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On the Role of Calcium in Chemotaxis and Oscillations of Dictyostelium Cells*

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Abstract. Migration of differentiated cells to a capillary containing cyclic AMP was enhanced in the presence of 1 mM $CaCl_2$ and was virtually absent in the presence of 1 mM EGTA. Furthermore, the cells contracted and extended pseudopods to a capillary filled with the calcium ionophore A 23187. At short distances, migration to the tip of the capillary was observed. The ionophore also induced transient decreases of the optical density of suspended cells indicating changes of cell shape. These findings support the hypothesis that cyclic AMP-binding to cell surface receptors causes a local influx of calcium ions. These in turn lead to an increase of the cytosolic calcium concentration and subsequently to an activation of cell migration. Perturbing pulses of the ionophore induced permanent phase shifts of free-running light scattering oscillations. This result indicates that cytosolic calcium is an intrinsic component of the oscillatory system.

Key words: Chemotaxis – Calcium – Oscillation – Dictyostelium – Ionophore

Aggregation of single amoebae to a multicellular organism is induced by pulses of cyclic AMP (for review see Gerisch 1982). The latter triggers an uptake of calcium ions (Wick et al. 1978; Bumann et al. in preparation) by binding to cell surface receptors (for review see Gerisch and Malchow 1976). Calcium stimulates contraction in actomyosin extracts (Condeelis and Taylor 1977), inhibits the phosphorylation of myosin heavy chains (Malchow et al. 1981) and affects the assembly of actin filaments (Brown et al. 1982). On the other hand calcium inhibits adenylate cyclase activity (Klein 1976; Loomis et al. 1978) and cyclic AMP synthesis by the cells (Gerisch et al. 1979). Our experiments were designed to test the following two hypotheses:
1.) Chemotactic orientation occurs at the receptor level. Receptors may be located along thin filopods thereby increasing spatial resolution. Those receptors

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exposed to the site of the cyclic AMP source are activated by cyclic AMP binding. A local influx of calcium leads to a local activation of movement. 2.) We examined whether calcium is involved in the oscillations occurring shortly before aggregation.

Material and Methods

The axenic strain of *Dictyostelium discoideum* was grown and induced to differentiate as described (Malchow et al. 1981). At 3–6 h after induction of differentiation (t_3-t_6) in Sørensen phosphate buffer pH 6.0 suspended cells were washed in the buffer specified in the legend. The light scattering technique was used as described by Gerisch and Hess (1974). The optical density was recorded at 500 nm using 2×10^7 cells per ml. For chemotaxis tests (Gerisch et al. 1975) cells were washed at t_{4-5}, diluted to 1 or 2×10^5 cells per ml and placed in a plastic chamber at 23° C.

Are Extracellular Calcium Ions Necessary for Chemotaxis?

Amoebae are attracted by a capillary filled with cyclic AMP (Gerisch et al. 1975). We tested this response in the absence and presence of calcium. In one experimental system we asked the question how many cells reach the tip of the capillary within 10 min. The distance from a group of cells was 75 μm. We found in six different experiments that in the average 5,6 cells collected at the tip in the presence of 1 mM $CaCl_2$, 3.6 in 10 μM $CaCl_2$ and 0.6 in 100 μM EGTA. In another setup we challenged single cells. Table 1 shows that most of the cells migrated three quarters of the distance to a capillary within 10 min if the medium contained 1 mM $CaCl_2$. In the presence of 1 mM EGTA however, no cell migrated further than one quarter of the distance. At 10 μm Ca^{2+} and 100 μM EGTA intermediate responses were obtained.

Table 1. Chemotaxis to cyclic AMP in the presence and absence of calcium. Migration of single cells towards the tip of a capillary filled with 100 μm cyclic AMP within 10 min. Cell numbers are shown, 100% ≙ 75 μm. The buffer contained 5 mM tricine, 10 mM KCL, 5 mM NaCl and calcium or EGTA as indiated, and was finally adjusted to pH 7.0. Migration of single cells starting at a distance of 75 μm from the tip of a capillary filled with 100 μM cyclic AMP was evalutated 10 min after insertion of the capillary using a calibrated ocular. Several experimental conditions were compared each day in order to account for variation in cell motility

Addition to the medium	0%	0–25%	25–50%	50–75%	75–100%
10^{-3} M $CaCl_2$	–	–	1	7	27
10^{-4} M $CaCl_2$	–	2	10	7	1
10^{-5} M $CaCl_2$	–	16	15	4	–
None	–	13	5	2	–
10^{-4} M EGTA	2	10	13	1	–
10^{-3} M EGTA	11	9	–	–	–

