A MODEL BASED ON RECEPTOR DESENSITIZATION FOR CYCLIC AMP SIGNALING IN *DICTYOSTELIUM* CELLS

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ABSTRACT We analyze a model based on receptor modification for the cAMP signaling system that controls aggregation of the slime mold *Dictyostelium discoideum* after starvation. The model takes into account both the desensitization of the cAMP receptor by reversible phosphorylation and the activation of adenylate cyclase that follows binding of extracellular cAMP to the unmodified receptor. The dynamics of the signaling system is studied in terms of three variables, namely, intracellular and extracellular cAMP, and the fraction of receptor in active state. Using parameter values collected from experimental studies on cAMP signaling and receptor phosphorylation, we show that the model accounts qualitatively and, in a large measure, quantitatively for the various modes of dynamic behavior observed in the experiments: (*a*) autonomous oscillations of cAMP, (*b*) relay of suprathreshold cAMP pulses, i.e., excitability, characterized by both an absolute and a relative refractory period, and (*c*) adaptation to constant cAMP stimuli. A two-variable version of the model is used to demonstrate the link between excitability and oscillations by phase plane analysis. The response of the model to repetitive stimulation allows comprehension, in terms of receptor desensitization, of the role of periodic signaling in *Dictyostelium* and, more generally, the function of pulsatile patterns of hormone secretion.

1. INTRODUCTION

Among the best understood of biological rhythms are metabolic periodicities due to oscillatory enzyme reactions (Hess and Boiteux, 1971; Goldbeter and Caplan, 1976; Berridge and Rapp, 1979). Several examples of such biochemical oscillations are known, but the one with the clearest physiological significance is that of the periodic synthesis of pulses of cyclic AMP (cAMP) in the slime mold *Dictyostelium discoideum* (Gerisch and Wick, 1975). These pulses, emitted with a period close to 10 min, control chemotaxis and differentiation during the interphase that leads to aggregation of the amebae after starvation (Bonner, 1967; Konijn et al., 1967; Alcantara and Monk, 1974; Loomis, 1975, 1982; Newell, 1977; Darmon and Brachet, 1978; Devreotes, 1982; Gerisch, 1982). Recent observations suggest that periodic cAMP pulses also play a role in the subsequent formation of a multicellular fruiting body in this and other slime mold species (Schaap and Wang, 1986).

In support of observations on agar (Shaffer, 1962; Gerisch, 1968), studies in cell suspensions show that the signaling system is capable of two types of dynamic behavior, namely, autonomous oscillations of cAMP (Gerisch and Hess, 1974; Gerisch and Wick, 1975; Gerisch et al., 1979) and relay of suprathreshold cAMP pulses, i.e., synthesis of a pulse of cAMP in response to cAMP stimulation of sufficient magnitude (Roos et al., 1975; Shaffer, 1975).

Previous analysis of an allosteric model proposed for the cAMP signaling system by Goldbeter and Segel (1977, 1980) showed that relay and oscillations represent two modes of dynamic behavior which result from the autocatalytic regulation of adenylate cyclase, the latter enzyme being activated upon binding of extracellular cAMP to a cell surface receptor (Roos and Gerisch, 1976; Gerisch and Malchow, 1976). The phenomenon of relay is closely associated with autonomous oscillations and reflects the excitability of the adenylate cyclase reaction in *Dictyostelium* (Goldbeter et al., 1978). A similar link between excitable and oscillatory behavior has been found in experimental and theoretical studies of the nerve membrane (Fitzhugh, 1961) and in experiments on chemical oscillatory reactions (Winfree, 1972; De Kepper, 1976).

For most parameter values, both the oscillations and the relay response were associated in the Goldbeter-Segel model with a significant variation in the substrate ATP. Although ATP compartmentation near the adenylate cyclase reaction site cannot be excluded, such a dependence on ATP variation appears to be ruled out by experiments that show that intracellular ATP remains practically constant during cAMP oscillations (Roos et al., 1977), and that adaptation of the relay response to constant stimuli occurs in the absence of ATP consumption when the cAMP receptor is uncoupled from adenylate cyclase upon incubation with caffeine (Theibert and Devreotes, 1983).

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These observations led us to consider an alternative mechanism for relay and oscillations of cAMP that is not based on the assumption that adenylate cyclase is an allosteric enzyme and does not require any significant variation in ATP. The mechanism rests on desensitization of the cAMP receptor upon binding of extracellular cAMP (Goldbeter and Martiel, 1980, 1985*a*; Martiel and Goldbeter, 1984).

In the classical model of Katz and Thesleff (1957), the phenomenon of desensitization, which accounts for a decrease in responsiveness upon prolonged stimulation, is explained by the transition of the receptor into a high-affinity state less or not coupled to the physiological response. Such a mechanism is exemplified by the acetylcholine receptor for which multiple conformational states possessing different affinities for the ligand have indeed been observed (Changeux, 1981). The receptor states may, however, differ not only by their conformation but also by their degree of covalent modification. A large number of receptors that play a role in the sensing of external stimuli are known to be covalently modified. Thus phosphorylation of the β-adrenergic receptor in turkey erythrocytes is associated with a decrease in adenylate cyclase activity (Sibley et al., 1984). In bacteria, stimulation by chemical attractants causes smooth swimming; adaptation of this response to constant stimuli is brought about by methylation of the chemoreceptors (Springer et al., 1979; Koshland, 1979).

Experimental evidence for covalent modification of the cAMP receptor has recently been obtained in *D. discoideum* by Devreotes and Sherring (1985) and Klein et al. (1985) who showed that the cAMP receptor undergoes a reversible phosphorylation upon stimulation by cAMP. As in bacterial chemotaxis, such modification appears to play a role in the adaptation of the signaling response to constant stimuli (Theibert and Devreotes, 1983). Moreover, cAMP oscillations are accompanied by a periodic alternance between the phosphorylated and unphosphorylated states of the cAMP receptor (Klein et al., 1985*a, b*).

We have previously reported the results of some numerical simulations of a model based on receptor modification and on the self-amplification properties of the cAMP signaling system in *D. discoideum* (Martiel and Goldbeter, 1984; Goldbeter et al., 1984; Goldbeter and Martiel, 1985*a*). The time evolution of the model in these studies was governed by a set of seven differential equations. Autonomous oscillations, relay of cAMP pulses, and adaptation to constant stimuli were demonstrated by computer simulations. More complex phenomena such as aperiodic oscillations (chaos), bursting, and the coexistence between two stable periodic regimes (birhythmicity) were later found (Goldbeter and Martiel, 1985*b;* Martiel and Goldbeter, 1985, 1987).

Here, we extend these preliminary, qualitative results and provide a detailed analysis of a three-variable version of the model for cAMP signaling based on receptor modification. Using experimentally available values for the model parameters, we compare theoretical results with numerous observations made under different experimental conditions. We indicate how a unified view of relay and oscillations can be gained from phase plane analysis when further reducing the model to only two variables. We also predict the behavior of the signaling system under repetitive stimulation and use the results to suggest an explanation for the function of periodic signaling in intercellular communication.

In Section 2, we present the model and show how to reduce the complete set of kinetic equations to a three-variable system governing the time evolution of intracellular and extracellular cAMP and the fraction of receptor in active state. Section 3 is devoted to cAMP oscillations and to the relay of cAMP pulses. Relay of suprathreshold cAMP pulses occurs for parameter values close to those that produce oscillations. We show how the existence of an absolute and a relative refractory period for relay correlates with receptor modification. The link between relay and oscillation in a two-variable version of the model is demonstrated by phase plane analysis.

The behavior of the signaling system under constant stimulation is analyzed in Section 4. In these conditions, the model accounts for a number of experimental results such as adaptation to step increases in extracellular cAMP (Devreotes and Steck, 1979; Dinauer et al., 1980*b, c*), the absence of a sharp threshold for relay (Devreotes and Steck, 1979) which contrasts with the threshold observed for stimulation by cAMP pulses, and the continuous activation of cAMP synthesis by successive step increases in extracellular cAMP (Devreotes and Steck, 1979). Finally, in Section 5, we determine the response of the model to square-wave stimulation. The results show that as in the case of pulsatile patterns of hormone secretion, the effectiveness of periodic signaling can be comprehended in terms of receptor desensitization.

2. MODEL AND KINETIC EQUATIONS

2.1. Description of the Model

The model based on receptor modification for the cAMP signaling system in *D. discoideum* is schematized in Fig. 1 and corresponds to the following sequence of reaction steps:

$$(a)\quad \mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}$$

$$(b)\quad \mathrm{R} + \mathrm{P} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{RP}$$

$$(c)\quad \mathrm{D} + \mathrm{P} \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{DP}$$

$$(d)\quad \mathrm{RP} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{DP}$$

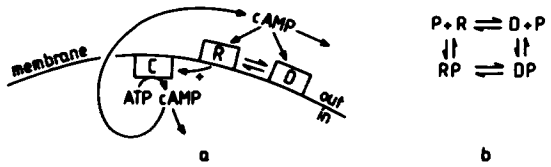

FIGURE 1 Model based on receptor desensitization for the cAMP signaling system of the slime mold *Dictyostelium discoideum*. Extracellular cAMP binds to the active state (R) of the receptor and thereby activates adenylate cyclase (C) which produces cAMP from intracellular ATP. The transition from the active to the desensitized state (D) of the receptor may in principle occur through a simple conformational change or though covalent modification. The latter situation appears to prevail in *D. discoideum* where the R and D states correspond to the dephosphorylated and phosphorylated forms of the cAMP receptor. Arrows indicate transport of cAMP into the extracellular medium and cAMP hydrolysis by the intracellular and extracellular forms of phosphodiesterase. *b* shows interconversions between the R and D states in the presence of extracellular cAMP (P).

$$(e)\quad 2RP + C \underset{d_3}{\overset{a_3}{\rightleftharpoons}} E$$

$$(f)\quad E + S \underset{d_4}{\overset{a_4}{\rightleftharpoons}} ES \xrightarrow{k_4} E + P_i$$

$$(g)\quad C + S \underset{d_5}{\overset{a_5}{\rightleftharpoons}} CS \xrightarrow{k_5} C + P_i$$

$$(h)\quad P_i \xrightarrow{k_i}$$

$$(i)\quad P_i \xrightarrow{k_t} P \xrightarrow{k_e}$$

$$(j)\quad \xrightarrow{v_i} S \xrightarrow{k'}. \qquad (1)$$

We assume that the monomeric receptor, located on the outer face of the plasma membrane, exists in two conformational states, R and D. Extracellular cAMP (P) binds to these two conformations with different affinities (steps *b* and *c*). The transitions between the two states (steps *a* and *d*) may represent a simple conformational change, or a process of covalent modification; in the latter case, we assume that the modifying enzymes operate in the first-order kinetic domain. Experimental evidence in *D. discoideum* suggests that these transitions are associated with desensitization through phosphorylation of the cAMP receptor (Devreotes and Sherring, 1985; Klein et al., 1985).

We make the hypothesis that upon diffusion in the membrane, two molecules of active complex RP bind to adenylate cyclase (step *e*) and thereby activate the enzyme. Whether such a process occurs in *Dictyostelium* remains a conjecture; however, receptor dimerization required for activation of the cellular response has been demonstrated for the gonadotropin-releasing hormone receptor (Conn et al., 1982; Blum and Conn, 1982; Blum, 1985). The reason for considering such cooperative process is that some form of nonlinearity is essential for oscillations (see, e.g., Nicolis and Prigogine, 1977). The cooperativity of step *e* replaces here the one previously located at the level of cAMP binding to a dimeric receptor (Martiel and Goldbeter, 1984). Although positive cooperativity in binding is suggested by some experiments (Coukell, 1981), it is not apparent in the observations made with constant stimuli (Devreotes and Steck, 1979). Both types of cooperativity yield similar results.

We consider a global step *e* without including a more detailed mechanism for adenylate cyclase activation by the cAMP–receptor complex. This process likely involves a GTP-binding protein as in the activation of adenylate cyclase in higher organisms (Van Haastert, 1984; Janssens et al., 1986; Ludérus et al., 1986). The nonlinearity of step *e* may thus be taken as reflecting also the amplification of the activation process due to interactions between the GTP-binding protein and adenylate cyclase.

In view of the results obtained for the β-adrenergic receptor (Sibley et al., 1984), we assume that the modified form DP of the receptor cannot couple to the cyclase, and therefore represents an inactive, desensitized state. Such a hypothesis holds with suggestions based on experimental observations (Lubs-Haukeness and Klein, 1982; Devreotes and Sherring, 1985; Klein et al., 1985). To account for the enhancement in cAMP synthesis that follows binding of extracellular cAMP, we consider that the receptor–adenylate cyclase complex E (step *f*) has a larger affinity for the substrate ATP (S) and/or a larger catalytic activity than the free form C of the enzyme (step *g*).

Part of intracellular cAMP (P_i) is hydrolyzed by an intracellular phosphodiesterase (step *h*), whereas part is transported across the plasma membrane into the extracellular medium where it is hydrolyzed by the membrane-bound and extracellular forms of this enzyme (step *i*). In agreement with the experiments (Dinauer et al., 1980*a*), these steps are each governed by an apparent first-order rate constant. A final assumption is that the substrate ATP is synthesized at a constant rate and used in a number of reactions besides that catalyzed by adenylate cyclase; a global rate constant k' characterizes the latter ATP consuming processes. The assumption of a constant rate of utilization in these processes would yield similar results (the ATP level will, in fact, be taken as constant after reduction of the model to a three-variable system).

