EXCITABILITY IN THE ADENYLATE CYCLASE REACTION IN *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

Cyclic AMP (cAMP) signals control chemotaxis and differentiation during the hours that follow starvation in the slime mold *Dictyostelium discoideum* [1–3]. The signalling system consists of a cell-surface cAMP receptor and of a functionally-coupled adenylate cyclase [4–6]. Experiments in cell suspensions show that this system can exhibit three types of behaviour:

(i) Relay of extracellular cAMP signals [7,8];
(ii) Autonomous generation of periodic cAMP pulses [9];
(iii) Lack of response to stimulation by cAMP.

Under relay conditions, a suprathreshold signal of extracellular cAMP elicits a pulsatory synthesis of intracellular cAMP of much larger magnitude. We analyze here this amplification process in a model [10] proposed for relay and oscillation of cAMP in *D. discoideum*, and show that it represents an excitable response of adenylate cyclase.

Excitability is the capability of a system, initially at a stable steady state, to amplify small perturbations in a pulsatory manner. This phenomenon has been analyzed in models for the nerve membrane [11], for an enzyme with autocatalytic pH-dependent kinetics [12], and for the Belousov-Zhabotinsky reaction [13,14]. Experiments on the latter type of reaction [15,16] exemplify a property common to all these excitable systems, their oscillatory capability. As shown below, the adenylate cyclase reaction in *D. discoideum* also belongs to the class of excitable and oscillatory chemical systems.

Besides allowing further predictions, the mechanism of excitability proposed for adenylate cyclase provides a unifying interpretation, in terms of phase plane analysis, of several results obtained in previous simulations [10], namely the existence of a threshold for stimulation by extracellular cAMP, the existence of a refractory period, and the necessary association of relay with sustained oscillations of cAMP. These properties account, on molecular grounds, for several observations made on the wavelike aggregation of *D. discoideum* amoebae [17–19], and substantiate the description of the aggregation fields as excitable media [20].

2. Results and discussion

The model analyzed for relay and oscillation of cAMP [10] is based on the observation that binding of extracellular cAMP to a cell surface receptor activates adenylate cyclase [21]; the latter enzyme transforms intracellular ATP into cAMP. The activation by cAMP could be mediated by an intracellular effector such as cyclic GMP [22] acting on a protein kinase [23], or calcium [24], though this ion seems to affect the basal activity of adenylate cyclase rather than the

oscillations [5]. To represent the regulation by cAMP, we made the simplest assumption that the receptor behaves as a regulatory subunit of adenylate cyclase; we further assumed that the catalytic and regulatory entities are dimers, and that the resulting complex obeys the concerted allosteric model [25] with exclusive binding of substrate and positive effector to the R state. We neglected the effect of diffusion, as most experiments on relay and oscillation are performed in continuously stirred suspensions of *D. discoideum* cells.

The system is described by the following differential equations [10]:

$$d\alpha/dt = \nu - \sigma\phi$$

$$(1/q)\ d\beta/dt = \sigma\phi - (k_t\beta/q)$$

$$d\gamma/dt = (k_t\beta/h) - k\gamma \qquad (1)$$

where

$$\phi = \alpha(1+\alpha)(1+\gamma)^2/[L+(1+\alpha)^2(1+\gamma)^2] \qquad (2)$$

The three variables α, β and γ denote the concentrations of intracellular ATP, intracellular cAMP, and extracellular cAMP divided by K_s, K_p and K_p, respectively, where K_s and K_p are the Michaelis constant of adenylate cyclase for ATP and the dissociation constant of the cAMP receptor. Parameters ν and σ relate to the constant ATP input and to the maximum cyclase activity, divided by K_s; $q = K_s/K_p$; k_t and k measure the assumed linear rates of cAMP transport across the cell membrane and of the phosphodiesterase reaction; L is the allosteric constant of adenylate cyclase; h is a dilution factor [10].

