Measurement of a Steady State Current in the Dark in Photoreceptors of the Barnacle

Christof C. Krischer and D. Hupp

Institut für Neurobiologie der Kernforschungsanlage Jülich

(Z. Naturforsch. **28 c**, 597–599 [1973] ; received June 21, 1973)

Barnacle, photoreceptors, dark current

Receptor potentials are measured extracellularly by means of an air gap method along the optic nerve of the median and the lateral photoreceptor of the barnacle. Both photoreceptors have an outward dark current (photoreceptor positive with respect to the nerve) which is diminished approximately 70% during the plateau phase of the receptor potential. Ouabain abolishes dark current and receptor potential in the same proportion. With a *Limulus* lateral eye preparation a dark current and light induced current changes were measured which had the same polarity and a similar magnitude as the receptor currents of the barnacle.

In the *vertebrate* retina *extracellular measurements* revealed that a current flows in the dark in the receptor cells (rods). The rhodopsin containing outer segment is negative with respect to the inner segment [1, 2]. Illumination with flashes causes a transient reduction of this steady "dark current" which is graded with respect to the flash intensity [1, 2].

In *invertebrate* photoreceptors most of the data were obtained by *intracellular measurements:* The steady state potential in the dark, *e. g.* cell inside negative with respect to the outside, is transiently diminished towards zero, depending on the intensity of the applied flashes [3].

It is the aim of this paper to show with a procedure which is comparable to the extracellular method used by Hagins *et al.* [1] that one can observe in the invertebrate photoreceptors of the barnacle a dark current (of opposite polarity) as in vertebrate photoreceptors. Some evidence for extracellularly measured dark potentials has already been reported by Stieve *et al.* [4, 5].

Methods

In a *plexiglas chamber* 2 pools of seawater were separated by a silicone greased partition of 1 mm diameter. The photoreceptor was placed in pool 1, and the proximal end of the nerve in pool 2. 1 mm of nerve connected the pools across the partition. The exposed part of the nerve was for the lateral photoreceptor about half way between ganglion and photoreceptor and somewhat closer to the eye, for the median photoreceptor. Ag/AgCl *wick electrodes* were placed in the pools, one in each. The nerve end containing pool was connected to a solid state amplifier in the follower configuration. The other pool was grounded. The bright spot of a Tektronix 5030 oscilloscope beam served as *light stimulus* which was delivered via a light guide of 5 mm diameter to the photoreceptor. The background light intensity during the experiments was 15 ± 10 lux.

The eye-nerve preparation of *Balanus eburneus* was directly dissected out of the animals after removal of the shells. The nerve was cut off approximately 2 mm distally from the ganglion. Preparations with the ganglion attached gave the same results. The preparation took approximately 10 min. The muscle fibers which accompany the median optic nerve were not removed. The measuring temperature was 21 – 23 °C.

Requests for reprints should be sent to Dr. C. C. Krischer, Institut für Neurobiologie der Kernforschungsanlage Jülich, *D-5170 Jülich.*

Results

Fig. 1 shows the extracellularly measured potential changes to saturating light intensities of the median and the lateral photoreceptors of the barnacle, respectively.

It is seen that the potential level E_d of the nerve end during the dark period is more negative than the short circuited zero potential $E' = 0$ (line in the center of the figures). This dark potential arises across the nerve resistance, R_{sh}. According to Ohm's law the equivalent dark current, i_d, equals E_d/R_{sh}. The resistance R_{sh} can be evaluated from the amplitude of the short negative pulses (Fig. 1) which are due to 1 nA current pulses. The dark current of the

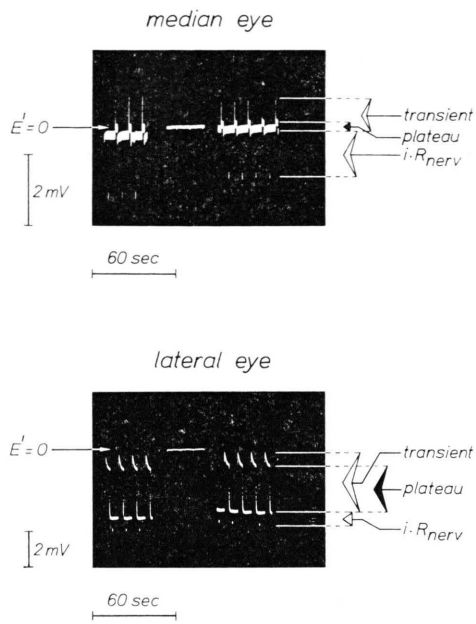

Fig. 1. Extracellular recordings of receptor potentials of the barnacle. Zero potential (chambers short circuited) is at $E'=0$ in the center of the recordings. Saturating light stimuli of 3 sec duration are applied every 10 sec. Note, the base line during the dark intervals is more negative than $E'=0$. During the dark intervals short current pulses of $i=1$ nA amplitude are applied. The corresponding potential drop $i \cdot R_{nerv}$ is indicated in the figure.

median photoreceptor is much smaller than the dark current of the lateral photoreceptor, Table I.

The effect of light is a change of current, Δi. During the transient phase the current of the median photoreceptor passes zero and becomes positive for a short time. During the plateau phase both receptors maintain a current level close to zero. The values of current changes (see Table I) are similar for the transient phase but differ considerably in the plateau phase; the median being much smaller than the lateral. The difference in relative values is more important than the difference in absolute values. The ratio of values is most likely independent of the method, whereas the absolute values critically depend on the relative position of the pools and the partition with respect to the nervous system of the animal. The ratio of dark current to plateau current change is with 1.33 and 1.43 about the same for the two photoreceptors. Intense illumination therefore abolishes in the steady state about 70% of the dark current.

