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Plasma Membrane Permeabilization by 60- and 600-ns Electric Pulses Is Determined by the Absorbed Dose

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Abstract

We explored how the effect of plasma membrane permeabilization by nanosecond-duration electric pulses (nsEP) depends on the physical characteristics of exposure. The resting membrane resistance (R_m) and membrane potential (MP) were measured in cultured GH3 and CHO cells by conventional whole-cell patch-clamp technique. Intact cells were exposed to a single nsEP (60 or 600 ns duration, 0–22 kV/cm), followed by patch-clamp measurements after a 2–3 min delay. Consistent with earlier findings, nsEP caused long-lasting R_m decrease, accompanied by the loss of MP. The threshold for these effects was about 6 kV/cm for 60 ns pulses, and about 1 kV/cm for 600 ns pulses. Further analysis established that it was neither pulse duration nor the E-field amplitude per se, but the absorbed dose that determined the magnitude of the biological effect. In other words, exposure to nsEP at either pulse duration caused equal effects if the absorbed doses were equal. The threshold absorbed dose to produce plasma membrane effects in either GH3 or CHO cells at either pulse duration was found to be at or below 10 mJ/g. Despite being determined by the dose, the nsEP effect clearly is not thermal, as the maximum heating at the threshold dose is less than 0.01 °C. The use of the absorbed dose as a universal exposure metric may help to compare and quantify nsEP sensitivity of different cell types and of cells in different physiological conditions. The absorbed dose may also prove to be a more useful metric than the incident E-field in determining safety limits for high peak, lowaverage power EMF emissions.

Keywords

dose effect; electroporation; cell membrane; patch-clamp

INTRODUCTION

High-voltage electric pulses of nanosecond duration have been shown to produce multifarious effects in living cells, including permeabilization of intracellular granules [Schoenbach et al., 2001], endocytosed vacuoles [Tekle et al., 2005], and cell plasma membrane [Pakhomov et al., 2007a,b]; cell shrinkage [Schoenbach et al., 2001] or cell swelling and blebbing [Pakhomov et al., 2007a]; damage to the nuclear DNA [Stacey et al., 2003]; intracellular calcium bursts [Vernier et al., 2003; White et al., 2004]; externalization of phosphatidylserine residues [Vernier et al., 2004]; necrotic or apoptotic cell death [Beebe et al., 2002; Schoenbach et al., 2002; Beebe et al., 2003; Vernier et al., 2003; Pakhomov et al., 2004]; and tumor growth

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inhibition [Beebe et al., 2002; Nuccitelli et al., 2006]. It is worth mentioning that many of these effects are essentially different from those caused by conventional electroporation with longer electric pulses [Neumann et al., 1989; Weaver, 2000, 2003; Zimmermann et al., 2000].

Any nsEP treatment is characterized by a number of physical parameters (pulse width, voltage, rise time, repetition rate, number of pulses in a train, etc.), resulting in an endless variety of possible combinations. In most studies referenced above, the choice of specific exposure parameters appears almost arbitrary, or it was dictated by such factors as the availability of the nsEP exposure devices and by the appearance of a particular effect being studied. In different studies, the pulse duration was 7, 10, 60, 300, or 600 ns; the E-field varied from about 10 to 300 kV/cm; some effects were elicited by single pulses, whereas others required trains of more than 1000 pulses; and so forth. As a result, numerous reports of biological findings with nsEP exposure are difficult or impossible to compare and analyze together.

Limited attempts to compare isolated combinations of the exposure parameters just confirmed what was intuitively expected, that is, that more pulses at a higher voltage cause more profound effects than fewer pulses at a lower voltage [e.g., Stacey et al., 2003; Vernier et al., 2006]. A physical model based on possible conformational changes of organelle membranes [Joshi et al., 2004] suggested that apoptotic effects of nsEP should be proportional to the product of E-field (E) and pulse duration (τ) (also referred to as an “Electric Impulse”), and this model has gained some experimental support [Beebe et al., 2004; Pakhomov et al., 2007b]. In separate studies, cell survival after exposure to trains of 10 ns pulses of different voltages was shown to be determined by the absorbed dose, which is proportional to E^2 [Pakhomov et al., 2004, 2007b]; however, these studies were not extended to compare the effects of different durations of nsEP.