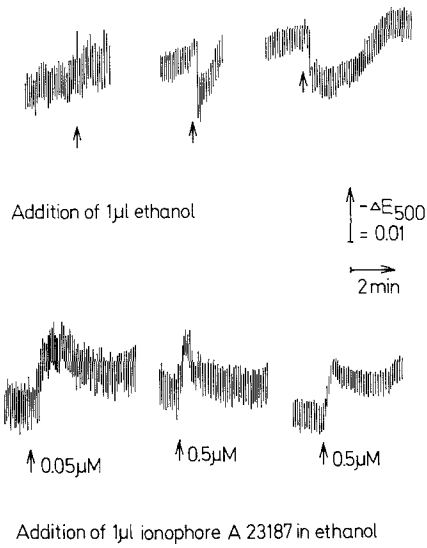


Fig. 1. Changes of light scattering of suspended cells induced by ethanol or the ionophore A 23187. Three different experiments are shown. The cells were suspended in 10 mM KCl, 0.1 mM $CaCl_2$. The addition of ethanol was either shortly before or after the addition of the ionophore shown below. The developmental age was t_4-t_6

Cyclic AMP addition to a cell suspension evokes a decrease of the optical density (Gerisch and Hess 1974). This decrease is attributed to a change of cell shape (Wurster et al. 1978). As shown in Figs. 1 and 3 addition of the calcium ionophore A 23187 induced a small but consistent decrease of the optical density. The addition of the same amount of solvent, ethanol,elicited an increase of light scattering. A second addition of the solvent, however, may cause no change.

Challenging amoebae by a capillary filled with 4 µM A 23187 caused a contraction and the formation of pseudopods in the direction of the capillary. In several cases, if the amoebae were close to the tip of the capillary, amoebae migrated to the tip (Fig. 2). Note the contraction of the cells which lie at some distance of the tip 30 s after the insertion of the capillary containing the ionophore. The frontal part was easier to stimulate than the rear end. The response, however, was much weaker than that evoked by cyclic AMP. The solvent, 0.1% DMSO, was a weak repellent.

These data seem to indicate: 1. External calcium is necessary for cyclic AMP-induced migration, with high levels of calcium increasing it. 2. An increase of the cytosolic calcium concentration elicited by the addition of the ionophore causes a change of cell shape, and a local application of the ionophore can mimic cyclic AMP mediated orientation. The following results of the literature support our conclusions: Mason et al. (1971) found an increase in the formation of aggregates with increasing external calcium concentration. Cone and Bonner (1980) observed that glassfibres coated with the ionophore A 23187 induced center formation in *P. violaceum* and in *D. discoideum* but in the latter to a much lesser extent. Finally Brachet and Klein (1977) and Loomis et al. (1978) reported that calcium ionophore addition accelerated the onset of aggregation and stimulated the precocious appearance of adenylate cyclase activity in *Dictyostelium.*

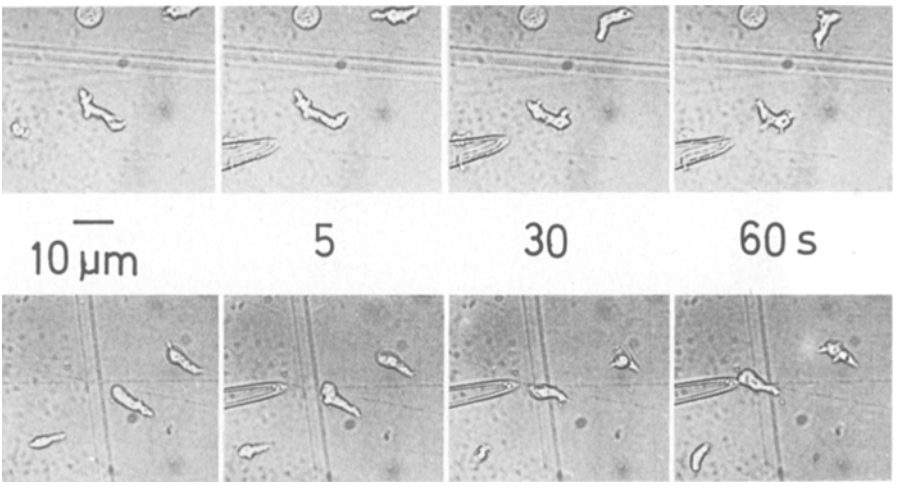


Fig. 2. Calcium-ionophore-induced migration of single cells towards the tip of a capillary. The latter contained either 0.1% DMSO (upper row) or 4 µM A 23187 in 0.1% DMSO (lower row). The ionophore was diluted freshly each time from a 4 mM stock solution in DMSO. t_{4-5} cells were tested either directly or following incubation at 4° C overnight. Cells were resuspended at 1×10^5 cells per ml in 10 mM KCl, 0.1 mM $CaCl_2$ or 5 mM tricine, 5 mM KCl, 0.1 mM $CaCl_2$ pH 7.0. No difference was observed between either condition

Our experiments, however, do not exclude the possibility that also for non-chemotactic migration extracellular Ca^{2+} is necessary as the studies on amoeboid movement of *Chaos carolinensis* by Taylor et al. (1980) seem to indicate.

Is Calcium Involved in Light Scattering Oscillations?

