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Rapid report

Photodynamic membrane damage at the level of single ion channels

Lars Kunz, Günther Stark *

Department of Biology, University of Konstanz, P.O. Box 5560 M638, D-78434 Konstanz, Germany

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Abstract

Illumination of cellular membranes by visible light in the presence of appropriate photosensitizers is known to inactivate specific ionic pathways and to increase the unspecific leak conductance of the membranes. While previous studies have concentrated on the macroscopic ionic currents, the present study separates the two phenomena at the microscopic level. Using opossum kidney (OK) cells as epithelial model system and photofrin II as sensitizer, the patch-clamp technique in inside-out configuration has been applied to show the inactivation of single ion channels immediately after start of illumination and the subsequent strong increase of the leak conductance. Inactivation is shown for two kinds of channels: the large-conductance Ca^{2+}-dependent K^+ channel (maxi-K_{Ca}) and the stretch-activated nonselective cation channel (SA-cat). © 1997 Elsevier Science B.V.

Keywords: Photosensitization; Photofrin II; Patch-clamp; Opossum kidney cell; Ion channel; Leak conductance

Cellular membrane modifications are currently considered as important primary steps of photosensitized cell inactivation [1–4]. This holds especially for hematoporphyrin and its derivatives such as photofrin II. As a consequence of photodynamic membrane damage, depolarization of the membrane potential as well as modifications of ion transport pathways have been observed, such as inactivation of K^+-, Na^+- and Ca^{2+}-currents and an increase of the unspecific ionic leak conductance [5,6]. So far inactivation of the currents has been investigated at the macroscopic level. Application of the patch-clamp technique in order to study inactivation of single ion channels has been found to be complicated by the strong current fluctuations accompanying the simultaneous increase of the leak conductance of the membrane [7]. Using opossum kidney (OK) cells as cellular model system and photofrin II as sensitizer, we have been able to separate the two phenomena. We show that channel inactivation clearly precedes the increase of the leak conductance, which is presumed to be due to photodynamic induced lipid peroxidation of the membrane.

The opossum kidney cell line has been repeatedly used as a model system to study different functions of proximal tubular epithelia. The plasma membrane of the cells contains several types of ion channels, which have been characterized in recent years [8–11]: a large-conductance Ca^{2+}-dependent K^+ channel (maxi-K_{Ca}), K^+ channels of lower conductance, stretch-activated nonselective cation channels (SA-cat) and Cl^- channels. The present communication concentrates on two types of channels, which have been found in a great variety of different cell types: the maxi-K_{Ca} and the SA-cat channels. The maxi-K_{Ca}

* Corresponding author. Fax: +49 7531 883183. E-mail: gunther.stark@uni-konstanz.de

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(or BK, i.e. big K^+ channel) has single channel conductance values in excess of 100 pS (under physiological conditions) [12–15]. It has been discussed in connection with different cellular functions, such as the regulation of neurotransmitter release and the control of arterial vessel tone [16]. Mechanosensitive or stretch-activated (SA) channels respond to membrane stress by changes in open probability [17]. The SA-cat studied appears to be involved in volume regulation of OK cells [8,12]. A role in cellular volume regulation has also been suggested for the maxi-K_{Ca} channel [17].

OK cells were maintained in culture at 37°C [8]. They were isolated from confluent monolayers with trypsin and plated on coverslips several hours before use. Subsequently the cells were incubated in the dark for 60–100 min with photofrin II (typically 0.1–1 $\mu g/ml$) in a standard high-NaCl solution (see below). Thereafter, the solution was exchanged for the same solution without sensitizer. The cells were transferred to a bath chamber mounted on an inverted microscope (Zeiss). Inside-out patch clamp recordings were performed with single unconnected and round cells [18]. Electric currents and potentials were measured using an EPC-9 patch clamp amplifier and the software PULSE (HEKA, Germany). The current records were sampled at 4 kHz and filtered at 1.33 kHz. Outward currents (from the cytoplasmic to the external side) were defined as positive currents and shown as upward deflections. Potentials refer to the cytoplasmic side. A HeNe-Laser (wavelength: 632.8 nm; range of applied intensities: 500–2000 W/m^2) was used as a light source. Illumination was started not later than 2 h after incubation. Experiments were performed at approximately 22°C in the following solutions (at pH 7.4): standard high-NaCl solution (145 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$), high-KCl solution (135 mM KCl, 20 mM NaCl), and high-KCl solution with 1 mM $CaCl_2$ (144 mM KCl, 20 mM NaCl, 1 mM $CaCl_2$). Additionally, all electrolyte solutions contained 1 mM $MgCl_2$, 20 mM HEPES, and 18 mM glucose.

