J. Membrane Biol. 166, 179–185 (1998)

The Journal of
Membrane
Biology

Photofrin II Sensitized Modifications of Ion Transport Across the Plasma Membrane of an Epithelial Cell Line: I. Electrical Measurements at the Whole-Cell Level

L. Kunz, G. Stark
Department of Biology, University of Konstanz, Box M638, D-78457 Konstanz, Germany

Received: 26 May 1998/Revised: 8 September 1998

Abstract. Photofrin II is a photosensitizer frequently applied in photodynamic therapy. Light-induced tumor cell inactivation observed in the presence of this substance has been suggested to start with modifications at the level of cellular membranes. In the present study electrophysiological techniques are applied in order to investigate the action of photofrin II on functional properties of the plasma membrane of opossum kidney (OK) cells (as an epithelial model system) and of fibroblasts. Illumination of the cells in the presence of photofrin II (or Zn-phthalocyanine) leads to comparatively fast depolarization of the membrane potential. It is caused by a strong change of the membrane conductance which proceeds in two phases. Both phases contribute to a loss of ion selectivity of the plasma membrane between K^+ and Na^+. In the first phase, specific pathways for K^+, which determine the resting potential under physiological conditions, are inactivated. The second phase is distinguished by a marked increase of a nonselective conductance. The increase of the latter — after light-induced initiation — continues in the dark. The conclusions are derived from light-induced, time-dependent changes of the membrane conductance and of the shape of the current-voltage relationship detected under different experimental conditions.

Key words: Photosensitization — Photofrin II — Patch-clamp — OK-cells — Membrane potential — Membrane conductance

Introduction

The light-induced oxidation of biological macromolecules and of cellular structures in the presence of photosensitizers is known to cause a multitude of functional changes in biological systems usually summarized as photodynamic effects (Foote, 1976; Straight & Spikes, 1985). The light energy absorbed by the photosensitizer is able to modify nearly all types of biologically relevant macromolecules via a redox- or energy-transfer reaction. In many cases this has been found to occur via singlet oxygen as a reactive intermediate (Moore et al., 1997; Ochsner, 1997). Though photosensitized reactions usually have deleterious (i.e., negative) cellular consequences, photodynamic effects are also of increasing (positive) clinical relevance (Henderson & Dougherty, 1992; van Hillegersberg et al., 1994; Schuitmaker et al., 1996). In the frame of the photodynamic therapy (PDT), the absorption of visible light is used for tumor destruction. In most of the clinical applications, photofrin II has been used as a photosensitizer. Therefore, studies about its mode of photodynamic action on cellular structures may contribute to an understanding of pathophysiological processes, and also to the development and improvement of therapeutic applications.

For most photosensitizers of practical importance, the plasma membrane and the membranes of cellular organelles (mitochondria) have been found to be important sites of photodynamically induced cellular damage (Penning & Dubbelman, 1994; Moore et al., 1997). This also holds for photofrin II, a purified active fraction of hematoporphyrin derivative and for Zn-phthalocyanine. Photofrin II is a mixture of products of a nonspecific oligomerization of hematoporphyrin (Bonnett, 1995). The present communication contains the first detailed electrophysiological study in the presence of photofrin II based on the patch-clamp technique. It is intended to analyze the functional modifications of the plasma membrane as detailed as possible, in order to understand the mode of action of membrane-active photosensitizers.

Early events in the photodynamically induced cellular damage are the depolarization of the membrane potential and an increase in cytoplasmic free calcium (reviewed by Ben-Hur & Dubbelman, 1993; Valenzeno

Correspondence to: G. Stark

& Tarr, 1995). Both phenomena result from the photomodification of cellular membranes and of their transport systems. Changes of the membrane potential and of other electrical membrane properties may be investigated by electrophysiological methods. Electrophysiological studies of photodynamic membrane effects have so far been performed for excitable cells (Kohli & Bryant, 1964; Pooler, 1987; Valenzeno, 1987; Tarr & Valenzeno, 1991; Schaffer et al., 1994; Valenzeno & Tarr, 1995), and for fibroblasts (Krammer-Reubel, 1992). Previous investigations have, however, been limited to the analysis of action potentials and macroscopic membrane currents. The present study for the first time combines the analysis at the macroscopic level of whole-cell currents (part I) with that at the microscopic level, i.e., at the level of the currents flowing through individual ion channels (part II).

In the frame of our photodynamic studies we have been using an established epithelial cell line as well as lipid membranes in the presence of well-known model systems for ion transport (Kunz et al., 1995; Koufen, Zeidler & Stark 1997; Kunz & Stark, 1997). Cultured cells from the opossum kidney (OK-cells) show essential properties of the proximal tubular epithelium and have several well-characterized ion channels (Ubl, Murer & Kolb, 1988; Kolb, 1990; Schwegler et al., 1990; Hollunder-Reese et al., 1991; Ubl, 1992). They represent a model well-suited for photomodification studies of epithelial cells, which are often the target for photochemotherapy.

