Two Different Bump Types of the Ventral Photoreceptor of *Limulus*

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The elementary responses, the "bumps", of the ventral photoreceptor of *Limulus polyphemus* were measured under voltage clamp conditions. We observed a type of bump which differs in size and time parameters from the "standard" bumps described previously. The average value of the amplitude of these "small, slow" bumps is about 20-fold smaller than that of the standard bumps (about 1 nA) and the average latency is about 25% longer. The duration of these bumps is 2–5-fold longer than that of a standard bump with the same amplitude. The small, slow bumps are probably light-induced and arise with about the same frequency after a light flash as standard bumps. The different kinetics of the two bump types can be explained by the assumption that the transduction chain branches into at least two pathways.

Materials and Methods

Limulus ventral nerves were dissected as described previously [1] and mounted in a test vessel, where they were continuously superfused with physiological saline (PS) at a flow rate of about 1 ml/min. The physiological saline (pH 7.5) contained (in mmol/l): Na^+ 480, K^+ 10, Ca^{2+} 10, Mg^{2+} 55, Cl^- 515, SO_4^{2-} 30, HEPES 10. The temperature was kept at 15 °C.

The membrane potential of the cell was clamped to the dark potential (about −45 mV), and the membrane current was measured with a single electrode voltage clamp (SC-100 Biologic, France) [2, 3]. The maximal error of the clamp voltage regulation was less than 1 mV. As light source a photoflash (Metz Mecablitz 60 CT-1) was used. The light stimuli with a half duration of 0.1 ms were filtered by a broadband interference filter of 540 ± 40 nm. Light-induced bumps were evoked by illuminating a dark-adapted photoreceptor with a very weak flash of light. The flash was repeated every 10 sec for 250–1000 times. The light energy was about 0.5×10^8 photons/cm^2, which evoked on the average about 0.75 light-induced bumps per flash. The experiments lasted 1 to 3 h. The sensitivity of the cells did not change significantly during this time. Spontaneous bumps were recorded in the time intervals between the light flashes when probably no more light-induced bumps occur (3rd to 10th second after flash).

The membrane current was digitized with a frequency of 1 kHz and stored on an IBM-compatible personal computer. The current traces were digitally filtered with a 50 Hz notch filter and a 70 Hz low pass filter [4]. Both filters had a 10th order Bessel characteristics. The bump parameters were evaluated by a computer program as described previously [5]. Each bump parameter was confirmed by visual inspection.

Results

Fig. 1 shows several recordings of the receptor current. The data presented here were obtained in one experiment. Similar results were found in 3 of 6 investigated photoreceptors.

Beside the large (amplitude = 0.38 nA) standard bumps described previously [5, 6], also very small fluctuations can be observed. The amplitudes of these fluctuations are about 25 pA. Their durations of about 230 ms are clearly longer than those of a very small standard bump (see trace 13 Fig. 1). In Fig. 1 only records with relatively small standard bumps are shown. This was done in order to illustrate the difference in the time course. Due to the small size and the long duration of these fluctuations we call them "small, slow" bumps.

Most of the records show both bump types, a small, slow bump and one or more (not shown) standard bumps. Very often the bumps are super-

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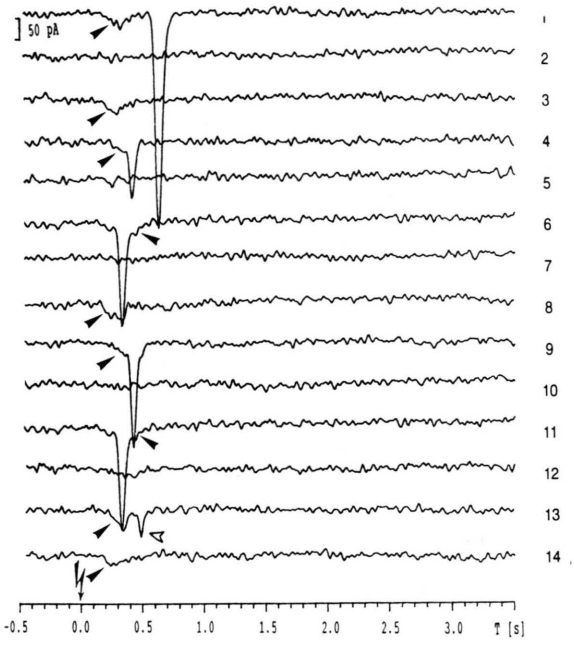

Fig. 1. Recordings of the current signal from the *Limulus* ventral photoreceptor in response to a flash at T = 0. Two different kinds of bumps are observed, standard bumps and small, slow bumps. The small, slow bumps are marked by arrows. The open arrow in trace 13 shows one very small standard bump. Because the amplitudes of most of the standard bumps are very large compared to the amplitude of small, slow bumps, only traces with small standard bumps were selected. The bump-evoking flash (2.7×10^7 photons/cm^2, 540 nm, duration 0.1 ms) was repeated every 10 sec. The membrane potential was clamped to −45 mV. Experiment HRE 140590, 15 °C.

