Normal chemotaxis in *Dictyostelium discoideum* cells with a depolarized plasma membrane potential

BERT VAN DUIJN\(^1,2\)*, SAKE A. VOGELZANG\(^1,2\), DIRK L. YPEY\(^2\), LOEK G. VAN DER MOLEN\(^1\) and PETER J. M. VAN HAASTERT\(^1\)†

\(^1\)Cell Biology and Genetics Unit, Zoological Laboratory, Leiden University, Kaiserstraat 63, NL-2311 GP Leiden, The Netherlands
\(^2\)Department of Physiology and Physiological Physics, Leiden University, PO Box 9604, NL-2300 RC Leiden, The Netherlands

* Author for correspondence at the second address
† Present address: Laboratory of Biochemistry, Department of Chemistry, Groningen University, Nijenborgh 16, 9747 AG Groningen, The Netherlands

Summary

We examined a possible role for the plasma membrane potential in signal transduction during cyclic AMP-induced chemotaxis in the cellular slime mold *Dictyostelium discoideum*. Chemotaxis, cyclic GMP and cyclic AMP responses in cells with a depolarized membrane potential were measured. Cells can be completely depolarized by two different methods: (1) by treatment with azide; this probably causes inhibition of the electrogenic proton pump, which was shown earlier to regulate plasma membrane potential in *D. discoideum*. (2) By electroporation, which causes the formation of large non-ion-selective pores in the plasma membrane.

It was found that in depolarized cells the cyclic AMP-mediated cyclic AMP accumulation was inhibited. In contrast, chemotaxis to a cyclic AMP source was normal; the cyclic AMP-induced accumulation of cyclic GMP, which is known to mediate the chemotactic response, was also not affected. We conclude that membrane-potential-regulated processes, such as voltage-gated ion channels, do not play an essential role in chemotaxis in *D. discoideum*.

Key words: membrane potential, chemotaxis, H⁺-ATPase, ion fluxes, *Dictyostelium discoideum*.

Introduction

Chemotaxis plays an important role in the life cycle of the cellular slime mold *Dictyostelium discoideum*. During the vegetative stage the amebae are chemotactic to folic acid and pterin (Pan *et al.* 1972, 1975), which are secreted by their food source, bacteria. When the food source is exhausted the amebae aggregate to form a multicellular slug, which differentiates into a fruiting body. During cell aggregation chemotaxis is mediated by the chemotactant cyclic AMP, which is secreted by the cells (Bonner, 1947; Konijn *et al.* 1967). The binding of cyclic AMP to the surface cyclic AMP receptor elicits various intracellular responses, including a rapid but transient activation of adenylate cyclase and guanylate cyclase (see Janssens and Van Haastert, 1987; McRobbie, 1986; Newell *et al.* 1987). Experiments with *D. discoideum* 'streamer F' mutants show that the cyclic GMP response is implicated in the chemotactic response (see Ross and Newell, 1981; Janssens and Van Haastert, 1987; Newell *et al.* 1987). However, the intracellular cyclic AMP response is not involved in chemotaxis (e.g. see Newell *et al.* 1987; McRobbie, 1986).

Although many cellular responses are known to be associated with chemotaxis (Devreotes and Zigmond, 1988; Janssens and Van Haastert, 1987; McRobbie, 1986), it is still largely unknown how chemotaxis is regulated in *Dictyostelium*. Various experiments suggest a role for ions in cyclic AMP signal transduction and chemotaxis. A decrease in extracellular calcium concentration and an increase in extracellular potassium concentration upon addition of cyclic AMP to a suspension of cells have been reported (Aeckerle *et al.* 1985; Bumann *et al.* 1984, 1986; Malchow *et al.* 1982). Furthermore, cyclic AMP induces changes in the extracellular (Malchow *et al.* 1978) and intracellular pH (Aerts *et al.* 1987).

It may be expected that these changes in the distribution of ions over the plasma membrane cause changes in the plasma membrane potential. On the other hand, several aspects of signal transduction could depend on the plasma membrane potential. The relationship between membrane potential, membrane conductance changes and chemotactic movement of cells has been investigated in other cell types. In *Paramecium* spp. ciliary movement induced by chemo-repellents and chemo-attractants is regulated by membrane potential changes and ion fluxes.
discoideum is mainly generated by an electrogenic proton membrane potential (Gallin and Gallin, 1977) have been reported. In the non-cellular slime mold Physarum polycephalum membrane potential deflections are related to chemoreception and chemotaxis (Ueda et al. 1975, 1985).

