PERIODIC CHANGES IN ADENYLATE CYCLASE AND cAMP RECEPTORS IN *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

Information basic to the construction of a multicellular organism is transmitted between starved *Dictyostelium discoideum* amebae as cAMP pulses [1–3]. In response to these signals, cells differentiate into aggregation-competent amebae [4] and chemotact toward centers [1] consisting, presumably, of cells which autonomously and rhythmically release cAMP [5]. The effects of cAMP pulses are probably mediated by plasma membrane receptors, as evidenced by cell-surface cAMP-binding sites [6]. Within the first few minutes after stimulation by a pulse of cAMP, cells transitorily increase their cellular cAMP levels [3] and excrete the cyclic nucleotide [2,3]. This relay of the cAMP pulses results in the co-ordinated aggregation of a large cell-population [1,5]. The unidirectional movement of amebae is explained by a refractory period, of a few minutes' duration, during which time cells are incapable of relaying the signal [7]. On the basis of computer analyses, Golbeter and Segel have advanced a model for autonomous oscillations and relay of cAMP in which extracellular cAMP, via its receptor, activates adenylate cyclase [8]. Recent support for this model has been obtained by Roos and Gerisch who have shown that a transient stimulation of adenylate cyclase activity can be evoked by an applied pulse of cAMP [9]. In the absence of applied cAMP pulses, differentiating amebae rhythmically synthesize and excrete cAMP [2,10]. In this communication we present evidence for spontaneous oscillations in adenylate cyclase. We also demonstrate periodic changes in binding of cAMP to its receptors. The relationship between these two phenomena is discussed.

2. Methods

Ax-2 amebae [11] were maintained as exponentially growing cultures in HL-5 media [11]. Differentiation was initiated by washing cells twice in 17 mM phosphate buffer, pH 6.2 and agitating them in that buffer at a density of 10^7 amebae/ml as described by Beug et al. [12]. After 5 h, cells were concentrated by centrifugation to 2×10^7 amebae/ml, oxygenated, and monitored in an ACTA III recording spectrophotometer for periodic changes in light scattering [13]. For adenylate cyclase determinations, replicate samples of 1.5 ml were withdrawn approximately every 2 min and centrifuged for 15 s in an Epindorff minifuge. Supernatants were decanted and pellets frozen in dry ice. The maximum time required for these manipulations was 45 s. Cells were lysed and immediately assayed for adenylate cyclase activity as described [14]. Cell assays were performed in triplicate. When levels of cAMP-binding were measured, 200 μl samples of cells were withdrawn every 30 s and incubated with 5×10^{-8} M [^{3}H]cAMP. After 15 s, aliquots were centrifuged through silicons layers [3] and the cell-associated radioactivity determined. Non-specific binding was determined by incubating cells with [^{3}H]cAMP and excess non-radioactive cAMP. A detailed description of the procedure is available elsewhere [15]. All experiments were performed at least three times.

3. Results and discussion

When cells are starved in spinner suspensions, they display rhythmic changes in optical density [13] which have been correlated with transitory changes in cAMP synthesis [10]. The experiment described in fig.1 shows that oscillations in adenylate cyclase activity can be measured in such cells. Amebae were starved for 5 h, at which time they generated the optical density changes shown in the upper graph. Oscillations occurred with a frequency of 4–5 min. The adenylate cyclase activities of these amebae are represented in the lower curve. Oscillations in enzymic activity of 3–4-fold were observed. The precise localization of the maximum activities of adenylate cyclase is limited by the time interval between cell-sampling. High levels of enzymic activity were observed just prior to the fall in optical density. This finding is consistant with the observation of Gerisch and Wick that maximum cAMP levels are observed during this period [10]. The degree of enzyme activation seen here is similar to that observed when adenylate cyclase is transitorily activated by an applied pulse of cAMP [9].

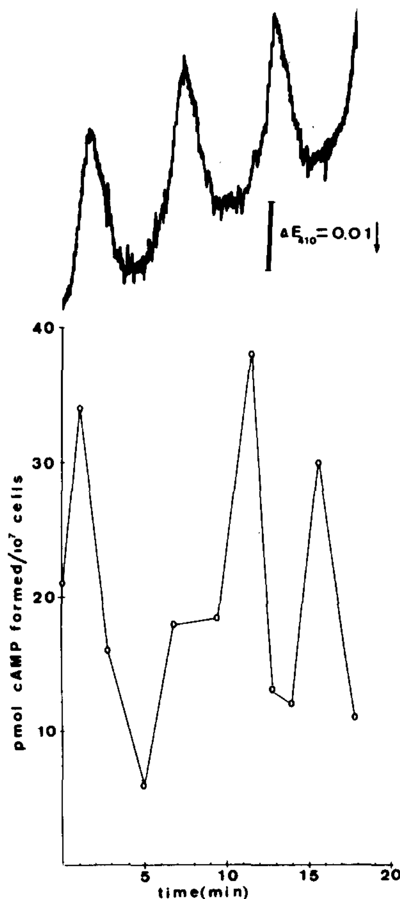

Fig.1. Oscillations in adenylate cyclase activity of differentiating amebae. Amebae were starved for 5 h, at which time they displayed the oscillatory behavior indicated by changes in optical density (upper curve). Adenylate cyclase activities (lower curve) were measured as described in Methods. Each value represents the average of triplicate determinations.

