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Control of wave propagation in a biological excitable medium by an external electric field

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Abstract

We present an experimental evidence of effects of external electric fields (EFs) on the velocity of pulse waves propagating in a biological excitable medium. The excitable medium used is formed by a layer of starving cells of *Dictyostelium discoideum* through which the waves of increased concentration of cAMP propagate by reaction–diffusion mechanism. External dc EFs of low intensities (up to 5 V/cm) are shown to speed up the propagation of cAMP waves towards the positive electrode and slow it down towards the negative electrode. Electric fields were also found to support an emergence of new centers, emitting cAMP waves, in front of cAMP waves propagating towards the negative electrode.

Keywords: cAMP waves; Electric field effects; Control of intercellular communication; *Dictyostelium discoideum*; Biological excitable medium; Pacemaker emergence

1. Introduction

Excitable media supporting the propagation of pulse waves can be found in many biological, biochemical, physiological and chemical systems [1–3] of which the Belousov–Zhabotinsky (BZ) reaction [4,5], oxidation of CO on a Pt surface [6], nerve axon [7], heart tissue [8] and developing slime mold *Dictyostelium discoideum* (DD) [9,10] are the most studied ones. In living organisms, pulse waves are the basis of many vital functions and thus the investigation of the wave dynamics, and especially the possibility of its control, has become an extensive field of research [11]. So far, the control of the wave propagation has mostly been studied in chemical media of BZ reaction type. The control has been performed by (i) varying concentrations of reaction species and temperature [4], (ii) varying either local or global illumination of photosensitive reaction mixtures [12–14] and (iii) imposing dc or ac electric fields (EFs) on the system [15–24]. In the first two cases, the control resides in modifying the excitability of local elements composing the medium, in the third case the control force affects the spatial coupling between excitable elements by differential flows of ionic reaction species.

In the BZ reaction medium, external EFs induce a variety of complex non-linear responses. They account for the full or partial wave annihilation and formation of wave fragments [15,21,22], development or termination of spiral waves [18,20,21], drift of spirals through the medium [19], back-firing of new waves from the wake of the existing one [16,17,21,22] and the reversal of the wave motion [16,17,24]. Direct current EFs also affect the propagation velocity, slowing down the waves propagating towards the negative electrode and speeding up those propagating towards the positive electrode [16,23].

In this paper, the effects of external dc EFs on the propagation of pulse waves are studied in the biological excitable system formed by a suspension of starving cells of slime mold DD spread on agar. In such layer, the circular or spiral waves of the increased concentration of cAMP (3′ 5′ - cyclic adenosine monophosphate) propagate after few hours

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of starvation having a twofold function: (i) to transfer the information about adverse living conditions from cell to cell and (ii) to induce chemotactic motion of cells against the gradient of cAMP [9,10]. The cells move towards the centers emitting cAMP waves where, later on, large numbers of them (~10^5 cells) form fruiting bodies carrying spores.

Waves of cAMP propagate by reaction–diffusion mechanism where the reaction covers the production of cAMP inside of individual cells triggered by superthreshold level of cAMP outside of the cell. Newly produced cAMP is secreted from the cell, where (i) it acts via positive feedback on the cell ability to synthesise more of cAMP [25] and (ii) diffuses to the neighbouring cells [26]. Since cAMP (an activator of the process) is present in the water suspension of cells as an anion, an external dc EF will modify its mass flow towards the neighbouring cells, and thus it will influence the propagation of cAMP waves.

Effects of dc EFs on the propagation of cAMP waves have been demonstrated in a mathematical model of spatially one- and two-dimensional, homogeneous layer of starving DD cells [27]. In the model, the production of cAMP was described by Martiel–Goldbeter reaction mechanism [25] and cAMP, excreted by cells, was assumed to subject to the mass flow by both the diffusion and electromigration. EFs were found to accelerate (decelerate) the propagation of cAMP waves towards the positive (negative) electrode, to annihilate cAMP waves propagating towards the negative electrode (at E>6 V/cm) and to evoke the spontaneous emergence of pacemakers in the wake of these pulse waves (for E>1.25 V/cm). Experiments reported in this paper affirm in certain extent the results obtained by mathematical modelling.