The two kinds of ion channels investigated can be clearly identified by their activation behaviour and by their single channel values [8,9,11]. The maxi-K_{Ca} channel has a mean single channel conductance of 190 pS (in high-KCl solution with 1 mM $CaCl_2$) and is only observed in the presence of sufficiently high Ca^{2+} concentration on the cytoplasmic side, while the typical fluctuations of stretch-activated (SA) channels (20 pS in standard high-NaCl or high-KCl solutions) appear on application of a pressure difference across the membrane. Activation of SA-cat channels may also be observed following the transition from the cell-attached to the inside-out configuration.

Fig. 1 shows the behaviour of the electric current across an inside-out patch in the presence of a single maxi-K_{Ca} channel. Before illumination — due to the high concentration of Ca^{2+} — the channel is preferentially in the open state (characterized by the downward deflection) and shows only brief flickering to the closed channel state (trace A). Upon illumination, the open probability is at first strongly reduced (trace B). Finally, channel activity disappears completely, i.e. the current level corresponds to that of the closed channel state (trace C). If illumination is stopped at this time, the current signal remains at the same level (as was found by independent experiments). The

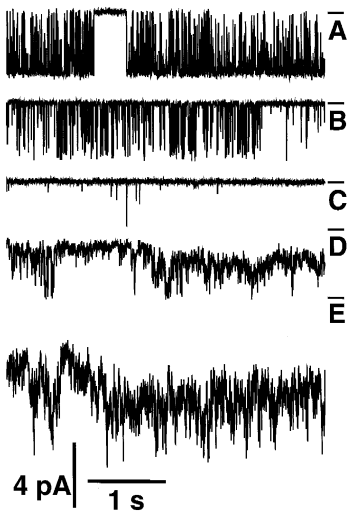

Fig. 1. Photodynamic inactivation of a single maxi-K_{Ca} channel. OK cells were incubated for 65 min with 0.5 $\mu g/ml$ photofrin II. The current traces were obtained in inside-out configuration at a constant holding potential of −20 mV (additionally filtered at 500 Hz). Pipette and bath contained high-KCl solution with 1 mM $CaCl_2$. (A) Typical single-channel trace before illumination (bar indicates closed state). (B) 80 s, (C) 110 s, and (D) 6.5 min after start of illumination. Trace E was taken from another experiment performed under the same conditions. The bars at the right hand side indicate the current values observed before illumination and in the absence of channel activity.

continuation of illumination leads to further characteristic changes, namely the appearance of burst-like current noise (which is clearly different from the characteristic channel fluctuations), and a continuous increase of the mean current flowing across the patch (traces D and E). This is believed to indicate an increase of the leak conductance of the membrane induced by photodynamically induced lipid peroxidation (see below). The current traces shown in Fig. 1 represent only part of the data collected at the experiment. The complete experiment (which is one out of 9 different experiments with largely identical results) is analysed in Fig. 2. The channel was found to be active for more than 10 min before illumination. Start of illumination results in a fast reduction to zero of the mean probability, P_0, in the open channel state (averaged over 20 s). In the dark, channel activity has been observed for 30 min at least, i.e. there is a clear correlation between the decay of P_0, and the onset of illumination. Photodynamically induced channel inactivation was also found at stretch-activated channels (10 independent experiments). Fig. 3 illustrates the continuous decrease towards zero in the number of the channels contributing to the current fluctuations. Trace 1 (obtained before illumination) is indicative of the simultaneous presence of 6–8 individual channels. The number of channels is reduced to 1–2 (trace 2) and finally to zero (trace 3) upon illumination. Inactivation of ion channels is followed by a strong increase of the leak conductance. This is demonstrated by the experiment shown in Fig. 4, where a comparatively high concentration of the sen-

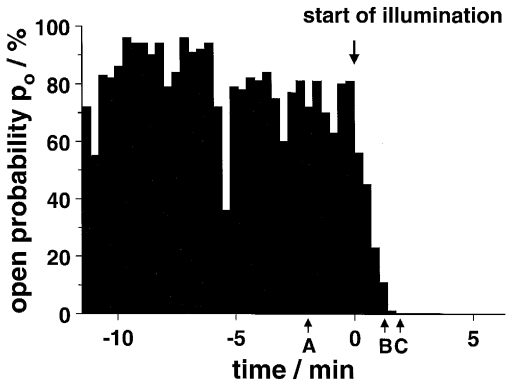

Fig. 2. Photodynamic induced decrease in open probability of the maxi-K_{Ca} channel. Current fluctuations shown in Fig. 1 were used to calculate the mean open probability (as the fraction of time spent in the open state) for intervals of 20 s. The threshold for event detection was set at 50% of the average channel amplitude. The labels A, B, and C mark the time of measurement of the current traces shown in Fig. 1. For further experimental conditions see legend to Fig. 1.