At present, there is an urgent need for systematic studies focused specifically on the analysis of how nsEP effects depend on different exposure parameters. As a first step in this direction, we compared the magnitude of plasma membrane permeabilization (as measured by its electrical resistance, R_m) in two different cell lines (GH3 and CHO), at two pulse durations (60 and 600 ns), and within a range of nsEP voltages (1-22 kV/cm). The effect of a long-lasting R_m decrease after a single nsEP can be reliably produced by E-field voltages several times lower than required for most other known nsEP effects, and it is likely one of the primary interaction mechanisms of nsEP with living cells [Pakhomov et al., 2007a,b].

The scope of this study was limited to formal quantitative analysis of nsEP effect dependence upon exposure parameters, without attempting to reveal or understand various physiological consequences or fine physical processes involved. It is worth noting, however, that a systematic comparison of bioeffects under different exposure conditions can, by itself, be a powerful tool to establish the underlying interaction mechanisms, as was the case in the classic ionizing radiation biology [Hall, 2000]. Furthermore, such analysis may prove useful to provide a scientific basis for safety guidelines in exposure to high-peak, low-average power EMF (see Discussion Section for more detail).

MATERIALS AND METHODS

Experiments described below were performed concurrently between September 2007 and March 2008 at Old Dominion University (ODU) in Norfolk, VA and at Air Force Research Laboratory (AFRL) at Brooks City-Base in San Antonio, TX. Consequently, the data were acquired with two similar, but not identical set-ups for nsEP exposure and patch-clamp recording. Experiments at the two locations employed the same cell lines, same media, and adhered to identical cell handling and nsEP exposure protocols; nonetheless, minor differences in procedures and measurements could not be excluded. To minimize potential impact of any

uncontrolled variables, each set of experiments that were run on a particular set-up within a certain time interval included a matching set of control experiments. These controls (sham exposures) were run on the same set-up, within the same time interval, and alternated randomly with actual nsEP exposures.

Cell Lines

We used GH3 (a murine pituitary) and CHO-K1 (Chinese hamster ovary) cell lines. These cell lines have distinctly different morphology and physiology, which could potentially affect their sensitivity to nsEP. Pituitary cells, including GH3, may express numerous plasma membrane channels, including tetrodotoxin-sensitive Na^+ channels, voltage-sensitive Ca^{2+} channels, transient and delayed rectifying K^+ channels and multiple Ca^{2+} -sensitive K^+ channel subtypes [Czarnecki et al., 2003; Stojilkovic et al., 2005]. In contrast, CHO cells express few endogenous channels [Gamper et al., 2005] and typically display no voltage-sensitive currents at all. Hence, comparison of nsEP effects in GH3 and CHO cells could help in understanding how various ion channels contribute to the change in plasma membrane properties after nsEP exposure.

Both cell lines, cell growth media and supplements were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in 75-cm² flasks at 37 °C with 5% CO_2 in air. GH3 cells were cultured in Ham's F12K medium supplemented with 2.5% fetal bovine serum (FBS) and 15% horse serum. CHO cells were propagated in Ham's F12K medium supplemented with 10% FBS. The growth media also contained 1% penicillin/streptomycin. Immediately preceding the experiments, cells were transferred onto glass cover slips pre-treated with poly-L-lysine (Sigma-Aldrich, St. Louis, MO) to improve cell adherence.

Patch-Clamp Set-Up and Data Acquisition

Pipettes for patch-clamp recording were manufactured from borosilicate glass (1B150F-4, World Precision Instruments, Sarasota, FL, or BF150-86-10, Sutter Instrument, Novato, CA). They were pulled to a tip resistance of 1.5-3 M Ω using a Flaming/Brown P-97 Micropipette puller (Sutter Instrument, Novato, CA).

Exposures of individual cells to nsEP and subsequent measurements of membrane resistance (R_m) were performed in a glass-bottomed chamber (Warner Instruments, Hamden, CT) mounted on a stage of an inverted microscope. The microscopes employed at ODU and at AFRL were, respectively, Olympus IX71 (Olympus America, Center Valley, PA) and Leica DMI4000 (Leica Microsystems, Wetzlar, Germany). A cover slip with cells was placed into the chamber filled with a bath buffer at room temperature, and an individual cell suitable for nsEP exposure and patch-clamp recording was selected. The nsEP-delivering electrodes and a glass micropipette were positioned next to the selected cell (as shown in Fig. 1) using MP-285 and MP-225 robotic micromanipulators (Sutter).

