Biochemical and Biophysical Research Communications 369 (2008) 1022–1026

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effects on capacitance by overexpression of membrane proteins

D. Zimmermann [a,b,*], A. Zhou [c], M. Kiesel [d], K. Feldbauer [a], U. Terpitz [a], W. Haase [e], T. Schneider-Hohendorf [d], E. Bamberg [a,b], V.L. Sukhorukov [d]

[a] Department of Biophysical Chemistry, Max-Planck-Institute of Biophysics, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany
[b] Department of Biophysical Chemistry, Chemistry and Pharmacy, Johann-Wolfgang-Goethe University, Max-von-Laue-Strasse 4, 60438 Frankfurt am Main, Germany
[c] Department of Cell Physiology, Max-Planck-Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany
[d] Department of Biotechnology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
[e] Department of Structural Biology, Max-Planck-Institute of Biophysics, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 23 February 2008
Available online 10 March 2008

Keywords:
Patch-clamp
Electrorotation
Freeze-fracture electron microscopy
HEK293
Overexpression
Membrane capacitance
Channelrhodopsin-2

ABSTRACT

Functional Channelrhodopsin-2 (ChR2) overexpression of about 10^4 channels/μm^2 in the plasma membrane of HEK293 cells was studied by patch-clamp and freeze-fracture electron microscopy. Simultaneous electrorotation measurements revealed that ChR2 expression was accompanied by a marked increase of the area-specific membrane capacitance (C_m). The C_m increase apparently resulted partly from an enlargement of the size and/or number of microvilli. This is suggested by a relatively large C_m of 1.15 ± 0.08 $\mu F/cm^2$ in ChR2-expressing cells measured under isotonic conditions. This value was much higher than that of the control HEK293 cells (0.79 ± 0.02 $\mu F/cm^2$). However, even after complete loss of microvilli under strong hypoosmolar conditions (100 mOsm), the ChR2-expressing cells still exhibited a significantly larger C_m (0.85 ± 0.07 $\mu F/cm^2$) as compared to non-expressing control cells (0.70 ± 0.03 $\mu F/cm^2$). Therefore, a second mechanism of capacitance increase may involve changes in the membrane permittivity and/or thickness due to the embedded ChR2 proteins.

The area-specific membrane capacitance C_m [$\mu F/cm^2$] of cells, which reflects the dielectric properties and the surface topology of a membrane, has been shown to respond to cell physiological changes [10]. Exocytosis may evoke drastic changes in membrane capacitance by increasing the membrane area after fusion of vesicles [3,15]. Furthermore, there were intensive studies on artificial bilayers to probe the effects of different lipid compositions on C_m. Pronounced effects were found when changing the membrane thickness by varying the length of the carbon chains [4]. The incorporation of proteins can also change the membrane capacitance due to an alteration of membrane topology. Proteins, thereby, may act as a compressor or a stretcher of the bilayer and, thus, evoke changes of the membrane's capability to act as a capacitor [17,18]. It has also been shown that the presence of proteins may have an impact on electroporation and -fusion of cells [20,21]. However, Gentet et al. [7] observed no changes in membrane specific capacitance of HEK293 cells expressing the Gly-receptor at physiological surface densities.

In this communication, we investigated the effects evoked by a high-scale overexpression of the membrane protein Channelrhodopsin-2 [12,19] on the specific membrane capacitance of HEK293 cells. Quantitatively, the level of expression was revealed by electrophysiological recordings using the patch-clamp technique and particle analysis of freeze-fracture electron microscopy images. A channel density of about 10^4 channels/μm^2 was observed. As revealed by patch-clamp measurement, ChR2 expression was accompanied by an increase in the specific membrane capacitance C_m of about 30%. An independent determination of the specific capacitance of individual cells by the non-invasive electrorotation technique [2,6,8,9,20] revealed similar results. The combination of techniques and, in particular, the comparison of isoosmolar and hypoosmolar conditions demonstrated that the observed increase in specific capacitance was linked to the protein expression level.