Materials and Methods

A stock solution (1 mg/ml) of water soluble photofrin II (Quadra Logic Technologies, Kattendijke, Netherlands) was kept at −20°C. Diluted solutions (33 μg/ml) were stored at +4°C for one month at maximum. Thereafter, changes of the UV/VIS-spectrum became apparent. A water soluble mixture of single- and double-sulfonated Zn-phthalocyanine ($ZnPcS_{1/2}$) was prepared according to the procedure of Ali et al. (1988).

OK-cells, kindly supplied by Dr. H. Murer (Zürich, Switzerland), were maintained in culture in a humidified 10% CO_2 atmosphere in DMEM/Hams F12 (1:1) culture medium (Sigma, St. Louis, MO) containing an additional 10% (v/v) fetal calf serum (Gibco, Eggenstein, Germany), 48 mM $NaHCO_3$, 43 IU/ml penicillin G, and 50 μg/ml streptomycin (both Serva, Heidelberg, Germany) (Ubl et al., 1988). The cells were isolated from confluent monolayers with trypsin and plated on coverslips several hours before being used. They were incubated for 0.5–8 hr (1 hr typically) in a high-NaCl solution (bath1, *see* Table) containing photofrin II (0.1–10 μg/ml; typically 0.33 μg/ml) at room temperature. Thereafter, cells were stored in the same solution without the sensitizer. Electrophysiological experiments were performed up to 3 hr after incubation. During this time interval, the photodynamic effect was found to be roughly identical. This indicates a rather constant membrane concentration of the sensitizer. At the experiments with the sulfonated Zn-phthalocyanine, the sensitizer was added to the pipette solution (10 nM–1 μM). This mode of sensitizer application was also successfully tried with photofrin II (25–50 μg/ml).

Some supplementary experiments were performed with fibroblasts. Rat embryo fibroblasts (REF) from a primary culture prepared from 13-day-old embryos of Fischer rats, line CDF(F344)/CRLBR (Charles River, Sulzfeld, Germany), were obtained with a CPD (Cumulative Population Doublings) of 10 and were further cultivated in DME with 5% (v/v) fetal calf serum (both Gibco, Eggenstein, Germany) using roughly the same procedure as with OK-cells. M1-cells are young REF (with CPD 2) immortalized by transfection with the c-myc oncogen (Land, Parada & Weinberg, 1983; Simm, Halle & Adam, 1994). Both types of fibroblasts were kindly supplied by the late Dr. G. Adam (Konstanz, Germany).

Table. Composition of bath and pipette solutions

Solution	[NaCl]/mM	[KCl]/mM	Solution	[NaCl]/mM	[KCl]/mM
Bath1	140	4	pip1	20	135
Bath2		154	pip2		150
Bath3	150		pip3	150	

All solutions contained in addition 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 (or 18) mM glucose and 10 (or 20) mM HEPES. The pipette solutions 1–3 contained 2 mM EGTA to ensure a free Ca^{2+} concentration of about 0.1 μM. The pH of the solutions was adjusted to 7.4.

After establishment of the patch-clamp configuration (*see below*), the cell was illuminated with a HeNe-laser (Melles Griot, Carlsbad, CA) at wavelength 632.8 nm. The laser, including its power supply, was arranged outside the Faraday cage of the patch-clamp setup. The laser light was focused via fiber optics (Spindler & Hoyer, Göttingen, Germany) onto the cells under an angle of 45° (mean irradiance 5200 W/m^2).

Whole-cell patch-clamp recordings (Hamill et al., 1981; Penner, 1995) were performed using an EPC-9 patch-clamp amplifier and the software Pulse (HEKA elektronik, Lambrecht, Germany). The facilities of this software were also used to measure the series resistance, R_s, and the membrane capacitance, C_m (*cf.* Penner, 1995). Patch pipettes were pulled from borosilicate glass tubings (GC 150F-15, Clark Electromedical Instruments, Pangbourne Reading, England) with a micropipette puller P-87 (Sutter Instruments, Novato, CA) and then heat-polished. Pipette resistance was in the range of 1–5 MΩ.

The resting potential, V_m^0, of the cells was determined in the current-clamp mode of EPC-9. The conductance, G_m, and the current-voltage relationship of the cells were measured by application of various voltage-pulse protocols. G_m refers to the resting potential and was obtained by application of voltage pulses of 100 msec length (and amplitude steps of 5 mV) in the range $V_m^0 \pm 10$ mV. Current-voltage curves were measured via the time dependence of the current after a series of voltage pulses between −140 and +140 mV (*see* Fig. 1). The steady-state current observed at the end of the voltage pulse was plotted as a function of voltage. Positive currents from the cytoplasmic to the external side were defined as outward and shown as upward deflections. Potentials refer to the cytoplasmic side.

