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A Model for cAMP-mediated cGMP Response in Dictyostelium discoideum

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In Dictyostelium discoideum extracellular cyclic AMP (cAMP), as shown by previous studies, induces a transient accumulation of intracellular cyclic guanosine-5'-monophosphate (cGMP), which peaks at 10 s and recovers basal levels at 30 s after stimulation, even with persistent cAMP stimulation. Additional investigations have shown that the cAMP-mediated cGMP response is built up from surface cAMP receptor-mediated activation of guanylly cyclase and hydrolysis of cGMP by phosphodiesterase. The regulation of these activities was measured in detail on a seconds time-scale, demonstrating complex adaptation of the receptor, allosteric activation of cGMP-phosphodiesterase by cGMP, and potent inhibition of guanylly cyclase by Ca2+. In this paper we present a computer model that combines all experimental data on the cGMP response. The model is used to investigate the contribution of each structural and regulatory component in the final cGMP response. Four models for the activation and adaptation of the receptor are compared with experimental observations. Only one model describes the magnitude and kinetics of the response accurately. The effect of Ca2+ on the cGMP response is simulated by changing the Ca2+ concentrations outside the cell (Ca2+ influx) and in stores (IP3-mediated release) and changing phospholipase C activity. The simulations show that Ca2+ mainly determines the magnitude of the cGMP accumulation; simulations are in good agreement with experiments on the effect of Ca2+ in electroporpermeabilized cells. Finally, when cGMP-phosphodiesterase activity is deleted from the model, the simulated cGMP response is elevated and prolonged, which is in close agreement with the experimental observations in mutant stmF that lacks this enzyme activity. We conclude that the computer model provides a good description of the observed response, suggesting that the main structural and regulatory components have been identified.

INTRODUCTION

The slime mold Dictyostelium discoideum lives in the soil where it feeds on bacteria. Upon food depletion, the unicellular amoebae organize in a multicellular slug, in which differentiation occurs. The cells in the anterior part develop into stalk cells, whereas the cells in the posterior part will become spores (Schaap and Wang, 1986). The development of Dictyostelium is triggered by cAMP, which is secreted by the amoebae upon starvation (Konijn, 1972). Neighboring cells are capable of responding to the cAMP gradient by means of cAMP receptors in the cell membrane (Malchow and Gerisch, 1974; Green and Newell, 1975; Henderson, 1975; Mato and Konijn, 1975). Stimulation of these receptors triggers a cascade of reactions, which finally results in cell movement towards the increasing concentration of cAMP (Gerisch et al., 1975).

Upon stimulation of the cAMP receptor the intracellular enzymes guanylyl cyclase and phospholipase C are rapidly activated (Mato and Malchow, 1978; Europe-Finner and Newell, 1987). Consequently the concentrations of cyclic guanosine-5'-monophosphate (cGMP), inositol 1,4,5-trisphosphate1 (IP3), and Ca2+ increase, myosin is phosphorylated, and actin polymerizes, eventually resulting in enhanced and directed cell motility (Malchow et al., 1981; McRobbie and Newell, 1984;

1 Abbreviations used: IP3, inositol 1,4,5-trisphosphate.
Europe-Finner and Newell, 1986a,b; Liu and Newell, 1988). *Dictyostelium* exhibits chemotaxis towards different chemotacticants like cAMP and folic acid (Konijn et al., 1967; Pan et al., 1972). The role of cGMP in chemotaxis has been emphasized in *strmF*, a mutant which, due to the absence of cGMP-specific phosphodiesterase, has an increased cGMP response and shows prolonged chemotactic movement towards cAMP and folic acid (Ross and Newell, 1981; Van Haastert et al., 1982). The conclusion that cGMP is involved in chemotaxis was recently confirmed in experiments with mutant K18. This mutant, with strongly reduced guanylyl cyclase activity, shows no chemotaxis to either cAMP or folic acid (Kuwayama et al., 1993).