We found earlier that the membrane potential of D. discoideum is mainly generated by an electrogenic proton pump (Van Duijn et al. 1988; Van Duijn and Vogelzang, 1989). This electrogenic proton pump might play a role in the cyclic AMP-induced ion fluxes, that were observed in Dictyostelium.

Two aspects of the relationship between ion fluxes, membrane potential and chemotaxis, can be considered. First, the membrane potential may be altered by chemotactant-induced ion fluxes. Second, chemotactant-induced alteration of the membrane potential may affect the function of membrane proteins (including ionic channels and pumps) that are involved in the chemotactic response. The second aspect was studied in Dictyostelium by measuring chemotaxis after artificially depolarizing the membrane potential with azide or by electroporation. The induction of non-ion selective pores in the plasma membrane by electroporation made it possible to study partly the role of ion fluxes in chemotaxis. The membrane potential depolarization by azide and electroporation was established using intracellular microelectrodes. We observed that cells with a depolarized membrane potential showed normal chemotaxis and that membrane potential regulated processes do not appear to be essential in chemotaxis of D. discoideum.

Materials and methods

Materials
All chemicals were obtained from Sigma Chemical Co., St Louis, USA. The Na+-saline solution consisted of 40 mM-NaCl, 5 mM-KCl, 1 mM-CaCl₂ and 5 mM-HEPES–NaOH (pH7.0). K+-saline solution consisted of 50 mM-KCl, 5 mM-NaCl, 1 mM-CaCl₂ and 5 mM-HEPES–KOH (pH7.0). The K+-free saline solution was the Na+-saline solution without addition of KCl.

Culture conditions
Dictyostelium discoideum NC-4 (H) was grown in association with Escherichia coli 281 on solid medium containing 3.3 g peptone, 3.3 g glucose, 4.5 g KH₂PO₄, 1.4 g Na₂HPO₄, 2H₂O and 15 g agar per liter. Vegetative cells are harvested with cold 10 mM-sodium/potassium phosphate buffer, pH6.5 (PB), and washed free of bacteria by three washes and centrifugations at 150 g for 2 min.

Starved cells were obtained by incubation of cells on non-nutrient agar (1.5% agar in PB), at a density of 1.5×10⁶ cells cm⁻² for 4.5 h at 22°C or 15 h at 6°C.

Membrane potential measurements
For membrane potential measurements the cells were deposited on glass coverslips with a thickness of 0.17 mm (about 5×10⁴ cells cm⁻²). The glass coverslips were mounted on an open-bottom teflon culture dish, which was placed on the stage of an inverted microscope (Ince et al. 1985), and cells were observed using 100× objective magnification with oil-immersion optics. During measurements the cells were bathed in a Na+-saline solution (see Materials), unless stated otherwise.

Membrane potential measurements were made with fine-tipped microelectrodes with wide-angle-tapers filled with 3 M-KCl. Electrode resistances were 57.0 MΩ (s.d. = 18.2 MΩ, n = 119), as measured in Na+-saline solution. The membrane potential recordings were stored on a digital oscilloscope using pre-trigger display (Nicolet 3091) and plotted afterwards, using an x–y plotter (Hewlett Packard 7035B).

The peak-value, Eₚ, of the fast potential transient observed within the first milliseconds upon microelectrode impalement of a Dictyostelium cell was considered to be the best available estimate of the membrane potential of D. discoideum (Ince et al. 1986; Van Duijn et al. 1988).

Chemotaxis
Cells were allowed to adhere to a glass coverslip mounted on a teflon culture dish. Chemotaxis towards a capillary (diameter 2–3 μm) filled with bath solution containing 10⁻⁵ M of the chemoattractant cyclic AMP was observed at 100× objective magnification. At t = 0 s a short pressure pulse was applied to the capillary. At different time intervals photographs of the cells were taken. The distances between the tip of the capillary and the cells were measured from the photographs. By subtraction of these distances from the distance at t = 0 the distance moved towards or away from the capillary by the different cells was calculated and plotted as a function of time.

Electroporation
Electroporation of D. discoideum cells was accomplished by applying two pulses of 7 kV cm⁻¹ with a RC-time of 210 μs, separated by a 2 s interval, to a suspension of 10⁶ cells ml⁻¹ (Van Haastert et al. 1989). For electrophysiological and chemotaxis experiments, 20 μl cell suspension was immediately placed in the teflon culture dish provided with a glass coverslip and filled with 1 ml Na+-saline solution. Adherence of cells to the glass coverslip occurred within 5 s after addition of the cell suspension.

