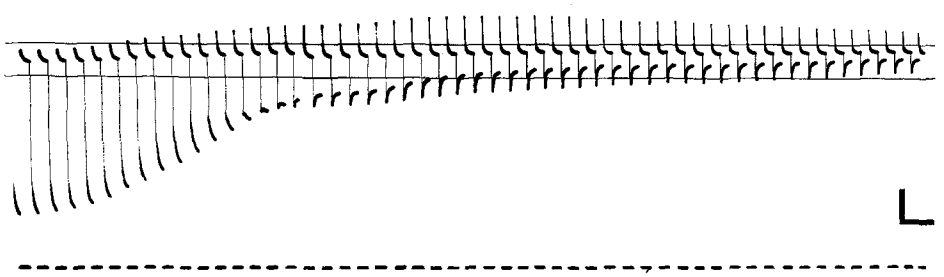

Fig. 3. The on-transient in the presence of alcohol. Butanol (0.9%) has been added to the bathing medium. The cell gradually depolarizes; the on-transient amplitude initially increases but then decreases despite a continued rise in the depolarization

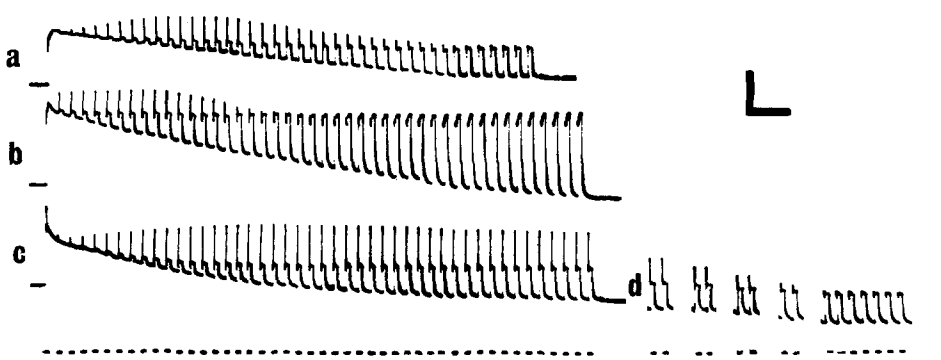

Fig. 4a–d. The effect of increased c_K^+ on the on-transient. **a** The PDA in normal Ringer. **b** The PDA in Ringer with 100 mM of the Na^+ replaced by choline. **c** The PDA in Ringer with 100 mM of the Na^+ replaced by K^+, increasing c_K^+ by about a factor of 10. **d** Washing out with normal Ringer. The high c_K^+ causes a sustained depolarization and a sustained increase in the on-transient

and the on-transients grew and declined a little earlier than in normal Na^+ (Fig. 4a). In high K^+ (Fig. 4c) there was some depolarization and the on-transients became larger and maintained their heights over a much longer period. Washing out with normal Ringer caused a decline of the on-transient (Fig. 4d).

Discussion

From Figures 1–4 one sees that various means of inducing depolarizations in the barnacle photoreceptor (PDA induction, background lights, alcohol, and increased external K^+) all enhance the amplitude of the on-transient. In Figures 1b, 3 and 4 there is a suggestion that depolarization beyond a certain level leads to a decline in the amplitude which does not seem to be solely a voltage or conductance saturation effect.

Two factors have been considered as sources of this behavior: The current-voltage characteristic of the cell, and (in Figures 1–3) the adapting effect of each stimulus on the background conductance.

Responses to current injection into current-depolarized cells (results not shown here) indeed exhibit on-transients whose amplitudes, although small, have the right qualitative dependence on depolarization; and the adapting effect should also have a maximum at intermediate depolarizations, since at resting level there is nothing to adapt, while in the presence of very strong background conductances the stimuli will be relatively too weak to have any effect.

These two factors may account for most of the present observations.

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