Spontaneous Bumps of the *Limulus* Photoreceptor Cell are Probably Triggered by the Spontaneous Activation of Single G-Protein Molecules

Helmut Reuß, Michael Rack, and Hennig Stieve

Institut für Biologie II, RWTH Aachen, Kopernikusstraße 16, D-W-5100 Aachen, Bundesrepublik Deutschland

Z. Naturforsch. **47c**, 932–936 (1992); received September 7, 1992

Limulus Ventral Nerve Photoreceptor, Spontaneous Bumps, Chemically Induced Bumps, Light-Induced Bumps, G-Protein

The elementary responses, the "bumps", of the ventral photoreceptor of *Limulus polyphemus* were studied under voltage clamp conditions. We compared spontaneous bumps with those induced by light and we applied the G-protein activator AlF_4^-. The amplitude of the spontaneous bumps is on the average 7–8 times smaller than that of the light-induced bumps. Bumps induced by AlF_4^- have identical size and time parameters when compared with spontaneous bumps. Thus at least a large part of the spontaneous bumps is probably triggered by the activation of single G-protein molecules. However, if the next step of the transduction chain is gainless, it is also possible the spontaneous bump originate from this step. The different size of spontaneous and light-induced bumps can be explained assuming that a light-activated metarhodopsin molecule leads to the activation of 3 to 12 G-protein molecules.

Introduction

When the photoreceptor of *Limulus* is illuminated with very dim light transient light responses, "bumps", can be observed. Bumps are the elementary responses of the photoreceptor which are evoked by single photons [1, 2]. A bump is caused by the successful absorption of a photon by a rhodopsin molecule which then starts a reaction chain leading to the opening up to 10^4 ion channels in the cell membrane [3].

Besides light-induced bumps spontaneous bumps can also be observed in the dark. As reported by several authors [4–7] spontaneous bumps are usually smaller than light-induced bumps. Our analysis shows that on the average the amplitudes of the spontaneous bumps for individual cells is 3- to 12-fold smaller than that of the light-induced bumps, with a mean of about 8 (5 cells).

It has been proposed that spontaneous discrete waves ("bumps") result from thermal isomerizations of visual pigment molecules [5]. By contrast, the reverse reaction – the conversion of metarhodopsin to rhodopsin – was proposed to contribute significantly to the generation of spontaneous bumps [4, 8]. The latter hypothesis, however, can hardly account for quantum bumps in vertebrates after sustained darkness, because vertebrate metarhodopsin is unstable [4, 9]. Our experiments suggest an alternative explanation for the origin of the spontaneous bumps and for the difference between spontaneous and light-induced bumps.

Bumps chemically induced by AlF_4^- were measured in the *Limulus* photoreceptor. According to the fact that chemically induced and spontaneous bumps are indistinguishable it seems plausible that a spontaneous bump is caused by the activation of only one G-protein molecule.

Materials and Methods

Limulus ventral nerves were dissected as previously described [10] and mounted in a test vessel. 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 saline containing in addition 10 mmol/l NaF and 10 µmol/l $AlCl_3$ was used to evoke chemically induced bumps. 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) [11, 12]. The maximum error of the clamp voltage regulation was less than 1 mV. As a light source a photoflash (Metz Mecablitz 60 CT-1) was used.

Abbreviations: F, bump area, current-integral of the bump over T_b; J_{max}, peak amplitude of receptor current; J_L, average J_{max} of the light-induced bumps; J_S, average J_{max} of the spontaneous bumps; J_{Se}, calculated J_S assuming an exponential function for the distribution of the bump amplitudes; λ, time constant fitted to the exponential decay; T_b, bump width, time from the detectable beginning of the bump until the current has returned to the base-line noise; T_{lat}, latency, the time from light flash to the first measurable deflection of the current from the base-line; T_{max}, Time-to-peak, the time from light stimulus to the maximum of the bump; T_r (= $T_{max} - T_{lat}$), rise time, duration of the rising phase of the bump.