The bath buffer contained (mM): NaCl, 140; KCl, 5; MgCl_2 , 4; HEPES, 10; glucose, 10; pH 7.4. The pipette solution for GH3 cells contained: NaCl, 5; KCl, 140; MgCl_2 , 1; HEPES, 10; pH 7.2. For CHO cells, the pipette solution was: NaCl, 5; KCl, 140; MgCl_2 , 1; HEPES, 10; mM CaCl_2 , 1, K-EGTA, 10; pH 7.2. The solutions' osmolality, as checked by a freezing point microosmometer (Advanced Instruments, Norwood, MA), was between 290 and 310 mOsm. All chemicals were purchased from Sigma-Aldrich.

Electrophysiology data were acquired using a Multiclamp 700B amplifier, Digidata 1322A or 1440A A-D converter, and pCLAMP10 software (MDS, Foster City, CA). As shown in our previous studies [Pakhomov et al., 2007a,b], nsEP exposure could be damaging to the gigaohmic seal between the cell membrane and the glass pipette, thereby effectively destroying the patch-clamp recording configuration. All cells in this study were exposed intact, and only

after the exposure was the glass pipette brought into contact with the cell membrane. This protocol effectively removed any concerns about possible artifacts caused by the presence of the glass pipette during the exposure, but caused a 70-120 s delay between nsEP application and data collection.

R_m was measured shortly after establishing the whole-cell recording configuration, in voltage-clamp mode, by applying 10- or 15-mV test pulses from a holding level of -70 or -60 mV. Resting membrane potential (MP) was measured by switching briefly from voltage to current clamp at $I=0$ A. Typical values of seal and access resistance were 4-15 G Ω and 4-10 M Ω , respectively. Measured MP values were corrected for respective junction potentials, as calculated with a pClamp10 utility.

Exposure to nsEP and local E-Field Modeling

The nsEP exposure device utilized at AFRL has been described previously [Pakhomov et al., 2007a,b]. In brief, a Blumlein line pulse generator [Kolb et al., 2006] produced nearly rectangular 60 ns pulses, typically accompanied by some faster components (caused by stray capacitance) and followed with smaller amplitude late components (caused by less than perfect impedance match between the line and the load). nsEP were delivered to a selected cell by a pair of tungsten rod electrodes (0.125 mm electrode diameter, 0.31 mm gap between the electrodes). These electrodes were positioned on the surface of the cover slip on either side of the selected cell, as shown in Figure 1.

At the ODU laboratory, rectangular nsEP were generated in a transmission line-type circuit. The electrical energy was stored in an RG 58 (50 Ω) coaxial cable and released in a pulse upon closing of a fast MOSFET switch (DE275-102N06A). The duration of the electric pulse equaled the round-trip time of the electromagnetic wave in the coaxial cable, and therefore was proportional to the length of the cable; to produce 600 ns pulses, the cable length was 60 meters. The amplitude of nsEP generated in this transmission line and measured across a matched 50 Ω load was one half of the charging voltage. For convenience, nsEP were triggered by external TTL pulses using pClamp software and the Digidata board. Similar to the AFRL set-up, nsEP were delivered to the selected cell via a pair of tungsten rods. However, the rod diameters were 0.1 mm, and the gap between them was reduced to 0.11-0.12 mm (Fig. 1).

In both set-ups, exact pulse shapes and amplitudes were captured and measured with 5-GHz TDS 3052 oscilloscopes (Tektronix, Beaverton, OR), as shown in Figure 1. The E-field between the electrodes was determined by 3D simulations with the finite element Maxwell equations solver Amaze 3D (Field Precision, Albuquerque, NM). At the location of the cell, 5 μ m above the cover slip, the calculated E-field values were about 25 kV/cm (AFRL) or 60 kV/cm (ODU) per 1 kV of the applied voltage. These numbers were uniformly used for dosimetry calculations throughout this study. In the plane parallel to the cover slip surface (5 μ m above it), the E-field was practically uniform (less than 10% change) within at least a 30 μ m radius from the central position, so potential inaccuracies (e.g., 10-15 μ m) in electrode placement with respect to the cell would have no appreciable effect on the efficacy of nsEP exposure. The variation of the E-field in the direction perpendicular to the cover slip surface also did not exceed 10% of the height of cells.

