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Birhythmicity in a model for the cyclic AMP signalling system of the slime mold *Dictyostelium discoideum*

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We demonstrate the coexistence of two simultaneously stable periodic regimes in a model based on receptor desensitization for the cyclic AMP signalling system of the slime mold *Dictyostelium discoideum*. These results provide the first example of autonomous birhythmicity in a biochemical model closely related to experimental observations. Whereas the transition from one stable mode of oscillations to the other can be elicited by suprathreshold stimuli, the two periodic regimes differ in their sensitivity to perturbations. That multiple oscillations occur in a model based on a single feedback loop suggests that the conditions for birhythmicity are widely satisfied in biological systems.

Biochemical oscillation *Birhythmicity* *Bistability* *Cyclic AMP signalling* Dictyostelium

1. INTRODUCTION

The property of birhythmicity confers on an oscillatory system the capability of operating in either one of 2 simultaneously stable periodic regimes [1,2]. This mode of dynamic behavior is the rhythmic counterpart of bistability [3] in which 2 stable steady states coexist under the same conditions, as observed experimentally in a number of biochemical systems [4–6]. Birhythmicity was first analyzed in biochemical models not directly based on experimental observations [1,2,7,8], and later observed in chemical oscillatory reactions [9–11]. The phenomenon has also been demonstrated in models of periodically driven oscillatory systems such as yeast glycolysis [12,13].

Autonomous (or endogenous) birhythmicity, i.e. the coexistence of 2 stable oscillations with different amplitudes and frequencies in the absence of periodic perturbation, has not yet been found experimentally in biological systems nor reported for biochemical models based on experiments. We demonstrate here the occurrence of autonomous birhythmicity in a model for the cyclic AMP (cAMP) signalling system of the slime mold *Dictyostelium discoideum*.

The present results show that the kinetic prerequisites for autonomous birhythmicity are not too stringent, so that the phenomenon may be of rather common occurrence in regulated biological systems. Our analysis indicates, however, that constraints on the experimental observation of birhythmic behavior may arise from the smallness of the appropriate parameter domain and from the fact that the 2 periodic regimes differ in their resistance toward perturbations. The latter difference markedly affects the propensity of the system to switch from one particular mode of oscillation to the other upon chemical perturbation.

2. MODEL FOR THE cAMP SIGNALLING SYSTEM BASED ON RECEPTOR MODIFICATION

After starvation, *D. discoideum* amoebae aggregate by a chemotactic response to cAMP signals emitted with a periodicity of several minutes by aggregation centers; the multicellular aggregates later develop into fruiting bodies (reviews [14,15]). The oscillatory synthesis of cAMP has also been observed in cell suspensions [16]. Besides glycolytic

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oscillations in yeast and muscle, cAMP oscillations in *Dictyostelium* represent the best-known example of biochemical periodicity at the cellular level [17,18].

The periodicity of cAMP signal generation originates from the autocatalytic regulation of cAMP synthesis in *D. discoideum* (see fig.1). Extracellular cAMP binds to a cell surface receptor and thereby activates adenylate cyclase. The cAMP synthesized is transported across the cell membrane into the extracellular medium where it is hydrolyzed by phosphodiesterase [14,15]. Evidence has recently accumulated showing that the cAMP receptor is modified covalently, probably through phosphorylation-dephosphorylation [19–21]. As in bacterial chemotaxis where receptor methylation is linked to sensory adaptation [22,23], modification of the cAMP receptor appears to be associated with adaptation of cAMP secretion in response to cAMP stimuli [19,20].

As an extension of a previous work [24], we have recently analyzed a model for the cAMP signalling system, taking into account both receptor modification and the positive feedback exerted by extracellular cAMP on adenylate cyclase via binding to the cAMP receptor [8,25]. This model, illustrated in fig.1, is governed by a set of 7 kinetic equations. It has been shown to account for oscillations and relay of cAMP signals, as well as for adaptation of cAMP secretion in response to constant cAMP stimuli [8,25]. The model also predicts the occurrence of bursting and of aperiodic oscillations of cAMP [26]. The latter phenomenon, commonly referred to as chaos, could account for the aperiodic signalling properties of the *D. discoideum* mutant Fr17 [26–28].