Bath and pipette solutions applied throughout the experiments are summarized in the Table. All experiments were performed at room temperature. Data reduction and analysis were performed using the software Fig.P (Fig.P software, Durham, NH). The number, *n*, of identical experiments is given in brackets. The error of the experimental quantities represents the standard deviation. For further experimental details *see* Kunz (1998).

Results and Discussion

The resting potential of OK-cells under physiological conditions (i.e., using solutions bath1 and pip1) was

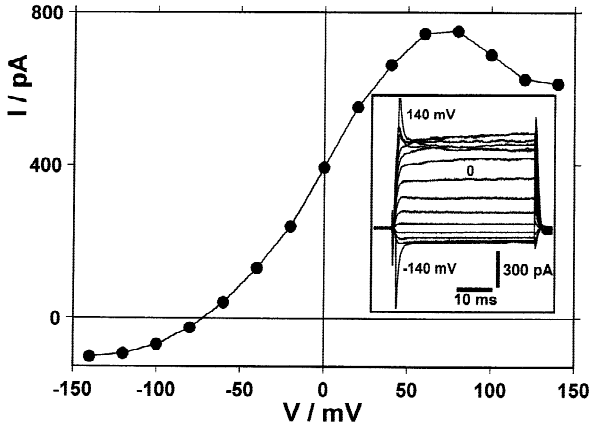

Fig. 1. Measurement of steady state current-voltage curves by application of voltage pulses with amplitudes from −140 to +140 mV (in steps of 20 mV). The holding potential (zero current potential) was −72 mV. The steady-state current observed within the last 5 msec of the voltage pulse (*see* inset) is plotted as a function of the membrane voltage. The experimental data were obtained from an OK-cell using solutions pip1 and bath1 (in the absence of photosensitizer).

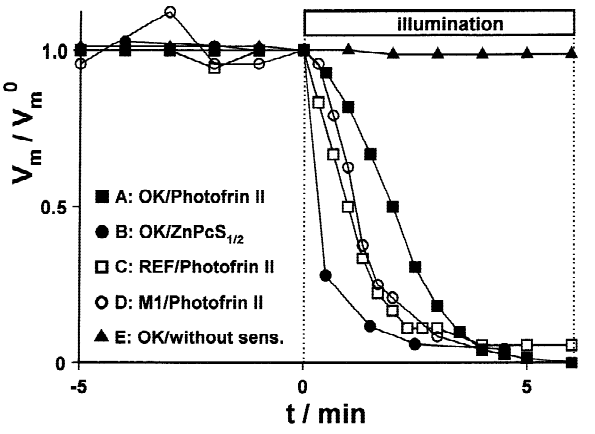

Fig. 2. The decay of the membrane potential following illumination of different cell types in the presence of either photofrin II or Zn-phthalocyanine. Solutions: pip1 and bath1. V_m^0 = resting potential, V_r, before illumination. (*A*) OK-cells after incubation with 0.33 μg/ml photofrin II for 60 min, V_m^0 = −76 mV. (*B*) OK-cells with 100 nM $ZnPcS_{1/2}$ in the pipette solution, V_m^0 = −72 mV. (*C*) REF-cells after incubation with 0.33 μg/ml photofrin II for 60 min, V_m^0 = −22 mV. (*D*) M1-cells after incubation with 1 μg/ml photofrin II for 60 min, V_m^0 = −28 mV. (*E*) OK-cells in absence of a sensitizer, V_m^0 = −83 mV.

found to be $V_m^0 = -77 \pm 7$ mV ($n = 67$). The current-voltage relationship shows outward rectification in this case (Fig. 1). This is due to the comparatively high K^+ permeability and the asymmetric distribution of K^+ ions (*see below*). The asymmetry of the current-voltage curves may be characterized by the ratio, $I(+)/I(-)$, of currents $I(+) = I(V_m^0 + \Delta V)$ and $I(-) = I(V_m^0 - \Delta V)$. For $\Delta V = 60$ mV, $I(+)/I(-) \approx 2$ is found under physiological conditions. A negative resistance is observed at potentials larger than +70 mV, i.e., there is a current decrease with increasing voltage. This is an indication for a voltage-dependent closing of ion channels at large positive potentials. The membrane conductance at the resting potential was found to be $G_m = 7.8 \pm 7.8$ nS ($n = 64$). If normalized to the membrane capacitance, C_m, (measured independently) values $G_c = G_m/C_m = 200 \pm 160$ *S/F* ($n = 57$) are obtained. The large standard deviation of G_m and G_c indicates a comparatively great variation (of about one order of magnitude) between individual cells. This is contrary to the resting potential, V_m^0, where rather constant values were found (*see above*).