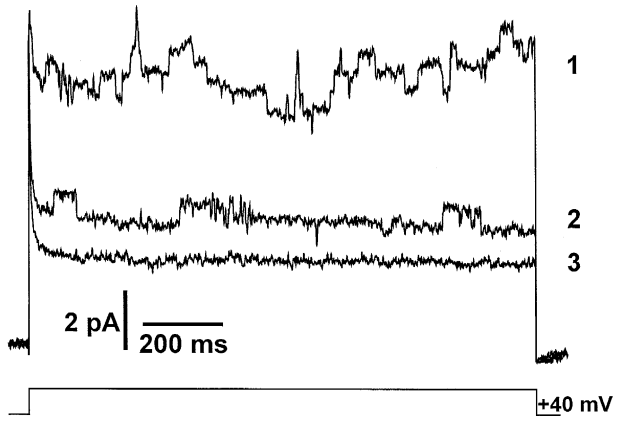

Fig. 3. Photodynamic inactivation of the stretch-activated cation channel (SA-cat). OK cells were incubated for 60 min with 0.2 μg/ml photofrin II. Channels are activated by the transition from the cell-attached to the inside-out configuration of the patch-clamp experiment and are detected via the response of the electric current following a series of depolarizing voltage steps of +40 mV amplitude (starting from a holding potential of 0 mV). The current signal was additionally filtered at 250 Hz. Pipette: high-KCl solution, bath: standard high-NaCl solution. (1) Before illumination, (2) 75 s, and (3) 115 s after start of illumination.

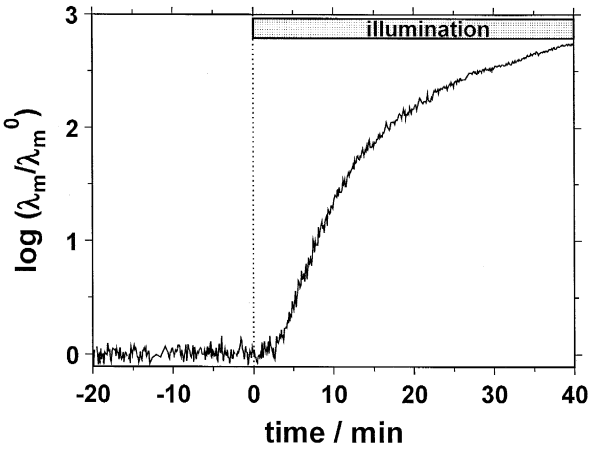

Fig. 4. Photodynamic induced increase of the nonspecific leak conductance. The normalized membrane conductance, λ_m / λ_m^0, of an inside-out patch was determined as a function of time before and during illumination. Standard high-NaCl solution was used in bath and pipette. Contrary to the experiments shown in Figs. 1 and 3, the pipette solution contained an additional high concentration of photofrin II (10 μg/ml).

sitizer was applied. An increase of almost 3 orders of magnitude in the membrane conductance was observed. At the end of illumination, the membrane conductance was found to be roughly equivalent to the conductance of the patch pipette. At standard sensitizer concentrations (cf. Figs. 1 and 3), the increase of the conductance was up to 2 orders of magnitude. Identical results were found at high-NaCl and high-KCl solutions.

The experiments shown in Figs. 1–4 indicate the presence of two different consequences of photodynamic membrane damage, which occur in different time domains after start of illumination: inactivation of ion channels and a strong increase of the leak conductance. The latter is presumably due to photosensitized lipid peroxidation, as has been concluded previously from experiments at the macroscopic level of membrane conductance [7]. An increase of the leak conductance similar to that shown in Figs. 1 and 4 has also been observed at planar lipid membranes [19]. Channel inactivation is clearly the more sensitive process. It might be caused by a photomodification of sensitive amino acids of the channel proteins, as in the case of the artificial channel formed by the peptide gramicidin A [20–22]. Alternatively, the two phenomena may have a common mechanistic basis, as was found throughout our studies on polyene channels in lipid membranes formed by the antibiotics amphotericin B or nystatin. In this case inactivation is caused by participation of polyene molecules in free radical-induced lipid peroxidation [23,24]. Independent of the detailed mechanism, our single channel experiments show that inactivation of the maxi-K_{Ca} channel is a multi-step phenomenon. There is a continuous reduction of the probability in the open channel state, as illustrated in Fig. 1 (traces B and C) and Fig. 2. This is contrary to the model channels mentioned above, for which an irreversible, direct transition from the open to the closed channel state was found.

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