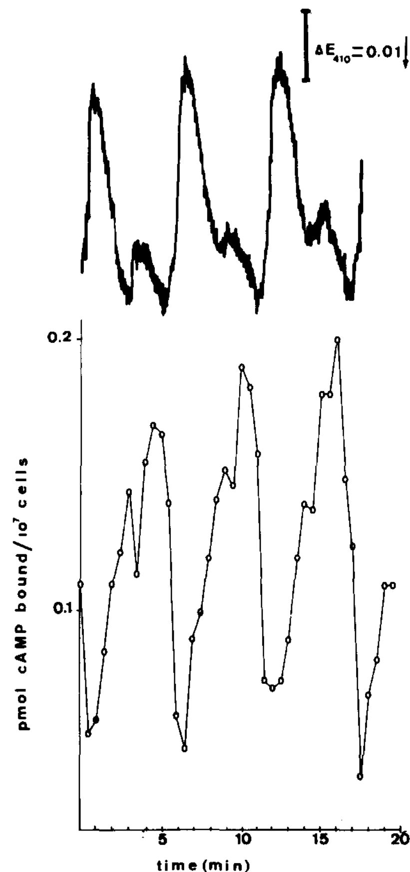

Fig.2. Oscilations in cAMP binding to differentiating amebae. Cells were starved for 5 h and levels of cAMP binding determined as described in the Methods section. Each point is the average of duplicate determinations. Upper curve, spontaneous oscillations in optical density. Lower curve, spontaneous oscillations in cAMP-binding.

The fact that changes in adenylate cyclase activity which occurred in intact cells could be measured in cell-free extracts suggests that such changes result from relatively stable alterations of the enzyme. Since adenylate cyclase is membrane-bound [16], it is possible that its activity is influenced by the association of the enzyme with other membrane components, in particular, the cAMP receptor. Therefore, we examined the possibility that binding of cAMP to the cell-surface also varied periodically. The results expressed in fig.2 show that 2-fold oscillations in cAMP-binding occurred with the same frequency as the oscillations in light scattering. Minimal binding just preceded or coincided with, the drop in optical density. Highest levels of binding were observed when optical density recordings were maximal. We are, as yet, unable to discern if the changes in cAMP-binding reflect isotopic dilutions due to the excretion of cAMP by the cells, or changes in cAMP receptor concentrations or affinity. The observations by Klein and Juliani that amebae down-regulate their number of cAMP binding sites in response to high external cAMP concentrations [17] suggest that the latter phenomenon may be responsible for the periodic changes in cAMP binding reported here. More detailed comparisons between adenylate cyclase activities, cAMP concentrations, and the levels of cAMP binding of wild-type amebae and developmental mutants should provide greater insight into the sequence of events involved in the oscillatory process.

Acknowledgements

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References

[1] Bonner, J. T. (19) in: The Cellular Slime Molds, 2nd ed Princeton University Press, Princeton, N.J.
[2] Shaffer, B. M. (1975) Nature 255, 549.
[3] Roos, W., Nanjundiah, V., Malchow, D. and Gerisch, G. (1975) FEBS Lett. 53, 139.
[4] Darmon, M., Brachet, P. and Pereira da Silva, L. (1975) Proc. Natl. Acad. Sci. USA 72, 3163.
[5] Robertson, A., Draye, D. J. and Cohen, M. H. (1972) Science 175, 333.
[6] Malchow, D. and Gerisch, G. (1974) Proc. Natl. Acad. Sci. USA 71, 2423.
[7] Cohen, M. H. and Robertson, A. (1971) J. Theoret. Biol. 31, 101.
[8] Goldbeter, A. and Segel, L. E. (1977) Proc. Natl. Acad. Sci. USA, in press.
[9] Roos, W. and Gerisch, G. (1976) FEBS Lett. 68, 170.
[10] Gerisch, G. and Wick, U. (1975) Biochem. Biophys. Res. Commun. 65, 364.
[11] Watts, D. J. and Ashworth, J. M. (1970) Biochem. J. 119, 171.
[12] Beug, H., Katz, F. E. and Gerisch, G. (1973) J. Cell Biol. 56, 647.
[13] Gerisch, G. and Hess, B. (1974) Proc. Natl. Acad. Sci. USA 71, 2118.
[14] Klein, C. (1976) FEBS Lett. 68, 125.
[15] Juliani, M. H. and Klein, C. (1977) Biochem. Biophys. Acta, in press.
[16] Klein, C. (1977) FEMS Microbiol. Lett. 1, 17–20.
[17] Klein, C. and Juliani, M. H. (1977) Cell, in press.