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Light-activated single channel currents in *Limulus* ventral nerve photoreceptors

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Abstract. Light-activated single channel currents were measured in *Limulus* ventral photoreceptors in the cell-attached configuration at 14 °C. The results show three channel types with conductances of 6.2, 10.4 and 28.7 pS. The most active channels have the 10 pS conductance; the open time histograms of these channels could be best fitted by the sum of two exponentials with time constants (and weights) of 0.58 ms (0.78) and 4.32 ms (0.22), suggesting two populations of channels or two open states. The mean open time was 1.38 ms. The open time histogram of the channels with the 29 pS conductance could be best fitted by a single exponential with a time constant of 3.35 ms. First latencies of the 10 pS channels were between 40 and 280 ms but those of the 29 pS conductance channels were ≥ 300 ms. These findings suggest that the two channel types are gated by two different intracellular transmitters or mechanisms.

Key words: Light activated channels – *Limulus* photoreceptor – Single channels

Introduction

In *Limulus* ventral nerve photoreceptors a conformational change of a rhodopsin molecule, caused by absorption of a photon, triggers an enzmye cascade resulting in an increase in the concentration of a putative intracellular transmitter (Cone 1973; Becker et al. 1988; Stieve 1989). Transmitter molecules bind to channel proteins, increase the open probability and depolarize the cell. So far, only one population of light-activated channels has been found and studied extensively in *Limulus* photoreceptors (Bacigalupo and Lisman 1983, 1984; Bacigalupo et al. 1986). Results presented here indicate that there are three light-activated cation channels, each with unique conductance and kinetic properties. It is suggested that two of these channels are triggered during a photoresponse by different transmitters.

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Material and methods

Desheathed ventral nerves of *Limulus* were treated with 2% pronase for one minute. Axons, connective tissues and glial cells covering the photoreceptor cells were removed by a suction electrode. The R- and A-lobe of denuded cells (Bacigalupo et al. 1986) could be distinguished and were used for experiments without further treatment. Patch pipettes (made as described elsewhere: Nagy et al. 1983) set on the R-lobe formed seals of 15 to 50 GOhm with moderate success ($\sim 20\%$ of pipettes). In about 10% of patches light-activated channels were found. Pipette and bath solutions were identical, containing (in m*M*): 425 NaCl, 10 KCl, 10 $CaCl_2$, 22 $MgCl_2$, 26 $MgSO_4$ and 15 HEPES. pH was adjusted to 7.8.

Photoreceptors were stimulated by white light of variable duration (max. intensity, $I_0 = 8$ mW/cm^2). Single channel currents were measured in the cell-attached configuration (Hamill et al. 1981) at 14 °C, at constant hyperpolarizing potentials (relative to the cells intracellular potential, which were between -45 and -36 mV). Currents were filtered at 2 kHz (-3 dB, four-pole Bessel filter) and stored in a pulse code modulated form (sampling frequency 44.1 kHz) on video tapes. 3 or 30 s segments of these records were selected, digitized at 10 or 1 kHz respectively, and used for off-line analysis. To construct the *i-V* curves the single channel current sizes were estimated from amplitude histrograms constructed from the 2 to 5 s range of records (digitized at 10 kHz). At this time the intracellular membrane potential (measured by an intracellular electrode in these experiments) had reached a constant value. Delay times of channel openings (measured from the onset of the light) and channel open times were measured with the accuracy of the sampling rate of 0.1 ms. In the early phase of illumination ($t < 500$ ms) the usual event detection with the half-amplitude threshold could not be applied owing to the change of the intracellular potential and so the base line current. In this range, a semi-automatic program was used, which detected events if the current jump was three times larger than the standard deviation of the preceding base line. The range of the base line was selected by the operator.