The peak specific absorption rate (SAR_p , kW/g) and absorbed dose (AD, mJ/g) were calculated using the following relationships:

$$SAR_p = \sigma \frac{E^2}{\rho} \quad (1)$$

$$AD=10^6 \times SAR_p \times \tau \quad (2)$$

where τ is the pulse duration (s), E is the electric field in the solution (kV/cm), ρ is the density of the solution ($\sim 1 \text{ g/cm}^3$), and σ is the conductivity of the solution (14.8 mS/cm for our bath buffer).

In this study, each cell was exposed to only a single nanosecond pulse, of either 60 or 600 ns duration, or was sham-exposed. All procedures and protocols for sham exposures were identical to actual nsEP exposures, but nsEP was not triggered.

Data Analysis

Patch-clamp measurements were performed on 146GH3 and 160CHO cells. Each cell was individually exposed to nsEP (or sham-exposed) just once, followed by patch-clamp measurements, and then discarded. Each treatment (nsEP at a given E-field intensity and pulse duration) was tested in 10-20 cells. All data below are presented as mean values $\pm$ SE.

As mentioned earlier, cells were exposed to nsEP prior to being “patched.” Hence, the individual properties of each cell prior to exposure were not known, and the quality of the whole-cell recording configuration could vary from one cell to another. Therefore, measured R_m and MP could be affected not only by nsEP exposure, but also by the unknown initial physiological state of the cell and by the quality of the whole-cell recording.

These additional factors posed little problem for GH3 cells, which form a fairly uniform population, where morphologically similar cells display similar R_m and MP. The success rate of forming a good whole-cell recording configuration in GH3 cells was close to 100%, so there was little need for any post hoc data conditioning.

In contrast, a culture of CHO cells is typically comprised of several subpopulations (e.g., see Fig. 1), and even cells that look similar may nonetheless manifest distinctly different physiological properties. Most of our control CHO cells had R_m of 1-2 G Ω and MP of -20 to -30 mV. However, in a fraction of cells, the MP was far more negative (about -70 mV) and R_m was only 0.1-0.3 G Ω , indicating that these cells are in a different physiological state. Furthermore, the rate of success of whole-cell patch clamp in CHO cells did not exceed 80-90%. Hence, in addition to analysis of the raw data from CHO cells, the same analysis was performed after data conditioning by: (i) disregarding data from all cells with unusually large negative MP, considering them as a different subpopulation, or (ii) disregarding data from 10%, 20%, 30%, or even 50% of cells with the lowest R_m values, considering them as potential failures for establishing a whole-cell recording. Obviously, these procedures were applied uniformly to all nsEP-exposed groups and to the shamexposed controls. Although we finally chose the method (A) to present findings in CHO cells, it is important to note that all of the above methods were tested and resulted in essentially the same plots and dependences. In other words, using raw or differently filtered data might somewhat affect the mean values and error bars, but would not have changed the overall conclusions of this study.

In each set of experiments, data from each exposed group were compared to the matching control using Student's *t*-test with Dunnet's correction for multiple groups [Dunnet, 1955].

RESULTS

Consistent with our earlier reports [Pakhomov et al., 2007a,b], exposure of GH3 cells to 60 ns electric pulses caused a long-lasting decrease of R_m , accompanied by MP loss (Fig. 2A). The

threshold for these effects was at or below 6 kV/cm, substantially less than for any other known effects of 60 ns pulses.

Exposure of GH3 cells to 10-fold longer (600 ns) pulses produced qualitatively similar changes, with the threshold as low as about 1 kV/cm (Fig. 2B). Hence, the E-field apparently was not the exposure metric that solely determined nsEP effect on the plasma membrane, but rather it was some combination of the E-field and the pulse duration.

Further analysis established that, for either pulse duration, the magnitude of R_m changes over the range of E-field intensities can be fit by a power function, which would appear linear on a double-logarithmic scale (Fig. 3A). However, the fit functions for 60 and 600 ns pulses were essentially different: to produce the same effect, the E-field (kV/cm) for 60 ns pulses needed to be some three- to sixfold higher than for 600 ns pulses. Likewise, plotting R_m against either the Electric Impulse (V ms/cm) or SAR_p (kW/g) also produced distinctly different curves for 60 and 600 ns pulses (Fig. 3B,C). Hence, none of the three exposure metrics (E-field, electric impulse, or SAR_p) would, by itself, be sufficient to predict the magnitude of bioeffects at different pulse durations of nsEP.