Not included in the present model is the possible effect of calcium ions on the signaling system. Although calcium can phase-shift the oscillations (Malchow et al., 1982), available data suggest that it is not needed for signal transduction to adenylate cyclase (Saito, 1979) and primarily affects the cGMP response that governs chemotaxis (Europe-Finner and Newell, 1984). Actin polymerization, required for the latter response, appears to be regulated by Ca ions liberated from intracellular stores, after the production of inositol 1,4,5-trisphosphate upon cAMP binding

to the receptor (Europe-Finner and Newell, 1986). A mechanism based on the putative inhibition of adenylate cyclase by intracellular calcium ions has recently been proposed for the signaling system, and applied to adaptation to constant stimuli (Rapp et al., 1985; Othmer et al., 1985). This model does not take into account the observed phenomenon of receptor modification, which plays here a central role in the dynamics of the signaling system. That calcium is not considered explicitly in the present model does not preclude its possible role in some of the processes listed in reaction sequence.

2.2. Reduction of a Three-Variable System

Eleven variables appear in reaction steps 1. Due to conservation relations for the receptor and for adenylate cyclase, the number of independent concentration variables reduces, however, to nine. Under spatially homogeneous conditions, which correspond to the experiments in continuously stirred suspensions of *D. discoideum* cells (Gerisch and Wick, 1975; Roos et al., 1975), the temporal evolution of the signaling system is thus governed by a set of nine ordinary differential equations listed as Eqs. A1 in Appendix A.

To simplify this system of nine differential equations, we consider all binding reactions *b, c, e–g* as much more rapid than the slow transitions between the unmodified and modified receptor states in steps *a* and *d*. Due to the simultaneous presence of fast and slow terms in some of the Eqs. A1, the obtainment of the quasi-steady-state equations is not straightforward (see Segel et al., 1986, for the analysis of a similar situation). In Appendix A we show how the quasi-steady-state regime of Eqs. A1 leads first to the following four-variable system in which ATP is still a variable:

$$\frac{d\rho_T}{dt} = -f_1(\gamma)\rho_T + f_2(\gamma)(1 - \rho_T)$$

$$\frac{d\alpha}{dt} = \upsilon - k'\alpha - \sigma\Phi(\rho_T, \gamma, \alpha)$$

$$\frac{d\beta}{dt} = q\sigma\Phi(\rho_T, \gamma, \alpha) - (k_i + k_t)\beta$$

$$\frac{d\gamma}{dt} = (k_t\beta/h) - k_e\gamma \qquad (2)$$

with

$$f_1(\gamma) = \frac{k_1 + k_2\gamma}{1 + \gamma}; \quad f_2(\gamma) = \frac{k_1L_1 + k_2L_2c\gamma}{1 + c\gamma}$$

$$\Phi(\rho_T, \gamma, \alpha) = \frac{\alpha(\lambda\theta + \epsilon Y^2)}{1 + \alpha\theta + \epsilon Y^2(1 + \alpha)}; \quad Y = \frac{\rho_T\gamma}{1 + \gamma}.$$

In the above equations, ρ_T denotes the total fraction of receptor in the active state; β and γ denote the intracellular and extracellular concentrations of cAMP divided by the dissociation constant K_R; α is the intracellular ATP concentration divided by the Michaelis constant K_m (see Table II).

We have kept in Eqs. 2 the level of ATP as a variable so as not to impose ATP constancy a priori, in order to determine whether the absence of significant variation in ATP observed in the experiments (Roos et al., 1977) can be accounted for by the model. Numerical simulations based on Eqs. 2 indicate that this is indeed the case (see the time course of variable α in Fig. 2 *a* below). This property allows us to further reduce the number of variables by considering α as a parameter in Eqs. 2. The dynamics of the cAMP signaling system is then governed by the three differential Eqs. 3, where the various functions and parameters remain defined as in Eqs. 2.

$$\frac{d\rho_T}{dt} = -f_1(\gamma)\rho_T + f_2(\gamma)(1 - \rho_T)$$

$$\frac{d\beta}{dt} = q\sigma\Phi(\rho_T, \gamma, \alpha) - (k_i + k_t)\beta$$

$$\frac{d\gamma}{dt} = (k_t\beta/h) - k_e\gamma. \qquad (3)$$

Eqs. 3 can be further reduced to a two-variable system for large values of q, k_t, and k_i. Then a quasi-steady-state hypothesis for β can indeed be made. The advantage of a two-variable system is that the link between relay and oscillations can be demonstrated by phase plane analysis. As the experimental values available for k_t and k_i, relative to q, are too low for such reduction to hold rigorously, we shall use the three-variable system governed by Eqs. 3 for studying the dynamics of the signaling system. Similar results are obtained, however, in the two-variable system, as discussed in Section 3.4 below.

3. AUTONOMOUS OSCILLATIONS AND RELAY OF cAMP PULSES

The synthesis of cAMP in *D. discoideum* possesses an autocatalytic nature as the production of intracellular cAMP is enhanced upon binding of extracellular cAMP to the receptor. This autocatalysis would give rise to a runaway process, were it not for the limiting effect imposed by receptor desensitization. The combination of these regulatory properties gives rise to the two most conspicuous modes of behavior of the signaling system, namely, autonomous oscillations and relay of suprathreshold cAMP pulses.

3.1. Autonomous Oscillations of cAMP

Linear stability analysis shows that, owing to the existence of a positive feedback loop in the synthesis of cAMP (Fig. 1), the steady state admitted by Eqs. 2 may become unstable under appropriate conditions, i.e., in a certain

range of parameter values (see below). Then the signaling system undergoes sustained oscillations around the unstable steady state (Fig. 2). These oscillations have been obtained by numerical integration of the four kinetic Eqs. 2, for parameter values close to those determined in the experiments. The range of values obtained experimentally for the parameters is given in Table I. Parameter values used for the numerical simulations of oscillatory behavior are listed in Table II.

Shown as a function of time in Fig. 2 *a* are the concentrations of intracellular and extracellular cAMP (on the same scale), the fraction of receptor in active state, ρ_T, and the concentration of ATP. The variation of extracellular cAMP is shown on an enlarged scale in Fig. 2 *b*, together with the fraction of receptor in the inactive state, δ_T, and the saturation function of the receptor, $\bar{Y}$, measuring total

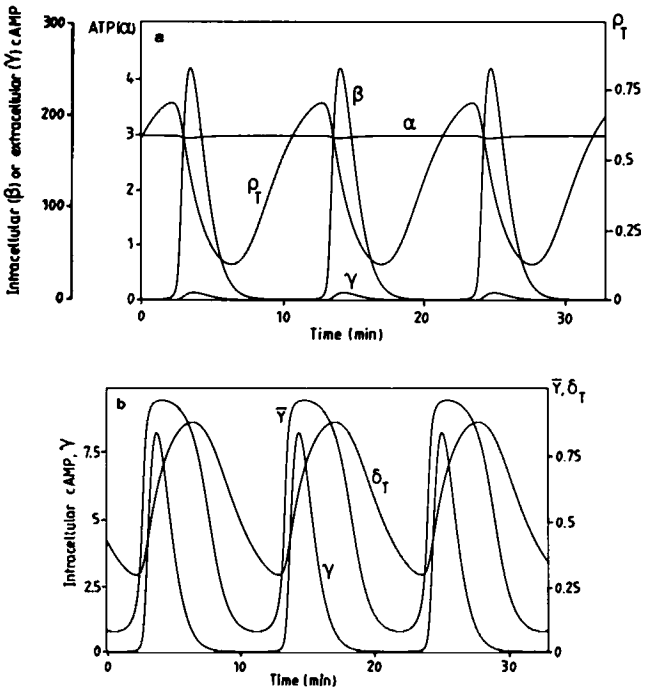

FIGURE 2 Autonomous oscillations of cAMP. Shown as a function of time in *a* are the normalized concentrations of intracellular (β) and extracellular (γ) cAMP, as well as the normalized concentration of ATP (α) and the total fraction of receptor in active state, ρ_T. The actual concentrations of cAMP and ATP can be obtained by multiplying the normalized concentrations by $K_R = 10^{-7}$ M and $K_m = 4 \times 10^{-4}$ M, respectively (see Table II). In b are shown the concomitant changes in the receptor saturation function $\bar{Y}$ (Eq. 5), and the total fraction of receptor in the desensitized (i.e., modified) state, δ_T; the concentration of extracellular cAMP is plotted again on an enlarged scale. The curves are obtained by numerical integration of the four-variable system governed by Eqs. 2. Similar results are obtained by integration of the three-variable system governed by Eqs. 3 with $\alpha = 3$, owing to the fact that α remains practically constant around this value in *a* (see Fig. 3 *a*). Parameter values are given in Table II, together with their experimentally determined range based on the data of Table I. Parameters v and k' are taken equal to 12 and 4 min^{-1}, respectively; these values correspond to cellular turnover rates for ATP of the order of 5 mM/min which are in the physiological range (see, e.g., Goldbeter and Koshland, 1987).

binding of cAMP to the R and D states:

$$\bar{Y} = \rho_T \frac{\gamma}{1+\gamma} + (1-\rho_T)\frac{c\gamma}{1+c\gamma}. \qquad (4)$$

The maximum in the fraction of unmodified, active receptor precedes the maximum in cAMP, whereas the maximum in the fraction of modified receptor follows the cAMP peak. Such periodic variation of the active and desensitized states of the receptor corresponds to the periodic alternance between the dephosphorylated and phosphorylated forms of the cAMP receptor in the course of oscillations in *D. discoideum* (Klein et al., 1985*a, b*). The phase relationships predicted by the model for receptor modification and cAMP synthesis agree with those observed in the experiments.

The relation between total cAMP binding and free extracellular cAMP in the course of oscillations is amenable to experimental observation. In the model, this relation strongly depends on parameter c, i.e., on the relative affinities of the two receptor states towards cAMP. Whereas the synthesis of cAMP results from the activation of adenylate cyclase through binding to the complex RP, $\bar{Y}(t)$ reflects binding of cAMP to both R and D forms of the receptor. If $c > 1$, as in Figs. 2 and 3, the decline in cAMP is followed only after a time lag by the decline in binding, since the decrease in autocatalysis is concomitant with the occupation of the high-affinity D state by cAMP. No time lag occurs when the active receptor state has the largest affinity, i.e., when $c < 1$.

A conspicuous feature of the oscillations in Fig. 2 is that ATP remains buffered around a mean value close to v/k'. Therefore ATP will from now on be considered as a parameter fixed at this value. The oscillations shown in Fig. 2 remain almost unchanged when the three-variable system 3 is integrated at fixed ATP concentration instead of the four-variable system 2 (see Fig. 3 *a*).

The agreement between model and experiments as to periodic behavior is qualitative and, in a large measure, quantitative. The oscillations obtained in the model for the parameter values taken from the literature match the observed oscillations for both the period, close to 10 min, and the amplitude: intracellular cAMP varies between 1 and 20 μM, extracellular cAMP closely follows intracellular cAMP and oscillates in a range smaller by one order of magnitude, whereas ATP remains practically constant around 1 mM (Gerisch and Wick, 1975; Roos et al., 1977).

The simulations of Fig. 2, as well as the subsequent ones below, have been performed with the value $K_m = 4 \times 10^{-4}$ M for adenylate cyclase (see Tables I and II). Given that the ATP concentration is of the order of 1.2 mM (Roos et al., 1977), we have set α, the ATP concentration divided by K_m, equal to 3 when keeping the ATP level constant. A smaller value of K_m, close to 20 μM, has also been reported (de Gunzburg et al., 1980). To obtain the ATP level of 1.2

TABLE I
EXPERIMENTAL RANGE OF PARAMETER VALUES

Parameter	Definition	Experimental range	Reference
K_R	Dissociation constant of cAMP–receptor complex in R state*	10^{-7} M	Klein (1969); Mullens and Newell (1978)
		3.6×10^{-8} M	Henderson (1975)
		10^{-8} M to 1.4×10^{-7} M	Coukell (1981)
		1.5×10^{-9} M	Devreotes and Sherring (1985)
K_D	Dissociation constant of cAMP–receptor complex in D state*	9×10^{-9} M	Mullens and Newell (1978)
		3×10^{-9} M to 9×10^{-9} M	Klein (1979)
		3×10^{-9} M	Devreotes and Sherring (1985)
k_1	Rate constant for modification step R → D	0.012 min^{-1}	Devreotes and Sherring (1985)
k_{-1}	Rate constant for demodification step D → R	0.104 min^{-1}	Devreotes and Sherring (1985)
k_2	Rate constant for modification step RP → DP	0.222 min^{-1}	Devreotes and Sherring (1985)
k_{-2}	Rate constant for demodification step DP → RP	0.055 min^{-1}	Devreotes and Sherring (1985)
cAMP receptor		10^5–10^6 molecules/cell	Gerisch and Malchow (1976)
Adenylate cyclase		2×10^4 molecules/cell	Gerisch and Malchow (1976)
Cell density		10^7 to 2×10^8 cells/ml	Gerisch and Malchow (1976)
Intracellular volume		10^{-12} liter	Gerisch and Malchow (1976)
		7.5×10^{-13} liter	Europe-Finner and Newell (1984)
Activity of adenylate cyclase	Basal rate	5.7×10^{-7} M/min	Loomis (1979)
	At maximum activation	2.3×10^{-5} M/min	Gerisch and Malchow (1976)
$K_{m,cyclase}$	Michaelis constant of adenylate cyclase	0.4 mM	Gerisch and Malchow (1976)
		0.2–0.5 mM	Klein (1976)
		17 μM and 0.4 mM	de Gunzburg et al. (1980)
k_i	Apparent first-order rate constant for intracellular phosphodiesterase	1.73 min^{-1}	Dinauer et al. (1980*a*)
$V_{m,PDE}$	Maximum activity for extracellular phosphodiesterase	10^{-5} M/min	Klein and Darmon (1976)
		5×10^{-5} M/min	Yeh et al. (1978)
$K_{m,PDE}$	Michaelis constant of extracellular phosphodiesterase for cAMP	4×10^{-6} M	Gerisch and Malchow (1976)
k_t	Apparent first-order rate constant for cAMP transport into extracellular medium	0.34–0.94 min^{-1}	Dinauer et al. (1980*a*)
ATP		1–1.5 mM	Gerisch and Malchow (1976) Roos et al. (1977)

*Some K_R or K_D values might pertain to cAMP receptors not related to adenylate cyclase. Values measured by Van Haastert (1984) in kinetic binding experiments on a time scale of seconds likely pertain to the formation of distinct receptor complexes during the phase of activation of adenylate cyclase, rather than to the later phase of adaptation mediated by receptor modification.

mM, the value of α should then be of the order of 60. However, as pointed out by de Gunzburg et al. (1980), compartmentation of ATP in eukaryotes may be such that ATP levels near the adenylate cyclase reaction site can be much lower than the millimolar range. Thus the value $\alpha = 3$ could still hold in these conditions. If the larger value of K_m and a lower ATP level are considered, the value of α would drop below unity. Therefore $\alpha = 3$ represents an intermediary value for the normalized ATP concentration.