Gerisch and Wick (1975) found that light scattering oscillations coincide with oscillations of the internal cyclic AMP concentration. Figure 3 shows perturbing pulses of 1 µM A 23187 to free running light scattering oscillations. As shown in Fig. 1 additions of the ionophore caused an immediate transient decrease in light scattering. Depending on the time of addition the ionophore also evoked phase shifts. A phase response curve (Fig. 4) summarizes the results: The ionophore caused phase advances twice during the period. Addition at 0.2 unit length of the period elicited phase advances which were manifest immediately. Addition at about 0.6 unit length of the period mostly reduced the amplitude of the following light scattering peak and resulted in phase advances of the subsequent peak. It would be possible to consider this phase shift obtained at 0.6 unit length of the period as a phase delay since the next light scattering peak in Fig. 3 was almost absent. However, we found that the reduction in amplitude of the latter was variable and therefore we scored these phase shifts as phase advances as well. Reduction in amplitude of the following peak was also observed if the ionophore was given around 0.75.

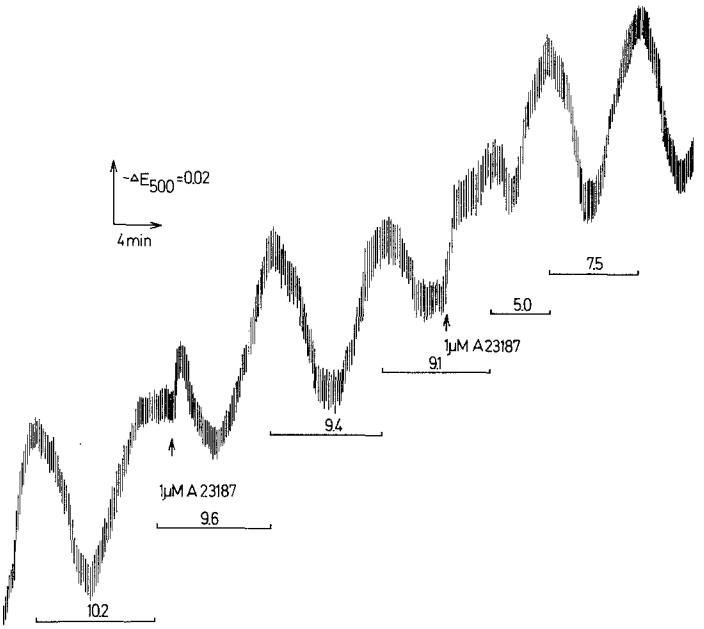
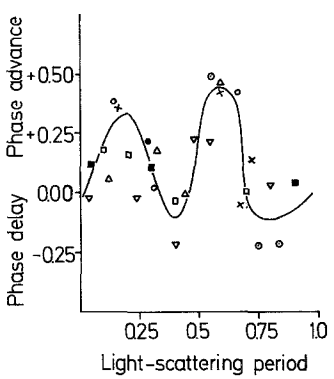


Fig. 3. Phase advance induced by the addition of the calcium ionophore to free running light scattering oscillations. 2×10^7 cells per ml differentiated in 5 mM tricine, 5 mM KCl pH 7.0 at 23° C. Changes of the optical density were examined at t_8 following the addition of 0.1 mM $CaCl_2$. The differentiation of suspended cells is delayed in tricine-buffer pH 7.0 as compared to Sørensen phosphate buffer pH 6.0

Fig. 4. Phase response curve to the ionophore A 23187 compiled from 8 separate experiments. Phase advances and delays were plotted as a function of sinusoidal light scattering oscillations at which the ionophore was added. All periods were normalized to 1. Phases 0 and 1 correspond to peaks of decreased light scattering. Phase shifts were evaluated immediately during the period in which the ionophore was added if the latter was given before 0.5 unit length of the period. If given later the following period was taken. The developmental age was t_5-t_9. The cells were suspended in 5 mM tricine, 5 mM KCl, 0.1 mM $CaCl_2$ pH 7.0 (×, ○, △, ⊙, ●); 10 mM KCL, 0.1 mM $CaCl_2$ (□, ▽) and Sørensen phosphate buffer pH 6.0, 0.1 mM $CaCl_2$ (■)



We found recently (Bumann et al. in preparation) that in suspensions of differentiated cells the extracellular calcium level oscillates as well. An inward flux of calcium is delayed slightly and is opposite in phase to previoulsy reported pH-oscillations (Malchow et al. 1978). It follows that the internal cytosolic calcium level is high at about 0.4 unit length of the light scattering period. A rise in the cytosolic calcium concentration induced by the ionophore caused phase advances just before and shortly after we expect the free internal calcium

concentration to be maximal. In addition, the calcium influx elicited at 0.6 unit length of the period seems to inhibit the next light scattering peak either directly by influencing changes of cell shape or indirectly by inhibition of cyclic AMP synthesis.

That oscillations in the calcium level are involved in light scattering oscillations is also indicated by experiments of Wick (1979). Addition of 1 mM EGTA reduced light scattering oscillations and readdition of calcium enhanced them.

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