The presence of the sensitizers photofrin II and Zn-phthalocyanine has no influence on V_m^0 as long as the membrane is kept in dark ($V_m^0 = -73 \pm 12$ mV ($n = 87$) and $V_m^0 = -72 \pm 10$ mV ($n = 34$), respectively). The same holds for the current-voltage curves, which are not affected by the sensitizers in the dark (*data not shown*). Illumination of the membrane in the presence of either of the sensitizers, however, leads to a virtually complete depolarization of the membrane potential, V_m, within several minutes (Fig. 2). The depolarization is clearly due to a photodynamic reaction, since there is no effect of light in the absence of the sensitizers (Fig. 2, trace E). Similar results are obtained with the fibroblasts REF and M1, which show lower resting potentials, V_m^0, in the absence of illumination. The depolarization is not due to an inactivation of the Na/K-ATPase, since the decay of V_m does not depend on the ATP concentration (0–5 mM in the pipette solution) and could not be influenced by adding the inhibitor ouabain to the bath (10–100 μM, $n = 5$). V_m was found to be fairly constant over (at least) a time range of 45 min in the absence and presence of ATP (and without photodynamic treatment). The experiments indicate that V_m — under conditions where the membrane separates two large ion reservoirs — is completely determined by the passive ionic permeabilities of the membrane, which are modified by the photodynamic effect. This is in line with a previous study by Specht and Rodgers (1991).

Additional tests were performed in order to exclude an influence of the photodynamic effect on the quality of the seal. As shown in Fig. 3, the series resistance, R_s, to the cell interior remains fairly constant throughout a typical experiment. The same holds for the membrane capacitance, C_m, apart from a significant increase towards the end of the experiment[1]. A deterioration of the seal

[1]This may be interpreted via a modified dielectric constant of the membrane interior induced by accumulation of products of (photodynamically induced) lipid peroxidation. This is supported by similar experiments performed at artificial lipid membranes (Sträßle et al., 1991). An increase of the membrane capacitance was observed after X-ray exposure, i.e., under conditions where lipid peroxidation was induced by free radicals of water radiolysis.

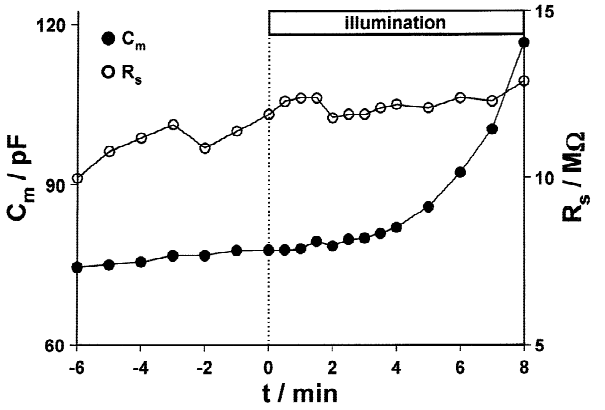

Fig. 3. The change of membrane capacitance, C_m, and of the series resistance, R_s, during illumination of an OK-cell in the presence of photofrin II. Experimental conditions *see* legend to Fig. 2 (trace *A*).

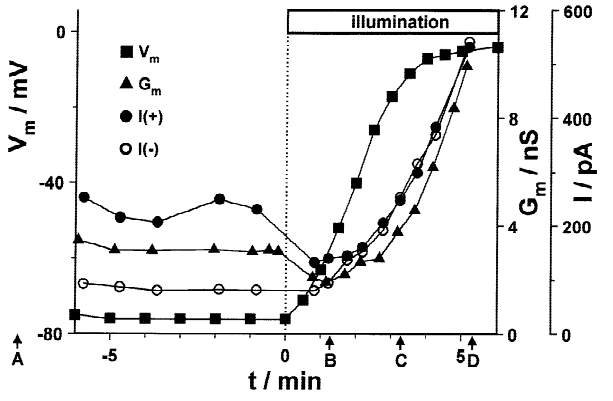

Fig. 4. Time dependence of the membrane potential, V_m, of the membrane conductance, G_m, and of the currents $I(+)$ and $I(-)$ (observed at depolarizing and hyperpolarizing potential steps of 60 mV, respectively) after illumination of OK-cells in the presence of photofrin II. Experimental conditions see legend to Fig. 2 (trace A). The letters A, B, C, and D indicate the times at which the current-voltage curves shown in Fig. 5 were measured.

would be accompanied by a decrease of C_m, which is in contrast to the experimental reality. Further evidence for an intact seal throughout a photodynamic treatment is provided by the fact that the whole-cell properties are observed in the cell-attached state after a photodynamic treatment (in the presence of 10–50 μg/ml photofrin II in the pipette solution only). Typical current-voltage curves may be measured in this way, which are identical to those obtained in the normal whole-cell configuration (e.g., Fig. 1). Addition of the sensitizer to the pipette solution lowers the resistance of the membrane patch only, while the whole-cell properties (including the seal resistance) are not affected. This may be applied as a new method to generate a light induced slow-whole-cell version (perforated patch) of the patch-clamp technique, which is described in more detail in part II of this series. A further test applies to the visual microscopic inspection of the cell under the patch. There was no evidence for a change of the cell shape induced by the photodynamic treatment.