Contrary to these three metrics, it was the absorbed dose that uniformly and adequately determined the effect of both 60 and 600 ns pulses (Fig. 3D): plotting the R_m against the AD has effectively brought the data for both pulse durations onto the same curve. The threshold AD to decrease R_m in GH3 cells was found to be at or below 10 mJ/g. The temperature change associated with this AD is calculated to be less than 0.01 °C ($\Delta T = AD/c_p$, where ΔT is the temperature change (°C) and c_p is the specific heat capacity of water, 4.2 J/g °C), clearly indicating the nonthermal nature of the nsEP effect. Above the threshold, the nsEP-induced change in R_m (for either 60 or 600 ns pulses) was best fit by the function $R_m(\text{exposed})/R_m(\text{control}) = 5.5AD^{-0.74}$, where AD is in mJ/g.

As a next step, we checked whether the discovered dependence of R_m changes upon AD would hold true for a different cell line. The same experiments were repeated in CHO cells, which are very different from GH3 cells in their origin, morphology, general physiology, and plasma membrane properties in particular. The raw data for CHO cells are presented in Figure 4, and normalized data for 60 and 600 ns pulses are plotted together in Figure 5. As with GH3 cells, CHO cells exposed at the equal absorbed doses displayed the same extent of R_m decrease, regardless of the exact duration of the nanosecond pulse applied. The slope of the best fit curve was somewhat less for CHO than for GH3 cells (−0.54 vs. −0.74), potentially indicating that CHO cells might be less susceptible to nsEP exposure, at least under the experimental conditions tested.

DISCUSSION

This study was the first attempt to explore the dependence of plasma membrane effects of nsEP on various parameters of exposure. We determined the E-field threshold for 60 and 600 ns pulses and demonstrated that, in fact, it is not the E-field itself, but rather the absorbed dose that appropriately takes into account both the E-field and pulse duration to determine the effect of the nsEP. The dose effect has been repeatedly and independently demonstrated in two different cell lines, and using two different exposure and data recording set-ups. The latter factors could have contributed to increased data variability; at the same time, they strengthened the findings of the dose effect by indicating that it was not affected by minor differences between the set-ups and nsEP exposure procedures at the two locations.

In earlier studies, we explored how the survival of Jurkat and U937 cells exposed to multiple 10 ns pulses depends on the exposure parameters [Pakhomov et al., 2004, 2007b]. We found that for different E-field values and different numbers of pulses, the survival rate was

proportional to the absorbed dose. The fact that both the cell survival and membrane effects described above were proportional to the absorbed dose, even under rather different experimental conditions, indicates that (a) the dose concept may be applicable to more categories of nsEP effects and (b) these effects could be mechanistically related to each other, for example, the extent of membrane permeabilization determines the rate of nsEP-induced cell death.

The dose-effect concept may potentially become a useful and universal tool to quantitatively compare nsEP effects in various cell lines and under different exposure conditions. The coefficients of the power fit equation may serve as a measure of cells' sensitivity to nsEP and how it is modified by cell media components, chemicals, temperature, phase of the cell cycle, etc.

At the same time, caution should be exercised when applying the dose concept to diverse nsEP effects reported in literature, as the primary mechanisms underlying these effects may be different from those explored in our study. At least in some cases, nsEP effects appeared proportional to $(Et)^{1/2}$, or just (Et) for a single pulse ($n = 1$) [Beebe et al., 2004; Pakhomov et al., 2007b]. At present, the limits of applicability of the dose concept are not known and need further exploration, using various cell lines, different pulse durations, and trains of multiple pulses.

Aside from fundamental questions related to mechanisms of nsEP effects, the dose concept may have notable implications for such applied areas as safety standards for high-peak, low-average power EMF emissions. At present, the safety limits for such emissions appear poorly justified and vary in different countries by orders of magnitude. For example, the E-field safety limit of 100 kV/m in air [IEEE Std C95.1., 1999] is not based on findings of a hazardous bioeffect at this or comparable E-field levels. On the contrary, it is based on the lack of consistent and reproducible findings of any potentially hazardous bioeffects that can be specifically tied to extremely high power emissions within the explored E-field limits. Most studies which aimed to reveal such effects reported that high E-fields produced no specific effects, or the threshold E-field intensity simply has not been reached [see for review Lu and DeLorge, 2000; Pakhomov and Murphy, 2000; Pakhomov et al., 2003]. However, “no-effect” studies can provide no inference on what the actual effect thresholds are (which can be just 10% or 100 times above the studied limits), and they provide no rationale for selecting one or other exposure metric to set the limit.

