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THE EFFECT OF 2,4-DINITROPHENOL ON DICTYOSTELIUM DISCOIDEUM OSCILLATIONS

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SUMMARY

During aggregation the cellular slime mold Dictyostelium discoideum emits pulses of cAMP about every 5 minutes. Only a small fraction of the aggregating cells produces the pulses autonomously, while most cells synthesize and release the nucleotide in a chain-reaction response to the autonomous signals (1). We report here that 2,4-dinitrophenol, KCN, and caffeine all inhibit the autonomous cAMP oscillations but do not interfere with the triggered response. Because of this, and other data (2), we question current models of the oscillatory synthesis of cAMP.

INTRODUCTION

Oscillatory phenomena are observed at all levels of biological organization, from the social to the molecular, with periods ranging from years to seconds (3). One of the simplest eukaryotes, Dictyostelium discoideum, displays oscillatory phenomena during the aggregation phase of its development. Time-lapse cinematography of amebae aggregating on a surface reveals that chemotaxis towards the aggregation center is not continuous, but occurs in pulses of movement. These pulses of inward movement spread as slow waves out from the center, new waves being produced with a period of about 5 minutes. This process is now understood to be initiated by the internally-controlled synthesis and release of the attractant, cAMP, from one or a few cells at the center of the territory (autonomous cells). These pulses are detected by adjacent cells which respond by chemotaxing towards the source, and also by emitting their own pulse of cAMP (relay-competent cells). Hence,

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two processes are involved in the generation of a cAMP wave, a triggering by autonomous cells, and signal transmission by relay-competent cells (1).

An important technical advance in studying the cAMP oscillations was the observation of Gerisch and Hess (4) that five- to seven-minute oscillations in scattered-light intensity occurred in illuminated suspensions of aggregation-phase D. discoideum amebae. Since these light-scattering oscillations are accompanied by oscillations in the extracellular and intracellular concentration of cAMP (5), it is assumed that they are due to the same basic mechanism that controls signal generation in cell layers on agar surfaces.

What this mechanism is at the molecular level remains a subject for speculation. The periodic production of cAMP by a population of cells has been modeled by Cohen (6) and by Goldbeter and Segel (7). Although differing substantially in details, both models utilize a limit-cycle oscillator that is identical for autonomous and relaying cells. We have initiated experimental studies of the oscillator by testing the effects of metabolic inhibitors, with the hope that they will point to controlling elements in the oscillator mechanism. Here we show that 2,4-dinitrophenol, KCN, and caffeine can suppress autonomous oscillations without affecting the relay competence of cells. This suggests that relay does not utilize the same basic oscillator as autonomous cAMP production.

METHODS

Cells of D. discoideum strain A3 (8) were grown in HL-5 medium, harvested and washed twice with cold distilled water as previously described (9). Cells were resuspended at a density of 2×10^7/ml in 16 mM MES·KOH [2-(N-morpholino)-ethane sulphonic acid] pH 6.0; or for pH measurements, in unbuffered 16 mM KCl. Because the cells alkalinize the unbuffered medium at a constant rate, a constant flow of HCl was used to maintain the extra-cellular pH between 6.0 and 6.2. Oscillations in light-scattering were observed by placing an aerated cell suspension in the light beam of a Zeiss PMQII spectrophotometer and recording transmitted light intensity at 540 nm. For cAMP measurements samples of cell suspension were rapidly transferred to equal volumes of 10% (w/v) trichloroacetic acid and vortexed. cAMP was purified and assayed as described previously (9) except that the alumina columns were omitted. Levels of cAMP obtained following an exogenous pulse were corrected for cAMP remaining from that pulse. This was done by including 4×10^4 cpm [^{3}H]cAMP (40