Physiological and biochemical assays
Determination of ATP levels. Cells were treated with 0.1 or 1 mM-azide (Na₃N₃) for 0–30 min at 20°C at a density of 10⁶ cells ml⁻¹ in PB; 100 μl of the suspension were added to 100 μl perchloric acid (3.5%, v/v). Samples were neutralized with 50 μl KHC₂O₃ (50% saturated at 20°C) and analysed by high performance liquid chromatography (HPLC) on a LiChrosorb 10RP18 column that was eluted with 5 mM-tri-butylammonium phosphate, 100 mM-KH₂PO₄, pH 4.5, 10% methanol.

Cyclic AMP and cyclic GMP response. Cells (10⁶ cells ml⁻¹) were treated with azide or subjected to electroporation. To measure the cyclic AMP response, cells were stimulated with 5 μM-2'-deoxy-cyclic AMP and 5 mM-dithiothreitol, and to measure the cyclic GMP response the stimulus was 0.1 μM-cyclic AMP. At the times indicated in Fig. 4 (below) 100 μl samples were taken, and cyclic AMP and cyclic GMP levels were measured by isotope dilution assays (Van Haastert, 1984; Van Haastert and Van der Heijden, 1983).

Results
Depolarization of the membrane potential
Membrane potential measurements. The peak-value, Eₚ,
Fig. 1. Effect of azide on the membrane potential of *D. discoideum*. A. Potential transient recorded upon microelectrode impalement (moment of impalement indicated by arrow) of a cell bathed in Na\(^+\)-saline solution. The peak-value of the potential transient, \(E_p\), is a more reliable measure than the semi-stationary potential, \(E_n\), for the true resting membrane potential prior to impalement. In cells preincubated with 100 \(\mu\)M-azide no potential transients were observed upon microelectrode penetration (recording indicated by *). B. Dependency of the mean peak-values of the potential transient, \(E_p\), on the azide concentration as measured in vegetative cells bathed in Na\(^+\)-saline solution (\(\bigcirc\), \(n=353, 26, 40, 35, 42, 40\) and 19 for azide concentrations of 0 to 500 \(\mu\)M, respectively). The \(E_p\) values measured in starved cells (\(\bullet\), \(n=68, 24\) and 11 for azide concentrations of 0 to 500 \(\mu\)M, respectively) were not different from the \(E_p\) values in vegetative cells. Bars represent ±S.E.

Membrane potential and chemotaxis in Dictyostelium

Table 1. Intracellular ATP concentrations after incubation of cells in different azide concentrations during different times

<table>
<thead>
<tr>
<th>Azide concn (M)</th>
<th>Incubation time (min)</th>
<th>%ATP concentration (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>(10^{-4})</td>
<td>15</td>
<td>49</td>
</tr>
<tr>
<td>(10^{-3})</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>(10^{-4})</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>(10^{-4})</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

Addition of azide to the cells. Kinetic experiments revealed that the plasma membrane was depolarized before the first time point, i.e. within 3 min after addition of azide (data not shown). Azide had similar effects on the membrane potential in vegetative cells and aggregative cells (Fig. 1B).

Membrane depolarization can be caused by several processes, among which are membrane conductance changes, ion concentration changes, and electrogenic ion pump activity changes; we have tried to exclude some of these effects. Azide is a well-known blocker of the respiratory chain phosphorylation in the mitochondria. We measured the effect of azide treatment on cellular ATP levels by means of HPLC. Table 1 shows that intracellular ATP levels are only slightly reduced at 3 min after addition of azide concentrations that resulted in complete depolarization of the membrane potential. This indicates that energy depletion is probably not the main cause of the azide-induced membrane depolarization.

Changes of intracellular sodium and/or potassium ion concentrations may play a role in the azide-induced depolarization. We therefore measured \(E_p\) in cells bathed in solutions with different concentrations of K\(^+\) and Na\(^+\). Cells bathed in K\(^+\)-saline solution still possess a large negative membrane potential (Fig. 2). In this solution the Na\(^+\) and K\(^+\) concentrations are approximately equal to the normal intracellular concentrations (Aeckerle et al. 1985; Maeda, 1983; Marin and Rothman, 1980). Addition of 100 \(\mu\)M-azide to the K\(^+\)-saline solution depolarized the cells (Fig. 2). Cells bathed in K\(^+\)-free
saline solution ($E_p = -20.5 \text{ mV}, \text{ s.d.} = 6.1 \text{ mV}, \ n = 15$) also showed depolarization upon addition of 100 μM-azide ($E_p = -5.1 \text{ mV}, \text{ s.d.} = 2.7 \text{ mV}, \ n = 18$). It thus appears that changes in sodium and potassium ion concentrations do not play a significant role in the azide-induced depolarization, which is supported by the observation that depolarization occurs immediately after azide addition.

