Effect of Defibrillation Pulses of Different Shapes on Biomembranes: Experimental Study

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We studied the effects of high-voltage single, double unipolar, and double bipolar electric pulses of exponential or sine shape on erythrocyte membranes. Either single or double (mono- or bipolar) pulses were used. All pulses electroporated the membranes, and the electroporation threshold did not depend on the pulse shape. Two successive pulses decreased erythrocyte number in a nonadditive way. Similar to defibrillation of the whole heart, the effect of two bipolar pulses on erythrocytes was more pronounced than the effect of two unipolar pulses.

Key Words: *membrane electroporation; bipolar pulses; defibrillation*

Sudden death caused by ventricular fibrillation is a still a pressing problem in clinical practice and experimental medicine. Further progress in this problem needs careful examination of the effects produced by electrical pulses on cell membranes and the search for optimal parameters of these pulses for cardiac defibrillation. Two types of defibrillating pulses are currently used: exponential (Edmark) and bipolar sine (Gurvich) waveforms. In clinical studies and experiments on animals [1,4,5], it was established that at the same energy of electric discharge bipolar pulses are more efficient [7,14]. However, there is no theory explaining this experimental fact.

In cardiac structures high-voltage pulse from the defibrillator generates electric field electroporating cell membranes and arresting fibrillation of the heart. However, it remains unclear, what is the optimal shape and amplitude of the electrical pulse, how monopolar and bipolar double pulses affect the cells, and what is the difference in their effects on the plasmalemma.

Our aim was to compare the effects of single and double (monopolar and bipolar) electrical pulses on cell membranes.

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MATERIALS AND METHODS

Experiments were performed on suspension of human erythrocytes in physiological saline (0.05 ml blood in 1 ml physiological saline, or 2.3×10^8 cell/ml). The optical density of the suspension (5 mm path) was equal to 1.

The concentration of erythrocytes in the suspension was calculated from photocolorimetric measurements of optical density of the suspension at λ=750 nm. The decrease in light intensity was caused by light scattering on erythrocytes. At low erythrocyte concentrations, optical density of suspension D is proportional to concentration n of red cells: $D=kn$. In other words, the kinetic curve of optical density $D(t)$ reflects the time course $n(t)$ of erythrocyte concentration. Decline of optical density referred here as kinetic curve $D(t)$ reflects hemolysis of erythrocytes caused by electric pulses.

Electric pulses were generated by Lifepak-7 or DI-03 clinical defibrillators and applied to titanium power electrodes submerged into quartz cuvette containing erythrocyte suspension in physiological saline. The electrodes completely covered the sides of the cuvette and generated homogenous electric field across the solution.

In all series of experiments, the external electric field strength was determined using measuring need-

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les. These needles were also used to assess homogeneity of the field in the cuvette. To this end, the standard alternating voltage was applied to the power electrodes, and voltage difference across the needles positioned in different sectors of the cuvette was measured.

Electric field strength and voltage in a solution depend on the energy of the applied pulse and impedance of the medium in the cuvette. The cuvette contained 3 ml suspension. The distance between electrodes was 17 mm, the width of the cuvette was 30 mm, and the height of suspension layer was 4 mm. Impedance of the saline was 100 Ω. For pulse energy of 230 J the voltage amplitude across the measuring needles was 2900 V, which corresponded to the field strength of 1700 V/cm.

Single monopolar pulse, two monopolar pulses, and two bipolar pulses were applied to erythrocyte suspension. Bipolar pulse had two sinusoidal half-waves of opposite polarity. In some experiments, two bipolar pulses with phase or antiphase amplitude ratio of 1:0.4 were used.

The pulse duration was 10 msec, and the interval between double pulses was 1 sec. In all series, the pulse amplitude was 2900 V.

In total, 87 experiments were carried out to study the effect of electric pulse train on suspension of erythrocytes.