cGMP levels start to increase at ∼1 s after stimulation of the cells with cAMP; peak levels are achieved 10 s later (Van Haastert, 1987a). Subsequently the concentration of cGMP declines to reach basal levels at ∼30 s (Mato et al., 1977). Several experiments suggest that the receptor-mediated cGMP response is regulated by complex mechanisms (Van Haastert and Van der Heijden, 1983). Although the peak values of the cGMP response depend on the stimulus concentration, the kinetics of the response is essentially independent with respect to the cAMP concentration. Extracellular cAMP is degraded by phosphodiesterase activity in the medium (Chang, 1968; Malchow et al., 1972; Panbacker and Bravard, 1972). The magnitude and kinetics of the cGMP response remain the same whether the cAMP stimulus is present for only 3 s or is not degraded at all (Van Haastert and Van der Heyden, 1983). Finally, when cells are stimulated twice at 30-s interval, they respond only to the second stimulus if the concentration is higher than that of the first stimulus (Van Haastert, 1983a). These experiments indicate that the receptor-mediated cGMP response is regulated by an adaptation mechanism.

Biochemically, the cGMP response is controlled at two points: synthesis by guanylyl cyclase and degradation by phosphodiesterase. Guanylyl cyclase is stimulated by the receptor (Mato and Malchow, 1978). Previous studies have indicated that adaptation of the cGMP response occurs upstream of guanylyl cyclase (Van Haastert, 1983a), presumably at the receptor or at the Ga2-protein (Okaichi et al., 1992). Detailed kinetic studies of cAMP binding to *D. discoideum* cells suggest that a subpopulation of surface receptors is involved in the activation of guanylyl cyclase and that adaptation is associated at the interconversions between active and inactive receptor forms (Van Haastert et al., 1986). Guanylyl cyclase activity is inhibited by Ca2+ ions (Janssens et al., 1989; Valkema and Van Haastert, 1992), suggesting that the cGMP response is regulated by receptor-stimulated Ca2+ uptake as well as by phospholipase C and IP3 via the release of Ca2+ from internal stores (Streb et al., 1983; Bumann et al., 1984; Van Haastert et al., 1989). Two classes of phosphodiesterases participate in intracellular cGMP degradation. Intracellular cGMP is degraded mainly by a cGMP-specific enzyme that is stimulated by cGMP at low concentrations. About 20% of intracellular cGMP is degraded by a less specific enzyme (Van Haastert et al., 1983). In summary, the cGMP response is controlled by a cGMP-stimulated phosphodiesterase and Ca2+-inhibited guanylyl cyclase, which is stimulated by a surface cAMP receptor that is subjective to adaptation. The contribution of each of these regulatory components to the final cGMP response is essentially unknown and can not easily be determined in experiments.

The kinetic values of nearly all biochemical reactions described above have been determined in previous experiments on the time scale of the cGMP response (seconds). To determine the contribution of receptor adaptation, Ca2+ inhibition of guanylyl cyclase and cGMP-stimulated phosphodiesterase activity to the final cGMP response we translated the observed reactions and kinetic values of all enzymes into a model. This model consists of five differential equations that describe the activated cAMP receptor, the changes in the concentration of cGMP, IP3 and Ca2+, and the activity of cGMP-specific phosphodiesterase, respectively. Different adaptation mechanisms were investigated, revealing that a specific adaptation regime is essential to describe the observed transient response. The model predicts that adaptation determines the appearance of the cGMP response curve, Ca2+ inhibition of guanylyl cyclase determines the magnitude of the response, whereas the cGMP stimulated phosphodiesterase determines the duration of the response. Finally the cGMP response in two signal transduction mutants was simulated by deleting phosphodiesterase activity and phospholipase C activity from the model; the predictions were similar to experimental data. We conclude that the model describes experimental data, suggesting that the main structural and regulatory elements of cGMP metabolism are included into the model.

**MATERIALS AND METHODS**

The relations between the different components that determine intracellular cGMP levels are presented in Figure 1. cGMP is degraded mainly by a cGMP-stimulated phosphodiesterase. Guanylyl cyclase produces cGMP; the enzyme is stimulated by an activated receptor (denoted by R*) and is inhibited by intracellular Ca2+ levels. The concentration of Ca2+ is controlled by receptor-stimulated IP3 levels and by receptor-stimulated Ca2+ uptake. The change of cGMP concentration is given by Eq. 1, where \( f \ SYN \) is the synthesis of cGMP and \( f \ DEG \) is its degradation.