Reprint requests to Prof. Dr. H. Stieve.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/1100–0932 $ 01.30/0

The light stimuli with a half duration of 0.1 ms were filtered by a broadband filter of 540 ± 40 nm. Light-induced bumps were measured by illuminating a dark-adapted photoreceptor with a very weak flash of light every 10 s for 250–1000 times. The light energy was about 0.5×10^8 photons/cm^2, which on the average evoked 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 periods of 15 to 30 min in which the photoreceptor was kept in the dark. Alternatively, they were measured in the time intervals between the light flashes when probably no more light-induced bumps occur (3rd to 10th second after flash) [13].

The signal of the membrane current was digitized with a frequency of 1 kHz and stored on an IBM-compatible personal computer. First the current traces were digitally filtered with a 50 Hz notch filter and a 70 Hz low pass filter [13]. Both filters had a 10th order Bessel characteristics. In the next step the bump parameters were evaluated as described previously [13, 14] by a computer program. Each bump was confirmed visually.

Results

The different size of spontaneous and light-induced bumps

Fig. 1 (left) shows the amplitude histograms of the spontaneous and the light-induced bumps of one cell. The average value and the shape of the distribution is clearly different for the two bump types. The amplitude histogram of the spontaneous bumps can be described by an exponential function. The amplitude histogram of the light-induced bumps has a "plateau"-like shape. The average amplitude value J_S of the spontaneous bumps is 2.4 times smaller than the value J_L of the light-induced bumps. Similar results were found in 4 other photoreceptor cells.

Table I shows the average values (arithmetical mean) of the amplitudes of the light-induced and

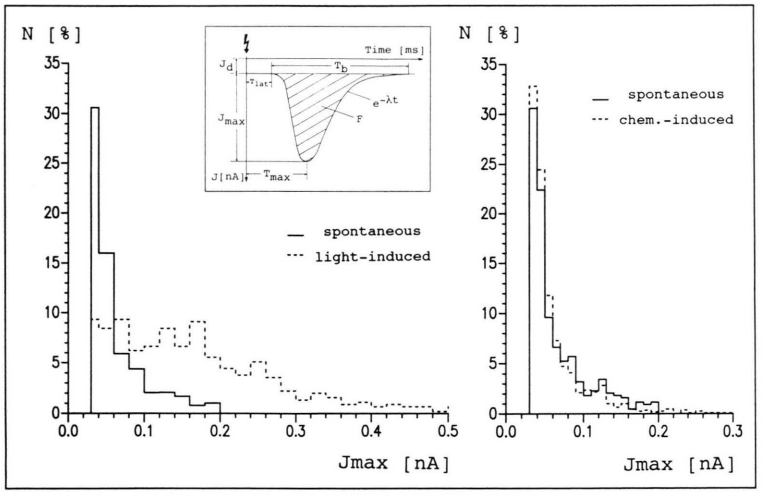

Fig. 1. Left: Amplitude distribution of light-induced (n = 440) and spontaneous bumps (n = 329) in physiological saline measured at a membrane potential of −45 mV. The amplitudes are calculated from apparently single bumps and the first bump from superimposed bumps. Mean values ± SEM, light-induced bumps: J_L = 164 ± 4.2 pA, spontaneous bumps: J_S = 69.3 ± 1.9 pA. The bump-evoking flash was repeated every 10 s.
Inset: Typical bump shape and the characteristic parameters: T_{lat}: latency, the time from light flash to the first measurable deflection of the current from the base-line. T_{max}: time-to-peak, the time from light stimulus to the maximum of the bump. T_r (= $T_{max} - T_{lat}$): rise time, duration of the rising phase of the bump. T_b: bump width, time from the detectable beginning of the bump until the current has returned to the base-line noise. J_{max}: amplitude of bump maximum. F: bump area, current-integral of the bump over T_b. λ: time constant fitted to the exponential decay.
Right: Amplitude distribution of spontaneous bumps (n = 329) recorded in physiological saline and chemically induced bumps (n = 1110) recorded in a saline containing additional 10 mmol/l NaF and 10 μmol/l $AlCl_3$. Only apparently single bumps (minimal amplitude 30 pA) were evaluated. Experiment: HRE 141190 (= cell No. 3 in Table I).