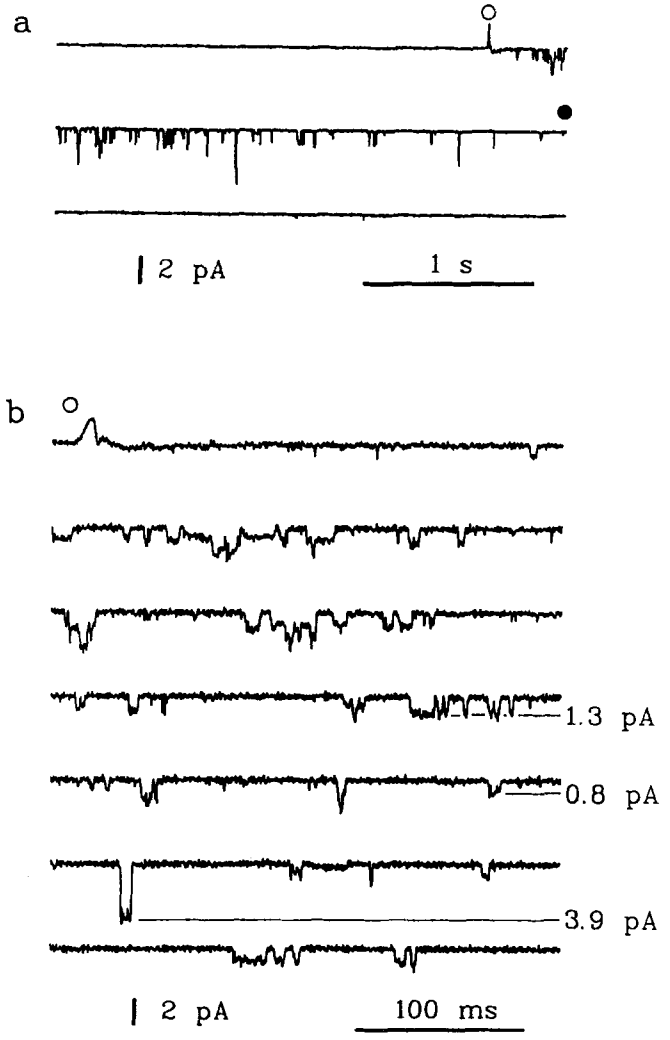

Fig. 1 a, b. Consecutive segments of two single channel currents recorded in *Limulus* ventral photoreceptor. **a** Inward currents (in negative direction) through light activated channels. The cell-attached patch was hyperpolarized by −70 mV. The intracellular potential was −11 mV at the end of the illumination. The sample frequency was 1 kHz. Open and filled circles indicate the switching on and off of the light, respectively. **b** Single channel currents with high resolution (10 kHz sampling frequency) at the same membrane potential as in **a**. The cell was illuminated continuously from the open circle. For **a** and **b**: the light intensity was $I_0/20$

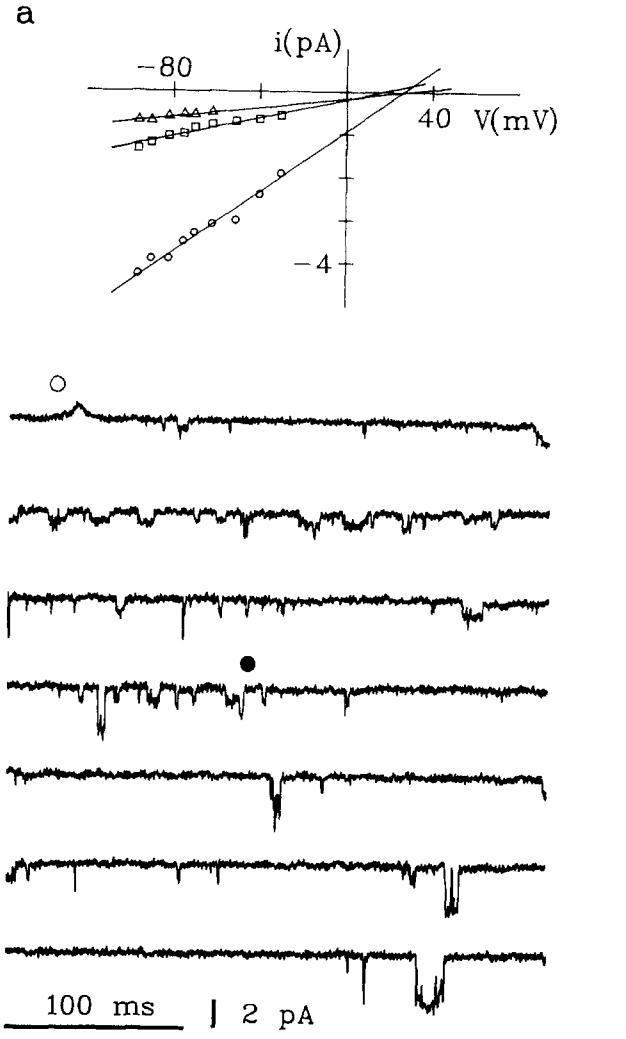

Fig. 2. a Single channel current amplitudes as a function of the membrane potential. The unitary conductance and reversal potential are: 5.1 pS and 30 mV for the triangles, 12.3 pS and 23 mV for the squares, 29.8 pS and 26 mV for the circles. The cell was stimulated for 5 s (light intensity: $I_0/50$). The intracellular potential was between −16 and −9 mV measured at the end of the illumination. **b** Segments of a single channel current record demonstrating short latencies of openings and the diminishing activity after stimulation. The patch was hyperpolarized by −50 mV. Sample frequency 10 kHz, light intensity I_0