The following experiments were performed in order to analyze the molecular events leading to the depolarization of the membrane potential. The latter is accompanied by a characteristic change of the membrane conductance and by a loss of the asymmetry of the current-voltage curves as shown in Figs. 4 and 5. Following the start of illumination, the conductance, G_m, after a transient decrease, shows a continuous increase. The clear difference of the currents $I(+)$ and $I(-)$ disappears completely as the time of illumination increases (Fig. 4). Both effects are confirmed by an analysis of the current-voltage relationship (Fig. 5). There is a decrease of the zero-current potential from about −70 mV to virtually zero, a transient decrease followed by an increase of the slope at the zero-current potentials and a continuous transition from an asymmetric to a symmetric shape of the curves with increasing duration of illumination (curves

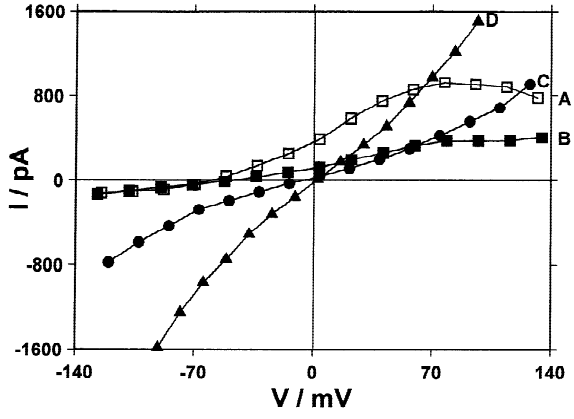

Fig. 5. Current-voltage curves observed before (A) and at times 1.3 min (B), 3.3 min (C) and 5.3 min (D) after start of illumination of an OK-cell in the presence of photofrin II. The data of Figs. 4 and 5 were obtained from the same experiment.

A–D). The negative resistance (at large positive potentials) disappears.

The results may be explained as follows. The membrane conductance, G_m, is normally governed by a comparatively large K^+-permeability. If the ionic pathways for K^+ are photodynamically inactivated, a reduction of two essential quantities is expected: of the absolute value of the membrane conductance, G_m, and of its ion selectivity expressed by the permeability ratio P_K/P_{Na} of the plasma membrane. The decay of the latter predicts a decrease of V_m, as is easily shown by application of Goldman's Equation (*see* part II). There is a second contribution to the loss of the ion selectivity (i.e., to the decay of V_m), namely the increase of the membrane conductance which follows the transient conductance de-

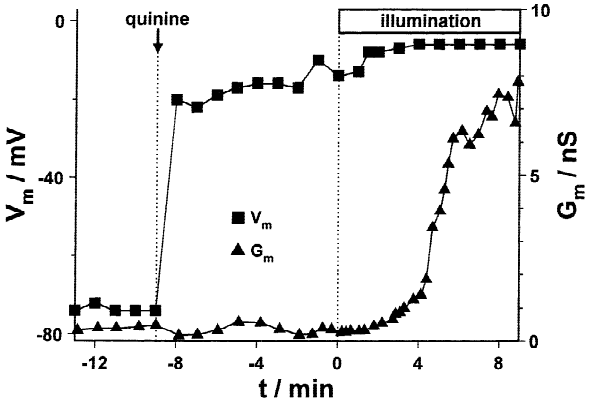

Fig. 6. The effect of 5 mM quinine and of illumination on the membrane potential, V_m, and on the conductance, G_m, of OK-cells. Cells were incubated with 0.33 μg/ml photofrin II for 60 min before the measurement (using solutions pip1 and bath1) was performed. Quinine was added at the arrow and was present throughout the rest of the experiment.

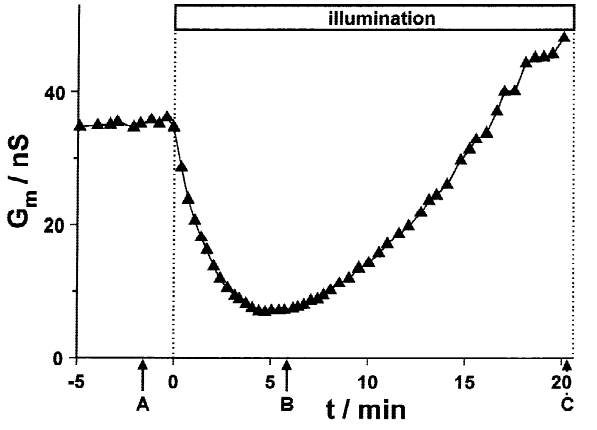

Fig. 7. Time dependence of the membrane conductance, G_m, under symmetrical concentrations of K^+ (solutions pip2 and bath2). OK-cells were incubated with 0.17 μg/ml photofrin II for 80 min. The letters A, B, and C indicate the times at which the current-voltage curves shown in Fig. 8 were measured.

crease (*cf.* Fig. 4). Fairly identical values for $I(+)$ and $I(-)$ are found throughout the second period of illumination, though asymmetric ionic concentrations were applied. This indicates a permeability ratio P_K/P_{Na} of close to 1 for the induced conductance. The conductance increase will be designated as a nonspecific leak conductance and will be further analyzed at the level of membrane patches (*see* part II).