The observation that $q \geqslant 100$ [10] suggests that the system of eq. (1) can be well approximated by the reduced system:

$$\beta = (q\sigma/k_t)\phi$$

$$d\alpha/dt = \nu - \sigma\phi$$

$$d\gamma/dt = k(\lambda\phi - \gamma) \qquad (3)$$

by means of a quasi-steady-state assumption for β, in the limit $q \to \infty$ with $\lambda = (q\sigma/hk)$ and (k_t/q) remaining finite. As in previous studies of excitable systems [11–14], the phase plane analysis of system (3) throws light on the phenomenon of excitability in the adenylate cyclase reaction. In the phase plane (α, γ), the steady state of system (3) is given by the intersection of the nullclines $\nu = \sigma\phi$ and $\gamma = \lambda\phi$ (see fig.1). One crucial point in the analysis is that the curve $\gamma = \lambda\phi$ is, under definite conditions, an S-shaped sigmoid; it then passes successively through a maximum and a minimum in α as γ increases (these extrema are denoted by A and D, respectively, in fig.1).

The stability properties of the steady state depend on its location on the sigmoid. These properties, in turn, govern the dynamic behaviour of adenylate cyclase. When the intersection of the two nullclines lies between A and D, i.e., in the region of negative slope on the sigmoid, the steady state is unstable. Sustained oscillations of the limit cycle type occur in these conditions [10]. When the intersection lies to the left or to the right of the oscillatory domain, the steady state is stable and excitability is mathematically possible. If the conditions are such that the steady state is located on the right branch of the sigmoid, a pulse of cAMP could, in principle, follow a transient decrease in extracellular cAMP. But relay consists in the amplification of an extracellular increase in cAMP.

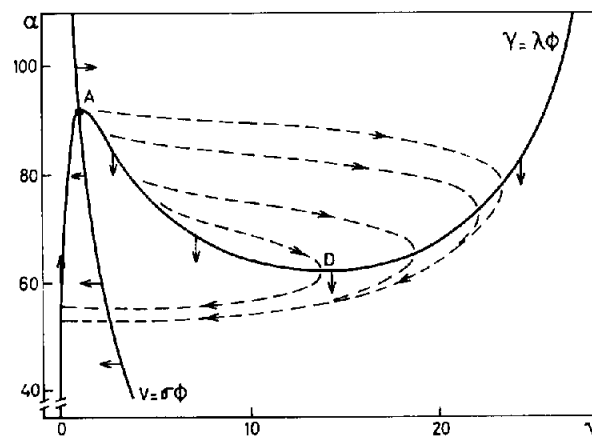

Fig.1. Excitability in the (α, γ) plane of system (3). The steady state (dot) is located at the intersection of the nullclines $\nu = \sigma\phi$ and $\gamma = \lambda\phi$ on which arrows indicate the local direction of the solution trajectory. Several trajectories (dashed lines) are drawn, corresponding to different suprathreshold initial conditions. Parameter values are: $\nu = 0.04\ s^{-1}$, $\sigma = 1.2\ s^{-1}$, $k = k_t = 0.4\ s^{-1}$, $L = 10^6$, $q = 100$, $h = 10$. These values yield semi-quantitative agreement with experiments on relay in cell suspensions [10].

Accordingly, we now show that relay can take place when the steady state lies on the left branch of the sigmoid, close to maximum A (see fig.1).

Several trajectories have been drawn in fig.1, showing the excitability properties of system (3). When the initial condition is such that γ exceeds a threshold value above the steady state, the system undergoes a large excursion in the phase plane across the right branch of the sigmoid, before returning to the stable steady state. Notice that the large excursion in the phase plane does not result from the existence of the two time scales often invoked in explanations of excitable behaviour [11–14] (two scales would exist in system (3) for large values of k). Excitability is here strictly related to the characteristics of function ϕ. To see this more clearly, note from fig.1 that relay is associated with trajectories that move in a quasi-horizontal fashion from near the central branch of the sigmoid before suddenly changing when they reach the vicinity of this curve's right limb. We thus seek a condition guaranteeing that $s = |d\alpha/d\gamma| < 1$ in the portion of the (α, γ) plane bounded below by the sigmoid. This is:

$$\phi > (\gamma - \nu/k)/(\lambda - \sigma/k) \qquad (4)$$

Because of the non-linear dependence of ϕ on γ, this inequality is satisfied in a large domain of γ values that extend close to the right branch of the sigmoid, provided α is not too small.