Results

Figure 1 a shows a single channel current record, which demonstrates that the openings of channels are elicited by light. No channel openings occur before the illumination. Channels open soon after the onset of the light and the activity disappears after switching of the light. The different current amplitudes suggest that light-activated channels with different conductances function in the patch. Figure 1 b shows a current record on an expanded time scale. For this record the most frequent current size is 1.3 pA and occasionally unitary currents of 0.8 pA and 3.9 pA are also observed.

Single channel current amplitudes were measured between −100 and −30 mV and plotted as a function of the membrane potential (Fig. 2 a). The single channel conductances and the corresponding reversal potentials (V_{rev}) of the three channel types are (from four patches): 10.4 (±2.1) pS. $V_{rev} = 22.8$ (±3.3) mV for the most active, 6.2 (±2.9) pS, $V_{rev} = 31.2$ (±5.6) mV for the channel with the smallest conductance and 28.7 (±3.4) pS, $V_{rev} = 28.1$ (±4.4) mV for that with the largest conductance. In all 9 patches used for the present analysis these three channels could be observed. (The 10 pS channel has sub-conductance states which will be described elesewhere.)

Figure 1 b suggests that the three channels differ not only in the unitary conductance, but also in kinetic properties. Channels with the most frequently observed current size (conductance 10 pS) opened with the shortest delay after the onset of the light (Fig. 2 b). Values for the

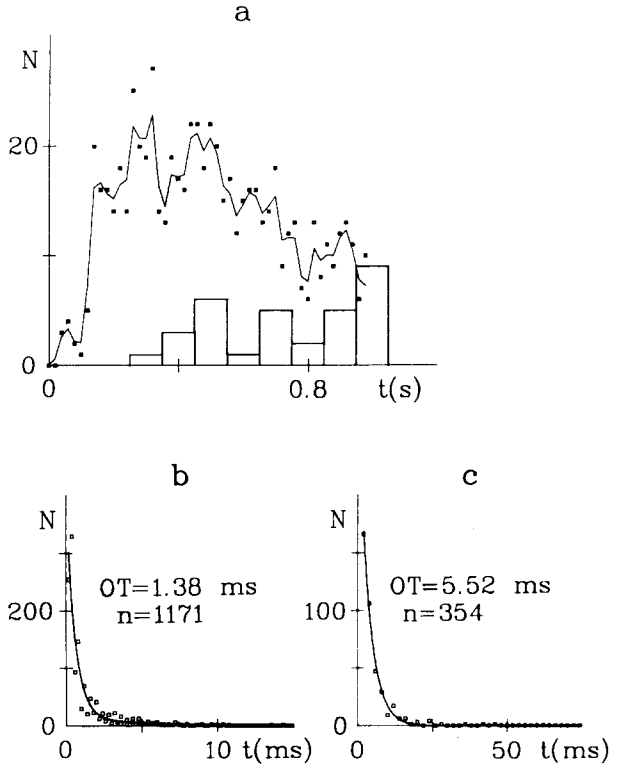

Fig. 3a. Latency histogram of single channel openings. Filled squares show the latencies of the openings of the 10 pS channels (continous line is the histogram after smoothing), bar graph shows the latency of openings of the 29 pS conductance channels. The patch was hyperpolarized by −40 mV, the cell was stimulated for 1 s from time 0, the light intensity was I_0. **b** Open time histogram of the most frequent channels. Time constants of the fit are 0.6 ms and 4.0 ms with weighting factors of 0.78 and 0.22, respectively. **c** Open time histogram of the large conductance channel. The fit resulted in a time constant of 3.5 ms. For **b** and **c**: the patch was hyperpolarized by −50 mV, light intensity was I_0. *OT* indicates the mean open time, *n* the number of events

first latencies were between 40 ms and 280 ms. The activity of this channel diminished after illumination (see Fig. 2b). The channel with the 29 pS conductance opened with a distinctly longer first latency (>300 ms) and remained active for a longer time after strong illumination (compare Fig. 1a and Fig. 2b). The first latency of the 6 pS conductance channel could not be determined with certainty owing to the small current amplitude in the early part of the illumination.