RESULTS

Electroporation with impulse electric field accelerated hemolysis of erythrocytes in comparison with control suspension. The pulse energy considerably affected parameters of the kinetic curves (Fig. 1, *a*). When this energy was below 100 J, the kinetic curves virtually did not differ from the control plot. Some insignificant changes in the shape of kinetic curves were observed

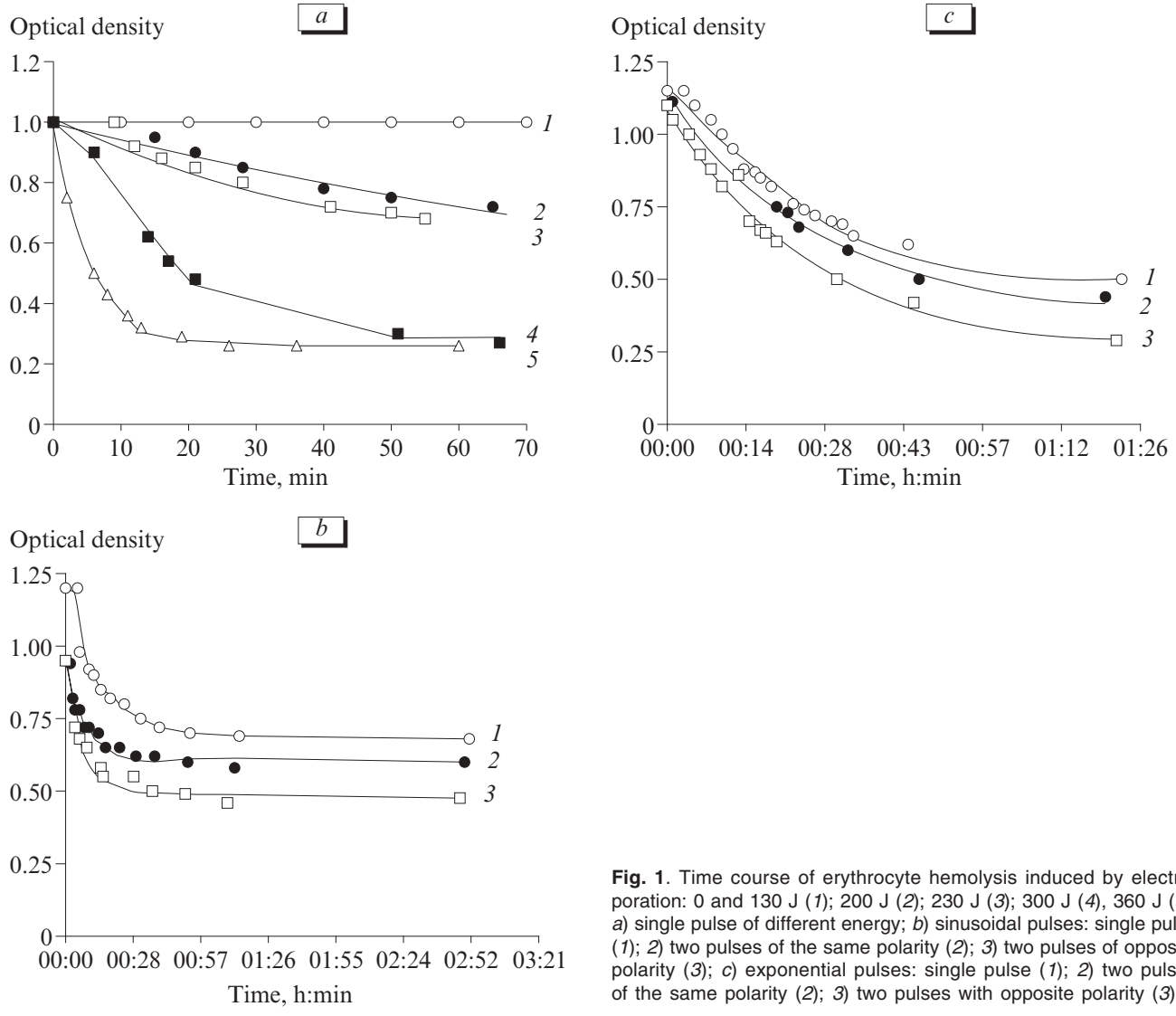

Fig. 1. Time course of erythrocyte hemolysis induced by electroporation: 0 and 130 J (*1*); 200 J (*2*); 230 J (*3*); 300 J (*4*), 360 J (*5*); *a*) single pulse of different energy; *b*) sinusoidal pulses: single pulse (*1*); *2*) two pulses of the same polarity (*2*); *3*) two pulses of opposite polarity (*3*); *c*) exponential pulses: single pulse (*1*); *2*) two pulses of the same polarity (*2*); *3*) two pulses with opposite polarity (*3*).

