Different Roles of Membrane Potentials in Electrotaxis and Chemotaxis of Dictyostelium Cells

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Received 6 April 2011/Accepted 27 June 2011

Many types of cells migrate directionally in direct current (DC) electric fields (EFs), a phenomenon termed galvanotaxis or electrotaxis. The directional sensing mechanisms responsible for this response to EFs, however, remain unknown. Exposing cells to an EF causes changes in plasma membrane potentials ($V_m$). Exploiting the ability of Dictyostelium cells to tolerate drastic $V_m$ changes, we investigated the role of $V_m$ in electrotaxis and, in parallel, in chemotaxis. We used three independent factors to control $V_m$: extracellular pH, extracellular [K$^+$], and electroporation. Changes in $V_m$ were monitored with microelectrode recording techniques. Depolarized $V_m$ was observed under acidic (pH 5.0) and alkaline (pH 9.0) conditions as well as under higher extracellular [K$^+$] conditions. Electroporation permeabilized the cell membrane and significantly reduced the $V_m$, which gradually recovered over 40 min. We then recorded the electrotactic behaviors of Dictyostelium cells with a defined $V_m$ using these three techniques. The directionality (directedness of electrotaxis) was quantified and compared to that of chemotaxis (chemotactic index). We found that a reduced $V_m$ significantly impaired electrotaxis without significantly affecting random motility or chemotaxis. We conclude that extracellular pH, [K$^+$], and electroporation all significantly affected electrotaxis, which appeared to be mediated by the changes in $V_m$. The initial directional sensing mechanisms for electrotaxis therefore differ from those of chemotaxis and may be mediated by changes in resting $V_m$.

Cells migrate directionally in response to many extracellular cues including chemical gradients (chemotaxis), topography, mechanical forces (mechanotaxis/durataxis), and electrical fields (EFs) (electrotaxis/galvanotaxis) (1, 3, 8, 15, 27). Electric fields have long been suggested to be a candidate directional signal for cell migration in development, wound healing, and regeneration. The mechanisms used by cells to sense the weak direct current (DC) EFs, however, have remained very poorly understood.

One of the immediate effects felt by a cell upon exposure to an EF is a change in the cell membrane potentials ($V_m$). In an EF, the plasma membrane facing the cathode depolarizes while the membrane facing the anode hyperpolarizes (17, 18). It has been proposed that the changes in $V_m$ may underlie electrotaxis. In a cell with negligible voltage-gated conductance, the hyperpolarized membrane facing the anode attracts Ca$^{2+}$ by passive electrochemical diffusion. This side of the cell then contracts, thereby propelling the cell toward the cathode. In a cell with voltage-gated Ca$^{2+}$ channels, channels near the cathodal (depolarized) side open, thereby allowing Ca$^{2+}$ influx. Intracellular Ca$^{2+}$ levels will rise both on the anodal side and on the cathodal side in such a cell. The direction of cell movement in this situation will depend on the balance between the opposing contractile forces (17). The role of $V_m$ in electrotaxis has not yet been directly tested.

In this report, we used Dictyostelium cells to test this directly. Dictyostelium cells show robust electrotaxis and tolerate significant changes in $V_m$, while maintaining good motility under conditions of different extracellular pH values and ion concentrations and even following electroporation (20, 25, 29). These features make Dictyostelium cells a unique testing model. We quantified electrotaxis and chemotaxis of cells with well-controlled $V_m$s by varying three independent factors. We found that the $V_m$ indeed regulated electrotaxis while having no effect on chemotaxis. We thus identified a contrasting role of $V_m$ between electrotaxis and chemotaxis which may underlie the mechanisms used by cells to sense weak dc EFs.

**MATERIALS AND METHODS**

**Cell culture and development.** Dictyostelium discoideum AX3 cells were grown axenically in HL5 medium. Vegetative cells were washed and starved in development buffer (DB) and then pulsed with 50 nM cyclic AMP (cAMP) every 6 min for an additional 4 h (29). All procedures were carried out at room temperature (~22°C).