Table I. Comparison of bump amplitudes for light-induced and spontaneous single bumps. J_L, J_S: average amplitude of light-induced and spontaneous (dark) bumps; J_{Se}: value of the amplitude of the dark bumps calculated by assuming an exponential distribution for the frequency distribution of the dark bumps. The evaluation limit for the smallest bump amplitude was 30 pA in cells 1–4 and 50 pA in cell 5.

	Light-induced bumps		Spontaneous bumps		Quotient	Calculated value	
Cell	N	J_L [nA]	N	J_S [nA]	J_L/J_S	J_{Se} [nA]	J_L/J_{Se}
1	437	0.38	364	0.11	3.5	0.080	4.8
2	708	0.57	328	0.096	5.9	0.048	11.9
3	440	0.17	405	0.069	2.3	0.026	6.4
4	1027	0.15	179	0.104	1.5	0.055	2.9
5	165	1.5	594	0.23	6.5	0.143	10.5
Mean		0.55		0.12	3.94	0.070	7.3
±SD		±0.49		±0.055	±1.97	±0.040	±3.4

spontaneous bumps calculated for 5 photoreceptor cells. The average amplitude of the bumps vary clearly from cell to cell. The largest average value of the bump amplitude (1.5 nA) is 10-fold larger than the smallest. The average value of the amplitude of the spontaneous bumps also varies from cell to cell. The largest spontaneous bumps were measured in cell number 5, in which also the largest value for the light-induced bumps was measured. The quotient J_L/J_S of the average bump amplitudes varies between 1.5 and 6.5, the mean value is about 4.

However, the correct relation between the amplitudes of the spontaneous and the light-induced bumps will be larger than these values because the average amplitude of the spontaneous bumps is easily overestimated. This is due to the fact that only bumps with amplitudes exceeding the noise level 2- to 3-fold can be evaluated correctly. In these experiments the minimal bump amplitude evaluated was about 30 pA (ref. Table I). The omittance of the bumps with the smallest amplitudes leads to a shift of the mean to greater values. To get a more correct mean of the amplitude of the spontaneous bumps the value J_{Se} was calculated assuming an exponential function for the distribution of the bump amplitudes. The calculated mean values of the amplitudes J_{Se} are about two times smaller than the measured values J_S because they contain all amplitudes including those less than 30 pA. Due to the larger amplitudes and the different form of the amplitude distribution, such a correction is not necessary for the mean amplitude of the light-induced bumps. In the latter case, the change in mean value by a correction is estimated to be less than 5%. With the calculated values J_{Se} the relations between the average amplitudes of the spontaneous and the light-induced bumps rise about 2-fold. The spontaneous bumps are 3- to 12-fold smaller than the light-induced bumps. On the average the corrected mean amplitude of the spontaneous bumps is 7.3-fold smaller than that of the light-induced bumps. According to the smaller bump amplitude also the time parameters of the spontaneous bumps are reduced [13].

Chemically induced bumps

To investigate the origin of the spontaneous bumps the following experiments were carried out. In dark-adapted photoreceptors spontaneous and light-induced bumps were measured for about 1–2 h. After that time the physiological saline was replaced by a saline containing in addition 10 mmol/l NaF and 10 µmol/l $AlCl_3$; AlF_4^- is formed in this solution [15]. In this saline also light-induced and spontaneous bumps were measured as described above. After saline exchange the number of spontaneous bumps rises from 0.1 s^{-1} up to 1–1.5 s^{-1}. Fig. 2 shows current traces recorded in the dark before and 1 h after the presence of AlF_4^- to the physiological saline. Table II presents a comparison of bump parameters of the spontaneous and the chemically induced bumps. The size and time parameters of the chemically induced bumps and the spontaneous bumps are al-