\[
\frac{dc(GMP)}{dt} = f \ SYN - f \ DEG
\]

**cGMP Synthesis**

The enzyme guanylyl cyclase hydrolyzes guanosine 5’-triphosphate to cGMP. In *Dictyostelium* this enzyme is likely a membrane-associated
protein (Mato and Malchow, 1978; Janssens et al., 1989). The rate of cGMP synthesis is given by

\[ f_{\text{SYN}} = \left[ 1 - \frac{[\text{Ca}^{2+}]^{p}}{[\text{Ca}^{2+}]^{p} + K_{\text{f}}} \right] \delta + \epsilon [\text{R}^{*}] \]  

(1a)

where \( \eta \) is the fraction of guanylyl cyclase that is sensitive to Ca\(^{2+}\) inhibition. In vitro all guanylyl cyclase activity is sensitive to Ca\(^{2+}\) inhibition (\( \eta = 1 \)); in electrophoresis cells ~20% of guanylyl cyclase activity remains active in the presence of 1 mM Ca\(^{2+}\) (\( \eta = 0.8 \)) (Van Haastert, unpublished results). \( K_{\text{f}} \) is the concentration of Ca\(^{2+}\) that induces half-maximal inhibition (\( K_{\text{f}} = 200 \) nM); inhibition of guanylyl cyclase by Ca\(^{2+}\) is a competitive process with a Hill coefficient \( n = 2.3 \) (Janssens et al., 1989; Valkema and Van Haastert, 1992). \( \delta \) and \( \epsilon \) represent the enzyme activity of guanylyl cyclase in basal and receptor-activated state, respectively. The values of these constants have been measured and are given in Table 1.

**cGMP Degradation**

The hydrolysis of cGMP to 5'-GMP is performed by two cyclic nucleotide phosphodiesterase activities: a small phosphodiesterase activity hydrolyzing cAMP and cGMP at approximately the same rate and a large enzyme specific for cGMP (Chang, 1968; Van Haastert et al., 1983). cGMP stimulates the latter enzyme threefold by decreasing the \( K_{\text{m}} \) of the enzyme at an unaltered \( V_{\text{max}} \) (Bulgakow and Van Haastert, 1983). The activity of phosphodiesterases in the model is designated by the following equation:

\[ f_{\text{DEG}} = (1 - \theta) \frac{V_{C}}{[\text{cGMP}]} + \theta \frac{V_{C}}{[\text{cGMP}]} + \frac{K_{\text{m}}}{[\text{cGMP}]} + \frac{K_{\text{m}}}{[\text{cGMP}]} + \frac{V_{A}}{[\text{cGMP}]} \]  

(1b)

In this equation \( V_{C} \) and \( V_{A} \) are the \( V_{\text{max}} \) of the cGMP-specific and the nonspecific enzyme, respectively; \( K_{\text{m}} \) and \( K_{\text{m}} \) are the Michalis-Menten constants of the cGMP-specific enzyme in the low and high active form, respectively. \( K_{\text{m}} \) is the Michaelis-Menten constant of the nonspecific phosphodiesterase. \( \theta \) is the fraction of the cGMP specific enzyme in the activated state, which is given by

\[ \frac{d\theta}{dt} = k_{4}[\text{cGMP}](1 - \theta) - k_{-4}\theta \]  

(1c)

\( k_{4} \) and \( k_{-4} \) are allosteric rate constants of activation and deactivation of the cGMP-specific phosphodiesterase. Detailed studies of cGMP degradation have provided the values of all constants (Van Haastert and Van Lookeren Campagne, 1984), which are given in Table 1.

**Regulation of Intracellular Ca\(^{2+}\) Levels**

Calcium ions inhibit guanylyl cyclase activity. Stimulation of the cAMP receptor induces influx of extracellular Ca\(^{2+}\) (Bumann et al., 1984) and activates phospholipase C whereby phosphatidylinositol-bisphosphate is hydrolyzed to IP\(_{3}\) and diacylglycerol. IP\(_{3}\) liberates Ca\(^{2+}\) from nonmitochondrial internal stores (Europe-Finner and Newell, 1986a). The IP\(_{3}\) concentration is given by

\[ \frac{d[IP_{3}]}{dt} = \alpha + \beta R^{*} - \gamma [IP_{3}] \]  

(2a)

where \( \alpha \) and \( \beta \) are the basal and receptor-stimulated activity of phospholipase C, respectively (Bolina et al., 1994), and \( \gamma \) is the first order rate constant of IP\(_{3}\) degradation (Van Lookeren Campagne et al., 1988).