The interpretation was tested by a series of additional experiments. The importance of the K^+ permeability for the membrane potential is demonstrated by Fig. 6. Addition of quinine, a blocker of K^+ channels (Castle, Haylett & Jenkinson, 1989; Ubl, 1992), leads to a strong depolarization of V_m and to a reduction of G_m by 76 ± 7% ($n = 6$) (in the absence of light). The experiment also indicates that the transient conductance decrease observed throughout the initial period of illumination (*cf.* Fig. 4) is due to an inactivation of K^+ channels. If K^+ channels are blocked by quinine, the transient decrease of G_m during illumination disappears (Fig. 6).

The role of K^+ channels for the photodynamic reduction of V_m is further substantiated by experiments performed with different salt solutions (Figs. 7–10). The membrane separates symmetrical solutions of either KCl (Figs. 7 and 8) or NaCl (Figs. 9 and 10). The transient conductance decrease is only observed in the presence of K^+, while the subsequent conductance increase is present in both cases. The corresponding current-voltage curves are symmetric in both cases (Figs. 8 and 10). The reduction of the slope, however, only exists in the presence of K^+ (*cf.* Fig. 8, curves A and B), whereas in the presence of Na^+ a continuous increase of the slope is observed (Fig. 10, curves A–D). The K^+ conductance shows voltage dependent inactivation at large potentials, as may be concluded from the current decay observed

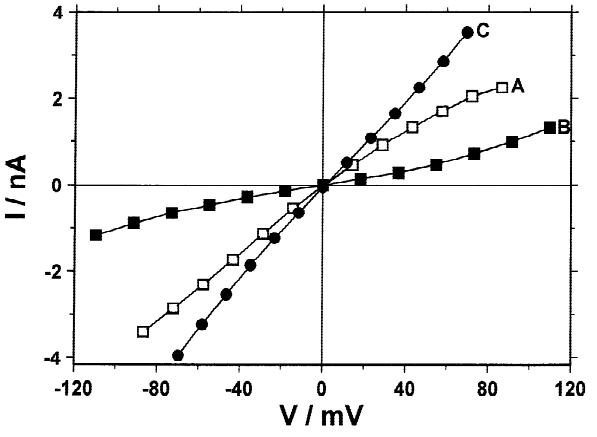

Fig. 8. Current-voltage curves observed before (A) and at times 5.5 min (B), and 20.4 min (C) after start of illumination of an OK-cell in the presence of photofrin II under symmetrical concentrations of K^+. The data of Figs. 7 and 8 were obtained from the same experiment.

after application of voltage pulses of sufficient amplitude and from the negative resistance at large positive potentials (*cf.* Fig. 1). All these properties, which are characteristic for the K^+ permeability of the plasma membrane, disappear after illumination in the presence of photofrin II.

All experiments are in line with the assumption of a twofold reason for the photodynamically induced loss of ion selectivity of the plasma membrane of OK-cells: an inactivation of ion channels specific for K^+, which largely determine the magnitude of the resting potential under physiological conditions, and a strong increase of an ionic pathway, which is nonspecific for the cations K^+ and Na^+. The contributions of the two important characteristics of photomodification may be separated by application of two subsequent, short periods of illumina-

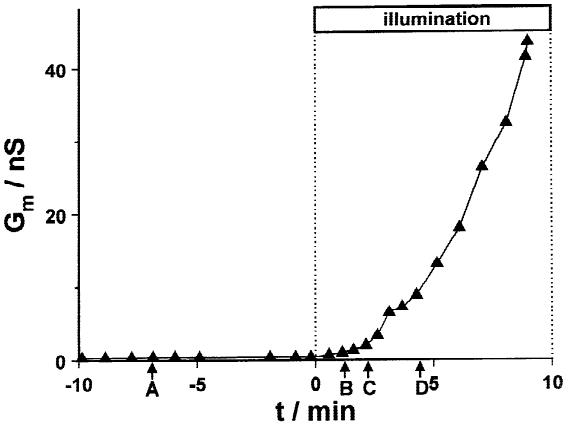

Fig. 9. Time dependence of the membrane conductance, G_m, under symmetrical concentrations of Na^+ (solutions pip3 and bath3). OK-cells were incubated with 0.33 μg/ml photofrin II for 60 min. The letters A, B, C, and D indicate the times at which the current-voltage curves shown in Fig. 10 were measured.