Methods and materials

Cell culture, molecular biology, and patch-clamp. Cell culture, molecular biology, expression of the fusion protein ChR2-YFP, patch-clamp measurements, as well as determination of the capacitance were carried out as described previously by Zimmermann et al. [19]. Mean capacitance values of the patch-pipettes were determined to be 6.9 ± 0.4 pF ($n = 5$) and subtracted from the capacitance values obtained in the whole-cell configuration. *Specific capacitance C_m:* the bath solution contained 140 mM NaCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$. The patch-pipette solution contained 110 mM NaCl, 10 mM EGTA, and 2 mM $MgCl_2$. Experiments were performed at 23 °C. *ChR2-mediated conductance:* the bath solution contained

* Corresponding author. Address: Department of Biophysical Chemistry, Max-Planck-Institute of Biophysics, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Hessen, Germany.
E-mail address: dirk.zimmermann@mpibp-frankfurt.mpg.de (D. Zimmermann).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.02.153

200 mM Guanidine (hydro)chloride (Gua-Cl), 2 mM $MgCl_2$, and 2 mM $CaCl_2$. The patch-pipette solution contained 1 mM Guanidine (hydro)chloride (Gua-Cl), 199 mM NMG-Cl, and 10 mM EGTA. Patch-clamp experiments were performed at 11 °C.

Additionally 10–20 mM HEPES were added to all solutions and the pH was adjusted to 7.4 by appropriate addition of NaOH, NMG-Cl or HCl. The osmolality of all solutions was measured using a Semi-Micro-Osmometer (Knauer, Berlin-Zehlendorf, Germany). All measurements were carried out under saturating light conditions and exclusively on cells with a diameter of 15 μm.

Freeze-fracture electron microscopy. Cells were fixed with 2.5% glutaraldehyde in sodium cacodylate buffer at room temperature, stepwise infiltrated with glycerol (up to 30%) and then frozen in ethane cooled with liquid nitrogen to −180 °C. Fracturing and shadowing were carried out in a BAF 060 freeze-fracture machine (BalTec, Liechtenstein). Shadowing was performed at an angle of 45° for Pt and at 90° for pure carbon. Acid cleaned freeze-fracture replicas were viewed in an EM208S electron microscope (FEI Company), and analyzed by eye and/or by a recognition software (ImageJ; http://rsb.info.nih.gov/ij/).

Electrorotation (*ER*). Sorbitol of highest purified grade was purchased from Sigma or Fluka (both Deisenhofen, Germany). Before electrorotation, the cells were washed 2–3 times with isoosmolar sorbitol solution (300 mOsm) and resuspended in iso- or hypoosmolar (100 mOsm) sorbitol solutions at a final cell density of $(1–2) \times 10^5$ cells/ml. Cells were incubated at least 3 min in hypoosmolar medium before measurement. The suspension conductivity (σ_e) was adjusted to ~10–70 μS/cm by addition of appropriate amounts of HEPES–KOH, pH 7.4. Conductivity and osmolality of the solutions were measured by means of a conductometer (Konduktometer 703, Knick GmbH, Berlin, Germany) and a Semi-Micro-Osmometer (Knauer), respectively.

The measurements of the field frequency f_{c1} inducing fastest anti-field rotation of cells were performed under low-conductivity conditions (σ_e < ~70 μS/cm) by the