The Ca\(^{2+}\) concentration of the cytosol is described by:

\[ \frac{d[Ca^{2+}]_{\text{cytosol}}}{dt} = \frac{V_{C}}{K_{m}^{c} + [Ca^{2+}]_{\text{out}}} + \frac{V_{A}[Ca^{2+}]_{\text{out}}}{K_{m}^{a} + [Ca^{2+}]_{\text{out}}} - 2C + D \frac{[IP_{3}]^{k}}{[IP_{3}]^{k} + [IP_{3}]^{q}} [Ca^{2+}]_{\text{store}} - E[Ca^{2+}]_{\text{cytosol}} - F[Ca^{2+}]_{\text{cytosol}} \]  

(2b)

The first part of the equation denotes the plasma membrane channels that transport Ca\(^{2+}\) to the cytosol, which follow Michaelis-Menten kinetics. Activation of the receptor alters the \( V_{\text{max}} \) and the \( K_{\text{m}} \) of the transport. The values of these constants have been measured (Milne and Coulk, 1991) and are presented in Table 1.

The second part of the equation represents the IP\(_{3}\)-mediated release of Ca\(^{2+}\) from nonmitochondrial stores (Europe-Finner and Newell, 1986a). Details of this reaction have not been determined in Dictyostelium; we assume values of reaction constants, which have been measured in mammalian cells (Streb et al., 1987; Champeil et al., 1989). The Ca\(^{2+}\) concentration in the IP\(_{3}\)-sensitive store is assumed to be 1 mM. The release of Ca\(^{2+}\) from the store by IP\(_{3}\) is assumed to occur in a coopervative way, with a Hill coefficient \( M = 2 \) and a half-maximal activity at \( q = 1.10 \) μM.

The third part of the equation denotes the Ca\(^{2+}\) pump activity E back to the extracellular medium and F back to the intracellular store. In unstimulated cells the influx of Ca\(^{2+}\) from the extracellular medium equals the efflux:

\[ \frac{V_{C}[Ca^{2+}]_{\text{out}}}{K_{m}^{c} + [Ca^{2+}]_{\text{out}}} = E[Ca^{2+}]_{\text{cytosol}} \]  

(2c)

Assuming a basal cytosolic Ca\(^{2+}\) concentration of 5 × 10\(^{-4}\) M (Abel et al., 1988) and an extracellular Ca\(^{2+}\) concentration of 10 μM (Bumann et al., 1984) implies \( E = 6 \) s\(^{-1}\). In unstimulated cells the influx from the extracellular Ca\(^{2+}\) store equals the flux of Ca\(^{2+}\) ions pumped back in this store yielding \( F = 6 \) s\(^{-1}\).

**Activation and Adaptation of the Surface cAMP Receptor**

Binding of cAMP to the surface receptor induces the accumulation of cGMP levels. The response is transient with maximal cGMP levels at 10 s and a recovery of basal cGMP levels after 30 s, even during persistent stimulation with cAMP. Partial desensitization could be provided by the Ca\(^{2+}\)-mediated inhibition of guanylyl cyclase and cGMP-stimulation of phosphodiesterase; this will be investigated in a model called simple adaptation. Several experiments suggest that desensitization is mediated by adaptation occurring at the level of the cAMP receptor (Van Haastert and Van der Heijden, 1983; Van Haastert, 1987b). Therefore alternative models were analyzed for different adaptation regimes.