A comparison of Tables I and II shows that we have used experimentally available values for all parameters except k_1, k_{-1}, k_2, k_{-2} for which we took values somewhat different from those determined experimentally by Devreotes and Sherring (1985) for the kinetics of phosphorylation and dephosphorylation of the cAMP receptor in *D. discoideum*. We have multiplied the experimental values of k_1, k_{-1}, and k_2 by a factor of 3, as the period of the oscillations would otherwise be three times larger than the value of 9 min observed in the experiments (Gerisch and Wick, 1975). Numerical simulations indicate that the period of oscillations is highly sensitive to the kinetic parameters that govern receptor modification. Given that the peak in cAMP synthesis is relatively brief (see Fig. 2), most of the period is devoted to the slower phases of modification and demodification of the receptor. As indicated by Eqs. 2 and 3, a decrease in k_1 and k_2 by a given factor, at constant L_1 and L_2, will produce a slowing down of receptor evolution and, hence, an increase in the period of oscillations by a roughly similar factor.

TABLE II
PARAMETER VALUES CONSIDERED IN NUMERICAL SIMULATIONS

Parameter	Definition or expression in terms of the parameters of scheme 1	Experimental range (see Table I)	Numerical value considered
K_R	d_1/a_1	1.5×10^{-9} to 1.4×10^{-7} M	10^{-7} M
K_D	d_2/a_2	3×10^{-9} to 9×10^{-9} M	10^{-8} M*
c	K_R/K_D	0.15–50	10
k_1		0.012 min^{-1}	0.036 min^{-1}
k_{-1}		0.104 min^{-1}	0.36 min^{-1}
k_2		0.222 min^{-1}	0.666 min^{-1}
k_{-2}		0.055 min^{-1}	0.00333 min^{-1}
L_1	k_{-1}/k_1	8.67	10
L_2	k_{-2}/k_2	0.25	0.005
K_m	$K_{m,cyclase} = (d_4 + k_4)/a_4$	17 μM to 0.5 mM	0.4 mM
q	K_m/K_R	120–3.3×10^5	4×10^3
σ	$V_{m,cyclase}/K_m = k_4 E_T/K_m$	0.05–1.35 min^{-1}	0.6 min^{-1}
k_i		1.73 min^{-1}	1.7 min^{-1}
k_e	$V_{m,PDE}/K_{m,PDE}$‡	2.5–12.5 min^{-1}	5.4 min^{-1}
k_t		0.34–0.94 min^{-1}	0.9 min^{-1}
α	ATP/K_m	2.4–70	3
h	Ratio of extracellular to intracellular volumes	5–100§	5
E_T	Total intracellular concentration of adenylate cyclase	3×10^{-8} M‖	3×10^{-8} M
R_T	Total extracellular concentration of cAMP receptor	1.5×10^{-9} to 3×10^{-7} M§	3×10^{-8} M
μ	E_T/R_T	0.1–10	1
η	R_T/K_R	0.02–200	0.3
θ_E	E_T/K_m	2×10^{-3} to 6×10^{-5}	Neglected
θ	K_m/K'_m (ratio of Michaelis constants for E and C forms of adenylate cyclase)	Not available	0.01
λ	k_5/k_4 (ratio of catalytic constants of forms C and E of adenylate cyclase)	Not available	0.01
ϵ	$R_T^2(a_3/d_3)$; coupling constant for activation of C by 2RP (step e)	Not available	1

*For $c \neq 10$, $K_D = 10^{-7}$ M/c.
‡This parameter includes the effect of the membrane-bound and extracellular forms of phosphodiesterase, which both act on extracellular cAMP.
§For a cell density ranging from 2×10^8 to 10^7 cells/ml.
‖Established for an intracellular volume of 10^{-12} liter.

3.2. Role of Receptor Desensitization in the Mechanism of cAMP Oscillations

The dynamics of oscillations based on receptor desensitization can be described as follows. Initially (see Fig. 2), the level of extracellular cAMP is minimum and the R state of the receptor predominates as the ratio R/D, equal to L_1 in the absence of ligand, is larger than unity. Upon binding of cAMP to the R state, the active cAMP–receptor complex RP forms and activates adenylate cyclase. This leads to an increase in intracellular and extracellular cAMP. Above a threshold level reached in the conditions of excitability and oscillations, the production of cAMP increases autocatalytically. As extracellular cAMP rises, however, the distribution between the active complex RP and the inactive complex DP is progressively shifted towards the latter (since $L_2 < 1$); this produces a decrease in enzyme activation. The level of cAMP then begins to decrease, owing to this progressive uncoupling and to cAMP hydrolysis by phosphodiesterase. At low external levels of cAMP the two complexes RP and DP dissociate and regenerate the free receptor molecules. A period of the oscillations is completed when the initial ratio of R vs. D is reestablished.

When desensitization occurs through covalent modification of the receptor, the passage into the inactive state is primarily caused by the ligand-induced transition from RP into DP. To characterize quantitatively the role of receptor modification in the dynamics of oscillations, it is useful to introduce the net modification fluxes ϕ_1 and ϕ_2 defined by Eqs. 5:

$$\phi_1 = -\rho_T \frac{k_1}{1+\gamma} + (1-\rho_T)\frac{k_1 L_1}{1+c\gamma}$$

$$\phi_2 = -\rho_T \frac{k_2\gamma}{1+\gamma} + (1-\rho_T)\frac{k_2 L_2 c\gamma}{1+c\gamma} \qquad (5)$$

with $d\rho_T/dt = \phi_1 + \phi_2$. The quantity ϕ_1 measures the net flux between the total pools of active and inactive receptor that occurs via the transition between R and D; the

quantity ϕ_2 measures the corresponding flux through the transition between RP and DP. Positive (negative) values of ϕ_1 and ϕ_2 indicate, respectively, that the net fluxes via the R $\rightleftharpoons$ D and RP $\rightleftharpoons$ DP transitions increase the pool of active (desensitized) receptor.

Oscillations based on ligand-induced receptor modification are exemplified in Fig. 3 *a*. Here, the large negative variation of ϕ_2, which accompanies the decrease in cAMP after a peak, shows that this decline is brought about by the ligand-induced modification of RP into DP. The subsequent variation in ϕ_1, which remains positive throughout a period, corresponds to the return from the desensitized to the active state through the transition from D to R.

This mechanism operates with $L_1 > 1$ (the R state predominates in the absence of ligand) and $L_2 < 1$ (the state DP predominates at ligand saturation). Moreover, the rate of receptor modification increases in the presence of ligand, i.e., $k_2 > k_1$. As indicated in Table I, such conditions hold for phosphorylation of the cAMP receptor in *D. discoideum* (Devreotes and Sherring, 1985). Oscillations may occur in these conditions for negligible values of k_1 and k_{-2} but require a finite value for k_{-1}. Indeed, the receptor must be capable of returning in finite time from D to R. An important property of this mechanism is that it produces oscillations for values of c either above or below unity.

Besides ligand-induced receptor modification, there exists a different mechanism capable of generating oscillations in the model. This second mechanism is based on conformational transitions in the absence of covalent modification. Then the microscopic reversibility condition $L_1 = L_2c$ applies in the cycle formed by the reactions between R, D, RP, and DP (see Fig. 1 *b*). This particular microscopic reversibility condition does not apply when the transitions from R to D and RP to DP occur via covalent modification; indeed, the direct and reverse steps of each reaction are then catalyzed by distinct enzymes operating with different cofactors (see further discussion of this issue in Segel et al., 1986).

Oscillations occur for purely conformational transitions only when the receptor possesses a high-affinity desensitized state, i.e., $c \gg 1$, with $L_1 > 1$ and, hence, $L_2 < 1$. As shown in Fig. 3 *b*, the decrease in cAMP after a peak is then brought about primarily by the pumping of the receptor into the high-affinity, inactive complex DP via the transition from R to D. This process corresponds to the

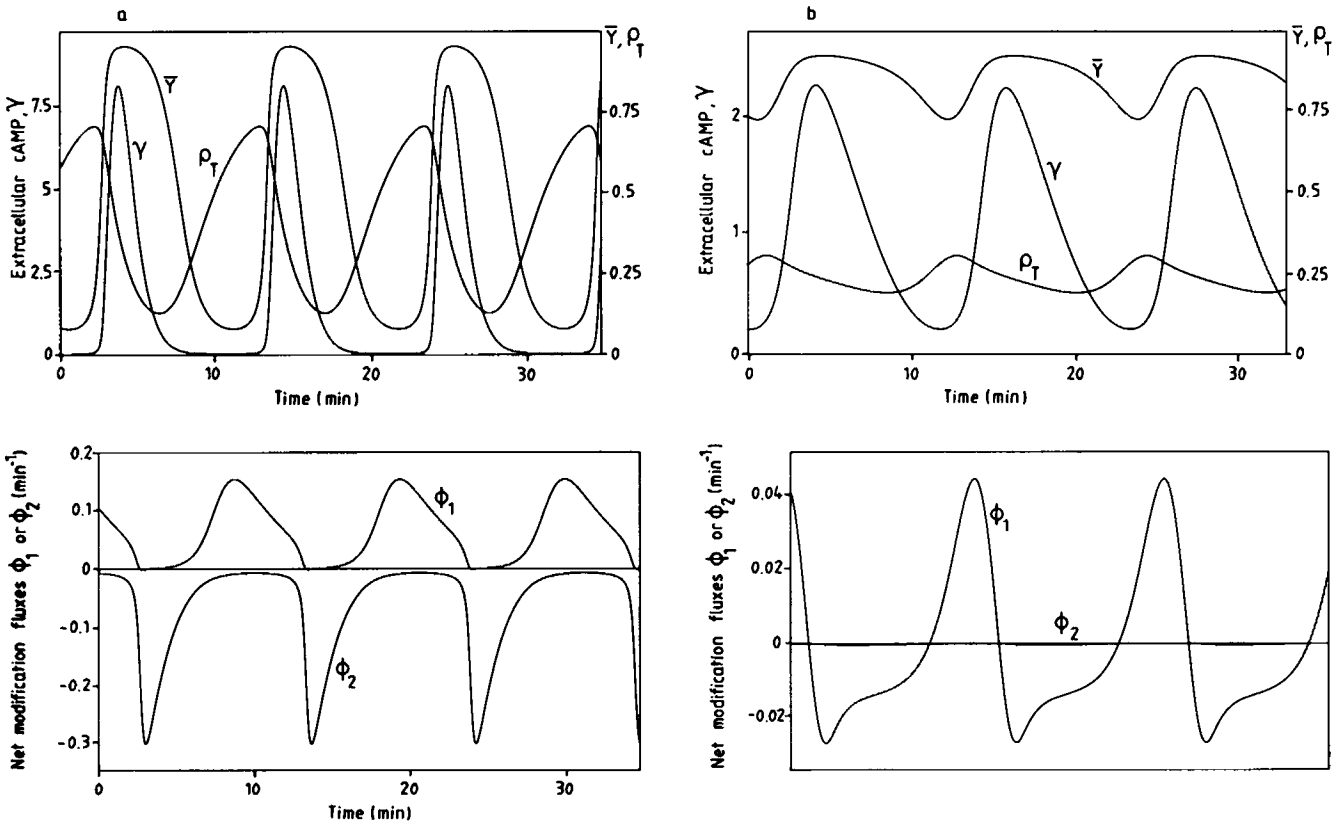

FIGURE 3 Evidence for two distinct oscillatory mechanisms based on receptor desensitization. In *a*, the transition from the active to the inactive state of the receptor primarily occurs through ligand-induced, covalent modification. In *b*, interconversion between the receptor states occurs by simple conformational changes in the absence of covalent modification. Shown in each case (*lower panels*) are the net modification fluxes ϕ_1 and ϕ_2 through the R $\rightleftarrows$ D and RP $\rightleftarrows$ DP transitions. The accompanying oscillations in extracellular cAMP (γ), in the receptor saturation function $\bar{Y}$, and in the total fraction of active receptor (ρ_T) are shown in the upper panels. The curves are obtained by integration of Eqs. 3 with $\alpha = 3$; Φ_1 and Φ_2 are determined according to Eqs. 5. Parameter values in *a* are as in Fig. 2; in *b*, $c = 100$, $L_2 = (L_1/c) = 0.1$, $k_1 = 0.4$ min^{-1}, $k_2 = 0.004$ min^{-1}.

sharp drop of ϕ_1 to negative values in Fig. 3 *b*; the later shift to positive values of ϕ_1 corresponds to the return of D to R at low cAMP levels. This mechanism can operate with negligible values of k_2 and k_{-2}, i.e., in the absence of ligand-induced modification, as shown by the reduced variation of ϕ_2 in this case.