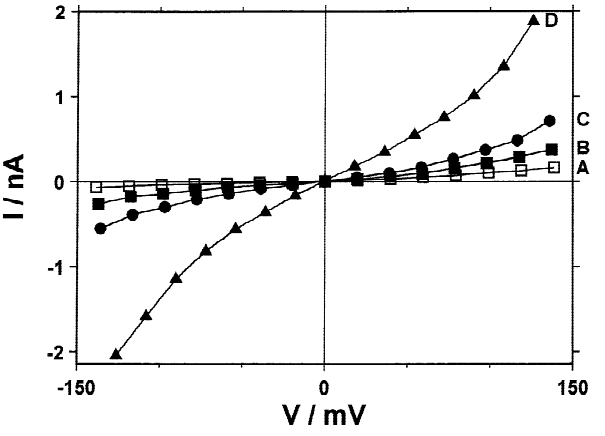

Fig. 10. Current-voltage curves observed before (A) and at times 1.2 min (B), 2.2 min (C) and 4.2 min (D) after start of illumination of an OK-cell in the presence of photofrin II under symmetrical concentrations of Na^+. The data of Figs. 9 and 10 were obtained from the same experiment.

tion (Fig. 11). During the first illumination period, V_m is reduced from −83 to about −35 mV, while G_m is reduced. The second period of illumination leads to further depolarization of V_m, while there is a pronounced increase of G_m. Figure 11 also shows that both the increase of the leak conductance and the concomitant decay of the membrane potential continue after stop of the second illumination interval. This is indicative of processes which, after light-induced initiation, proceed in the dark and finally lead to the enhanced leak conductance observed experimentally.

The present study analyzes the sources of the decay of the membrane potential following photomodification of the plasma membrane and is based on detailed experi-

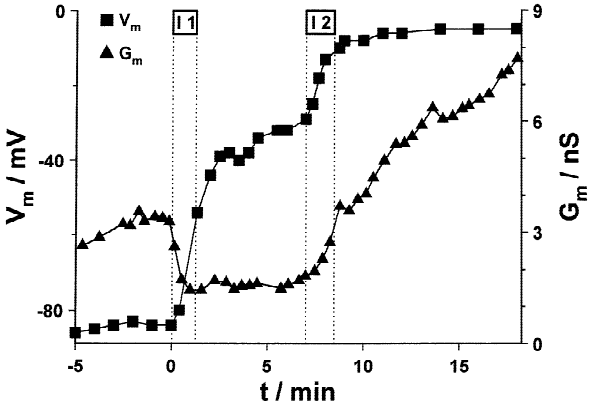

Fig. 11. The effect of fractionation of illumination on the membrane potential, V_m, and on the conductance, G_m, of OK-cells. Cells were incubated with 0.33 μg/ml photofrin II for 100 min before the measurement (using solutions pip1 and bath1) was performed. The cell was illuminated throughout the periods I1 and I2.

ments with OK-cells. We think, however, that the main conclusions are also valid for other cell types such as fibroblasts. A decay of the K^+ current (and of Na^+ and Ca^{2+} currents) and an increase of the leak conductance was also observed at photomodification of cardiac myocytes induced by rose bengal (Tarr & Valenzeno, 1991; Valenzeno & Tarr, 1995). It appears that photodynamically induced ion channel inactivation and enhancement of the leak conductance is a general phenomenon of biological membranes, which — on the microscopic level — is studied in part II of this study.

References

Ali, H., Langlois, R., Wagner, J.R., Brasseur, N., Paquette, B., van Lier, J.E. 1988. Biological activities of phthalocyanines — X. Syntheses and analyses of sulfonated phthalocyanines. *Photochem. Photobiol.* **47:**713–717

Ben-Hur, E., Dubbelman, T.M.A.R. 1993. Cytoplasmic free calcium changes as a trigger mechanism in the response of cells to photosensitization. *Photochem. Photobiol.* **58:**890–894

Bonnett, R. 1995. Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy. *Chem. Soc. Rev.* **24:**19–33

Castle, N.A., Haylett, D.G., Jenkinson, D.H. 1989. Toxins in the characterization of potassium channels. *TINS* **12:**59–65

Foote, C.S. 1976. Photosensitized oxidation and singlet oxygen. *In:* Free Radicals in Biology, Vol. II, W.A. Pryor, editor. pp. 85–133. Academic Press, New York

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Henderson, B.W., Dougherty, T.J. 1992. How does photodynamic therapy work? *Photochem. Photobiol.* **55:**145–157

van Hillegersberg, R., Kort, W.J., Wilson, J.H.P. 1994. Current status of photodynamic therapy in oncology. *Drugs* **48:**510–527

Hollunder-Reese, F., Mohrmann, M., Bleich, M., Greger, R. 1991. K^+-, Cl^-- and nonselective channels in opposum kidney cells. *Cell. Physiol. Biochem.* **1:**238–250

Kohli, R.P., Bryant, S.H. 1964. Photodynamic effect of dye on frog muscle fibre using microelectrodes. *Experientia* **20:**368–369

Kolb, H.-A. 1990. Ion channels in opossum kidney cells. *Renal Physiol. Biochem.* **13:**26–36.