TABLE 1. Effect of Polarity of Two Electric Pulses on Erythrocyte Hemolysis Rate (min^{-1})

Pulse shape		Series											
		1	2	3	4	5	6	7	8	9	10	11	12
Single pulse	V (10 min)	0.018	0.01	0.01	0.009	0.005	0.005	0.005	0.005	0.01	0.01	0.005	0.005
Two pulses of the same polarity	V_{sp} (10 min)	0.02	0.022	0.016	0.025	0.02	0.02	0.005	0.014	0.031	0.02	0.008	0.024
Two pulses of opposite polarity	V_{op} (10 min)	0.03	0.031	0.025	0.030	0.021	0.034	0.023	0.025	0.032	0.022	0.018	0.031

Note. V=0.008±0.003, V_{sp}=0.018±0.005, V_{op}=0.027±0.003 min^{-1}.

only at pulse energy >130 J. Significant changes occurred at 200 J and higher energy. Thus, experimental data agree with general view that electroporation is a threshold phenomenon.

Increasing pulse energy led to an increase in the fraction of erythrocytes hemolyzed over 1 h and decrease in half-time $T_{0.5}$ (time corresponding to 2-fold decrease in concentration of erythrocytes).

For numerical description of the experiments, we used hemolysis rate $V=dn/dt$, *i.e.* the number of cells hemolyzed per time unit. The hemolysis rate was a non-linear function of time $V=V(t)$. The hemolysis rate during the first 10-30 min was significantly higher than at later terms.

The kinetic curves were used to show the results of application of a single monopolar pulse with the energy E=230 J (Fig. 1, *c*, *1*), two pulses of the same polarity (Fig. 1, *c*, 2), and two pulses of the opposite polarity (Fig. 1, *c*, *3*). Figure 1 (*b*) shows the corresponding kinetic curves obtained after application of bipolar pulses from DI-03 defibrillator. This device generates a bipolar sine pulse with amplitude ratio of 1:0.4. The breakdown transmembrane potential was formed by first (higher) half-wave. The electroporation threshold (2900 V) corresponded to similar parameter for a single pulse of Lifepak-7 defibrillator.

The kinetic curve (*3*) for two bipolar pulses always run below the curve (*2*) for two monopolar pulses of the same duration and amplitude (Fig. 1, *b*, *c*). Two antiphase sine pulses generated by DI-03 defibrillator induced more rapid hemolysis than two similar pulses applied in the same phase.

The rate of erythrocyte hemolysis induced by double pulse was higher than in experiments with single pulse (Table 1). In 75% cases, the hemolysis rate in experiments with two pulses of opposite polarity (V_{op}) was higher than the hemolysis rate induced by two pulses of the same polarity (V_{sp}). In 25% cases, the values of hemolysis rates were virtually identical. In these experiments, the mean values of hemolysis rates were determined for t=10 min; all differences were significant at p=0.05.

For 12 chosen (from 80 series, 10 min) the normalized rate of erythrocyte hemolysis was higher in the experiments with double pulses of opposite polarity than for those with monopolar pulses (Fig. 2). In these experiments, the amplitudes of both monopolar and bipolar pulses were identical. Prolongation of stimulation to t=20 min did not change the observed ratios (Fig. 2).

In some experiments, the non-additive effects of double pulses were observed. In most cases, this phe-