As previously indicated, it is the mechanism of ligand-induced receptor modification that obtains for periodic cAMP signaling in *D. discoideum*. That such a mechanism is less dependent on the relative affinities of the R and D states for the ligand than the mechanism based on conformational transitions is confirmed by the stability diagrams established as a function of L_1 and L_2 for different values of c. These diagrams (Fig. 4) show that oscillations based on covalent modification can occur at large values of L_1 for values of c extending from below to above unity. Large values of c nevertheless favor oscillations as they correspond to a larger domain of instability in parameter space (Fig. 4 *a*).

In the nonphysiological conditions $L_1 < 1$, $L_2 > 1$, a coexistence between two stable steady states is observed in the model (Fig. 4). In these two states, the predominant form of the receptor is either D or RP, and the level of cAMP is, respectively, low and large. Such bistability has not been observed so far in the experiments.

3.3. Relay of Suprathreshold cAMP Pulses: Excitability

When the system admits a single, stable steady state, it always returns to this state after addition of a pulse of extracellular cAMP. The return to the asymptotic state is generally immediate, regardless of the magnitude of the perturbation. However, in a parameter domain contiguous to that of sustained oscillations, the qualitative behavior of the system becomes highly sensitive to the amplitude of the cAMP pulse. In such a situation, the system is excitable as it amplifies in a pulsatory manner suprathreshold cAMP pertubations. The latter perturbations correspond to the instantaneous addition of a given amount of extracellular cAMP in the experiments on relay in cell suspensions (Roos et al., 1975). The pulsatory increase in cAMP is accompanied by a transient decrease in the amount of active receptor (see response to first stimulus in Fig. 5).

The existence of a threshold for excitation is illustrated by the dose-response curve of Fig. 6 where the maximum of the peak of intracellular cAMP synthesized in response to a pulsatory stimulus is plotted as a function of magnitude of stimulation. Also indicated for comparison in Fig. 6 is the initial value of the saturation function $\bar{Y}$ of the cAMP receptor. The latter curve possesses no threshold and simply reflects Michaelian binding to the two states of the monomeric receptor. As indicated in Section 4 below, the sharpness of the threshold for relay of cAMP pulses is not due to the assumption of nonlinear coupling between the active receptor and the cyclase. Rather, this threshold reflects the excitability of the signaling system. This property accounts for the relay of suprathreshold cAMP pulses that takes place during *D. discoideum* aggregation (Shaffer, 1962; Robertson and Drage, 1975).

The threshold for relay of cAMP pulses predicted by Fig. 6 is close to 9×10^{-9} M, i.e., well below the value of

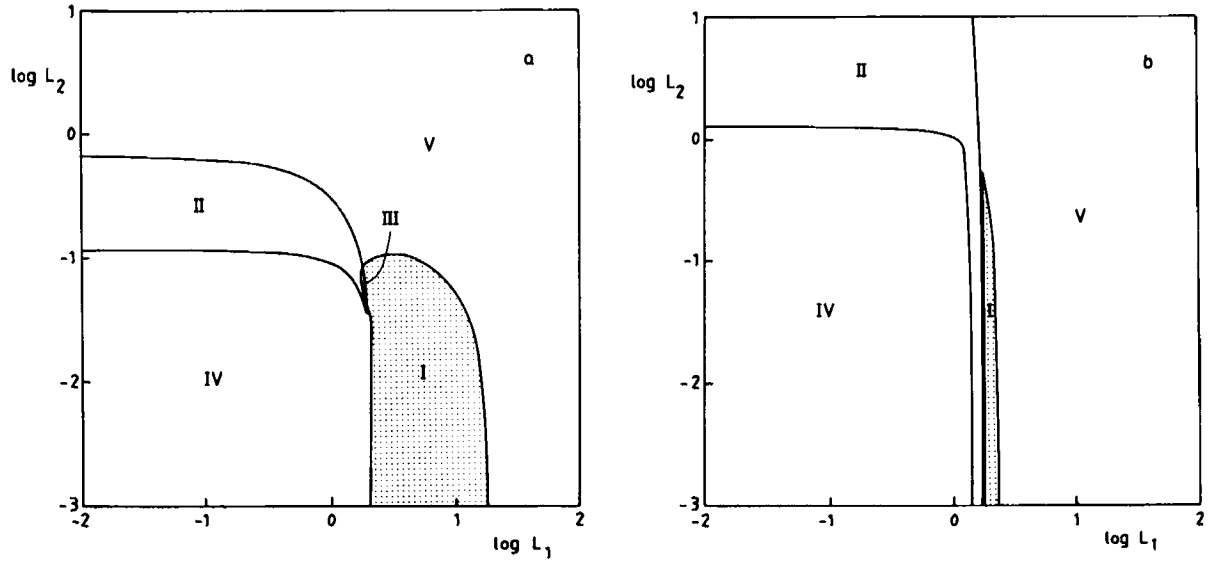

FIGURE 4 Stability diagrams showing the various behavioral domains as a function of L_1 and L_2, for $c = 10$ (*a*) and $c = 0.1$ (*b*). Other parameter values are as in Fig. 2. The dotted region (I) corresponds to sustained oscillations around a unique, unstable steady state. Region II corresponds to the coexistence between two stable steady states in which cAMP is either at a low or a high level. In the small domain (III), three steady states exist, only one of which is stable. In these conditions, a stable limit cycle around a higher cAMP level may coexist with a stable steady state in which the cAMP level is low (hard excitation). Outside these domains, a unique, stable steady state exists in which the level of cAMP is either low (IV) or large (V). The various domains are determined by numerical simulations and linear stability analysis around the steady-state solution(s) admitted by Eqs. 3.

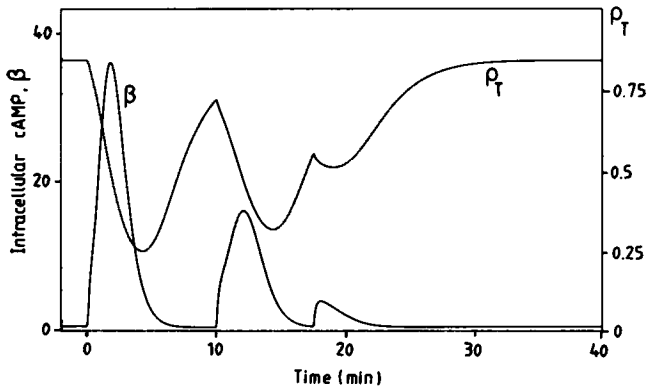

FIGURE 5 Relay response to cAMP pulses. The signaling system is stimulated at time zero by a perturbation increasing γ by 0.3 unit (this corresponds to the addition of a pulse of 3×10^{-8} M of extracellular cAMP, for $K_R = 10^{-7}$ M). 10 min after the first pulse, the system is perturbed by a second stimulus of the same magnitude. The smaller amplitude of the second peak in intracellular cAMP, β, is due to the fact that the fraction of active receptor, ρ_T, has not reached a sufficiently high value on its return to the steady state at the time of the second stimulation. A third stimulus of similar magnitude, delivered 7.5 min after the second pulse, elicits an even smaller response. The curves are obtained by integration of Eqs. 3 for the parameter values of Fig. 3 *a*, with $\sigma = 0.57$ min^{-1}, $k_e = 3.58$ min^{-1}, $k_i = 0.958$ min^{-1}, $\epsilon = 0.108$.

10^{-7} M taken for the dissociation constant of the cAMP–receptor complex (Table I). The former value is in good agreement with the stimulation threshold determined experimentally for the relay response, which is comprised between $2 \cdot 10^{-9}$ and 10^{-8} M (Grutsch and Robertson, 1978).

A second feature of the relay response to suprathreshold pulses of cAMP that is accounted for by the present analysis is the existence of a refractory period in the response to successive stimuli (Durston, 1974*a*; Robertson and Drage, 1975). A numerical simulation of the response to successive cAMP pulses of similar magnitude is illustrated in Fig. 5. When the response to the first stimulus has subsided, a second pulse elicits a smaller response because the fraction of active receptor ρ_T has not returned to its steady-state level. A third stimulus delivered after a shorter time interval elicits an even smaller response.

Let us denote by β_{m1} and β_{m2} the peak concentrations of cAMP reached in response to the first and second stimuli. Fig. 7 shows how the ratio β_{m2}/β_{m1} varies as a function of the time interval separating the second stimulus from the peak of the first response. The curve shows the existence of an absolute refractory period (ARP) of some 4 min in the case considered; during this period no second response can be elicited. Thereafter, during a relative refractory period (RRP), which extends over several minutes, the second response is measurable but smaller than the first; half-maximum recovery occurs some 5 min after the end of the absolute refractory period. When a sufficient time has elapsed before the second stimulus is applied, the ratio β_{m2}/β_{m1} returns to unity. The predicted value of the absolute refractory period for relay agrees with that observed in the experiments, which varies from 7 to 2 min in the course of aggregation on agar (Durston, 1974*a*).

To understand the molecular basis of the absolute and relative refractory periods, we have redrawn in Fig. 7 the time evolution of the fraction of active receptor, ρ_T, after a single stimulation. The curve starts at the value of ρ_T corresponding to the peak of the first response in cAMP in Fig. 5. The comparison of the evolution of ρ_T and β_{m2}/β_{m1}

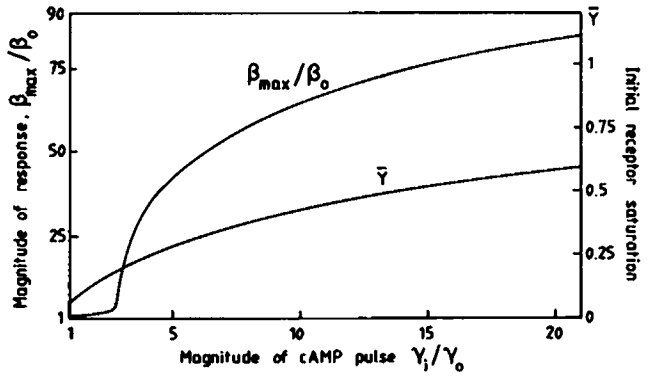

FIGURE 6. Threshold for relay of cAMP pulses. Shown is the amplitude of the relay response, measured by the maximum of the peak in intracellular cAMP (β_m) divided by the steady-state level (β_0), as a function of the magnitude of the cAMP pulse measured by the ratio of initial (γ_i) to steady-state (γ_0) extracellular cAMP. Also indicated is the initial value of the receptor saturation function, $\bar{Y}$ (Eq. 4). The curve for the relay response is obtained by integration of Eqs. 3 for the parameter values of Fig. 5; the steady-state level of extracellular cAMP is then $\gamma_0 = 0.0284$. The threshold for relay, close to $\gamma_i = 3\gamma_0$, corresponds to a pulse magnitude slightly below 9×10^{-9} M when taking the value $K_R = 10^{-7}$ M for the dissociation constant of the RP complex (see Table II).

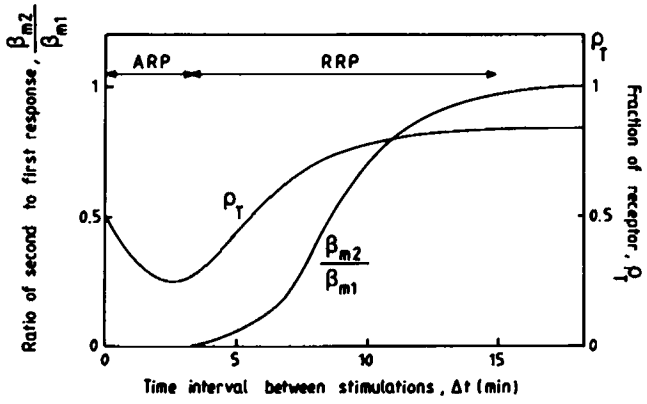

FIGURE 7 Refractoriness in the relay of cAMP pulses. The ratio β_{m2}/β_{m1} of the amplitude of the response to two successive cAMP pulses of similar magnitude (see Fig. 5) is shown as a function of the time interval separating the second stimulation from the peak of the first response. An absolute refractory period (ARP) of 3.5 min is followed by a somewhat longer relative refractory period (RRP) during which the magnitude of the second response progressively increases up to that of the first response. Correlation of this behavior with the recovery from receptor desensitization is indicated by the curve showing the evolution of the fraction of receptor in active state, ρ_T, after the first cAMP pulse, in the absence of the second stimulation. The curves start at the peak of the first response, in the conditions of Fig. 5.

shows that the absolute refractory period corresponds to the time during which the level of active receptor decreases. The relative refractory period corresponds to the progressive return of the receptor into the active state. The second response becomes identical to the first when the level of active receptor has reached its steady-state value. Furthermore, as observed for other excitable systems, the threshold for excitation in the relative refractory period decreases progressively as the system approaches the steady state.