Let us now examine the excitable behaviour of the real system governed by eq. (1), for the same set of parameter values. Figure 2 presents a trajectory in the (α, β, γ) space projected on the (α, γ) plane. To simulate the experiments on relay of a cAMP pulse in cell suspensions, we initially set the concentrations α and β at their steady state value and impose an instantaneous increase in γ above the threshold, which is 1.85 in the case considered. The relay response obtained by integration of eq. (1) yields satisfactory agreement with the phase-plane analysis of the reduced system (fig.1). As shown in the inset to fig.2, the ample trajectory in the (α, γ) plane corresponds to the synthesis of a pulse of intracellular cAMP, with an amplification factor close to 20.

Threshold values obtained for different steady states are represented by the black triangles in fig.2. The line joining these values defines a threshold locus

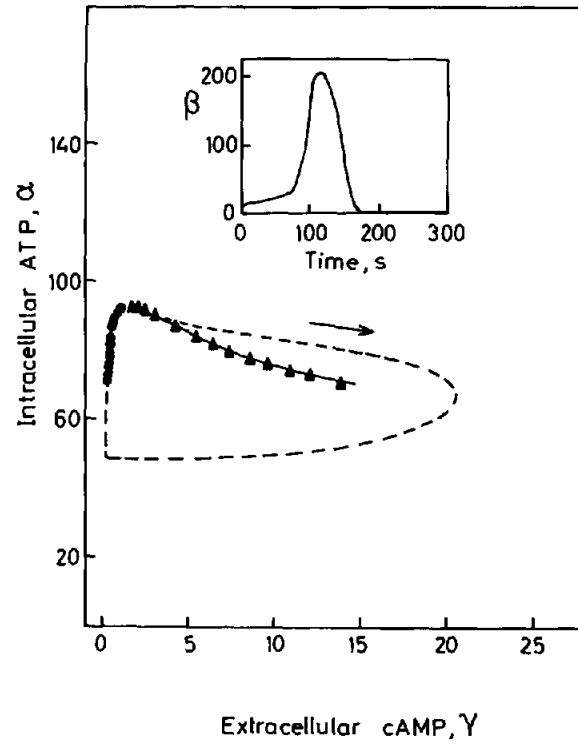

Fig.2. Excitability in system (1). Following a suprathreshold stimulation by extracellular cAMP, the system undergoes a large excursion (dashed line) before returning to the steady state. This projected trajectory corresponds to a pulsatory synthesis of intracellular cAMP, β (inset). Initial conditions are $\alpha = 92.366$, $\beta = 10$, $\gamma = 2$. To obtain the threshold locus (line joining the triangles), we took as initial conditions the steady states (dots) obtained upon decreasing ν and determined, for each of them, the value of γ to be exceeded for excitation. Parameter values are as in fig.1.

(the 'threshold separatrix' [11]): all trajectories initiated to the left of this curve return immediately to the corresponding steady state, whereas trajectories that begin to the right of the curve first undergo the large excursion associated with excitable behaviour. Dose–response curves that link the amplitude of relay to the magnitude of the external signal exhibit a sharp threshold when the steady state lies in the vicinity of maximum A (see fig.5 in [10]). The all-or-none response becomes graded when the steady state lies further apart from A; the triangles in fig.2 then represent quasi-threshold [11] values given by the

inflection points of the dose–response curves.

Besides accounting for the existence of a threshold for relay [19], the phase-plane analysis provides an explanation for the fact that *D. discoideum* cells are refractory, during a period which lasts several minutes, after relay of a cAMP signal [17–19]. Here, we should draw the distinction, as in the study of the nerve membrane [11], between an absolute and a relative refractory period. The model predicts that during the synthesis of the pulse the system is unable to respond to further stimulation and is in a state of absolute refractoriness. After this phase, the system can be excited by sufficiently large cAMP pulses; the threshold for these signals decreases and the amplitude of relay increases as the system approaches the steady state.