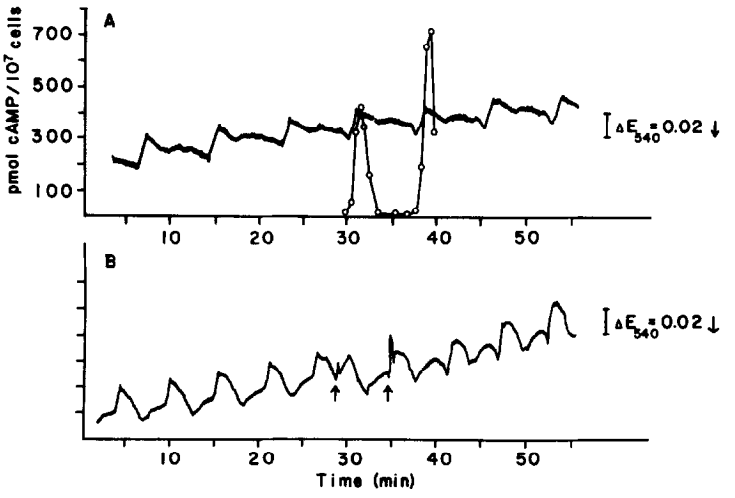

Fig. 1. cAMP and light-scattering oscillations.
A) Cells were prepared and cAMP (o) measured as described in Methods.
B) Arrows indicate when 1 mM cAMP was added to the cell suspension to a final concentration of 1 μM. ΔE refers to absorbance; the arrow indicates increasing absorbance.

Ci/mmol, New England Nuclear) per ml cell suspension and determining by chromatography (9) the amount remaining as cAMP. Extracellular pH was monitored with a Beckman glass pH probe.

RESULTS AND DISCUSSION

Typical light-scattering and cAMP oscillations for an illuminated suspension of D. discoideum are shown in Fig. 1A. Also shown is the effect of pulsing the suspension with cAMP (Fig. 1B).

The light-scattering oscillations cease and the transmitted light intensity decreases within 15 s of adding 33 μM DNP to a suspension of oscillating cells (Fig. 2). This decrease in intensity is followed by an increase which slowly levels off. Similar effects occur for DNP concentrations greater than 15 μM; below this concentration no effects are observed.

Total cAMP levels stop oscillating after addition of 33 μM DNP (Fig. 2A). However, addition of 1 μM cAMP to DNP-treated cells elicits changes in

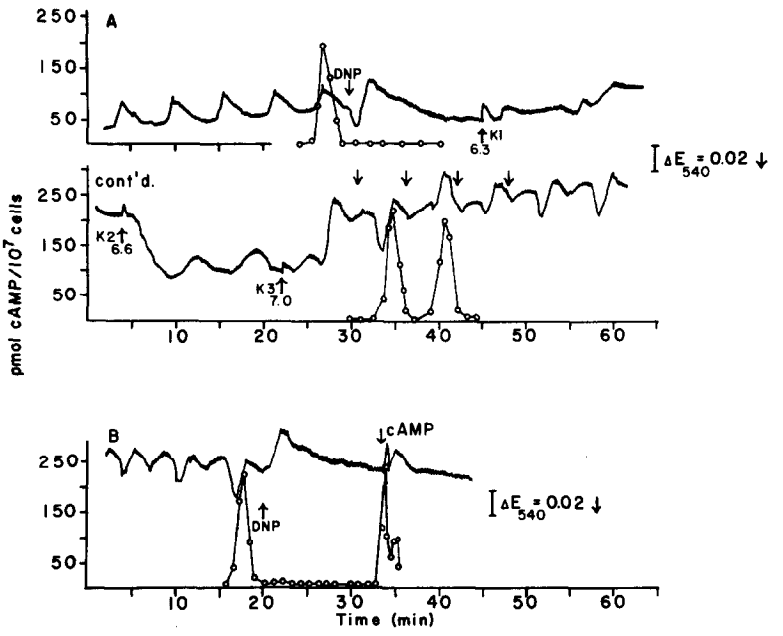

Fig. 2. Effect of DNP on light-scattering and cAMP oscillations and on relay competence.