3.4. Relay and Oscillations in a Two-Variable System

For sufficiently large values of k_t, k_i, and q, a quasi-steady-state assumption for the fast variable β can be made in system 3, so that the kinetic equation for β reduces to the algebraic relation

$$\beta = (q\sigma\Phi)/(k_i + k_t). \tag{6}$$

The signaling system is then governed by the following set of two kinetic equations for the total fraction of active receptor and the extracellular cAMP concentration:

$$\frac{d\rho_T}{dt} = -f_1(\gamma)\rho_T + f_2(\gamma)(1 - \rho_T)$$

$$\frac{d\gamma}{dt} = q'\sigma\Phi(\rho_T, \gamma) - k_e\gamma, \tag{7}$$

with $q' = qk_t/h(k_i + k_t)$. This two-variable system can be viewed as the core of the signaling mechanism, given that it also possesses the properties of excitable and oscillatory behavior.

The reduction to the two-variable system 7 is not justified on analytical grounds when one uses the experimental values listed for q, k_t, and k_i in Table I. Indeed, although $q \gg 1$, k_t and k_i are only of the order of 1. Therefore, most numerical simulations in the preceding and following sections were conducted with Eqs. 3. The numerical integration of Eqs. 7 nevertheless shows that the two-variable reduction provides a reasonably good approximation of the three-variable system governed by Eqs. 3.

For the parameter values used in Figs. 2 and 3 *a*, we represent by solid lines in Fig. 8 the time course of ρ_T and γ obtained by integration of Eqs. 7. Represented in dashed lines are the corresponding curves obtained when using Eqs. 3. It appears that the reduction to a two-variable system, although not mathematically rigorous for the parameter values considered, yields not too severe discrepancies with respect to the actual behavior of the three-variable system. The period, close to 10.7 min in the latter, decreases to 7.5 min, whereas the maximum amplitude of γ passes from 7.8 to 10.3. Thus, in the situation of Fig. 8, the error on the period and amplitude with the reduced system is of the order of 25–30%.

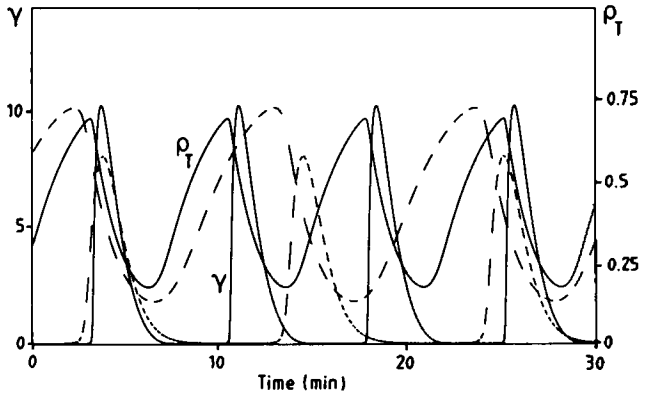

FIGURE 8 Comparison of oscillatory behavior in the two-variable system (7) (*solid lines*) and in the three-variable system (3) (*dashed lines*). The curves, obtained for the parameter values of Fig. 3 *a*, are drawn so that the first maximum in γ occurs at the same time in the two systems.

Besides its greater simplicity, the two-variable system is particularly useful for gaining insight from phase plane analysis into the link between relay and oscillations. The phase portrait corresponding to the oscillations of Fig. 8 is shown in Fig. 9. Drawn are the two nullclines of system 7; their intersection in the phase plane (ρ_T, γ) defines the steady state which, in these conditions, is unstable. The system then evolves to a stable limit cycle enclosing the unstable steady state. In the inset are shown the limit cycles obtained in the two- and three-variable versions of the model. The comparison of the two curves indicates that the only significant discrepancy in the form of the trajectory is a larger maximum in γ in the two-variable system.

The steady state is unstable when it lies in a region of sufficiently negative slope $d\rho_T/d\gamma$ on the sigmoidal nullcline $\dot{\gamma} = 0$. When located in a region of positive slope on this nullcline, just to the left of the oscillatory domain, the

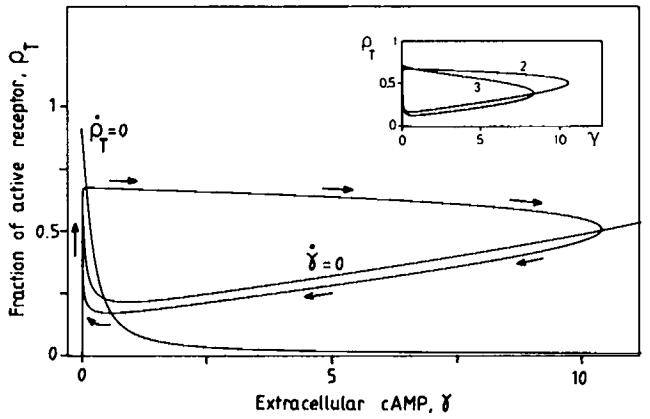

FIGURE 9 Phase plane dynamics associated with autonomous oscillations of cAMP. The closed curve represents the limit cycle reached in the two-variable system (ρ_T,γ) governed by Eqs. 7. Parameter values are as in Fig. 8. The nullclines $\dot{\rho}_T = 0$ and $\dot{\gamma} = 0$ are also indicated; they intersect at the unstable steady state. (*Inset*) Comparison of the limit cycle obtained in the two-variable system (7) with that obtained by projection in the (ρ_T,γ) plane in the three-variable system (3).

steady state is stable but excitable (Fig. 10). In the phase plane, after a pulse of extracellular cAMP, the system is displaced horizontally, to the right of the steady state. The addition of low amounts of cAMP does not produce any significant increase in intracellular (and extracellular) cAMP as the system returns immediately to the stable steady state (see inset of Fig. 10). When the perturbation exceeds a threshold, however, the system amplifies the stimulus by producing a large pulse of cAMP. As shown by the phase portrait of Fig. 10, this amplification corresponds to a large excursion of the system towards the right limb of the cAMP nullcline.

The phase portraits of Figs. 9 and 10 make clear that a close link exists between excitable and oscillatory behavior. As in the theoretical study of the nerve membrane, due to Fitzhugh (1961), both modes of behavior originate from the same nullcline structure, in slightly different conditions, i.e., for closely related parameter values. A similar conclusion was drawn in the phase plane analysis of our previous two-variable model for the cAMP signaling system (Goldbeter et al., 1978), but the receptor nullcline was replaced there by the nullcline for the substrate ATP.

4. ADAPTATION TO CONSTANT STIMULI

Devreotes and co-workers (Devreotes and Steck, 1979; Dinauer et al., 1980*b*, *c*) have conducted a series of elegant experiments in which *Dictyostelium* cells are subjected to constant cAMP stimuli. These experiments showed that cells respond to a step increase in extracellular cAMP by a transient increase in cAMP synthesis. Although the external stimulus remains constant at a higher level, cells adapt to such stimuli as the level of intracellular cAMP returns to the pre-stimulus value. Adaptation to constant stimuli is also observed for the cAMP-induced accumulation of cyclic GMP (Van Haastert and Van der Heijden, 1983). A similar phenomenon of exact adaptation to constant stimuli occurs in bacterial chemotaxis. There, as in the case of cAMP secretion in *Dictyostelium*, adaptation is brought about by receptor modification, but phosphorylation is replaced by reversible methylation (Koshland, 1979; Springer et al., 1979).

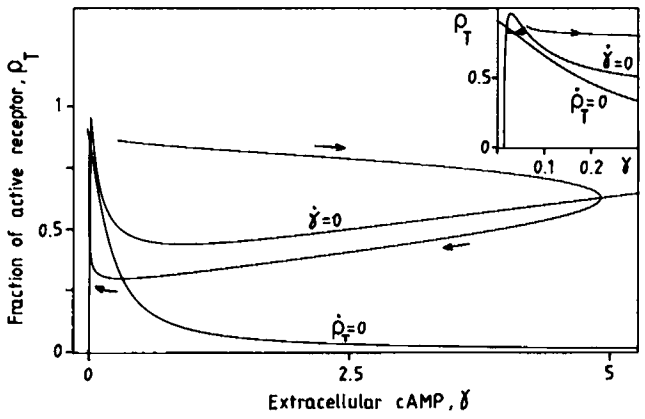

FIGURE 10 Phase plane dynamics associated with relay of suprathreshold pulses of extracellular cAMP in the two-variable system governed by Eqs. 7. Here the steady state is stable and lies immediately to the left of the oscillatory domain, which corresponds to the region of sufficiently negative slope ($d\rho_T/d\gamma$) on the sigmoid cAMP nullcline. The addition of a pulse of extracellular cAMP is simulated by instantaneously increasing the normalized cAMP level from the steady-state value $\gamma_0 = 0.0193$ to the initial value $\gamma_i = 0.3$. The system amplifies this suprathreshold perturbation in a pulsatory manner before returning to the stable steady state. (*Inset*) Enlargement of the phase portrait showing the response to both a subthreshold ($\gamma_i = 0.056$) and a suprathreshold ($\gamma_i = 0.06$) pulse of cAMP. The curves are obtained for $\sigma = 0.57$ min^{-1}, $k_e = 4$ min^{-1}, $k_i = 1$ min^{-1}, $\epsilon = 0.15$; other parameter values are as in Fig. 8.

To account for the experiments on adaptation to constant stimuli, we must control the concentration of extracellular cAMP, i.e., γ, in system 3. The evolution of the signaling system is now governed by the two kinetic Eqs. 8:

$$\frac{d\rho_T}{dt} = -f_1(\gamma)\rho_T + f_2(\gamma)(1 - \rho_T)$$

$$\frac{d\beta}{dt} = q\sigma\Phi(\rho_T, \gamma, \alpha) - (k_i + k_t)\beta, \qquad (8)$$

where γ is a parameter.

Maintaining the level of extracellular cAMP constant changes the dynamic properties of the signaling system. The positive feedback loop in cAMP synthesis is indeed suppressed; this abolishes the source of instability that leads to sustained oscillations. The steady state of Eqs. 8 is unique and always stable; it exists even in the absence of external stimulation ($\gamma = 0$).

As long as the external signal remains constant or nonexistent, the system remains in a stable steady state. A step increase in the external concentration of cAMP from γ_i to γ_f triggers the relay response. In Fig. 11, the relay response to a step increase from zero to 10^{-8} M (*a*), 10^{-7} M (*b*), and 10^{-6} M (*c*) is represented for the parameter values listed in Table II and used in Fig. 2. The synthesis of intracellular cAMP increases in response to these stimuli, but only in a transient manner: although extracellular cAMP is raised to a new, higher level, intracellular cAMP returns to a level close to that prevailing before stimulation.

Adaptation to constant stimuli results from receptor desensitization. Initially, since the active state of the receptor predominates, the step increase in extracellular cAMP enhances binding to this state with the subsequent activation of adenylate cyclase. Prolonged exposure to the stimulus induces the transition of the receptor into the desensitized state, and the activation of adenylate cyclase progressively decreases, whereas the level of modified receptor reaches a higher steady-state value (see inset to Fig. 11), in agreement with experimental observations (Devreotes and Sherring, 1985). Here, as in bacterial chemotaxis, receptor modification acts as a counterweight

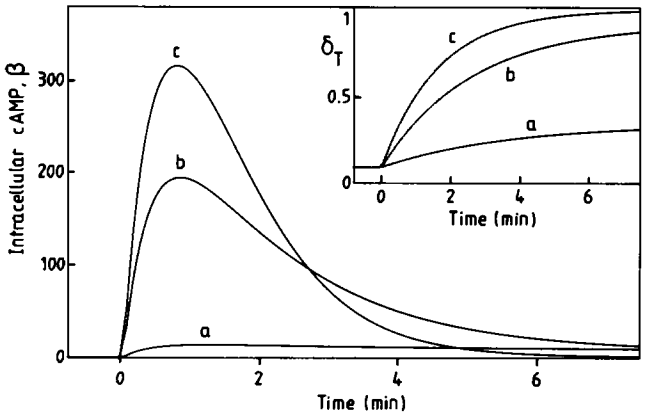

FIGURE 11 Adaptation to constant stimuli. At time zero, the signaling system is subjected to a step increase in the concentration of extracellular cAMP bringing γ from zero to 0.1 (*a*), 1 (*b*), and 10 (*c*). For $K_R = 10^{-7}$ M, these stimuli correspond to increases in cAMP from 0 to 10^{-8} M, 10^{-7} M, and 10^{-6} M, respectively. The variation in the fraction of modified receptor (δ_T) associated with the response to these three stimuli is shown in the inset. The curves are obtained by integration of Eqs. 8 for the parameter values considered for oscillatory behavior in Fig. 3 *a*.

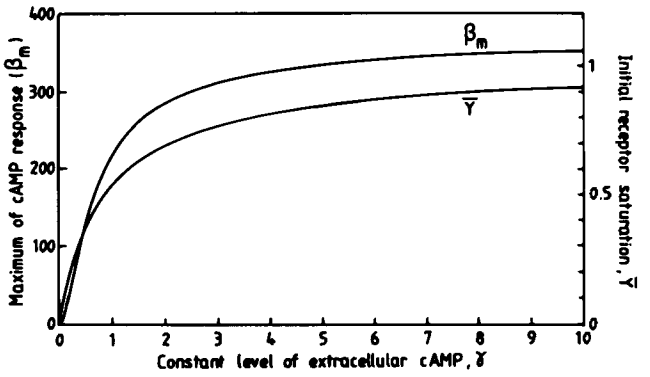

FIGURE 12 Dose-response curve for constant stimuli. The maximum of the peak in intracellular cAMP (β_m) after a step increase in stimulus is shown as a function of the final level of extracellular cAMP. The slight sigmoidicity of the response curve is due to the assumption of nonlinear coupling between the active cAMP–receptor complex and adenylate cyclase (step *e* in Eqs. 1). Also shown is the initial value of the saturation function $\bar{Y}$ of the receptor, given by Eq. 4. The curves are obtained as indicated in Fig. 11 for the same set of parameter values.

to changed external conditions (Koshland, 1980). In the model as in the experiments (Devreotes and Sherring, 1985), the receptor returns to its pre-stimulus level of modification upon removal of extracellular cAMP.