2. Experimental

The experiments were performed with AX2 strain of slime mold *D. discoideum* obtained from the laboratory of Dr. P. Folk, Charles University, Prague. The cells were grown in a liquid HL5 medium [28] in a dark room at temperature 21 °C and harvested when the cell density reached the value between 2 and 6×10^6 cells/ml. After centrifugation and double washing with a phosphate buffer (0.001 M Na_2HPO_4, 0.015 M KH_2PO_4, pH=6.14) the cells were spread over a nutrition-free agar (1% w/v of agar, 0.002 M caffein, phosphate buffer, pH=6.14.) on a Petri dish. The cell density on the agar was approximately 5×10^5 cells/cm^2 in all experiments. Petri dishes were then kept at temperature 21 °C until cAMP waves appeared on the layer (approximately 3−4 h after the onset of starvation).

An electric voltage was applied on the cell layer in Petri dish via two salt bridges made by two strips of filter paper (3×10 cm^2) soaked with the phosphate buffer and attached by one of their ends to the surface of the agar (see Fig. 1). The other ends of the salt bridges were placed in electrolytic chambers outside the Petri dish. A dc electric voltage was applied on Pt electrodes immersed in the phosphate buffer in the electrolytic chambers. The actual voltage drop (U) between the ends of salt bridges on the agar was measured by voltmeter at the end of each experimental run. The intensity of an applied electric field (E) was calculated as $E=U/d$ where d is the actual distance of salt bridges in the related experimental run. The electric current (I) passing through the cell layer was continuously monitored during the experiment and found to drop for 5% during an experimental run (lasting approximately 2.5 h).

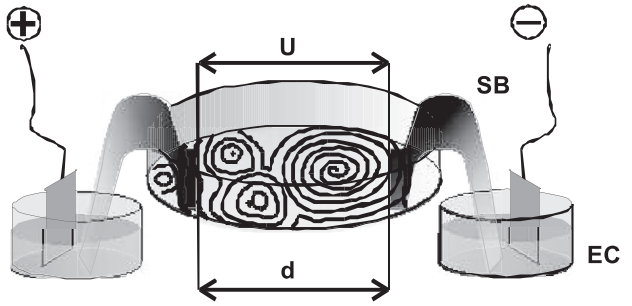

Fig. 1. Sketch of an experimental apparatus. SB—salt bridges, EC—electrolytic chambers, U—voltage difference measured, d—distance between salt bridges.

The propagation of cAMP waves was observed using dark-field photography [29]. The course of experiments was monitored and recorded, using a CCD camera connected to a PC. Images were grabbed every 10 s and processed later by commercial software LUCIA [30]. To evaluate propagation velocities, an “observation window” was chosen in the grabbed images and the propagation of cAMP waves through this window was traced constructing time–space plots. The window was placed on the images in such a way that the velocity vector of a wave being followed was parallel both to the length of the window and to the vector of an applied electric field.

3. Results

Effects of dc EFs on the velocity of propagation of cAMP waves were investigated by switching the external voltage on and off (in approximately 10-min intervals) and by alternating the polarity of the voltage between two subsequent switched-on time intervals. The propagation velocity of each wave was evaluated in each individual time interval from the slopes of a wave trace in the time–space plot (see Fig. 2). Six independent measurements of a slope in every time interval were taken in order to calculate the average velocity of the cAMP wave and its standard deviation. Average values of propagation velocities of four successive cAMP waves (shown in Fig. 2) in respective time intervals are summarised in Table 1. The variations in the values of velocities of different waves under the same “electric field” conditions are sometimes large as it is usually