A) Cells were prepared and cAMP (o) measured as described in Methods. The first arrow indicates when 100 mM DNP was added to the suspension to a final concentration of 33 μM. The three subsequent arrows, labeled K1, K2, K3, indicate additions of 1 M KOH to final increments of 3 mM each. Numbers under these arrows indicate extracellular pH. The arrows over the trace of restored oscillations indicate where cAMP peaks would appear were phase preserved.

B) Cells were prepared and cAMP (o) measured as described in Methods. DNP was added as above. To test for relay competence, cAMP was added to a final concentration of 1 μM, where indicated, from a 1 mM stock solution. The first time point was taken 15 s after addition of cAMP. ΔE refers to absorbance; the arrow indicates increasing absorbance.

light-scattering (Fig. 2B) which are identical to those recorded when cAMP is added to a suspension of untreated cells (Fig. 1B). This response can be reproducibly obtained upon subsequent additions of cAMP. In some experiments, in which the cells were several hours older, spontaneous light-scattering oscillations returned after two to three additions of 1 μM cAMP. The level of total endogenous cAMP after stimulation of DNP-treated cells with 1 μM cAMP is indicated in Fig. 2B. As can be seen, addition of exogenous cAMP stimulates a very rapid synthesis of cAMP by the cells.

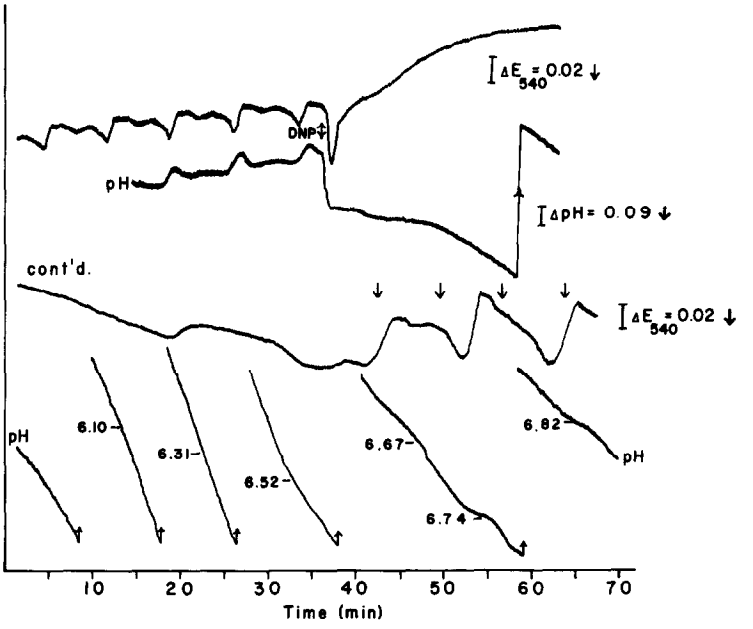

Fig. 3. Effect of DNP on extracellular pH oscillations. Cells were prepared as described in Methods for pH measurements. DNP was added as in Fig. 2 where indicated. After DNP addition the HCl source was removed to allow the cells to alkalinize the suspension. Numbers next to the pH trace indicate the extracellular pH at those points. The arrows over the trace of restored oscillations indicate where cAMP peaks would appear were phase preserved. ΔE refers to absorbance; the arrow indicates increasing absorbance. The extracellular pH increases in the direction indicated.

In unbuffered cell suspensions we have observed spontaneous oscillations in extracellular pH that are in phase with cAMP oscillations [also reported by Nanjundiah and Malchow (10)]. Observation of these pH oscillations is obscured unless the alkalinization of the medium is counterbalanced by constant addition of HCl. Like the oscillations in cAMP concentration and light-scattering, the pH oscillations are eliminated by DNP (Fig. 3). If the flow of HCl is stopped after DNP addition, the alkalinization of the medium by the cells reverses the DNP inhibition, allowing light scattering and pH oscillations to resume (Fig. 3). The reversal of DNP inhibition by the increased extracellular pH is probably due to a change in the partition coefficient of DNP across the cell membrane (11). If the pH is restored to 6.0 all oscillations are again inhibited (data not shown).