**Micropipette chemotaxis assay.** Chemotaxis experiments were performed as reported (4, 10). Briefly, 20 μl of cells (1 × 10$^4$ to 4 × 10$^4$ cells/ml) in DB were seeded onto a coverslip chamber. Bathing solutions with different pH values or different K$^+$ concentrations were then introduced. A Femtotip microinjection needle filled with 10 μM cAMP was placed into the field, and a positive pressure of 25 lb/in$^2$ was applied via a connected microinjector. Chemotaxis was recorded by time-lapse video using an inverted microscope (CKX41; Olympus) with a 10× objective lens. Images were taken every 30 s for 30 min.

**Electrotaxis assay.** Electrotaxis experiments were carried out as described previously (21, 28, 29). Developed cells were seeded into an electrotactic cham-

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† Published ahead of print on 8 July 2011.
FIG. 1. Extracellular pH and K⁺ concentration regulate the membrane potential (Vₘ) of Dictyostelium cells. Developed Dictyostelium cells were bathed in DB with different pH values and K⁺ concentrations. Vₘ was measured by microelectrode impalement. (A) Typical peak-shaped potential transient that was recorded upon microelectrode penetration of a D. discoideum cell bathed in DB with different pH values or K⁺ concentrations. (B and C) Continuous recording of Vₘ with an extra-fine electrode showed stable stationary Vₘ of D. discoideum. The dotted vertical line indicates the time when the bathing solution was replaced with a buffer of a different pH value or different K⁺ concentration. (D) Averaged Vₘs from 21 cells (pH 5.0), 16 cells (pH 6.5), 16 cells (pH 7.5), and 16 cells (pH 9.0). *, P < 0.001 compared to that in pH 7.5; #, P < 0.001 compared to that in pH 6.5. (E) Averaged Vₘs from 13 cells (5 mM K⁺), 16 cells (25 mM K⁺), and 24 cells (50 mM K⁺). *, P < 0.001 compared to that in buffer with K⁺ concentration of 0.5 mM K⁺; #, P < 0.001 compared to that in K⁺ concentration of 5 mM K⁺.

Membrane potential (Vₘ) measurements. Cells were seeded on a sterilized glass coverslip and observed with a 60× objective. Vₘ measurements were conducted using fine-tipped glass pipette microelectrodes. Two types of recording, transient and continuous, were used to verify each other. The pipettes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) using a PP-830 pipette puller (Narishige International, Inc., New York, NY), and the resistance was ~20 MΩ when the pipettes were filled with 3 M KCl solution, as measured in the DB. Recordings were performed using a GeneClamp 500 amplifier (Axon Instrument/Molecular Devices, Union City, CA). The signals were digitally filtered at 1 kHz and digitized at 2 kHz using a Digidata 1322A digitizer and pClamp, version 9.0, software (Axon Instrument/Molecular Devices).

DB solution was used as the standard recording solution. The solutions with higher or lower pH values were obtained by adding HCl or NaOH. The solutions with different K⁺ concentrations were made by using 3 M KCl and normal DB. All experiments were conducted at room temperature, and the recording was repeated in 16 or more cells.

Both transient impalement recording and continuous recording yielded similar Vₘ, which were consistent with previously published results (24).

Modulation of membrane potential (Vₘ). The first method used to control Vₘ was to maintain cells in four bathing solutions with pH values of 5.0, 6.5, 7.5, and 9.0. All solutions were autoclaved and stored at room temperature until use. Before measurement of Vₘ or chemotaxis and electrotaxis experiments, cells were bathed in a defined solution for ~10 min. The second method modulated Vₘ by adjusting the recording buffer [K⁺] at three concentrations (5 mM, 25 mM, and 50 mM). The K⁺ concentrations were verified with an ion-selective probe. All solutions were autoclaved. The third method was electroporation, which was carried out as previously described (7, 11). Electroporation was performed in a Gene Pulser Xcell Electroporation System (Bio-Rad) with two pulses of 0.85 kV/25 μF with a resistance-capacitance (RC) time of 1 ms, separated by a 5-s interval. For Vₘ measurements, 20 μl of cell suspension was immediately taken out of the electroporation chamber and placed in a petri dish in DB and measured 10 min later.

Statistics. Pearson’s correlation coefficient and a chi-square test were performed when pertinent. All data points were presented as means ± standard errors of the means (SEM) averaged from at least three measurements.