These measurements indicate that the azide-induced depolarization is not caused by energy depletion, sodium/potassium ion concentration changes or membrane permeability changes for sodium and potassium ions. The membrane potential is largely generated by an electrogenic proton pump (Van Duijn et al. 1988; Van Duijn and Vogelzang, 1989), and it therefore seems likely that the azide-induced depolarization is due to inhibition of the proton pump activity by an unknown mechanism.

Recovery from azide-induced depolarization was measured in cells bathed in K⁺—saline solution after washing the cells free of azide (Fig. 2). Cells were incubated in 100 μM-azide for 30 min, and then the azide was removed. The time-constant of recovery was about 15 min, but recovery was never complete. Recovery from 100 μM-azide-induced depolarization in cells bathed in Na⁺—saline solution was not different from that of cells bathed in K⁺—saline solution (data not shown).

**Electroporation-induced depolarization.** High-voltage pulses can be used to make the plasma membrane of Dictyostelium permeable; under the conditions used large molecules such as inositol, but not ATP and inositol 1,4,5-trisphosphate, can cross the plasma membrane (Van Haastert et al. 1989). The introduction of large non-selective conductances should cause a depolarization of the membrane. Measurements in electroporated cells show that the membrane is completely depolarized immediately after electroporation (Fig. 3). All cells measured within 10 min after electroporation had an $E_p$ value less negative than −10 mV. Recovery of the membrane potential is complete in about 40 min after electroporation. Recovered cells showed a much more negative $E_p$ value as compared to control cells before electroporation (Fig. 3). This is a result of the increase in cell size due to electrosorption. In these enlarged cells $E_p$ is closer to the true membrane potential (Van Duijn et al. 1988).

The experiments described above provide two different methods to achieve membrane potential depolarization: one causing a large increase in non-ion-selective membrane permeability (electroporation) and one causing no changes in membrane conductivity (azide). This offers the possibility of studying (1) the necessity of the membrane potential and (2) the role of ion fluxes in cell function.

**Signal transduction**

Chemoattractant-induced activation of guanylate cyclase is implicated in chemotaxis of Dictyostelium, but not the activation of adenylate cyclase (see Janssens and Van Haastert, 1987; Newell et al. 1987). The response of adenylate cyclase and guanylate cyclase upon stimulation with cyclic AMP was measured in suspensions of starved D. discoideum cells. Addition of 100 μM-azide before stimulation did not alter the cyclic GMP response (Fig. 4). However, the cyclic AMP response was absent in azide-treated cells (Fig. 4).

We also measured the cyclic AMP-mediated cyclic GMP and cyclic AMP response of electroporated cells at 30 s after the electroshock when cells still have a depolarized membrane potential. Fig. 4 shows that the cyclic GMP response is still present in electroporated cells. The cyclic AMP response, however, is absent in azide-treated cells (Fig. 4).

**Chemotaxis in depolarized cells**

We next studied the influence of both azide and electroporation on chemotaxis towards a cyclic AMP source. Fig. 5 shows the chemotactic response of cells bathed in Na⁺—saline solution. Control cells rapidly move to the microcapillary filled with cyclic AMP upon application of
a pressure pulse (Fig. 5A,B). Quantitative data are presented in Fig. 6. The chemotactic response of cells in 100 μM azide, which completely depolarized the membrane potential, is identical to the response of the control cells (Fig. 6). After more than 30 min in 100 μM-azide chemotaxis was still unaffected (data not shown). Chemotaxis was also measured in electroporated cells. Fig. 5C–F demonstrates that these cells respond chemotactically towards the cyclic AMP source. The quantitative data (Fig. 6) reveal that chemotaxis was not significantly (Student's t-test, 95% level) different from the response of control cells.

Discussion

In the present study chemotaxis and signal transduction were investigated in *D. discoideum* cells with depolarized membrane potentials. The experiments show that both azide and electroporation induce membrane depolarization in *D. discoideum* cells. The main result of this study is the presence of normal chemotaxis towards cyclic AMP in completely depolarized *D. discoideum* cells.

Membrane depolarization

Membrane depolarization can be achieved either by treatment with azide or by electroporation. Azide-induced depolarization does not depend on the K⁺ or Na⁺ concentration in the extracellular medium, which indicates that depolarization is not caused by azide-induced changes in sodium and/or potassium ion permeability and/or ionic gradients. Also in *Neurospora* azide does not affect membrane permeability (Slayman, 1965).