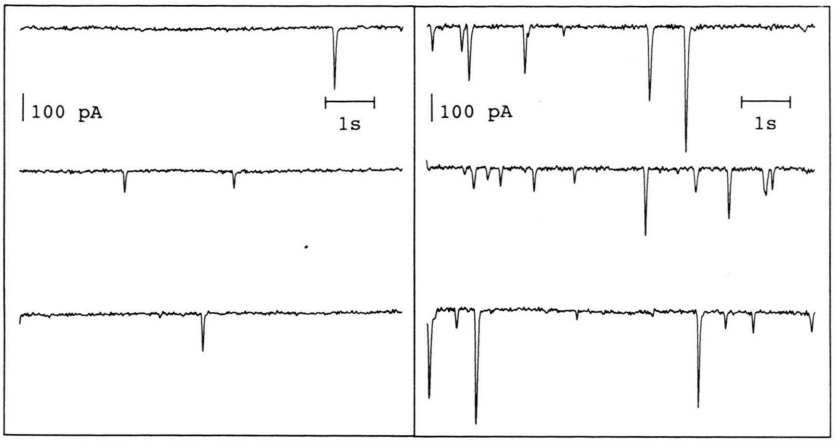

Fig. 2. Spontaneous and chemically activated current bumps measured at a membrane potential of −45 mV. Left: Bumps occurring in the dark in physiological saline. Right: Bumps measured in the dark in physiological saline containing 10 mmol/l NaF and 10 μmol/l $AlCl_3$. Experiment: HRE 141190.

Table II. Comparison of bump parameters (mean values ± SEM) for spontaneous (dark) bumps, light and chemically induced bumps. Bump parameters refer inset Fig. 1; N: number of evaluated single bumps. Two experiments: HRE 141190 (top) and HRE 231090 (bottom).

Bump type	N	J_{max} [pA]	F [pAs]	T_r [ms]	T_b [ms]	λ [s^{-1}]
Dark	329	69.3 ± 1.9	3.7 ± 0.17	44 ± 1.3	108 ± 3	52.9 ± 1.8
Chemical	1110	65.4 ± 1.3	3.4 ± 0.13	39 ± 1.1	102 ± 3	50.0 ± 1.8
Light	440	164 ± 4.2	9.4 ± 0.25	50 ± 0.8	126 ± 2	45.6 ± 0.7
Dark	98	114 ± 1.4	5.5 ± 0.83	38 ± 1.6	87 ± 4	69.7 ± 3.4
Chemical	871	135 ± 1.7	6.1 ± 0.93	36 ± 1.3	82 ± 3	71.3 ± 2.7
Light	218	647 ± 3.7	37.8 ± 2.43	52 ± 1.0	109 ± 2	38.3 ± 1.3

most identical. A second experiment with another photoreceptor cell showed similar results.

Beside the average values also the shape of the bump parameter distributions are not distinguishable for the spontaneous and the chemically induced bumps. In Fig. 1 (right) the amplitude histograms for the different bump types are compared. The size and the time parameters of the light-induced bumps are not influenced by AlF_4^-. The average value of the amplitude is 169 ± 9 pA (mean ± SEM).

As can be seen in Fig. 2, only a relatively small part of the spontaneous bumps reach a size as those induced by light. This indicates that only a small part of the spontaneous bumps may arise from the activation of rhodopsin molecules.

Discussion

The spontaneous bumps and bumps chemically induced by AlF_4^- are identical in size and time parameters. Thus it seems reasonable to assume that the spontaneous bumps originate from the same signal transduction chain. AlF_4^- can permeate cell membranes and activate G-proteins [15, 16]. An activated G-protein can start the transduction chain and in turn causes a chemically induced pump. It was already observed that these events tend to be smaller than those induced by light [17]. Superfusing locust photoreceptors with NaF, Payne [18] has observed a chemically induced noise, that was so small, that it could not be resolved into bumps. Kirkwood *et al.* [19] have shown that the intracellular injection of the G-protein inhibitor GDP-β-S reduces the number of the AlF_4^--induced bumps. Light adaptation reduces the size of the spontaneous bumps [6] as well as that of the chemically induced bumps [20]. These findings and the results presented here, that spontaneous and chemically induced bumps cannot be distinguished in size and time parameters confirm the hypothesis that spontaneous bumps

are triggered by the spontaneous activation of single G-protein molecules [3]. However, if the next step of the transduction chain is gainless, it is also possible the spontaneous bump originate from this step. The difference in size and time parameters between the light-induced and the spontaneous bumps can be explained by the assumption that one light-activated metarhodopsin activates on the average about 7–8 G-protein molecules thereby causing a larger bump. This is in agreement with the value of 8 for the gain of the first step of transduction estimated by Kirkwood *et al.* [19].