The latencies of all openings of the medium and large conductance channels are plotted in Fig. 3a, measured with 1 s illumination. The histogram shows that the 29 pS conductance channels (bar graph in Fig. 3a) are activated later than the 10 pS channels. The activity of the 10 pS conductance channel increases in the first 300 ms, but decreases after about 600 ms. The mean open time of the 10 pS channel was 1.38 ms ($n=1171$) and of the 29 pS conductance channel was 5.52 ms ($n=354$). Open time histograms of the 10 pS channels (Fig. 3b) could be best fitted by the sum of two exponentials resulting in time constants of 0.58 ms and 4.32 ms, and relative weights of 0.78 and 0.22. The open time histogram of the 29 pS conductance channel (Fig. 3c) could be best fit by a single exponential with a time constant of 3.35 ms. The averaged value of the open time (OT = 5.52 ms) is larger than the time constant of the fit indicating a slow component (note events at $t>25$ ms). However, owing to the small weight of these events a two exponential function did not result in a better fit than a single exponential.

Discussion

Results presented here show that there are three different light-activated channels in *Limulus* ventral nerve photoreceptors. The channel currents have similar reversal potentials (between 22 and 31 mV), but different unitary conductances and kinetics. The reversal potentials are close to those obtained for the macroscopic currents (18 mV, Brown and Mote 1974; 10–15 mV; Milecchia and Mauro 1969). Results are different from those published for the light-activated channels of the same preparation by Bacigalupo and Lisman (1983, 1984) and Bacigalupo et al. (1986). These authors reported a conductance of 43 pS for the most active channels and a very long and variable first latency, up to 5 s. Results presented here show ~10 pS conductance and first latency times between 40 and 280 ms. These latencies are in the range of the first latency of single photon responses, quantum bumps (Stieve 1985) and of macroscopic currents (Milecchia and Mauro 1969). Bacigalupo and Lisman (1983) also occasional observed a small conductance channel or substate.

The discrepancy between the present and the earlier results may be due to the different method of preparation. Bacigalupo and Lisman (1983, 1984), and Bacigalupo et al. (1986) mildly sonicated the nerves before the experiments in contrast to the present investigations. Sonication may result in some damage to the membrane which could change the local ion concentration and perhaps the pathway of the transmitter. A change in the ion concentration, for instance, might modify the single channel conductance (see Horn and Patlak 1980) and the reversal potential. Another explanation could be that different regions of the cell membrane are free for seal formation on sonicated as compared with non-sonicated cells. This would explain why different channel types were not reported earlier.

The conductance and latency of the 29 pS channel presented here are close to the parameters of the channels described by Bacigalupo and Lisman (1983, 1984), and Bacigalupo et al. (1986) and therefore, it may be the same channel. However the 10 pS conductance channel, which was most active in the non-sonicated preparations reported here, was not found in sonicated preparations. The open time histogram of the 10 pS channel could be best fitted by the sum of two exponentials. This suggests the presence of either two populations of channels having a similar conductance or one channel type with two kinetically distinct open states.

The mean open time of the 10 pS channel was 1.38 ms in contrast to the value of ~4 ms reported for the same potential range by Bacigalupo et al. (1986). The longer

value published by these authors may be explained by the lower cut-off frequency of their recording system (~420 Hz, calculated from the published rise time of 0.8 ms; see Colquhoun and Sigworth 1983), which resulted in impaired detection of short events.

The 29 pS channel has a longer latency than the 10 pS channel. This suggests that they are triggered by different transmitters or by different intracellular mechanisms. One of the pathways is probably through inositol 1,4,5-trisphosphate (IP_3), which releases calcium ions from intracellular stores (Fein et al. 1984). Calcium which causes desensitization (light adaptation) may, in addition, be the transmitter which evokes the opening of one channel type. Injection of IP_3 into the photoreceptor cell elicited a transient current. This response, however, could be inhibited by intracellular EGTA, in contrast to the photoresponse (Payne et al. 1986). Therefore, it was suggested that either light has access to a pathway for opening of channels which is not accessible for IP_3, or light releases a further transmitter. The present results support the idea of two transmitters and suggest that the current components blocked or not blocked by EGTA are due to the two channel types present. The two components of the macroscopic currents (Lisman and Brown 1971; Maaz et al. 1981; Helrich et al. 1988) may also be elicited by these channels.

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