RESULTS

Extracellular pH and K⁺ concentration regulated Vₘ in Dictyostelium cells. A typical negative peak potential was transiently observed upon impalement of a Dictyostelium cell with a microelectrode. Vₘ transiently reached a peak value within several milliseconds of impalement; the potential quickly decreased due to leakage (Fig. 1A). The initial peak value was used to reflect the Vₘ, as in previous studies (24, 25). The values were verified with the continuous measurement. We achieved continuous recording using microelectrodes with an extremely fine tip (resistance up to 30 MΩ) that penetrates a cell so the Vₘ can be reliably monitored continuously for a
**Chemotaxis and electrotaxis in bathing solutions with different pH values.** To test the effects of depolarizing $V_m$, on chemotaxis, we used a needle chemotaxis assay and quantitatively analyzed the directional cell migration. Without cAMP gradient, cells bathed in different solutions showed similar patterns of migration in random directions. In buffer solutions of pH 5.0, pH 6.5, and pH 9.0, no significant differences in cell morphology, behavior, or trajectory speed were observed among cells in response to the cAMP gradient (Fig. 2A and D; Table 1). This is consistent with a previous report (25).

The electrotaxis of *Dictyostelium* cells, however, is significantly altered by the pH of the bathing solution. The chemotactic indexes for cells bathed in DB of pH 5.0, pH 6.5, and pH 9.0 were 0.56 ± 0.01, 0.58 ± 0.01, and 0.52 ± 0.01, respectively. However, electrotaxis was significantly affected by bathing solution pH (Fig. 2C and D). Cells in solutions of pH 6.5 had more negative $V_m$s and showed the best electrotaxis. Cells in pH 5.0 or pH 9.0 showed significantly reduced electrotaxis, with directedness values of 0.51 ± 0.01 or 0.30 ± 0.01, respectively (Fig. 2D).

**Chemotaxis and electrotaxis of K$^+$-induced depolarized *Dictyostelium* cells.** We then examined the effect of extracellular [K$^+$] on chemotaxis and electrotaxis. Cells bathed in buffer of 5 mM K$^+$ moved toward cAMP with a typically polarized morphology. In buffer of 50 mM K$^+$, cells near the micropipette tip moved toward the cAMP source. Cells further away from the tip appeared to move less directionally toward the pipette. Nonetheless, directional migration was evident (Fig. 3A). Cells in 50 mM K$^+$ migrated with a reduced chemotactic index of 0.30 ± 0.01, compared to that of 0.58 ± 0.01 for cells in 5 mM K$^+$ (Fig. 3D).

Extracellular [K$^+$] significantly affected electrotaxis. Cells bathed in buffer with 5 mM K$^+$ showed robust electrotaxis with a directedness value of 0.93 ± 0.01 (Fig. 3D). The directedness gradually decreased to 0.64 in 25 mM K$^+$ and 0.24 in 50 mM K$^+$ (Fig. 3D). Cells bathed in buffer with 50 mM K$^+$ had a significantly lower directedness value, representing a decrease of 73%. Although increasing extracellular [K$^+$] appeared to inhibit both chemotaxis and electrotaxis, it seemed to have a more significant effect on electrotaxis (Table 2).

**Electroporation depolarized $V_m$ and abolished electrotaxis.** To further verify the role of $V_m$ in electrotaxis, we used electroporation to depolarize *Dictyostelium* cells. Electroporation with high-voltage pulses permeabilizes the cell plasma membrane, thus significantly depolarizing $V_m$ by causing a large increase in non-ion-selective membrane permeability. Measurements in electroporated cells showed that the membrane is

![FIG. 2. Extracellular pH plays different roles in chemotaxis and electrotaxis in *Dictyostelium* cells.](http://ec.asm.org/)

**TABLE 1. Effects of extracellular pH and K$^+$ on chemotaxis**

<table>
<thead>
<tr>
<th>Developing buffer</th>
<th>Trajectory speed (μm/min)</th>
<th>Displacement speed (μm/min)</th>
<th>Chemotactic index</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>4.69 ± 0.03</td>
<td>3.34 ± 0.03</td>
<td>0.56 ± 0.01</td>
<td>0.79 ± 0.01*</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>5.01 ± 0.02</td>
<td>3.06 ± 0.02</td>
<td>0.58 ± 0.01</td>
<td>0.70 ± 0.01</td>
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<tr>
<td>pH 9.0</td>
<td>3.89 ± 0.02†</td>
<td>2.07 ± 0.02†</td>
<td>0.52 ± 0.01</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td>5 mM K$^+$</td>
<td>5.01 ± 0.02</td>
<td>3.06 ± 0.02</td>
<td>0.58 ± 0.01</td>
<td>0.70 ± 0.01</td>
</tr>
<tr>
<td>50 mM K$^+$</td>
<td>4.26 ± 0.03</td>
<td>1.62 ± 0.03†</td>
<td>0.30 ± 0.01*</td>
<td>0.65 ± 0.01</td>
</tr>
</tbody>
</table>