An alternative explanation for the origin of the spontaneous bumps was proposed by Lisman [4], based on the finding that the rate of spontaneous bumps depends on the concentration of metarhodopsin in the *Limulus* median photoreceptor. In his hypothesis the spontaneous bumps are triggered by a spontaneous backward reaction of metarhodopsin from the inactive into an active state. To explain the difference of size between the spontaneous and the light-induced bumps two active states of the metarhodopsin had to be postulated. Thus it is possible that the measured spontaneous bumps are partly evoked by the spontaneous activation of single G-protein molecules and by some spontaneous backward reactions of metarhodopsin molecules.

Lederhofer *et al.* [21] assumed that the light-induced and the spontaneous bumps are identical and explained the measured difference as an artefact caused by a number of light-induced bumps which cannot be recognized because of a high degree of overlap in time. For the data presented here this theory can be excluded, because the energy of the light stimuli in our experiments was so weak that on the average one flash evokes 0.75 bump.

Acknowledgements

We like to thank Dr. K. Nagy for stimulating discussions. This work was supported by the Deutsche Forschungsgemeinschaft (Sti 16/13).

[1] A. Borsellino and M. G. F. Fuortes, J. Physiol. **196,** 507–539 (1968).
[2] H. Stieve, in: The Molecular Mechanism of Photoreception (H. Stieve, ed.), Dahlem-Konferenzen, pp. 199–230, Springer Verlag, Berlin, Heidelberg, New York, Tokio 1986.
[3] K. Nagy, Quarterly Review of Biophysics **24,** 165–226 (1991).
[4] J. Lisman, J. Gen. Physiol. **85,** 171–187 (1985).
[5] R. Srebro and M. Behbehani, J. Physiol. **224,** 349–361 (1972).
[6] H. Stieve and M. Bruns, Biophys. Struct. Mech. **9,** 329–339 (1983).
[7] S. Yeandle and J. B. Spiegler, J. Gen. Physiol. **61,** 552–572 (1973).
[8] T. D. Lamb, Vision Res. **21,** 1773–1782 (1981).
[9] D. Baylor, G. Matthews, and K.-W. Yau, J. Physiol. **309,** 591–621 (1980).
[10] H. Stieve, M. Pflaum, J. Klomfaß, and H. Gaube, Z. Naturforsch. **40c,** 278–291 (1985).
[11] A. Deckert and H. Stieve, J. Physiol. **433,** 467–482 (1991).
[12] H. Stieve and S. Benner, Vision Res. **32,** 403–416 (1992).
[13] H. Reuß, Bumps, die elementaren Reizantworten der Photorezeptorzelle des *Limulus polyphemus,* Thesis, RWTH Aachen (1991).
[14] H. Stieve, H. Reuß, H. T. Hennig, and J. Klomfaß, Z. Naturforsch. **46c,** 461–486 (1991).
[15] L. Stryer and H. R. Bourne, Ann. Rev. Cell Biol. **2,** 389–417 (1986).
[16] J. Bigay, P. Deterre, C. Pfister, and M. Chabre, FEBS Lett. **191,** 181–191 (1985).
[17] A. Fein and D. W. Corson, Science **204,** 77–79 (1979).
[18] R. Payne, J. Physiol. **325,** 261–279 (1982).
[19] A. Kirkwood, D. Weiner, and J. E. Lisman, Proc. Natl. Acad. Sci. (U.S.A.) **86,** 3872–3876 (1989).
[20] D. W. Corson and A. Fein, J. Gen. Physiol. **82,** 639–657 (1983).
[21] R. Lederhofer, J. Schnakenberg, and H. Stieve, Z. Naturforsch. **46c,** 291–304 (1991).