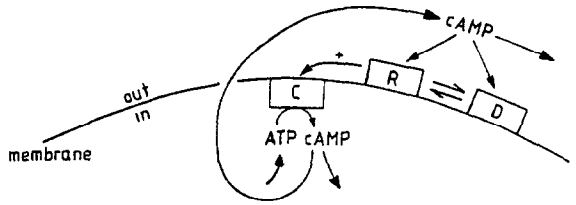

Fig.1. Model based on receptor modification for the cAMP signalling system of *D. discoideum*. Binding of extracellular cAMP to the active form of the receptor (R) elicits activation of adenylate cyclase (C). Arrows indicate synthesis of ATP, transport of cAMP into the extracellular medium and cAMP hydrolysis by the intracellular and extracellular forms of phosphodiesterase. Decrease in cAMP synthesis follows from the passage of the receptor into a desensitized state (D) uncoupled from adenylate cyclase [8,25,26].

In view of the difficulty of handling the complete system of 7 kinetic equations we have obtained, by means of a series of quasi-steady-state hypotheses, the following reduced 3-variable system which retains all qualitative properties of the original equations (J.L. Martiel and A. Goldbeter, in preparation):

$$d\alpha/dt = \nu - \sigma\phi(\alpha,\varrho_T,\gamma)$$

$$d\varrho_T/dt = -\varrho_T\mu(\gamma) + (1-\varrho_T)\eta(\gamma)$$

$$d\gamma/dt = q'\sigma\phi(\alpha,\varrho_T,\gamma) - k_e\gamma \qquad (1)$$

where $q' = qk_t/h(k_i + k_t)$,

$$\mu(\gamma) = (k_1 + k_2\gamma^2)/(1+\gamma^2),$$

$$\eta(\gamma) = (k_1L_1 + k_2L_2c^2\gamma^2)/(1 + c^2\gamma^2),$$

$$\phi(\alpha,\varrho_T,\gamma) = \frac{\alpha[\lambda\theta + \epsilon\varrho_T\gamma^2/(1+\gamma^2)]}{(1+\alpha\theta) + [\epsilon\varrho_T\gamma^2/(1+\gamma^2)](1+\alpha)}.$$

In these equations, α denotes the ATP concentration divided by the K_m for adenylate cyclase; ϱ_T denotes the total fraction of receptor in the active state R; γ is the extracellular cAMP concentration divided by the dissociation constant for the R state of the receptor (see [8,25] for further details and definition of the parameters).

All the modes of dynamic behavior observed in the complete 7-variable model for the signalling system [8,25,26] have been recovered by numerical integration of the reduced system (eqn 1). Conversely, the results on birhythmicity reported below, which were obtained by integration of eqns 1, are also obtained with the complete set of equations.

An alternative model for the signalling system has been proposed [29,30], based on the putative control of adenylate cyclase by Ca^{2+}. This model, in contrast to the present one, does not rely on receptor modification.

3. BIRHYTHMICITY: COEXISTENCE OF TWO STABLE PERIODIC REGIMES

For parameter values close to those that produce a single regime of bursting or aperiodic oscilla-