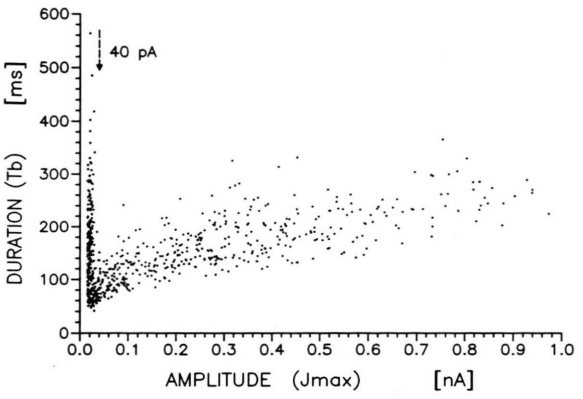

Fig. 2. The correlation plot of bump amplitude J_{max} and bump duration T_b shows two different populations. All bumps with amplitudes larger than 40 pA are standard bumps. The small, slow bumps have amplitudes less than 40 pA, moreover their duration is longer than those of small standard bumps. 761 single bumps evoked by 900 flashes were evaluated. Using the criteria described in the text, 473 standard bumps and 131 small, slow bumps can be selected. For 157 bumps it is not possible to decide to which of the two types they belong. Experiment: HRE 140590.

imposed (trace No. 1 shows one exception). However records containing only one bump type can also be observed. Records No. 3 and 14 show two examples where the light flash is followed by a small, slow bump only. Traces with only standard bumps were also measured, but very rarely (not shown). In this case it is not clear whether the record contains no small, slow bump or whether one is hidden in the noise or superimposed on the standard bump. The average noise amplitude (RMS) was about 5 pA in this experiment.

Duration

The different time course of the two bump types is clearly visible in a correlation plot of bump duration (T_b) *versus* bump amplitude (J_{max}). In Fig. 2 two different populations of bumps are distinguishable. The two bump types can be distinguished by the following criteria: All bumps with amplitudes larger than 40 pA are regarded as standard bumps (n = 571). All bumps with amplitudes less than 40 pA and durations larger than 160 ms are regarded as small, slow bumps (n = 131). The number of bumps with amplitudes less than 40 pA and durations less than 160 ms consist of a mixture of the two types. Bump-like fluctuations appearing with amplitudes less than 15 pA were not evaluated because they are not distinguishable from large noise peaks. So it is possible that some of the bumps are not recognized by us.

On the average the duration of the standard bumps increases with their amplitude (Fig. 2). The average bump duration can be described approximately by the following linear equation $T_b = A + B \cdot J_{max}$. The parameters A = 114 ms and B = 157 ms/nA were calculated by linear regression analysis for the experiment shown in Fig. 2. Using this equation we calculated the extrapolated average duration of a standard bump with an amplitude of 20 pA to be 117 ms. In contrast the average observed duration of small, slow bumps with an amplitude of about 20 pA is 226 ms.

Amplitude

The amplitude of the small, slow bumps is less than 40 pA. Using the criteria described above, the calculated average amplitude is 20 pA. Because only bumps larger than 15 pA can be evaluated the average amplitude of these bumps is probably still smaller. The average amplitude of the recognized light-induced standard bumps in this experiment was 0.38 nA. That means that the small, slow bumps are about 20-fold smaller than the standard bumps.

Latency

The latency (T_{lat}) is the time from the light flash to the first measurable deflection of the current from the base-line. Fig. 3 shows a comparison of the latency distributions for the two bump types. The latency distributions of the small, slow bumps and the standard bumps have similar asymmetrical shapes. The average latency of the small, slow bumps is 109 ms longer (about 25%) than that of the standard bumps. Because more than 90% of the small, slow bumps follow the light flash in less than a second it seems probable that they are induced by light like most of the standard bumps.

Spontaneous bumps

The number of 571 standard bumps contains a fraction of about 15% of spontaneous bumps which occur most probably independently of light with a rate of about 0.15 s^{-1} in this experiment. Spontaneous and small light-induced standard bumps have a similar time course and are not distinguishable in the correlation plot. The average amplitude of the spontaneous bumps in this experiment was 0.11 nA.