*The data represent means ± SEM, *P < 0.001, compared to buffer using 5 mM K$^+$; † P < 0.01, compared to buffer at pH 6.5; ‡ P < 0.001, compared to buffer at pH 6.5.
Electrotaxis in electroporation-induced depolarized cells was lost. Batch-matched control cells had good directional migration with a directedness value of $0.96 \pm 0.01$ within 10 min in an EF, which was maintained through 20 to 30 min (Fig. 4C). Following electroporation, electrotaxis was completely inhibited (Fig. 4B). In the first 20 min following electroporation, the directedness of cells in an EF was almost zero. At 30 to 40 min, some directedness reappeared as $V_m$ recovered to a certain degree (Fig. 4D).

We analyzed the relationship between these parameters and $V_m$. We found that $V_m$ correlates significantly well with electrotaxis, with a correlation coefficient of $-0.77$ (Fig. 5A), whereas chemotaxis did not appear to correlate with the changes in $V_m$ (Fig. 5B).

**DISCUSSION**

We tested the effects of extracellular pH and [K+] on electrotaxis using *Dictyostelium* cells, which have the unique property of tolerating changes in extracellular pH, [K+], and even electroporation, while maintaining good motility. We found that (i) changes in extracellular pH and [K+] and electroporation significantly affected $V_m$ and that (ii) reduced $V_m$ in response to these three factors significantly inhibited electrotaxis. The inhibitory effect on electrotaxis correlated well with the reduced $V_m$, but chemotactic effects did not.

In developed *Dictyostelium* cells, cAMP binds G protein-coupled receptors, activates Go2βγ, small GTPase, and class 1 phosphatidylinositol-3-kinases (PI3K), thereby phosphorylating phosphatidylinositol-3,4-bisphosphate [PI(3,4)P2] into phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3], and finally induces F-actin polymerization, resulting in pseudopod development. Several other pathways may also contribute to chemotaxis (9, 22). We demonstrated that *Dictyostelium* cells also show robust electrotaxis and are a good model for dissecting the molecular/genetic basis of electrotaxis (19, 29).

Extracellular pH, [K+], and electroporation significantly affected $V_m$ and correspondingly reduced or abolished electrotaxis. When $V_m$ recovered, electrotaxis was restored. $V_m$ in *Dictyostelium* cells is mainly generated by electrogenic proton pumps (24, 25). By varying extracellular pH, we controlled the $V_m$ with good reproducibility. The $V_m$ values were smaller than those reported previously (24, 25). We used two different recording methods to confirm the measurements. The difference in $V_m$ values may be due to other modifications: (i) the AX3 strain was used here whereas NC4 was used before; (ii) we

<table>
<thead>
<tr>
<th>Developing buffer</th>
<th>Trajectory speed (μm/min)</th>
<th>Displacement speed (μm/min)</th>
<th>Directedness</th>
<th>Persistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>9.27 ± 0.02*†</td>
<td>4.79 ± 0.02*†</td>
<td>0.51 ± 0.01*†</td>
<td>0.52 ± 0.01*†</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>14.79 ± 0.08*†</td>
<td>10.17 ± 0.07*</td>
<td>0.95 ± 0.01*</td>
<td>0.73 ± 0.02*</td>
</tr>
<tr>
<td>pH 7.5</td>
<td>11.68 ± 0.06*†</td>
<td>9.20 ± 0.06*†</td>
<td>0.97 ± 0.01*</td>
<td>0.78 ± 0.01*</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>9.05 ± 0.02*†</td>
<td>4.42 ± 0.02*†</td>
<td>0.30 ± 0.01*†</td>
<td>0.50 ± 0.01*†</td>
</tr>
<tr>
<td>5 mM K+</td>
<td>13.09 ± 0.05*†</td>
<td>8.55 ± 0.04*</td>
<td>0.93 ± 0.01*</td>
<td>0.66 ± 0.01*</td>
</tr>
<tr>
<td>25 mM K+</td>
<td>6.15 ± 0.03*†</td>
<td>3.57 ± 0.02*†</td>
<td>0.64 ± 0.01*†</td>
<td>0.59 ± 0.01*</td>
</tr>
<tr>
<td>50 mM K+</td>
<td>6.46 ± 0.01*†</td>
<td>2.95 ± 0.01*†</td>
<td>0.24 ± 0.01*†</td>
<td>0.46 ± 0.00*†</td>
</tr>
</tbody>
</table>