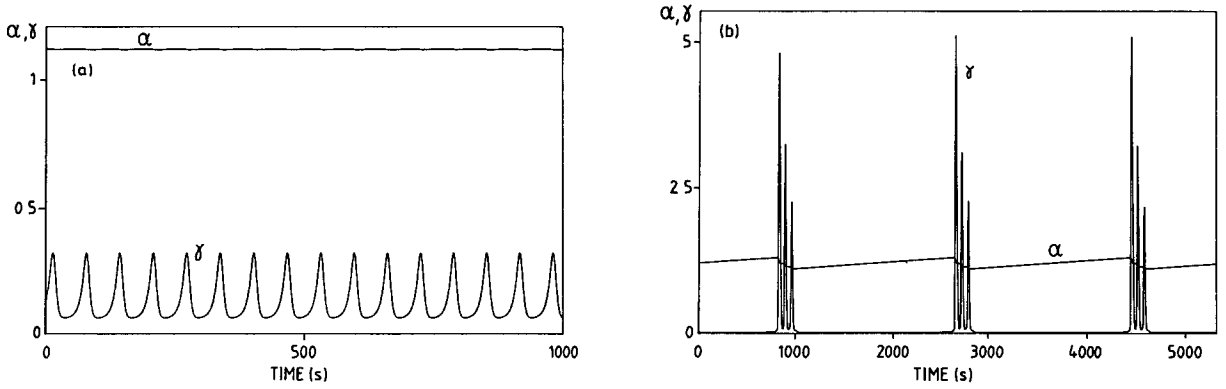

Fig.2. Birhythmicity. Depending on initial conditions, the cAMP signalling system evolves into either one of 2 stable periodic regimes, for the same set of parameter values: $\lambda = \theta = 10^{-2}$, $\sigma = 0.1\ s^{-1}$, $c = 10^2$, $L_1 = k_{-1}/k_1 = 85.507$, $L_2 = k_{-2}/k_2 = 8.551 \times 10^{-3}$, $k_1 = 0.15\ s^{-1}$, $k_2 = 0.06\ s^{-1}$, $\epsilon = 0.2$, $h = 5$, $q = 4 \times 10^3$, $k_e = 0.35\ s^{-1}$, $k_i = 0.6\ s^{-1}$, $k_t = 0.4\ s^{-1}$, $v = 1.4125 \times 10^{-4}\ s^{-1}$. The curves are obtained by numerical integration of eqns 1 for 2 different sets of initial conditions.

tions, eqns 1 give rise to birhythmicity: for the same set of parameter values, 2 stable modes of sustained oscillations coexist (fig.2). The evolution towards either one of these 2 oscillatory regimes solely depends on initial conditions. The first periodic regime (fig.2a) corresponds to a very slight variation in the substrate ATP (α) accompanied by small-amplitude oscillations of cAMP (γ). The second regime (fig.2b) corresponds to a more significant variation in α and to large-amplitude bursting oscillations in γ. The oscillatory regimes of fig.2a and b further differ in the period which is close to 1 and 30 min, respectively.

In the phase space formed by the concentrations of the 3 variables (fig.3), the 'folded' limit cycle [31] corresponding to the bursting oscillations of fig.2b can readily be compared with the smaller limit cycle associated with the oscillations of fig.2a. The 2 stable limit cycles are separated by 2 unstable cycles (not shown).

The passage from one periodic regime to the other can be elicited upon suprathreshold perturbation in any of the 3 variables, at the appropriate phase. Thus switching from the small-amplitude to the large-amplitude oscillations is shown to occur in fig.4 upon addition of a suprathreshold cAMP pulse. To achieve the reverse transition by means of a similar perturbation, it is necessary to choose a point on the large-amplitude limit cycle which is

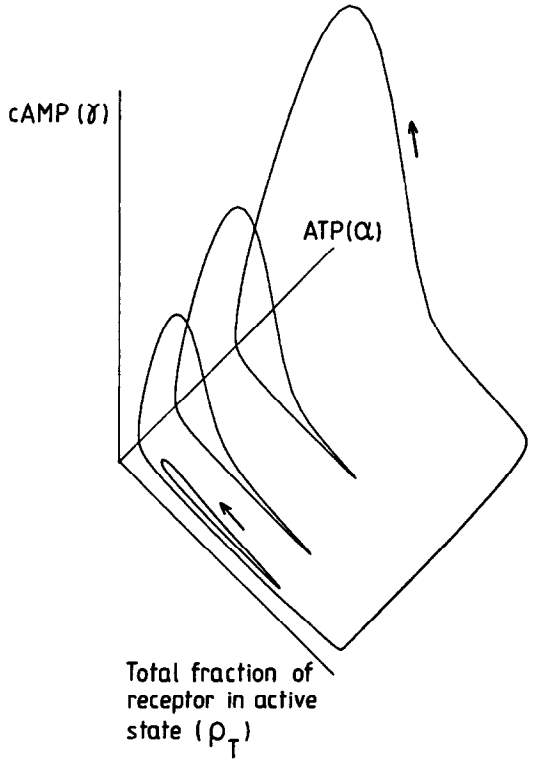

Fig.3. Phase space representation of birhythmicity. The 2 stable periodic trajectories corresponding to the oscillations of fig.2a,b are shown in the space formed by the concentrations of the 3 variables. The range of variation is 1.1–1.3 for α, 0–1 for ρ_T, and 0–5 for γ.