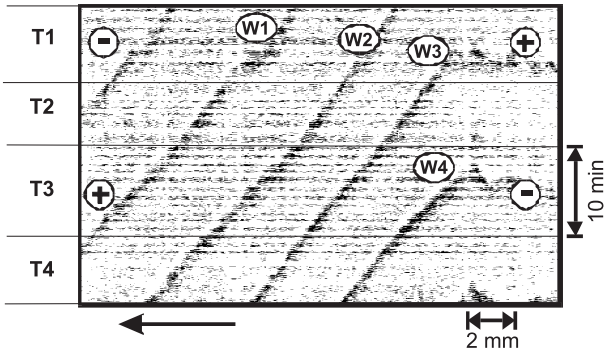

Fig. 2. Time–space plots tracing the propagation of four successive cAMP waves (W1–W4). Images were enhanced by image subtraction and by converting them to the negative. In the time intervals T2 and T4, no electric field was applied, the polarity of the electric field in time intervals T1 and T3 is opposite as denoted. E=3.5 V/cm, I=1.8 mA, the arrow denotes the direction of waves propagation.

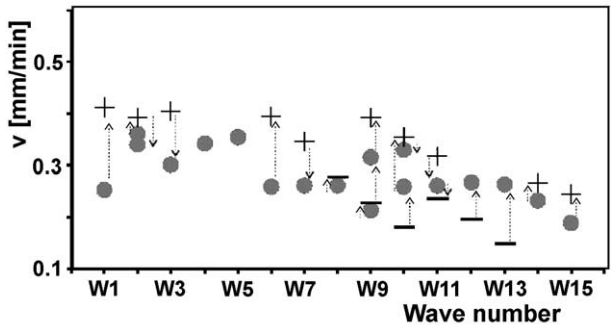

Fig. 3. Propagation velocities of 15 successive waves with and without applied EF. E=5 V/cm, I=3.2 mA. Arrows denote the succession of switching the electric field on and off, (●) the wave velocities with no EF applied, (+ (−)) the velocity of a wave propagating towards the positive (negative) electrode.

found in other experimental measurements of propagation velocities of cAMP waves in the layer of DD cells [29].

Changes of average velocities of 15 successive waves upon switching on and off an EF of the intensity E=5 V/cm are illustrated in Fig. 3 where the arrows trace the time order of switching the EF on and off. For example, wave 10 propagated first towards the negative electrode (in other words in the negative EF) at velocity nearly 0.2 mm/min (marked by − in Fig. 3). Then the EF was switched off and the velocity of wave 10 increased to the value 0.25 mm/min. Then the field was switched on again, now with the positive electrode facing the wave, and the velocity increased to the value of approximately 0.35 mm/min. After switching the field off, the velocity decreased a little.

Closely inspecting Fig. 3, one can see that sometimes the propagation velocity of a wave changes remarkably after switching the EF on as, e.g., in the case of waves 1 and 6; sometimes the change in the propagation velocity is not so convincing as, e.g., in the case of waves 2, 8 and 14. To assess whether the difference between the propagation velocities of a chosen wave in two successive time intervals is statistically significant (at the level of significance α=0.05) and whether the velocity has increased or decreased, the "Two-Sample t-Test for Small Sample Size" (n1=n2=6 are numbers of measurements of velocities of a chosen wave in two successive intervals) [31] was applied on measured data. For example, wave W1 (see Table 1) propagates in the time interval T1 towards the negative electrode (E<0) at velocity $v_{E<0}$=0.215±0.005 mm/min. When the field is switched off during the time interval T2, the velocity increases to v_0=0.28±0.01 mm/min. The t-test has shown that the difference between $v_{E<0}$ and v_0 is statistically significant, suggesting that the propagation of cAMP waves in the negative EF is slower than in the EF-free situation. In the time interval T3, the wave W1 propagated towards the positive electrode at velocity $v_{E>0}$=0.39±0.03 mm/min. t-Test showed that the difference between v_0 and $v_{E>0}$ is also statistically significant suggesting that the positive field can accelerate the propagation of cAMP waves. However, for example, the difference between the velocities of the second wave (W2) in the time intervals T1 and T2 was found not to be statistically significant.