The model accounts for other experimental observations on the response to constant cAMP stimuli. First, in agreement with experimental studies (Devreotes and Steck, 1979), the dose-response curve for relay (Fig. 12) lacks the sharp threshold observed for the amplification of cAMP pulses (compare with Fig. 6). This difference is due to the suppression of the positive feedback during constant stimulation. The data of Fig. 12 indicate that half-maximum relay occurs in the model for the value $\gamma = 0.7$ which, for $K_R = 10^{-7}$ M, corresponds to a cAMP stimulus of 7×10^{-8} M. This value compares well with that of 5×10^{-8} M observed by Devreotes and Steck (1979) in these conditions.

Second, it is possible, as in the experiments of Devreotes and Steck (1979), to elicit the continuous secretion of cAMP by increasing the cAMP stimulus from 10^{-12} to 10^{-5} M by successive doublings at 90-s intervals (Fig. 13). The time course of the relay response in such conditions compares well with that observed in the experiments. Fig. 13 predicts that the half-maximum amount of cAMP secreted occurs 27 min after the beginning of the increase from 10^{-12} M, at an extracellular cAMP level of 8×10^{-8} M. The corresponding experimental values in Fig. 9 of Devreotes and Steck (1979) are 25 min and 10^{-7} M, respectively.

Finally, the model shows that adaptation can proceed in the absence of activation of adenylate cyclase, as demonstrated by Theibert and Devreotes (1983). These authors uncoupled the cAMP receptor from adenylate cyclase by using caffeine and showed that no response to cAMP occurs upon removal of caffeine after cells have been incubated during 5 min with both caffeine and cAMP. The simulations of Fig. 14 indicate that this result can be explained in terms of receptor modification. When the coupling between the receptor and the cyclase is restored, the receptor has already reached the steady-state level of modification corresponding to the cAMP stimulus. Therefore the level of intracellular cAMP also corresponds to steady-state conditions. As this level is close to that prevailing before stimulation, no significant cAMP synthesis will occur.

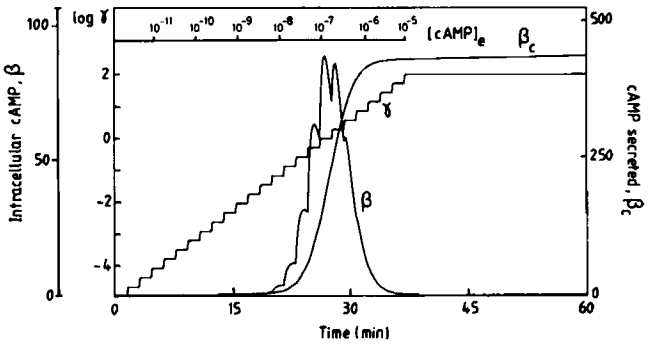

FIGURE 13 Continuous activation of cAMP synthesis by serial increments in cAMP stimulation. The curves are obtained in a manner similar to that used in the experiments of Devreotes and Steck (1979): the extracellular cAMP level is varied from 1.192×10^{-12} M up to 10^{-5} M by doubling the cAMP concentration in 25 steps lasting 90 s each. The stimulus magnitude is given as $\log(\gamma)$ (the corresponding extracellular cAMP level is indicated at the top of the graph, when K_R is taken as 10^{-7} M). Shown are the instantaneous (β) and cumulated (β_c) concentrations of intracellular cAMP; β_c represents the total amount of cAMP secreted upon stimulation minus the quantity secreted at the basal steady-state rate in the absence of external stimulus. The curves are obtained by integration of Eqs. 8 for the parameter values of Fig. 11. β_c is given by the equation $\beta_c = k_t \int_0^t (\beta - \beta_0)dt$, where $\beta_0 = 0.241$.

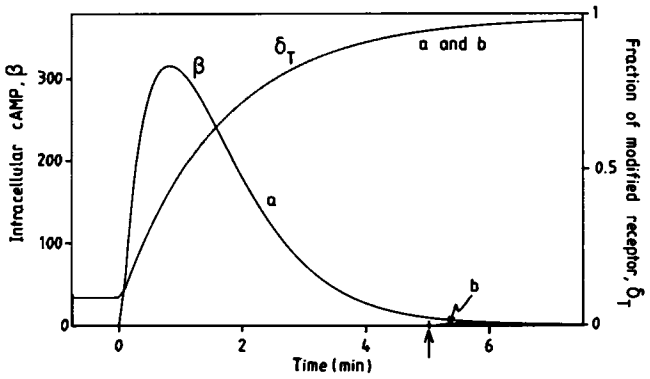

FIGURE 14 Adaptation in the absence of adenylate cyclase activation. Upon increasing extracellular cAMP at time zero in a stepwise manner ($\gamma = 0 \rightarrow 10$), the signaling system responds by synthesizing a pulse of intracellular cAMP (β) (curve *a*). This curve is the same as curve *c* in Fig. 11. If the coupling between the receptor and adenylate cyclase is prevented during 5 min by setting $\epsilon = 0$ in Eqs. 8 (thus mimicking the effect of caffeine in the experiments of Theibert and Devreotes [1983]) and restored thereafter (arrow), no significant synthesis of cAMP takes place (curve *b*). This is due to the fact that the fraction of modified receptor δ_T has reached its higher steady-state value corresponding to the increased stimulus, even in the absence of receptor coupling to the cyclase. The evolution of δ_T is thus the same in situations *a* and *b*. The curves for β and δ_T are obtained by integration of Eqs. 8, taking into account the relation $\delta_T = 1 - \rho_T$. Parameter values are as in Fig. 11.

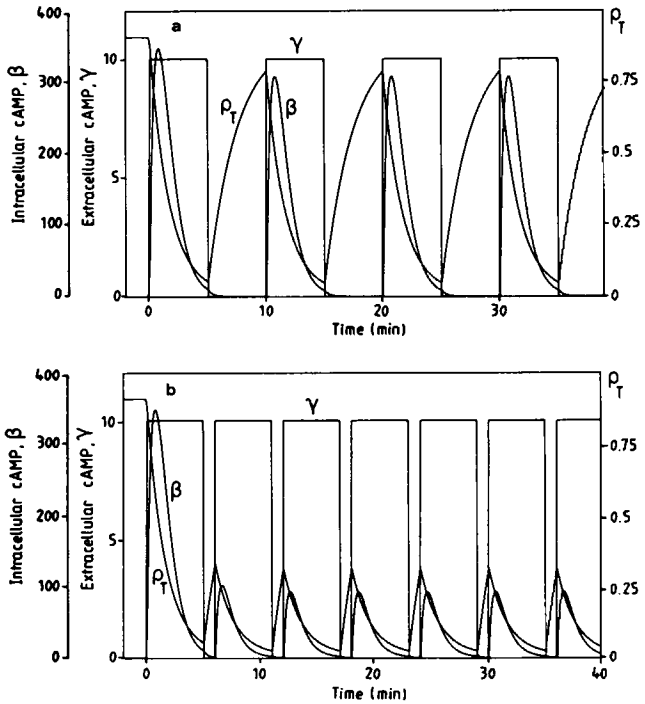

FIGURE 15 Response of the signaling system to repetitive stimulation. The stimulus consists in a square-wave variation in extracellular cAMP whose level increases from $\gamma = 0$ to 10, and stays at this high value during 5 min before returning to zero. The interval between successive square pulses is 5 min in *a* and 1 min in *b*. Also shown are the time course of the fraction of active receptor, ρ_T, and the variation in intracellular cAMP, β. The curves are established by integration of Eqs. 8 for the parameter values of Fig. 11.

5. RESPONSE TO PERIODIC STIMULATION

We have already shown how the model behaves in response to repetitive stimulation in conditions where positive feedback makes it excitable (Fig. 5). We now wish to determine the response of the signaling system to periodic stimulation when the positive feedback is suppressed by control of the extracellular cAMP level. The interest of determining such response is twofold. First, corresponding experiments have not yet been carried out in *Dictyostelium*, so that the theoretical results will have predictive value. Second, a number of hormones are known to exert their physiological effects only when delivered in a periodic manner (see Discussion). The present model allows one to address the question of the effectiveness of periodic hormone signaling in terms of receptor desensitization.

We shall consider the simplest situation in which the level of extracellular cAMP is controlled as in Section 4, but repetitively, i.e., in a square-wave manner. The parameters characterizing this periodic signal are the time interval separating two square pulses (T_{off}), the duration of a pulse (T_{on}), the basal level of γ between pulses (γ_{off}), and the level of γ at the height of a pulse (γ_{on}).

Shown in Fig. 15 *a* is the response to square pulses of saturating magnitude ($\gamma_{on} = 10$) when the duration of the pulse and the interval between two successive stimuli are both equal to 5 min. Then the amplitude of the elicited pulse in cAMP slightly decreases after the first response but reaches a steady-state level close to the initial amplitude. The time evolution of ρ_T shows that the fraction of active receptor has enough time to largely recover from the desensitized state between two stimuli so that the response to successive pulses of extracellular cAMP is nearly maximum.

In Fig. 15 *b*, the interval between successive square pulses has been reduced from 5 to 1 min, whereas the duration of each stimulation remains unchanged. The response to the first stimulus is, of course, the same as in Fig. 15 *a*, but it rapidly declines for the next pulse and settles at a markedly reduced amplitude. The curve for ρ_T indicates that this refractoriness originates from the fact that the interval between successive stimuli is too short for the receptor to recover significantly from the desensitized state.

6. DISCUSSION

The comprehension of a biological rhythm largely reduces to finding the mechanisms responsible for both its rising and decreasing phases over a period, as well as the manner in which these mechanisms are coupled so as to generate self-sustained periodic behavior. In *D. discoideum*, the increase in cAMP synthesis originates from the positive feedback exerted by extracellular cAMP through binding to the cAMP receptor and subsequent activation of adenylate cyclase. To avoid a "runaway" phenomenon, autocatalytic control must induce its own limitation. The

limitation can, in principle, result from different processes such as a depletion of substrate upon transformation into the reaction product (Goldbeter and Segel, 1977), or inhibition of adenylate cyclase by an intracellular effector such as calcium, as hypothesized by Rapp et al. (1985). We have shown that the limitation that induces the decreasing phase of cAMP synthesis during oscillations may originate from another molecular mechanism that uncouples the receptor from adenylate cyclase.

Role of Receptor Desensitization in cAMP Relay and Oscillations

Our model for the cAMP signaling system in *D. discoideum* is based on the assumption that the receptor exists in two states, R and D, which differ by the fact that only the R state can couple to adenylate cyclase. When extracellular cAMP increases, it binds to the predominant R state and thereby enhances the synthesis of cAMP. The decrease in cAMP synthesis is brought about by the cAMP-induced modification of the R state of the receptor into the D state uncoupled from adenylate cyclase. This assumption holds with the observation that adaptation in *D. discoideum* occurs through cAMP-induced phosphorylation of the cAMP receptor (Klein et al., 1985*a, b*; Devreotes and Sherring, 1985).

The present analysis has uncovered a second way in which a model based on receptor desensitization may, in principle, give rise to autonomous oscillations. While in the above mechanism of ligand-induced receptor modification the R and D states may or may not differ by their affinity for cAMP, the second mechanism, based on pure conformational transitions, requires that the desensitized, inactive D state has a much higher affinity for cAMP than the R state, which still predominates in the absence of ligand. Then, as the cAMP level rises, the ratio of R to D is shifted towards the D state, owing to the higher affinity of the latter for cAMP. As the D state cannot activate adenylate cyclase, cAMP synthesis decreases and a new cycle begins when the initial R/D ratio is reestablished.

The distinction between the two mechanisms of periodic behavior was highlighted by quantifying in each of them the net fluxes of the R $\rightleftharpoons$ D and RP $\rightleftharpoons$ DP interconversions in the course of oscillations (Fig. 3). An important difference between the two oscillatory mechanisms is that the second one, based on conformational transitions, requires that the D state possess a higher affinity for cAMP. The constraint of such a tight-binding state is not required in the mechanism based on covalent modification. Expenditure of energy in the form of ATP hydrolysis accompanies receptor phosphorylation in the latter conditions.

Receptor modification similarly plays a major role in the dynamics of the relay response. When the system has been stimulated by a cAMP pulse, a certain time must elapse before it can relay a second pulse of similar magnitude. We have shown that this refractory period corresponds to the time required for the receptor to return from the desensitized to the active state after stimulation. The refractory period is at first absolute, i.e., no significant response to the second pulse is observed as long as the level of receptor in active state decreases as a result of the first stimulation. Then, as the level of active receptor rises up to the pre-stimulus value, there is a relative refractory period during which the threshold for relay of cAMP pulses progressively diminishes and the response to the second stimulus increases until it finally reaches the magnitude of the first response.

Comparison with Experiments

Excitable and oscillatory behavior of the cAMP signaling system account, respectively, for the relay of cAMP pulses and for autonomous oscillations of cAMP observed in *D. discoideum* amebae during the hours that follow starvation, both in cell suspension experiments and on agar.

Most numerical simulations have been performed after reducing from nine to three the number of kinetic equations governing the time evolution of the model. Although the further reduction to a two-variable system is not rigorously justified for the experimental values considered for the parameters, we have shown that the qualitative properties of the three-variable system are conserved in the two-variable version, and that the quantitative discrepancies due to the reduction from three to two variables are of the order of 30% for both the period and amplitude of oscillations. As to relay of cAMP pulses, the dose-response curve exhibits an even sharper threshold in the two-variable system.