Until now, we have considered a system where excitation is elicited by a pulse of extracellular cAMP. We can replace such a signal by a continuous input of extracellular cAMP, which has been shown to interfere with the oscillatory system [26,27]. A dose–response curve similar to that found for pulsatory signals is obtained (see fig.3): excitation occurs only when the constant source of external cAMP exceeds a threshold. This result is in agreement with [28] where the existence of a threshold for relay was shown, using both a pulsatory and a continuous cAMP signal. The value of the threshold predicted by fig.3 for a constant cAMP input is $10^{-2}K_p\ s^{-1}$, i.e., $10^{-9}\,M\,s^{-1}$ when taking for the dissociation constant of the cAMP receptor the upper value [10] $K_p = 10^{-7}$ M; larger values are found when the steady state is not as close to the oscillatory domain. In fig.2, the threshold for relay in response to a cAMP pulse ranges from $2–10\ K_p$, i.e., from $2.10^{-7}–1.10^{-6}$ M. These values compare well with the data obtained in fields of *D. discoideum* amoebae on agar [28], and could be checked in cell suspensions.

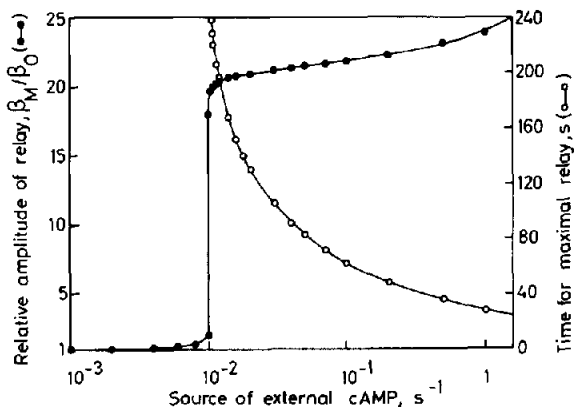

Fig.3. Dependence of relay on the magnitude of a constant signal of extracellular cAMP. The amplification factor is given as the maximum of the intracellular cAMP peak, β_M, divided by the steady state level β_0. The second curve shows the time at which β_M is reached after beginning of stimulation. The curves are obtained for the parameter values of fig.1, by integration of eq. (1) with a constant source term, divided by K_p, inserted in the evolution equation for γ.

The above model for relay and oscillation of cAMP in *D. discoideum* will have to be modified in some measure as further information accumulates on the different steps of the signal mechanism. To a large extent, however, the present analysis is independent of the details of the activation process: in a two-variable phase plane, the activation of adenylate cyclase by extracellular cAMP, direct or indirect, should result in a sigmoid nullcline for an intracellular metabolite (ATP in the present case) as a function of extracellular cAMP; such an S-shaped sigmoid is a prerequisite for the obtention of sustained oscillations and excitability for closely related values of the parameters. An analogous phase plane behaviour has been obtained in a qualitative study of the cAMP signalling system based on a different regulation of cAMP synthesis [29]. A similar mechanism of excitability also holds in a model for the phosphofructokinase reaction [30]. As the models for phosphofructokinase and adenylate cyclase are of similar nature, indirect support for the mechanism of relay of cAMP signals could come from the demonstration, in glycolyzing yeast or muscle extracts, of an amplification of ADP pulses in conditions close to those that produce glycolytic oscillations [30].

The preceding results on excitability apply to the adenylate cyclase reaction in *D. discoideum*. Do they apply to other hormone-activated adenylate cyclases? This might be the case, and could provide a means of amplifying small extracellular hormone signals by translating them into a large pulse of intracellular cAMP. The clear reason, however, for which the phenomenon occurs in *D. discoideum* is that cAMP itself acts there as the extracellular hormone which activates adenylate cyclase. This autocatalytic loop is

responsible, as in many other chemical systems [31,32], for excitable and oscillatory behaviour.

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