Results of t-tests applied to a large number of velocities of cAMP waves in two successive time intervals are summarised in Table 2. When EF of the intensity E=3.5 V/cm was applied, the velocities of cAMP waves in the positive field were larger than their velocities in EF-free situation in 68% of cases while they were lower in only 6% of cases. In 26% of cases, the changes in the velocities were not statistically significant. Negative fields have increased the velocities of cAMP waves, compared to the zero field conditions, in only 10% of cases, while it decreased the

Table 1
Propagation velocities of cAMP waves both with and without an applied electric field

Time interval	Electric field	Propagation velocities v_0 or v_E (mm/min)			
		W1	W2	W3	W4
T1	E<0	0.215±0.005	0.22±0.02		
T2	OFF	0.28±0.01	0.24±0.02	0.281±0.008	
T3	E>0	0.39±0.03	0.37±0.03	0.32±0.01	
T4	OFF	0.31±0.04	0.35±0.02	0.286±0.007	0.303±0.004

E=3.5 V/cm. E<0 (E>0) means that waves propagate towards the negative (positive) electrode.

Table 2
Percentage of accelerated (v_E>v_0) and decelerated (v_E<v_0) waves by dc electric fields

Comparison of velocities	|E|=3.5 V/cm		|E|=5 V/cm	
	E>0	E<0	E>0	E<0
v_E>v_0	68%	10%	91%	17%
v_E<v_0	6%	59%	5%	72%
$v_E \cong v_0$	26%	31%	4%	11%
Number of cases	49	29	144	92

E<0 (E>0) means that waves propagate towards the negative (positive) electrode. v_E and v_0 denote velocities of a wave with and without an external electric field, respectively.

Table 3
Average propagation velocities

Propagation velocity	$\|E\|$=3.5 V/cm	$\|E\|$=5 V/cm
v_0 (mm/min)	0.29±0.04	0.30±0.06
$v_{E>0}$ (mm/min)	0.34±0.05	0.39±0.08
$v_{E<0}$ (mm/min)	0.27±0.06	0.23±0.05

v_0, and $v_{E>0}$ ($v_{E<0}$) denote the wave velocity in zero EF, and the velocities of waves propagating towards the positive (negative) electrode, respectively.

wave velocity in 59% of cases. In 31% of cases, the velocity change was not statistically significant.

The effects of a stronger electric field (E=5 V/cm) on the propagation velocity of cAMP waves were more pronounced. In 91% of cases, the propagation of waves towards the positive electrode accelerated, and in 72% of cases, the propagation of waves was decelerated when propagating towards the negative electrode. The percentage of statistically insignificant changes in propagation velocities is substantially lower than in the case when electric field of intensity E=3.5 V/cm was applied (see Table 2). Also, the change of the propagation velocity of a wave increases with the increasing value of the EF intensity as it is illustrated by average values of wave propagation velocities listed in Table 3.

Applied EFs strongly influence also the shape of cAMP waves and the number of random pacemakers as it is illustrated in Fig. 4. In the figure, several long, continuous wave bands are seen to propagate from left to right, in the negative (Fig. 4A) and the positive (Fig. 4B) electric fields. One can see that the waves propagating in the negative field are thinner and coarser than the waves propagating towards the positive electrode. One can also notice a large number of new pacemakers, emitting circular cAMP waves, arising in front of the long continuous wave bands if those propagate towards the negative electrode.

4. Discussion

The experimental observations and statistical evaluation of the obtained data clearly show that the velocity of cAMP waves propagation can be considerably altered by applied, direct current electric fields of low intensities. Applied EFs were found to increase (decrease) the velocity of waves propagating towards the positive (negative) electrode comparing to the EF-free wave velocity. The average velocities at a given intensity and polarity of the EF, calculated from experimental data, show that the acceleration and deceleration increases with the intensity of an applied EF (see Table 3).