Most of the small, slow bumps are probably light-induced, but also spontaneous slow, small bumps may occur. Their rate of 0.008 s^{-1} is about 20-fold lower than that of the spontaneous standard bumps. If the spontaneous small, slow bumps are, as the spontaneous standard bumps, smaller than the light-induced bumps, most of them should be hidden in the noise.

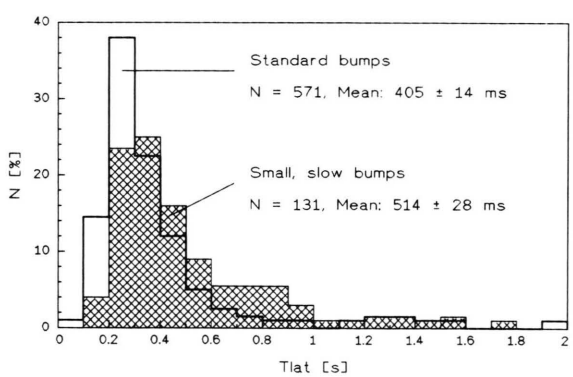

Fig. 3. Comparison of latency distributions for standard bumps and small, slow bumps. The different bump types were selected using the following conditions: a) small low bumps: $J_{max} < 40$ pA and $T_b > 160$ ms; b) standard bumps: $J_{max} > 100$ pA. Only single bumps were evaluated. Experiment: HRE 140590.

Conclusions

The small, slow bumps are different from the standard bumps in the following three features:

1. Their average latency is about 25% longer than that of the standard bumps.
2. Their average bump duration is about 2-fold longer than that of standard bumps with the same amplitude.
3. Their average amplitude is 20 pA or less. This is only 5% of the value of the standard bumps.

Similar results were observed in 3 of 6 cells. In the cells in which small, slow bumps could be detected the average noise amplitude (RMS) was less than 5 pA, in the other ones it was larger, about 5–20 pA. It is possible that the small, slow bumps are not recognizable in all photoreceptor cells, because they are hidden in the noise in some cells. Most of the small, slow bumps were probably triggered by light, but also spontaneous events were measured.

Discussion

The two different bump types seem to arise independently of each other. We observed records containing both bump types as well as such containing only one bump type in the same cell. Moreover, there are significant differences in the amplitudes and the time parameters of two bump types. Further evidence for more than one bump type was provided by Contzen *et al.* [7]. These results together with findings described in the literature (see below) are difficult to explain if the opening of the light-activated ion channels is assumed to be controlled by a linear transduction chain.

The light-induced current is composed of three components, which can be distinguished by varying the light intensity and the state of light adaptation [8]. Patch clamp investigations of the *Limulus* ventral photoreceptor have shown that there are three types of light-activated ion channels [9, 10]. These channels and macroscopic currents are different in the reversal potential as well as in the activation and deactivation kinetics. Furthermore there is controversial evidence that the light-induced conductance increase in the *Limulus* photoreceptor cell might be mediated either by an IP_3-calcium pathway [11, 12] or by cGMP [13, 14]. In the squid photoreceptor three different light-activated G-proteins were found [15]. Also for the *Limulus* photoreceptor there are indications that more than one G-protein is involved in phototransduction [16].

These and other results [3, 17] lead to the working hypothesis that the transduction chain of the *Limulus* photoreceptor is branched into two or probably even into three pathways [17]. Light-activated rhodopsin may activate three different types of G-proteins which in turn may start three branches of the transduction chain (see Fig. 4). The different types of G-proteins may even be activated by different types of rhodopsin.

A definite correlation between the different bump types and the different ion channels as well as to the different components of the macroscopic receptor current is not yet possible with these data, because they were taken under very different conditions (*i.e.* light intensity and state of dark adaptation).

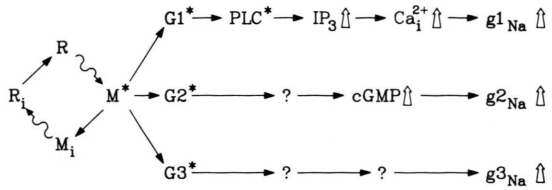

Fig. 4. Working hypotheses: trifurcated transduction sequence of the photoreceptor cell of *Limulus* [12]. R, rhodopsin; M*, active metarhodopsin; M_i, inactivated metarhodopsin; R_i, inactivated rhodopsin; G 1, G 2, G 3, three different types of G-proteins; PLC, phospholipase C; IP_3, inositol-trisphosphate; cGMP, cyclic guanosine monophosphate; $g1_{Na}$, $g2_{Na}$, $g3_{NA}$, three light-induced sodium conductances. Asterisks symbolize activated states, open arrows increases.

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