Koufen, P., Zeidler, U., Stark, G. 1997. Photodynamic inactivation of ion channels formed by the polyene antibiotic amphotericin B in lipid membranes. *J. Photochem. Photobiol. B: Biol.* **38:**129–135

Krammer-Reubel, B. 1992. Transmembrane potential measurements of normal and transformed human fibroblasts following photodynamic laser therapy. *Bioelectrochem. Bioenergetics* **27:**19–22

Kunz, L. 1998. Photodynamische Wirkungen von Photofrin II auf Ionenkanäle und Leckleitfähigkeit der Plasmamembran. Doctoral thesis, University of Konstanz, Konstanz, Germany

Kunz, L., Stark, G. 1997. Photodynamic membrane damage at the level of single ion channels. *Biochim. Biophys. Acta* **1327:**1–4.

Kunz, L., Zeidler, U., Hägele, K., Przybylski, M., Stark, G. 1995. Photodynamic and radiolytic inactivation of ion channels formed by gramicidin A: oxidation and fragmentation. *Biochemistry* **34:**11895–11903

Land, H., Parada, L.F., Weinberg, R.A. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304:**596–602

Moore, J.V., West, C.M.L., Whitehurst, C. 1997. The biology of photodynamic therapy. *Phys. Med. Biol.* **42:**913–935

Ochsner, M. 1997. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J. Photochem. Photobiol. B: Biol.* **39:**1–18

Penner, R. 1995. A practical guide to patch clamping. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 3–30. Plenum Press, New York

Penning, L.C., Dubbelman, T.M.A.R. 1994. Fundamentals of photodynamic therapy: cellular and biochemical aspects. *Anti-Cancer Drugs* **5:**139–146

Pooler, J.P. 1987. Photodynamic modification of excitable cell function. *In:* Light-activated pesticides. J.R. Heitz, editor, pp. 109–121. ACS symposium series, Vol. 339. American Chemical Society, Washington, DC

Schaffer, P., Ahammer, H., Müller, W., Koidl, B., Windisch, H. 1994. Di-4-ANEPPS causes photodynamic damage to isolated cardiomyocytes. *Pfluegers Arch.* **426:**548–551

Schuitmaker, J.J., Baas, P., van Leengoed, H.L.L.M., van der Meulen, F.W., Star, W.M., van Zandwijk, N. 1996. Photodynamic therapy: a promising new modality for the treatment of cancer. *J. Photochem. Photobiol. B: Biol.* **34:**3–12.

Schwegler, J.S., Steigner, W., Heuner, A., Silbernagl, S. 1990. pH_i-Dependent membrane conductance of proximal tubule cells in culture (OK): differential effects on K^+- and Na^+-conductive channels. *J. Membrane Biol.* **117:**243–251

Simm, A., Halle, J.-P., Adam, G. 1994. Proliferative and metabolic capacity of rat embryo fibroblasts immortalized by c-myc depends on cellular age at oncogenic transfection. *Eur. J. Cell Biol.* **65:**121–131

Specht, K.G., Rodgers, M.A.J. 1991. Plasma membrane depolarization and calcium influx during cell injury by photodynamic action. *Biochim. Biophys. Acta* **1070:**60–68

Straight, R.C., Spikes, J.D. 1985. Photosensitized oxidation of biomolecules. *In:* Singlet Oxygen, Vol. IV. A.A. Frimer, editor, pp. 91–143. CRC Press, Boca Raton

Sträßle, M., Wilhelm, M., Stark, G. 1991. The increase of membrane capacitance as a consequence of radiation-induced lipid peroxidation. *Int. J. Radiat. Biol.* **59:**71–83.

Tarr, M., Valenzeno, D.P. 1991. Modification of cardiac ionic currents by photosensitizer-generated reactive oxygen. *J. Mol. Cell. Cardiol.* **23:**639–649

Ubl, J. 1992. Ionentransportsysteme in einer Nierenzellinie (OK-Zellen) und deren Beteiligung an der Volumenregulation. Doctoral thesis, University of Konstanz, Konstanz, Germany

Ubl, J., Murer, H., Kolb, H.-A. 1988. Ion channels activated by osmotic and mechanical stress in membranes of opossum kidney cells. *J. Membrane Biol.* **104:**223–232

Valenzeno, D.P. 1987. Photomodification of biological membranes with emphasis on singlet oxygen mechanisms. *Photochem. Photobiol.* **46:**147–160

Valenzeno, D.P., Tarr, M. 1995. Mechanisms of cellular photomodification. *In:* Light-Activated Pest Control. J.R. Heitz, editor, pp. 24–33. ACS symposium series, Vol. 616, American Chemical Society, Washington, DC