Table I. Photoreceptor currents of the barnacle [nA]. Mean values ± s.d. of mean.

	Dark current i_d	Light induced current changes Δi		$i_d/\Delta i_p$
		Transient Δi_t	Plateau Δi_p	
median eye $n=9$	0.97 ± 0.29	3.47 ± 1.03	0.68 ± 0.20	1.43
lateral eye $n=7$	4.3 ± 0.98	4.18 ± 0.93	3.23 ± 0.73	1.33

Preliminary experiments were carried out in addition:

1. Ouabain, a poison which abolishes sodium pump activity, was placed in 10^{-3} M concentration (in seawater) into the eye pool. After 8 – 12 min the dark current and the light induced current change were both diminished to 50% of the original value. For median and lateral photoreceptors, the proportion of dark current to light induced current changes remained approximately constant during the time course of the experiment.

2. A preparation of the *Limulus* lateral eye with attached optic nerve gave, under the same experimental conditions as described above, qualitatively the same results as for the barnacle. A negative dark current, i_d, was measured at the nerve end. Light diminished it to about 50 – 80%. The nerve resistance was 60 – 100 kΩ, $i_d = 1 - 4$ nA.

Discussion

The analogy between extracellularly measured vertebrate and invertebrate dark currents is obvious: 1. The magnitude of the dark current determines the amplitude of light induced current changes during the plateau phase. The ratio of $i_{dark}/\Delta i_{plateau} \approx 1.4$, and is apparently independent of the magnitude of i_{dark}. 2. The polarity of the light induced current change is such that the "dark potential gradient" between photopigment and nerve side of the photoreceptors is diminished by illumination.

Intracellular measurements revealed that on illumination, *vertebrate* outer segments hyperpolarize and decrease conductance of the outer membrane [6]. Contrary to this, intracellular measurements with many *invertebrate* photoreceptors such as the barnacle appear to react to light the opposite direction; the receptor depolarizes and the conductance increases [3, 7]. The experiments of this paper showed that the dark current also has the reverse polarity.

In the dark the current flows in the same direction as an injury current, leaking inward into the nerve. The same direction of current flow was measured at the synapse-end of vertebrate rod cells [1, 8]. Measurements with completely uninjured photoreceptors and nerves are, however, not yet possible for vertebrate and invertebrate photoreceptors.

Photoreceptors can be described as a source-sink system (like a radio transmitter-receiver system). The photoreceptive membrane system represents the source and the neuronal membrane the sink. For vertebrate photoreceptors the microelectrodes only penetrate into one part of the system, the outer segment, *e. g.* the source, or the inner segment, the sink.

In the invertebrate photoreceptors the electrodes are impaled into the short circuited source-sink system. The rhodopsin containing microvilli and the nerve membrane are in proximity in a pear shaped arrangement [9]. The wider portions are populated by microvilli and the narrow portions by nerve membrane.

In this system the dark current measurable at the nerve end of the "pear" flows the same direction as the dark current at the synapse end of rod cells [8]. Because of the short circuit arrangement of source and sink in the soma of the cell, individual contributions of the current and impedance changes cannot be measured.

The magnitude of light induced current change referred to unit volume of photoreceptive membrane system (rod outer segment of vertebrates and microvilli of invertebrates) is also comparable. For half-saturating response the transient current change, Δi, for one rod of the rat is quoted as approximately 30 pA [1], and for a barnacle lateral receptor it is approximately 20 nA [3]. The receptive membrane volumes V are $3.3 \cdot 10^{-10}$ cm^3 [1] and $4 \cdot 10^{-7}$ cm^3 [10% of the receptor volume = microvilli volume [9, 10]] respectively. The ratio $\Delta i/V$ is $9 \cdot 10^{-2}$ A/cm^3 and $5 \cdot 10^{-2}$ A/cm^3, for vertebrate and invertebrate, respectively.

The specialization of the barnacle photoreceptors is such that the small, fast responding median receptor [11] has a large transient and a small steady state phase. For the large, slow responding lateral receptor the reverse holds; a relatively small transient phase is coupled with a relatively large steady state output. It is to be checked whether this size-response relation is a general feature of photoreception.

The authors are indebted to Prof. Dr. H. Stieve for suggesting the *Limulus* experiment and his help in the experiment. The investigation is supported by Deutsche Forschungsgemeinschaft SFB 2000/160.

[1] W. A. Hagins, R. D. Penn, and S. Yoshikami, Biophys. J. **10**, 380 [1970].
[2] R. D. Penn and W. A. Hagins, Biophys. J. **12**, 1073 [1972].
[3] H. Mack Brown, S. Hagiwara, H. Koike, and R. W. Meech, Federat. Proc. **30**, 69 [1971].
[4] H. Stieve and Chr. Wirth, Z. Naturforsch. **26 b**, 457 [1971].
[5] H. Stieve, Helgol. Wiss. Meeresunters. **7**, 149 [1960].
[6] J.-I. Toyoda, H. Nosaki, and T. Tomita, Vision Res. **9**, 453 [1969].
[7] R. Millecchia and A. Mauro, J. gen. Physiol. **54**, 331 [1969].
[8] R. Zuckerman, Biophys. Soc. Abstr. p. 101a, Toronto 1972.
[9] W. H. Fahrenbach, Z. Zellforsch. Mikroskop. Anat. **66**, 233 [1965].
[10] C. C. Krischer, Z. Naturforsch. **26 b**, 1326 [1971].
[11] C. C. Krischer, Verh. dtsch. Zool. Ges. **1970**, 165.