In *Neurospora* and yeast cells azide-induced depolarization is thought to be due to azide-induced ATP depletion. However, azide-induced membrane depolarization precedes azide-induced reduction of oxygen consumption in both *Neurospora* and yeast cells (Slay-
Some of these ATPases can be inhibited by azide in vivo (Van Duijn and Vogelzang, 1984; Blanco, 1982). However, the use of electroporated cells in strongly buffered extracellular environments (Pogge von Strandmann et al. 1984; Nishimura et al. 1985) that the intracellular cyclic AMP concentration is most likely not directly due to membrane potential depolarization. This depolarization can also be induced by incubating cells at low extracellular pH (Van Duijn and Vogelzang, 1989); however, this treatment does not inhibit cyclic AMP-induced cyclic AMP accumulation (P. Devreotes, personal communication). Dinauer and coworkers (1980) suggest that azide can block a step between binding of cyclic AMP to the plasma membrane receptor and activation of adenylyl cyclase. A role for calcium in the activation of adenylyl cyclase cannot be excluded.

Chemotaxis is a complex process that includes the detection of gradients of chemoattractant, the transduction of temporo-spatial information about the chemotactic gradient and the analysis of this information in terms of directed pseudopod formation. In this study we have shown that cells with depolarized plasma membrane treated and electroporated cells used in the chemotaxis and signal transduction experiments all have a completely depolarized membrane potential, and that electroporation will induce non-ion selective fluxes across the plasma membrane.

Chemotaxis and signal transduction

Many physiological responses, among which are ionic events, are associated with chemoreception and chemotaxis (see Devreotes and Zigmond, 1988). In different amoeboid cells chemotactant-induced membrane potential changes have been reported (Gallin and Gallin, 1977; Ueda et al. 1975, 1985). Our experiments show that cells depolarized by azide or by electroporation exhibit normal chemotaxis in a cyclic AMP gradient. We conclude that membrane potential-regulated processes are not significantly involved in regulation of chemotaxis in D. discoideum. Hence, if the ion fluxes observed upon chemotactic stimulation (Bumann et al. 1984, 1986; Malchow et al. 1978, 1982; Böhme et al. 1987; Ackers et al. 1985; Aerts et al. 1987) are necessary for chemotaxis, these fluxes will not be under the control of the membrane potential. Since large non-ion-selective conductances are present in electroporated cells and intracellular Na$^+$ and K$^+$ concentrations are not strongly buffered in D. discoideum (Maeda, 1983), the data also suggest that K$^+$ and Na$^+$ fluxes are not important in the regulation of chemotaxis. The present experiments do not inform us about a possible role of calcium fluxes in chemotaxis, since the intracellular calcium concentration is strongly buffered and regulated, as in other eukaryotic cells. The use of electroporated cells in strongly buffered calcium solutions may permit a study of the role of intracellular calcium in chemotaxis.

Both azide- and electroporation-depolarized cells have a normal cyclic GMP response upon stimulation with cyclic AMP. Therefore, membrane potential-regulated processes are not involved in the cyclic GMP response. In contrast, the cyclic AMP-mediated cyclic AMP response is inhibited in both azide-treated and electroporated cells. This confirms the general finding (e.g. see Newell et al. 1987; McRobbie, 1986) that the intracellular cyclic AMP response is not involved in chemotaxis. This absence of cyclic AMP relay in electroporated and azide-treated cells is most likely not directly due to membrane potential depolarization. This depolarization can also be induced by incubating cells at low extracellular pH (Van Duijn and Vogelzang, 1989); however, this treatment does not inhibit cyclic AMP-induced cyclic AMP accumulation (P. Devreotes, personal communication). Dinauer and coworkers (1980) suggest that azide can block a step between binding of cyclic AMP to the plasma membrane receptor and activation of adenylyl cyclase. A role for calcium in the activation of adenylyl cyclase cannot be excluded.

Electroporation also causes membrane depolarization. Immediately after electroporation all cells measured were completely depolarized. Electroporated cells in suspension show uptake of inositol up to 10 min after applying the high voltage (Van Haastert et al. 1989). Therefore, we conclude that the electroshock-induced depolarization is due to the introduction of non-ion-selective pores in the plasma membrane.

From the measurements we conclude that the azide-
potential show a normal chemotactic response. These results suggest that voltage-dependent processes and fluxes of ions over the plasma membrane do not play an important role in chemotaxis.

The authors thank Louis C. Penning and Martinus J. De Vries for signal transduction measurements in electroporated cells, and Theo M. Konijn and Pauline Schaap for critically reading the manuscript. This study was supported by a grant to P. J. M. Van H. from the C. and C. Huygens Fund, which is subsidized by the Netherlands Organization for Scientific Research (NWO).

References


(Received 18 August 1989 – Accepted 16 October 1989)