The interest of the two-variable system is that it allows direct visualization of the link between excitability and oscillations by phase plane analysis (Figs. 9 and 10) and permits isolation of the core of the signaling mechanism. Furthermore, a two-variable system should prove useful in the study of concentric and spiral patterns which develop during the wavelike aggregation of *D. discoideum* amebae on agar (Gerisch, 1968; Alcantara and Monk, 1974; Tomchik and Devreotes, 1981). Two-variable reductions have previously been used to analyze the propagation of waves in chemical systems such as the Belousov-Zhabotinsky reaction (Tyson and Fife, 1980; Fife, 1984).

We have already discussed in Section 3 the qualitative and, in a large measure, quantitative agreement between theoretical and experimental results with respect to the period and amplitude of oscillations, and with respect to the characteristics of relay. Such agreement was obtained by using available experimental values for most parameters of the model. As previously mentioned, the main changes required for reaching an appropriate value for the period bear on the rate constants for receptor modification k_1, k_{-1}, and k_2, which were multiplied by a factor of 3.

Another change with respect to experimentally determined parameter values pertains to the dephosphorylation rate constant k_{-2} for which we took a value smaller by a

factor of 16 (see Tables I and II). Given the change in k_2 this yields the value of 5×10^{-3} for L_2, which is fifty times smaller than that reported by Devreotes and Sherring (1985). The reason for taking such a low value of L_2 is that it yields more complete transition into the inactive state DP and, hence, better agreement with the experiments on adaptation to constant stimuli (see below). However, as indicated in Fig. 4, oscillations can occur in the model for values of L_2 as large as 0.1, close to the value 0.25 reported for L_2.

That we had to change the values of the phosphorylation and dephosphorylation rate constants to obtain oscillations of the appropriate period is not necessarily worrying. The experimental values were determined at a given stage of development. It may well be, however, that the activity of the kinase and phosphatase that control modification of the cAMP receptor vary during the interphase that leads to aggregation. Such developmental changes do occur for adenylate cyclase, phosphodiesterase, and the cAMP receptor (Henderson, 1975; Klein, 1976; Klein and Darmon, 1975, 1977; Gerisch and Malchow, 1976; Loomis, 1979).

With respect to relay of cAMP pulses, the excitable nature of the signaling system accounts for the existence of a sharp threshold for amplification of such stimuli. The value predicted by the model (Fig. 6) compares well with that observed for the threshold in experiments with cells aggregating on agar (Shaffer, 1962; Robertson and Drage, 1975; Grutsch and Robertson, 1978). A threshold has also been demonstrated in cell suspension experiments (Roos et al., 1975) but it should be desirable to establish a complete dose-response curve to determine more precisely its value in these conditions. We also showed that relay of suprathreshold pulses is associated with the existence of an absolute and a relative refractory period, both of which last several minutes as observed in the experiments (Durston, 1974*a*; Robertson and Drage, 1975). These properties are shared by other excitable systems, as exemplified by the nerve membrane (Fitzhugh, 1961) and chemical oscillatory reactions (Winfree, 1972; De Kepper, 1976).

Adaptation to Constant Stimuli

When subjected to constant stimuli, the cAMP signaling system becomes analogous to other sensory systems in that it exhibits then the property of adaptation. Besides the present model, specific to the *D. discoideum* signaling system, various models for adaptation based on receptor modification have been considered, the first being that of Katz and Thesleff (1957), who introduced the concept of a two-state model for desensitization of the acetylcholine receptor. Models based on receptor methylation have been analyzed for exact adaptation in bacterial chemotaxis (Goldbeter and Koshland, 1982; Block et al., 1983; Asakura and Honda, 1984). Segel et al. (1986) have recently analyzed the conditions in which binding of a ligand to a two-state receptor system may trigger a physiological activity that always returns to the same steady-state level, regardless of the magnitude of the step increase in stimulus. Knox et al. (1986) applied these results to exact adaptation in bacterial chemotaxis and in the cAMP relay response to constant stimuli in *D. discoideum*. On the other hand, a model based on feedback regulation of adenylate cyclase by calcium ions has been proposed for adaptation in the latter system (Rapp et al., 1985; Othmer et al., 1985).

Here, as in the analysis of Segel et al. (1986) and Knox et al. (1986), adaptation is brought about by receptor modification but the coupling of the receptor to adenylate cyclase is considered explicitly. Only partial adaptation occurs in the three-variable model governed by Eqs. 9 as the signaling system does not always return to the same intracellular cAMP level at steady state. When the substrate ATP is maintained constant, the final cAMP level indeed depends on the magnitude of the constant stimulus; the variability, however, may be quite small, especially for large stimuli. Exact adaptation can occur in the model in a robust manner when the difference between the rate of synthesis of ATP and its rate of consumption in reactions other than that catalyzed by adenylate cyclase is taken as a constant (Martiel and Goldbeter, 1984; Goldbeter and Martiel, 1985*a*). Alternatively, exact adaptation might well occur for particular values of the coupling constants of the four receptor states with adenylate cyclase (Segel et al., 1986). In the present analysis, no coupling takes place for the states R, D, DP as only the form RP can activate the cyclase.

That no threshold exists for the relay response to constant stimuli (Devreotes and Steck, 1979) in contrast to the response to cAMP pulses (Robertson and Drage, 1975) is well explained by the analysis of the model in these distinct conditions. Under the natural conditions that prevail during slime mold aggregation, the self-amplification properties of the signaling system give rise to excitability, i.e., relay of suprathreshold cAMP pulses, and autonomous oscillations. The positive feedback in cAMP synthesis is suppressed when extracellular cAMP is artificially held constant. Then the system becomes analogous to those in which adenylate cyclase is under hormonal control. There, indeed, the level of external activator, i.e., the hormone, is independent from the product of the reaction, cAMP.

The model predicts that the cAMP level yielding half-maximum response to constant stimulation is close to 7×10^{-8} M (see Fig. 12), i.e., one order of magnitude above the sharp threshold for relay of cAMP pulses (see Fig. 6). As pointed out above, these results are in agreement with experimental observations. A further difference between stimulation by cAMP pulses and by constant cAMP stimuli is that in the former case the relay response displays both an absolute and a relative refractory period (Fig. 7), whereas in the second situation only a relative refractory period is found, both in the experiments and in the model.

Qualitative and quantitative agreement with the experiments also obtains for the effect of successive doublings of the cAMP stimulus from 10^{-12} to 10^{-5} M (Fig. 13) and for the absence of response to cAMP stimulation (Fig. 14) when the receptor has been uncoupled from adenylate cyclase by prior treatment with caffeine (Theibert and Devreotes, 1983).

Predictions of the Theoretical Analysis

Besides explaining in a unified manner a large number of experimental observations on the signaling system, the present analysis offers a number of predictions that might be tested experimentally. We have already alluded to the possible changes in receptor kinase and phosphatase activities in the course of *Dictyostelium* development after starvation. Any change in the properties of the kinase and phosphatase, due to mutations or to the use of specific inhibitors or activators, should alter the characteristics of oscillatory behavior. At constant phosphatase/kinase activity ratios, i.e., at constant L_1 and L_2 values, decreasing the phosphorylation rate should increase the period of oscillations. The model predicts, however, that there exists a definite range of L_1 and L_2 values producing oscillations. Fig. 4 shows that if the system initially operates in the oscillatory domain, a sufficient increase in L_1 and L_2 should bring the system into a stable steady state characterized by a high level of cAMP (domain V). Conversely, a concomitant decrease of appropriate magnitude in L_1 and L_2 should move the system towards a stable steady state characterized by a low cAMP level (domain IV). The changes in L_1 and L_2 may originate from changes in the kinetic properties of either the receptor kinase or the phosphatase. Both constants increase with enhanced phosphatase activity and decrease with enhanced kinase activity.

Another type of prediction relates to the response to periodic stimuli in conditions where the positive feedback loop is suppressed by control of the extracellular cAMP level. Numerical simulations indicate (Fig. 15) that the amplitude of the cAMP response should reach a steady-state value upon repetitive stimulation. Moreover, the response amplitude should diminish in a sigmoidal manner when the interval between successive pulses progressively decreases.

The molecular mechanisms of activation of adenylate cyclase and of desensitization to cAMP stimuli are currently being unraveled in further detail. Biochemical advances on the phosphorylation–dephosphorylation reactions and on the role of the GTP-binding protein in signal transduction (Janssens et al., 1986; Ludérus et al., 1986; Van Haastert, 1987) will undoubtedly add or modify steps in scheme 1 on which our model is based. The question arises as to how these changes will affect the predictions of the model. Our analysis should prove fundamentally robust with respect to such changes. Indeed, as suggested by the good agreement of our theoretical predictions with a great amount of data obtained in different experimental conditions for measured parameter values, the model already takes into account the most conspicuous properties of the signaling system, namely, self-amplification and desensitization through uncoupling of the receptor from adenylate cyclase. Excitability, oscillations, and adaptation to constant stimuli will necessarily result from these regulatory processes in a manner that is relatively independent of their detailed molecular implementation. Changes resulting from modifications of the basic steps and, hence, of the kinetic equations should rather bear on quantitative predictions, although the latter are eventually dictated in large measure by the affinity of the receptor towards cAMP.

Unified Mechanism for Different Modes of cAMP Signaling

In physiological conditions where extracellular cAMP is allowed to vary, the signaling system of *D. discoideum* amebae after starvation undergoes transitions from a state of no relay to relay of suprathreshold cAMP signals, and then to autonomous oscillations of cAMP. These sequential changes in dynamic behavior obtain in the model when the kinetic equations incorporate the continuous increase in the number of receptor sites and in the activity of key enzymes of the signaling system which is observed over a 6-h period after starvation (Goldbeter and Martiel, 1987). The model thus provides a plausible molecular basis for the developmental transitions in cAMP signaling observed in cell suspensions. That excitability and oscillations occur in the model for closely related values of the parameters also explains why in the course of aggregation cells may either relay signals or produce periodically cAMP pulses in an autonomous manner. Aggregation centers would thus be the first cells to enter the oscillatory domain of the signaling system in parameter space (Goldbeter, 1975; Goldbeter and Segel, 1980). Moreover, for constant cAMP stimuli, the model displays the property of adaptation and loses the threshold characteristic of the relay response to cAMP pulses. The model based on receptor desensitization therefore permits unification of the various modes of dynamic behavior of the cAMP signaling system observed under different experimental conditions.

We have previously predicted the occurrence of yet another mode of signaling, namely aperiodic oscillations of cAMP (i.e., chaos), in a seven-variable version of this model (Martiel and Goldbeter, 1985). Such behavior could account for the aperiodic signaling properties of the *D. discoideum* mutant Fr17 (Durston, 1974*b*). Chaos here is dependent on a variation in the substrate ATP. The occurrence of such phenomenon in the four-variable version of the model (Eqs. 2) is currently under investigation.

Role of Periodic Signaling in Intercellular Communication

The present analysis suggests an explanation for the periodic nature of the signaling process that governs aggregation of *D. discoideum* cells. Besides enlarging the size of

the aggregation territory—species such as *Dictyostelium minutum* which lack relay and oscillations in the course of aggregation indeed form much smaller aggregates (Gerisch, 1968)—the use of a periodic signal may represent the most efficient way to deliver a stimulus in conditions of maximum cellular responsiveness. A constant cAMP stimulus would indeed reduce responsiveness through receptor modification and desensitization. The simulations performed in conditions of excitability (Figs. 5 and 7) as well as in the absence of positive feedback (Fig. 15) both indicate that cAMP pulses delivered at too short intervals, before the receptor has fully recovered from the adapted state, elicit a markedly reduced response, much as constant stimuli. Such results explain in terms of receptor desensitization why a constant cAMP stimulus (Darmon et al., 1975) or cAMP pulses applied at intervals of 2 min (Wurster, 1982) fail to induce cell differentiation, in contrast to cAMP pulses delivered once every 5 min (Darmon et al., 1975; Gerisch et al., 1975).

A similar explanation in terms of avoidance of receptor desensitization may hold (Goldbeter, 1987) for the effect of periodic pulses of hypothalamic gonadotropin-releasing hormone (GnRH) on the release of the luteinizing hormone (LH) and the follicular stimulating hormone (FSH) by the pituitary (Belchetz et al., 1978; Knobil, 1980). The constant input of GnRH fails to induce the release of LH and FSH that is brought about by GnRH pulses delivered at the physiological period close to 1 h. Pulses of GnRH applied with a shorter period are also ineffective (Knobil, 1980). The efficiency of intermittent, pulsatile release of GnRH has been exploited successfully for induction of ovulation in treatments of some hypothalamic-dependent cases of infertility (Leyendecker et al., 1980; Reid et al., 1981).

Periodic signaling by both GnRH and cAMP pulses can be regarded as optimal modes of intercellular communication. Sharp periodic pulses delivered at appropriate time intervals indeed elicit a maximum number of high-amplitude responses, without inducing permanent receptor desensitization, in systems that nevertheless retain the capability of adapting to constant stimuli. In *D. discoideum*, the adequacy of the period of cAMP signaling with the refractory period of the receptor system is ensured by the fact that both are dictated by the same mechanism of receptor modification.