Faster propagation of waves towards the positive electrode than towards the negative one is reflected also in the respective differences in the wave band widths (see Fig. 4). The dark bands are a result of the subtraction of two grabbed images being Δt seconds apart from each other and thus the width of a band is proportional to the distance a wave has travelled during the Δt time interval. Broader bands of waves in Fig. 4B thus correspond to the larger velocities of waves propagating towards the positive electrode comparing to the velocities of waves propagating towards the negative electrode.

Effects of dc EFs on the propagation velocity can be understood on the basis of the mechanism of cAMP waves propagation [2,25–27] and the fact that cAMP is present in the water-based environment of cell population as a negatively charged ion. The velocity of cAMP wave propagation depends both on the rate of synthesis and release of cAMP and on the intensity of the cAMP mass flow in the extracellular space. The mass flow determines how quickly cAMP reaches the neighbouring cells to trigger the cAMP synthesis there. Under EF-free conditions the mass flow of cAMP occurs only by diffusion. When a dc EF is applied the intensity of cAMP flow is enhanced by electromigration of cAMP anion. If the electromigration flow and the diffusive flow are of the same direction, which happens when a cAMP wave propagates towards the positive electrode, cAMP reaches the neighbouring cells earlier and cAMP synthesis is triggered earlier than under EF-free conditions. This leads to the increase of the velocity of cAMP waves propagation towards the positive electrode. On the other hand, when a cAMP wave propagates towards the negative electrode, the electromigration and diffusive flows of cAMP anions are of the opposite directions, and thus the electromigration decreases the diffusive supply of

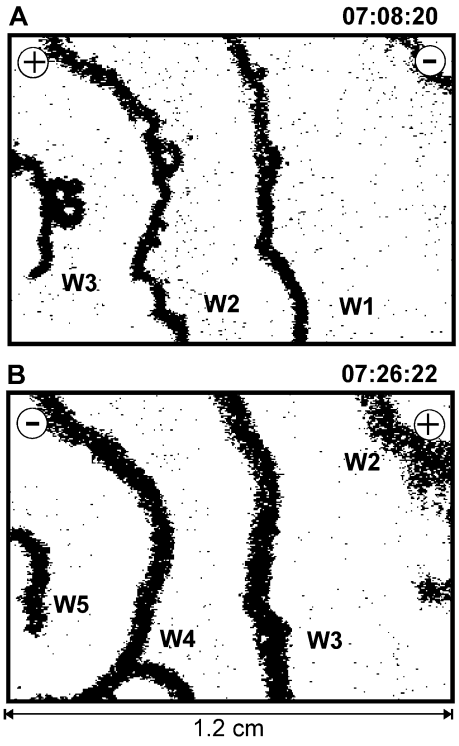

Fig. 4. Enhanced images of waves propagating towards the (A) negative and (B) positive electrode. Time is measured from the beginning of starvation. Area shown is 8.3×12 mm (E=5 V/cm, I=3.2 mA).

cAMP to the neighbouring cells. As a result, the propagation of the cAMP wave is slowed down.

The tendency of cAMP waves to speed up towards the positive electrode and to slow down towards the negative electrode was also observed in the mathematical model of the propagating cAMP waves in the aggregating layer of DD cells [27]. Another phenomenon, predicted by the model, particularly the annihilation of waves by negative EFs, was not observed in our experiments. However, it is possible that EFs of larger intensities than those used in our experiments are necessary to cause the waves to annihilate.