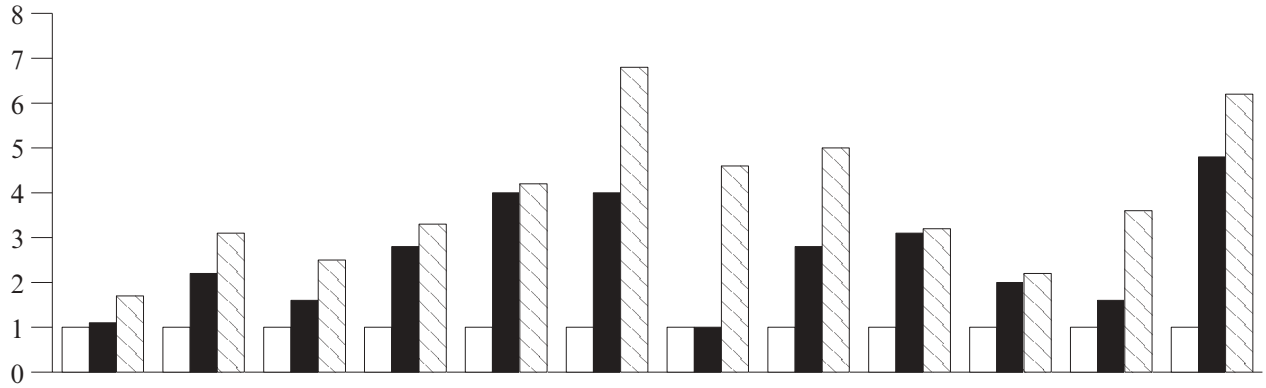

Fig. 2. Effect of electric pulse shape on erythrocyte hemolysis rate. Open, solid, and hatched bars represent baseline normalized hemolysis rate V/V=1, normalized rate for pulses of the same (V_{sp}/V) and opposite (V_{op}/V) polarity, respectively.

nomenon characterized the effect of two pulses with opposite polarity. For 10-min stimulation, non-additivity was observed in 7 of 12 cases of double pulses with the same polarity ($V_{sp}>2V$) and in 11 of 12 cases of double pulses with opposite polarity ($V_{op}>2V$). For 20-min stimulation, non-additivity was observed in one of 12 cases of double pulses with the same polarity and in five of 12 cases of pulses with opposite polarity.

The experiments showed that the hemolysis rate starts to increase (in comparison with that observed in physiological saline without stimulation) only at certain threshold strength of electric field E in solution. The transmembrane potential Df_i induced on erythrocyte is determined by Maxwell's formula $\Delta\varphi_i=1.5E_O R\cos\theta$, where R is radius of the cell and θ is the angle between vector E_O and radius-vector. For non-hemolyzed erythrocytes with R=4 μ, the threshold $\Delta\varphi_i$ is about 450 mV. At smaller values of $\Delta\varphi_i$ electroporation is insignificant. At larger values of $\Delta\varphi_i$, an avalanche electroporation develops: the number of pores and their diameter increase persistently [8,9]. For given R, electroporation is determined only by E_O. Therefore, both exponential (Edmark) pulse and bipolar sine (Gurvich) pulse yield the same threshold potential value, although the energy of these pulses can differ by 1.5-2 times at the same field strength E_O.

In some experiments, the radius of produced pores was assessed by the method of hemolysis inhibition with glucose molecules [11]. After 10-min stimulation with electric pulses, the pore radius did not surpass 0.7 nm.

The effect of paired impulses is not a twofold effect of single pulses. This feature results from the action of several different factors: normal distribution of parameters of examined cells, variation of erythrocyte orientation in the electric field, and changes in membrane composition after first stimulus. The state of cell population before the second pulse differs from that before the first stimulus, which explains the observed aftereffect.

The most important result was obtained when erythrocyte suspension was stimulated with two pulses of opposite polarity: V_{op} was higher than V_{sp}, although the first pulse in both modes of stimulation has the same (positive) polarity. Thus, the prehistory for the second pulse was identical in both modes of stimulation, but its effect was more pronounced if its polarity changed in respect to the first pulse: in this case, the number of intact erythrocytes after 10-min stimulation was smaller. This effect was more pronounced in erythrocyte suspension prepared no earlier than 10 min before stimulation, but was smoothened in erythrocyte suspension prepared 10-15 min before stimulation. It is known that transfer of potassium and sodium ions during the first 10 min of erythrocyte incubation is low, but later cationic permeability of erythrocyte membrane increases [3]. In our experiments, erythrocyte membrane was electroporated by the first pulse inducing a step-like increase in membrane permeability. During the second pulse of opposite polarity, the direction of electrical current altered. This pulse induced different changes in local transmembrane currents in comparison with those induced by the pulse of the same polarity.

The pulses of opposite polarity induce asymmetrical electroporation in the opposite sites of the cells: at these sites, the number and diameter of the pores are different [12,13]. Similar phenomena were observed in the integral myocardium [6,9]. The non-homogenous distribution of proteins and lipids in membrane also produces a significant effect on asymmetrical formation of the pores by electric pulses [2,15].

The aftereffects are also related to relaxation processes of surface and space-charge polarization of the cells induced by pulsed electric field. In this case, the action of the second pulse of alternative polarity is not identical to that induced by the second pulse of the same polarity.

In our experiments, the nature of observed effects was statistical, which corresponds to the action of bipolar pulses during defibrillation in clinic [7].

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