As an alternative to no-effect studies, one can start with experiments at E-field levels so high that bioeffects are easily detectable, as is the case with nsEP effects on plasma membrane. Once these effects have been clearly defined, we gradually decreased the E-field to establish their thresholds, and explored whether these effects were in fact determined by the E-field or by other exposure metrics. Importantly, we found that it is the absorbed dose rather than the E-field itself that determines the effect. While it is clearly understood that there is a substantial gap between *in vitro* studies in individual cells and safety limits for human exposure, our data suggest that the E-field-based safety limits for pulsed emissions may be potentially misleading and need to be replaced by absorbed dose limits.

Coincidentally, the ICNIRP safety standard already uses the absorbed dose criterion to limit exposure to pulsed radiofrequency emissions [ICNIRP Guidelines, 1998]. In the frequency range 0.3-10 GHz and for localized exposure of the head, in order to limit or avoid auditory effects caused by thermoelastic expansion, the absorbed dose (also called “specific absorption”) is limited to 10 mJ/kg for workers and 2 mJ/kg for the general public, averaged over 10 g tissue. The rationale for choosing the absorbed dose as the exposure metric was

guided by the fact that the auditory effect is determined by absorbed dose, not just by E-field [Lin, 1978].

The question of whether the potential annoyance of the auditory effect is relevant to EMF exposure safety has been widely debated, but is beyond the scope of our article. In contrast to the auditory effect, long-lasting plasma membrane permeabilization and the loss of membrane potential could have profound physiological consequences. Note that the measured absorbed dose threshold for membrane permeabilization is about three orders of magnitude greater than for the auditory effect; however, this number may require correction for multiple pulses, for potentially more vulnerable types of cells, and for in-tissue exposure conditions (rather than single cells in a saline buffer). In addition, more research is required on the efficiency of pulsed E-field coupling from air into tissues: the estimates by published models vary from just a minor field change to a decrease by 2-4 orders of magnitude [with the latter estimates probably being more reliable; see Lu and DeLorge, 2000]. These topics will be the subject of our future studies.

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Fig. 1.

Exposure of individual cells to nanosecond electric pulses (nsEP). On the left is a representative picture of the dimensions and positions of the nsEP-delivering electrodes and a glass micropipette in contact with exposed cell (see Materials and Methods Section for more detail). To the right is a representative oscilloscope trace of a 600 ns pulse.

Fig. 2.

The decrease in membrane resistance and the loss of membrane potential in GH3 cells caused by a single 60 ns (**A**) or 600 ns (**B**) electric pulse as a function of the E-field intensity. The E-field of zero corresponds to sham exposure. The data were collected by whole-cell patch clamp within 2-3 min after the exposure, and presented here as the mean $\pm$ SE values for 10-20 independent experiments in most of the groups. Asterisk (*) indicates $P < 0.05$ when compared to the matching sham-exposed group.

Fig. 3.

The effect of 60 and 600 ns pulses on membrane resistance in GH3 cells plotted against different parameters of nsEP exposure: E-field (A), Electric Impulse (B), peak specific absorption rate (C), and absorbed dose (D). The same data as presented in Figure 2 were normalized to the respective control groups and plotted on a double-logarithmic scale. The horizontal axis is broken to allow for display of control values; a slight offset added to visualize control data for 60 and 600 ns pulses separately. The data were fit by two different power functions for 60 and 600 ns pulses (A-C) or by a single power function (D), with resulting coefficient of determination $R^2 = 0.91$.

Fig. 4. The decrease in membrane resistance in CHO cells as a function of the E-field intensity for 60 ns (**A**) and 600 ns (**B**) electric pulses. Other designations are the same as in Figure 2.

Fig. 5. The effect of 60 and 600 ns pulses on membrane resistance in CHO cells plotted against the E-field (**A**), Electric Impulse (**B**), peak specific absorption rate (**C**), and absorbed dose (**D**). Other details are the same as in Figure 3.