The changes of the propagation velocity in a dc EF can explain the increased emergence of pacemakers and small circular waves in front of waves propagating towards the negative electrode (cf. Fig. 4). In zero field situation, pacemakers, producing circular waves, arise at random places during early stages of the aggregation. The pacemakers have different periods of cAMP production and the emissions of waves by different pacemakers are shifted in phase. Arising circular waves mutually interact and can either annihilate or join. Annihilation can lead to the formation of free-end waves, as, e.g., the one on the left side of Fig. 4A,B, while joining leads to the formation of large continuous wave bands. The propagation of large waves synchronises the periodic synthesis of cAMP in individual cells by entraining their internal cycles to the wave period. However, when the propagation of large waves is slowed down by the negative EF (Fig. 4A), some cells can adopt back their internal periods and the pacemakers can emerge again. On the other hand, the acceleration of the wave propagation in the positive EF (Fig. 4B) enhances the process of synchronization of periodic cycles of cells, and less pacemakers are formed.

The emergence of new pacemakers anywhere along the main wave band cause a coarsening of the wave front in the negative EF. Soon after emerging, the pacemakers and the circular waves of small diameters merge with the approaching large wave band making it to seem coarse. On the other hand, the acceleration of waves towards the positive electrode prevents new pacemakers to arise and the waves are smoother than the waves in the negative and zero EFs.

When effects of applied EFs on cAMP waves are compared with those on BZ waves, one can see that the change of propagation velocity is the only effect observed in both systems. The waves are accelerated by positive EFs (or decelerated by negative EFs) since, in both systems, the applied EF facilitates (or suppresses) the flow of activator from a wave into the medium ahead of the wave. The effect of EF in the DD system is direct—increasing (or decreasing) the mass flow of cAMP anion ahead of the wave—while in the BZ system, the effect is indirect—decreasing (or increasing) the mass flow of negatively charged inhibitor (Br^-) against the wave [16].

Wave splitting, reversal and annihilation, observed only in the BZ system, result from the interplay of differential electromigration flows of the three main wave components—the activator ($HBrO_2$), the catalyst ($Fe(phen)_3^{2+}$) and the inhibitor (Br^-) [16,17,22]. In the DD system, it is only the activator (cAMP anion) that was subjected to electromigration flow in low EFs used in our study. The inhibitor (cAMP receptors on DD cell membrane) is bound to the cells whose movement is not directly affected by low EFs used in our experiments.

However, when a somewhat stronger EFs will be used, the electrotaxis of DD cells, i.e., the enhanced cell migration towards the negative electrode [32] can combine with chemotaxis in a nontrivial way. Though the electrotactic motion is approximately 10 times slower than the chemotactic motion (velocity of electrotaxis at E>10 V/cm is 3 μm/min [32], while chemotactic velocity is 30 μm/min [29]), the electrotaxis takes place all the time the EF is switched on, while chemotaxis occurs only when a passing cAMP wave provides an increasing cAMP gradient for cells. When chemotaxis takes place, the electrotaxis is insignificant but electrotaxis overtakes when cells stop moving chemotactically. While both, chemotaxis and electrotaxis, will have the same direction when cAMP waves will propagate towards the positive electrode, they will act in mutually opposite directions when cAMP waves will propagate towards the negative electrode. Thus, larger negative EFs can lead to interesting, highly nonlinear effects on the cAMP wave propagation. However, these investigations require the construction of a thermostated reactor that would allow for more intensive cooling of the layer of DD cells on agar than the present experimental setup.

5. Conclusions

Considering the observed effects of EFs on the velocity of cAMP waves, a question on what could be the impact of the velocity changes on the aggregation itself arises. The duration of aggregation depends on the overall speed of cell chemotaxis towards the aggregation centers and on the emergence of new pacemakers—the new centers of aggregation. The faster the cells move, the shorter the duration of aggregation. As suggested by numerical simulations, cAMP waves propagating towards the positive electrode have larger amplitudes but less steep gradients of cAMP than the zero field waves [27]. Since the larger amplitude will result to the faster cell chemotaxis, while the less steep gradient of cAMP will slow it down, it is not trivial to predict the overall effects of changed velocities of cAMP waves on the duration of aggregation. The waves propagating towards the positive electrode can also slow down the progress of aggregation stage suppressing the emergence of new pacemakers. Oriented electrotaxis of cells in stronger EFs can further complicate both the cAMP wave propagation and aggregation process.

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