The data in Fig. 2 indicate that the cellular cAMP synthesizing system (adenylate cyclase, ATP) and cAMP receptors are functional after DNP addition. The cessation of cAMP oscillations after DNP addition must therefore be due to an effect on the autonomously oscillating cells. One possibility is that the basal oscillator in the autonomous cell itself is destroyed while another is that the basal oscillator remains intact, but its driving link to the periodic efflux of cAMP is disrupted. Data presented in Figs. 2 and 3 argue against the latter possibility. Were the basal oscillator to remain intact, it should maintain its phase in the presence of DNP. This prediction can be tested as the effects of DNP are reversed if the extracellular pH is increased to about 7.0. In unbuffered suspensions (Fig. 3) the cells themselves alkalinize the medium; in buffered suspensions it can be achieved by addition of KOH (Fig. 2A). As shown in these figures, the phase of the restored oscillations differs from that existing prior to DNP addition. Control experiments showed addition of KOH alone does not cause phase-shifting. It is still possible that the basal oscillator remains intact, but that DNP changes its period in addition to uncoupling it from cAMP production. This type of model is difficult to test experimentally.

The initial signal leading to activation of the adenylate cyclase differs in autonomous cells and relaying cells; in the former the signal comes from the basal oscillator, whereas in the latter it comes from binding of cAMP to the external cell membrane. However, it is unknown if the two signals subsequently share common steps in their processing. The fact that DNP disrupts autonomous cAMP oscillations but not cAMP relay suggests that at least some facets of the basal oscillator in autonomous cells are not shared by the relay system in relay competent cells. This finding argues against models in which a unified mechanism for autonomous oscillations and relay is proposed (7).

In conjunction with studies to be published elsewhere (2), we extend our reservations concerning the unified oscillator models to question whether

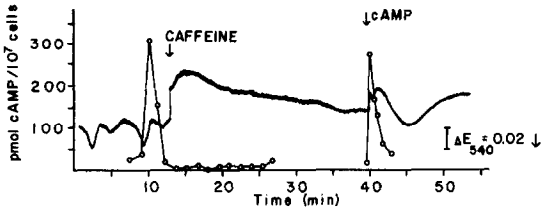

Fig. 4. Effect of caffeine on light-scattering and cAMP oscillations and on relay competence.
Cells were prepared and cAMP (o) measured as described in Methods. The first arrow indicates when 100 mM caffeine was added to the suspension to a final concentration of 5 mM. The second arrow indicates when relay competence was tested by addition of cAMP as in Fig. 2B. ΔE refers to absorbance; the arrow indicates increasing absorbance.

production of cAMP by relaying cells involves an oscillator at all. The models of both Goldbeter and Segel (7) and of Cohen (6) are predicated on oscillations of control metabolites that occur with the same period, but different phase, as cAMP. We have found no evidence for such oscillations of metabolites during light-scattering oscillations (2). We suggest, therefore, that metabolite oscillations occur only in autonomous cells. Because only about one cell in 10^4 is autonomous (1), oscillations in their metabolites would not be measurable against the background of non-autonomous cells.

At this point we do not know how DNP acts to inhibit oscillations. Other compounds, which are thought to have different metabolic effects, give similar patterns of inhibition as DNP. Like DNP, caffeine inhibits spontaneous oscillations, but has no effect on signal relay (Fig. 4). In D. discoideum caffeine is not an inhibitor of cAMP phosphodiesterase (12); possibly it alters the intracellular distribution of Ca^{++} as has been found for muscle cells (13). Although its effects on cAMP levels were not determined, 50 μM KCN has the same effect on light-scattering oscillations as DNP and caffeine; autonomous oscillations are suppressed, but the response to exogenous cAMP is normal (data not shown, but see Fig. 2 or 4 for similar traces).

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