*The data represent means ± SEM. *P < 0.001 compared to buffer at pH 7.5; †, P < 0.001 compared to buffer at pH 6.5; ‡, P < 0.001 compared to that in buffer with 5 mM K+.**
used DB buffer while Van Duijn and coworkers used a Na+/H11001-, saline (40 mM NaCl, 5 mM KCl, 1 mM CaCl₂ and 5 mM HEPES-NaOH, pH 7.0); (iii) we used different development protocols (24, 25). The concentration of extracellular K⁺ affects Vᵐ (26). Different extracellular K⁺ concentrations regulated Vᵐ: the higher the K⁺ concentration, the lower the Vᵐ (Fig. 1).

At 50 mM K⁺, electrotaxis was significantly inhibited (Fig. 3). Depolarization of cells following electroporation abolished the electrotactic response while recovery of Vᵐ restored the electrotactic response (Fig. 4). Chemotaxis of the cells with an altered Vᵐ, modulated by changes in extracellular pH or [K⁺], was largely unaffected. This is consistent with a previous report (25). Collectively, these results support the theory that the inhibition of electrotaxis by changes in extracellular pH, [K⁺], and electroporation appears to be a specific effect caused by changes to Vᵐ. The genome of Dictyostelium cells shows at least two possible transient receptor potential (TRP) channel genes, a Ca²⁺ channel gene, and several K⁺ channel genes (14). Several signal transduction pathways related to electrotaxis could depend on Vᵐ caused by the interactions between ion channels and other signaling proteins such as integrins (2, 5, 6, 12, 13, 16, 23). It may involve different membrane proteins, such as ion channels, transporters, receptors, and the actin cytoskeleton, and may also involve Ca²⁺ signaling (20). The reduced Vᵐ might inhibit Ca²⁺ signaling and thereby affect electrotaxis. Another possibility is that Vᵐ may control the sensors that detect the EFs. We are currently using a
high-throughput strategy to screen for such sensing molecules in electrotaxis. In conclusion, changes in extracellular pH, [K\textsuperscript{+}], and electroporation all had significant effects on electrotaxis. When the \( V_m \) was depolarized, electrotaxis was significantly inhibited. Extracellular pH, [K\textsuperscript{+}], and electroporation all had significant effects on electrotaxis, which appeared to be mediated by the changes in \( V_m \). The initial directional sensing mechanisms for electrotaxis therefore differ from those in chemotaxis and may be mediated by changes in \( V_m \).

ACKNOWLEDGMENTS

This work was supported by a grant from the NSF (MCB-0951199 to M.Z. and P.N.D.). M.Z. is also supported by NIH grant 1R01EY019101, California Institute of Regenerative Medicine grant RBI-01417, and the University of California at Davis (UC Davis) Dermatology Developmental Fund. This study was supported in part by an unrestricted grant from Research to Prevent Blindness, UC Davis Ophthalmology, and Yunnan Province Talented Recruiting Program (2009C127). We thank the Wellcome Trust for continuous support (WT082887MA to M.Z.).

We thank Lin Cao and Kristen Swaney for sharing technical expertise and other members of the Zhao and Devreotes laboratories for their help. We thank Tsung-Yu Chen for kindly providing the electrophysiological equipment and Liping Nie for kindly helping us to use her electroporation device.


We declare that we have no conflicts of interest.

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FIG. 5. Significant correlation is between \( V_m \) and electrotaxis but not chemotaxis. (A) Scatter plot shows electrotactic directedness and resting membrane potential (\( r = -0.77 \)). The best-fit line is shown. (B) Scatter plot shows chemotactic index and membrane potential (\( r = -0.51 \)).