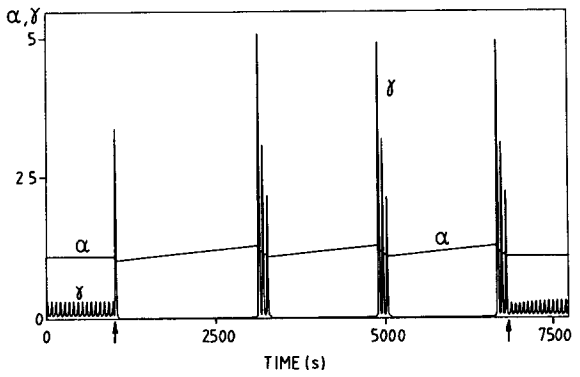

Fig.4. Reversible transition between 2 simultaneously stable periodic regimes. The signalling system oscillating in the small-amplitude periodic regime is perturbed (first arrow) at time t = 1000 s (where ϱ_T = 0.167, α = 1.113) by an instantaneous increase in γ from 0.318 to 0.418, and thereafter switches to the large-amplitude oscillatory regime. The threshold for inducing the transition to the latter regime is γ = 0.4096 in this point, which corresponds to a 30% increase in the instantaneous value of γ. The large-amplitude oscillations are perturbed (second arrow) at time t = 6800 s (where ϱ_T = 0.774 and α = 1.111) by increasing γ from 0.048 to 0.058, this stimulation bringing the system back to the small-amplitude oscillations. The range of addition of γ eliciting the switch in this point extends from 0.056 to 0.062; this corresponds to a 15–30% increase in the instantaneous value of γ.

as close as possible to the smaller limit cycle; this occurs just after completion of the last burst in γ in fig.2b. Then, a suprathreshold addition of cAMP brings about the transition to the latter regime (fig.4). However, this perturbation has to be finely tuned as there exists a second, higher threshold above which cAMP perturbation of the larger limit cycle keeps the system in the attraction basin of this periodic regime (see legend to fig.4).

4. DISCUSSION

We have obtained evidence for multiple oscillations in the form of birhythmicity in a model based on receptor modification for the cAMP signalling system of *Dictyostelium* cells. Previous analyses of the coexistence between 2 simultaneously stable periodic regimes in biochemical systems were carried out in models not directly related to experiments [1,2,7,8,32]. The present results provide the first example of autonomous birhythmicity in a realistic biochemical model closely related to experimental observations.

How do multiple oscillations arise in the model of fig.1? Birhythmicity as well as chaos is favored by the interplay between 2 oscillatory mechanisms, as shown by the analysis of a model for 2 autocatalytic enzyme reactions coupled in series [1,2]. Similarly, in the case of the periodic forcing of an oscillatory system, multiple oscillations and chaos can be viewed as originating from the coupling of an exogenous to an endogenous periodicity [12,13]. Here, birhythmicity originates from the interplay between 2 endogenous oscillatory mechanisms coupled in parallel in the cAMP signalling system. Both mechanisms share the same positive feedback loop exerted by extracellular cAMP but differ in the process limiting cAMP autocatalysis (see fig.1). Limitation is brought about alternatively by substrate depletion or receptor desensitization as the receptor progressively passes into the inactive state upon incubation with extracellular cAMP. As for aperiodic oscillations [8,26], the interplay between the 2 oscillatory mechanisms gives rise to birhythmicity when both limiting effects acquire comparable importance. A unique regime of periodic oscillations occurs whenever one of these effects predominates; a single oscillatory mechanism is then active whereas the other remains 'silent'.

The present results indicate that birhythmicity does not require a multiplicity of instability-generating feedback processes. The fact that the phenomenon occurs in a realistic biochemical model based on a single positive feedback and on a process as ubiquitous as receptor desensitization suggests that the conditions for birhythmicity are likely satisfied at the different levels of biological organization, e.g. in neuronal dynamics.

Birhythmicity could be of physiological significance in enhancing the sensitivity of an oscillatory system that becomes capable of switching back and forth between different periodic regimes upon appropriate stimulation. Experimental evidence for birhythmicity in *D. discoideum* as well as in other biological systems is presently lacking. Theoretical analyses such as the present one define the conditions for the occurrence of birhythmicity and should thereby facilitate an experimental search for the phenomenon. Such a search may not be easy; in-

deed, the present model, previous theoretical results [1,7,8], as well as experiments in chemical systems [9–11], all indicate that birhythmicity generally occurs in a parameter domain much smaller than that corresponding to a unique regime of oscillations.

Theoretical studies point to another aspect that bears on the experimental search for birhythmicity. In a 2-variable model analyzed for the phenomenon [7], phase plane analysis indicated a marked asymmetry in the sensitivity of the 2 oscillatory regimes toward perturbations. Here also it appears (see fig.4) that the switching from a low-amplitude to a large-amplitude oscillatory regime takes place rather easily and occurs once the perturbation exceeds a threshold, at the appropriate phase. Switching from the large-amplitude to the small-amplitude oscillatory regime requires much finer tuning both with respect to the magnitude of the perturbation and to the phase at which it is applied.

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