APPENDIX A

Kinetic Equations and Reduction to a Three-Variable System

The time evolution of the concentration of the various species appearing in the kinetic scheme 1 is governed by the following differential equations:

$$\frac{d\rho}{dt} = k_1(-\rho + L_1\delta) + d_1(-\rho\gamma + x)$$

$$\frac{d\delta}{dt} = k_1(\rho - L_1\delta) + d_2(-\delta c\gamma + y)$$

$$\frac{dx}{dt} = k_2(-x + L_2 y) + d_1(\rho\gamma - x) + (2\mu/h)d_3(-\epsilon x^2\bar{c} + \bar{e})$$

$$\frac{d\bar{c}}{dt} = d_3(-\epsilon x^2\bar{c} + \bar{e}) + (d_5 + k_5)(-\bar{c}\alpha\theta + \overline{cs})$$

$$\frac{d\bar{e}}{dt} = d_3(\epsilon x^2\bar{c} - \bar{e}) + (d_4 + k_4)(-\bar{e}\alpha + \overline{es})$$

$$\frac{d\overline{es}}{dt} = (d_4 + k_4)(\bar{e}\alpha - \overline{es})$$

$$\frac{d\alpha}{dt} = v - k'\alpha - \sigma(\overline{es} + \lambda\overline{cs}) + \theta_E[(d_4 + k_4) \cdot (-\bar{e}\alpha + \overline{es}) + (d_5 + k_5)(-\bar{c}\alpha\theta + \overline{cs})]$$

$$\frac{d\beta}{dt} = q\sigma(\overline{es} + \lambda\overline{cs}) - (k_i + k_t)\beta$$

$$\frac{d\gamma}{dt} = (k_t\beta/h) - k_e\gamma + \eta[d_1(-\rho\gamma + x) + d_2(-\delta c\gamma + y)]. \quad \text{(A1)}$$

In these equations $\rho = R/R_T$, $\delta = D/R_T$, $x = RP/R_T$, $\bar{e} = E/E_T$, $\overline{es} = ES/E_T$, $c = C/R_T$, and $\overline{cs} = CS/R_T$, where R_T and E_T represent the total amount of receptor and of adenylate cyclase; β and γ denote respectively the concentrations of intracellular and extracellular cAMP divided by the dissociation constant $K_R = d_1/a_1$; α is the intracellular level of ATP normalized by the Michaelis constant $K_m = (d_4 + k_4)/a_4$. Moreover, $L_1 = k_{-1}/k_1$ is the equilibrium ratio of the states R and D in the absence of ligand, whereas $L_2 = k_{-2}/k_2$; $c = K_R/K_D$ with $K_D = d_2/a_2$ is the nonexclusive binding coefficient of extracellular cAMP for the two receptor states; $v = v_i/K_m$; h is the dilution factor (see Table II for definition of other parameters).

In deriving Eqs. A1, we made use of the two conservation relations for the receptor and for adenylate cyclase:

$$R_T = R + D + RP + DP + (2/h)(E + ES)$$
$$E_T = E + ES + C + CS. \quad \text{(A2)}$$

The assumption that R_T and E_T remain constant holds in first approximation, given that the time scale for the variation of these parameters is much longer than the time scale for relay and oscillations. The conservation relations (A2) take into account the fact that the receptor and adenylate cyclase concentrations are defined with respect to the extracellular and intracellular volumes, respectively. Eqs. A2 yield the following expressions for y and $\overline{cs}$, which supplement Eqs. A1:

$$y = 1 - \rho - \delta - x - (2\mu/h)(\bar{e} + \overline{es})$$
$$\overline{cs} = 1 - \bar{c} - \bar{e} - \overline{es}. \quad \text{(A3)}$$

In the limit of fast binding of extracellular cAMP to both forms of the receptor and fast association between RP and C, C and S, E and S, the following inequality on the rate constants for the reaction steps 1 holds

$$(k_1,k_{-1},k_2,k_{-2},k_i,k_t,k_e,\sigma,k') \ll (a_1,d_1,a_2,d_2,a_3,d_3,a_4,d_4,a_5,d_5). \quad \text{(A4)}$$

As Eqs. A1 contain both fast binding and slow modification terms, we introduce new variables to separate the nine differential Eq. A1 into two sets, one associated with the slower time scale governing the interconversion of the receptor forms, and another associated with the faster time scale governing the binding reactions.

Let us define ρ_T and δ_T as the total fractions of the receptor in the active and inactive (modified) states, $\overline{Y}$ as the total fraction of the receptor forms

bound to cAMP, A as the total concentration of intracellular ATP (free plus bound to the two forms of adenylate cyclase), and Γ as the normalized total concentration of extracellular cAMP (free plus bound to the two receptor states). These new variables are expressed as a function of the old ones by Eqs. A5.

$$\rho_T = 1 - \delta_T = \rho + x + (2\mu/h)(\bar{e} + \overline{es})$$
$$\bar{Y} = x + y + (2\mu/h)(\bar{e} + \overline{es})$$
$$A = \alpha + \Theta_E(\overline{cs} + \overline{es})$$
$$\Gamma = \gamma + \eta(1 - \rho - \delta) = \gamma + \eta\bar{Y}. \qquad \text{(A5)}$$

The evolution equations for these new variables can now be obtained by taking their time derivative from Eqs. A5 and inserting into the resulting relations the relevant equations from Eqs. A1. This procedure yields the new set of Eqs. A6 in which the first four equations govern the evolution of slower variables, while the remaining equations relate to fast variables (system A6 can be complemented by one of the three equations for ρ, δ, or x in A1 but these equations contain both fast and slow terms and will therefore not be used in the subsequent reduction).

$$\frac{d\rho_T}{dt} = k_1(-\rho + L_1\delta) + k_2(-x + L_2 y)$$
$$\frac{dA}{dt} = v - k'\alpha - \sigma(\overline{es} + \lambda\overline{cs})$$
$$\frac{d\beta}{dt} = q\sigma(\overline{es} + \lambda\overline{cs}) - (k_i + k_t)\beta$$
$$\frac{d\Gamma}{dt} = (k_t/h)\beta - k_e\gamma$$
$$\frac{d\bar{Y}}{dt} = d_1(\rho\gamma - x) + d_2(\delta c\gamma - y)$$
$$\frac{d\bar{c}}{dt} = d_3(-\epsilon x^2\bar{c} + \bar{e}) + (d_5 + k_5)(-\bar{c}\alpha\Theta + \overline{cs})$$
$$\frac{d\bar{e}}{dt} = d_3(\epsilon x^2\bar{c} - \bar{e}) + (d_4 + k_4)(-\bar{e}\alpha + \overline{es})$$
$$\frac{d\overline{es}}{dt} = (d_4 + k_4)(\bar{e}\alpha - \overline{es}), \qquad \text{(A6)}$$

where y and $\bar{c}$ are still given by Eqs. A3.

We now require that, after an initial transient phase, the differential equations for the fastest variables $\bar{Y}$, $\bar{c}$, $\bar{e}$, and $\overline{es}$ reduce to algebraic equations corresponding to the quasi-steady-state hypothesis for these receptor and enzyme forms. This condition leads to Eqs. A7.

$$d_1(\rho\gamma - x) + d_2(\delta c\gamma - y) = 0$$
$$\bar{e}\alpha - \overline{es} = 0$$
$$\bar{c}\alpha\Theta - \overline{cs} = 0$$
$$\epsilon x^2\bar{c} - \bar{e} = 0. \qquad \text{(A7)}$$

In the first of these equations each of the two terms in parentheses vanishes since, when the quasi-steady-state regime holds, the set relations A7 must remain independent of the actual values of d_1 and d_2. The five algebraic relations obtained from A7, plus the four kinetic equations for the slow variables in A6, correspond to the nine degrees of freedom of the initial system A1.

Taking into account the conservation relations A3 we obtain Eq. A8 for $\bar{c}$ as a function of α and x; similar relations are obtained for $\bar{e}$, $\overline{es}$, and $\overline{cs}$.

$$\bar{c} = [1 + \alpha\Theta + \epsilon x^2(1 + \alpha)]^{-1}. \qquad \text{(A8)}$$

The evolution equations for the remaining slow variables can now be transformed according to relations A7 and A8, yielding the four-variable differential system A9:

$$\frac{d\rho_T}{dt} = k_1(-\rho + L_1\delta) + k_2(-\rho\gamma + L_2\delta c\gamma)$$
$$\frac{dA}{dt} = v - k'\alpha - \sigma\alpha(\lambda\Theta + \epsilon\rho^2\gamma^2)/[1 + \alpha\Theta + \epsilon\rho^2\gamma^2(1 + \alpha)]$$
$$\frac{d\beta}{dt} = q\sigma\alpha(\lambda\Theta + \epsilon\rho^2\gamma^2)/[1 + \alpha\Theta + \epsilon\rho^2\gamma^2(1 + \alpha)] - (k_i + k_t)\beta$$
$$\frac{d\Gamma}{dt} = (k_t/h)\beta - k_e\gamma. \qquad \text{(A9)}$$

To express in these equations the old variables in terms of the new ones, we use the definitions (A5) which take the form

$$\rho_T = \rho(1 + \gamma) + (2\mu/h)[\epsilon\rho^2\gamma^2(1 + \alpha)]/[1 + \alpha\Theta + \epsilon\rho^2\gamma^2(1 + \alpha)]$$
$$A = \alpha + \Theta_E[\alpha(\theta + \epsilon\rho^2\gamma^2)]/[1 + \alpha\Theta + \epsilon\rho^2\gamma^2(1 + \alpha)]$$
$$\Gamma = \gamma + \eta(\rho\gamma + \delta c\gamma) + (2\mu/h)[\epsilon\rho^2\gamma^2(1 + \alpha)]/[1 + \alpha\Theta + \epsilon\rho^2\gamma^2(1 + \alpha)]. \qquad \text{(A10)}$$

As experimentally observed (see Tables I and II), the parameters (μ/h), Θ_E, and η are much smaller than unity. Neglecting the terms multiplied by these factors in Eqs. A10, we obtain the simpler expressions for the new variables ρ_T, A, and Γ as a function of the original ones ρ, α, and γ:

$$\rho_T = \rho(1 + \gamma); A = \alpha; \Gamma = \gamma. \qquad \text{(A11)}$$

Inserting the expressions A7 into A11 and taking into account the conservation relations A3, we get

$$\rho = \rho_T/(1 + \gamma); \delta = (1 - \rho_T)/(1 + c\gamma). \qquad \text{(A12)}$$

The four-variable system (A9) takes the final form (A13):

$$\frac{d\rho_T}{dt} = -\rho_T[(k_1 + k_2\gamma)/(1 + \gamma)] + (1 - \rho_T)[(k_1L_1 + k_2L_2c\gamma)/(1 + c\gamma)]$$
$$\frac{d\alpha}{dt} = v - k'\alpha - \sigma\Phi(\rho_T, \gamma, \alpha)$$
$$\frac{d\beta}{dt} = q\sigma\Phi(\rho_T, \gamma, \alpha) - (k_i + k_t)\beta$$
$$\frac{d\gamma}{dt} = (k_t/h)\beta - k_e\gamma \qquad \text{(A13)}$$

with $\Phi(\rho_T, \gamma, \alpha) = \alpha(\lambda\Theta + \epsilon Y^2)/[1 + \alpha\Theta + \epsilon Y^2(1 + \alpha)]$; $Y = \rho_T\gamma/(1 + \gamma)$. These equations are identical to the four-variable system (2) analyzed in the text. System 2 further reduces to the three-variable

system (3) when considering that the ATP level (α) does not vary in time.

Eqs. A13 are valid in the limit of negligible (μ/h), Θ_E, and η (see Eqs. A11). The values of the two first pararameters are generally smaller than that of the latter, which may reach 200 (see Table II). It may therefore be of interest to determine the behavior of the system when the cAMP bound to the receptor is taken into account (this amounts to keeping the term $\eta[\rho\gamma + \delta c\gamma]$ in Eqs. A10). In the limit $(\mu/h) \to 0, \Theta_E \to 0$ and finite η we obtain, instead of Eqs. A11:

$$\rho_T = \rho(1 + \gamma); A = \alpha; \Gamma = \gamma + \eta(\rho\gamma + \delta c\gamma). \quad \text{(A14)}$$

The differential system A13 takes the form

$$\frac{d\rho_T}{dt} = -\rho_T f_1(\gamma) + (1 - \rho_T) f_2(\gamma)$$

$$\frac{d\alpha}{dt} = v - k'\alpha - \sigma\Phi(\rho_T, \gamma, \alpha)$$

$$\frac{d\beta}{dt} = q\sigma\Phi(\rho_T, \gamma, \alpha) - (k_i + k_t)\beta$$

$$\frac{d\Gamma}{dt} = (k_t/h)\beta - k_e\gamma, \quad \text{(A15)}$$

with $\Phi(\rho_T, \gamma, \alpha) = \alpha(\lambda\Theta + \epsilon Y^2)/[1 + \alpha\Theta + \epsilon Y^2(1 + \alpha)]$; $Y = \rho_T\gamma/(1 + \gamma)$; $f_1(\gamma) = (k_1 + k_2\gamma)/(1 + \gamma)$; $f_2(\gamma) = (k_1 L_1 + k_2 L_2 c\gamma)/(1 + c\gamma)$; $\Gamma = \gamma + \eta[\rho_T\gamma/(1 + \gamma) + (1 - \rho_T)c\gamma/(1 + c\gamma)]$. γ is given by the unique positive root of the third-degree polynomial:

$$-c\gamma^3 + (c\Gamma - 1 - c - \eta c)\gamma^2 + [(1 + c)\Gamma - 1 - \eta c - \eta\rho_T(1 - c)]\gamma + \Gamma = 0, \quad \text{(A16)}$$

which must be solved in the course of the numerical integration of Eqs. A15. When $K_R = 10^{-7}$ M, the value of η is 0.3 (see Table II) and the approximation (A13) can be made without resorting to the more complicated expressions A14 and A16, as indicated by comparative integration of Eqs. A13 and A15.

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