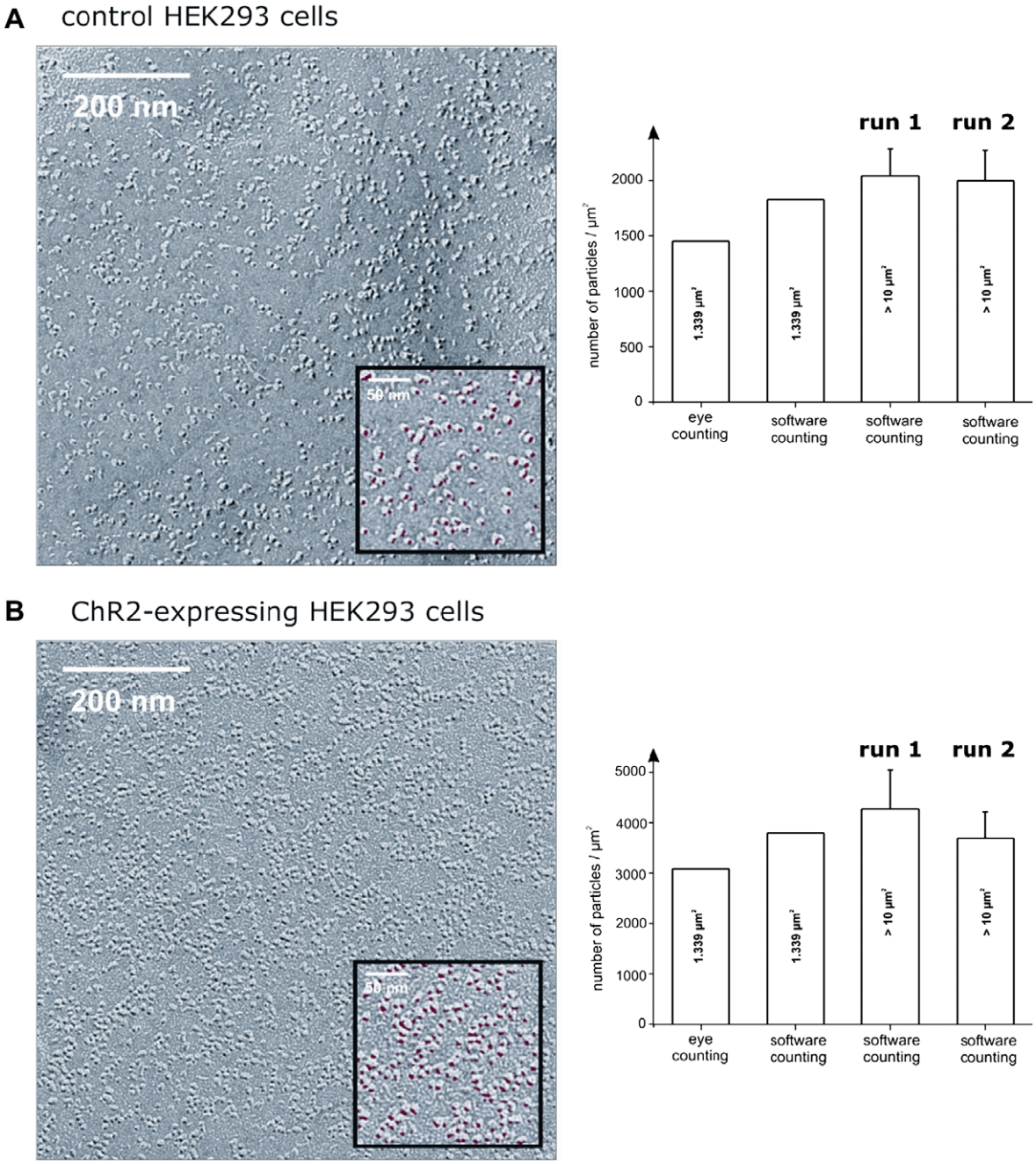

Fig. 1. In (A) and (B): freeze-fracture electron microscopy images of control HEK293 cells and HEK293 cells expressing ChR2-YFP are shown, respectively. Mean particle (p) densities in (A) and (B) were determined by use of recognition software to be 2042 ± 243 and 4277 ± 773 p/μm² (counting by eye: 1453 and 3090 p/μm², respectively). Red dots in the enlarged images highlight the particles scored by the recognition software. *Note:* particle size has become more homogeneous in the images obtained from ChR2-expressing HEK293 cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)