**Simple Adaptation.** The binding of cAMP to the receptor is a simple bimolecular reaction, and the occupied receptor remains in the activated state (Scheme 1). Adaptation does not occur at the receptor, but intracellularly at the level of cGMP synthesis or degradation. The differential equation for the occupied activated receptor R\(^{L}\) is

\[ \frac{dR^{L}}{dt} = k_{4}[cAMP](1 - R^{L}L) - k_{-4}R^{L} \]  

(3a)

**Linear Adaptation.** This model introduces the adapted occupied receptor state R\(^{0}\)L, which is formed from the activated occupied receptor R\(^{L}\) (Scheme 2). The differential equation for the activated occupied receptor R\(^{L}\) and for the occupied receptor R\(^{0}\)L are

\[ \frac{dR^{L}}{dt} = k_{4}[cAMP](1 - R^{L} - R^{0}L) - k_{-4}R^{L} + k_{2}R^{L}L + k_{3}R^{L}L \]  

\[ \frac{dR^{0}L}{dt} = k_{2}R^{L}L - k_{3}R^{0}L \]  

(3b)
Box-model. The receptor-box model is based on a study on the activation of adenyl cyclase in *Dictyostelium* (Goldbeter and Koshland, 1982; Knox et al., 1986). The model assumes two interconvertible forms of the receptor R0 and R1, respectively. Each form of the receptor can be associated with the ligand cAMP, yielding R°L and R1L, respectively (Scheme 3). All four receptor states possess a specific activity a. The total receptor activity R is denoted as follows:

\[
R^* = a_1R^* + a_2R^*L + a_3R^*L + a_4R^D
\]  

Experimental data indicate that the association of ligand to the receptor is much faster than the interconversion between the receptor forms, thus:

\[
\frac{dR^D}{dt} = k_3R^2 - k_5R^D
\]

\[
\frac{dR^*L}{dt} = k_5R^*L[cAMP] - k_3R^*L[cAMP]
\]

Cycle-model. The cycle-model describes the adaptation process as a series of sequential interconversions of receptor forms. This model was based on kinetic studies of the interaction between cAMP and a subpopulation of receptors that are supposed to be involved in the activation of guanylyl cyclase (Van Haastert et al., 1986) (Scheme 4). cAMP binds reversibly to the receptor, yielding RL. This receptor form converts with the rate k4 to the activated state of the receptor R°L. k2 is not a constant, but declines with time according k2 = 0.22e-°7 s-1. The active receptor R°L then converts to a desensitized state R°L with a rate constant k4 = 0.17 s-1. R°L slowly converts back to the inactive receptor RL with k0 = 7.3 × 10-3 s-1 (Van Haastert et al., 1986; Van Haastert, 1987b). The differential equations for the different receptor forms are

\[
\frac{dR^*L}{dt} = k_1RL - k_3R^*L
\]

\[
\frac{dR^*L}{dt} = k_0R^*L - k_3R^*L
\]

\[
\frac{dRL}{dt} = k_3[cAMP](1 - RL - R°L - R°L) - k_4RL - k_5RL
\]
### Table 1. Kinetic values of enzymes involved in the cAMP-induced cGMP response in *Dictyostelium discoideum*

<table>
<thead>
<tr>
<th>Constant</th>
<th>Unit</th>
<th>Value</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\eta$</td>
<td></td>
<td>0.8</td>
<td>Fraction of GuCy that is sensitive for $\text{Ca}^{2+}$</td>
<td>[1]</td>
</tr>
<tr>
<td>$n$</td>
<td></td>
<td>2.3</td>
<td>Hill-coefficient of inhibition of GuCy by $\text{Ca}^{2+}$</td>
<td>[2, 3]</td>
</tr>
<tr>
<td>$K_0$</td>
<td>M</td>
<td>$2 \times 10^{-7}$</td>
<td>[Ca$^{2+}$] giving half-maximal inhibition of GuCy</td>
<td>[2, 3]</td>
</tr>
<tr>
<td>$\delta$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$4.0 \times 10^{-8}$</td>
<td>Basal activity of GuCy</td>
<td>[4]</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$1.7 \times 10^{-6}$</td>
<td>Activity of stimulated GuCy</td>
<td>[4]</td>
</tr>
<tr>
<td>$V_G$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$2 \times 10^{-6}$</td>
<td>Hydrolytic activity of cGMP-specific PDE</td>
<td>[5]</td>
</tr>
<tr>
<td>$V_{IH}$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$2 \times 10^{-7}$</td>
<td>Hydrolytic activity of nonspecific PDE</td>
<td>[6]</td>
</tr>
<tr>
<td>$K_{aL}$</td>
<td>M</td>
<td>$5.4 \times 10^{-6}$</td>
<td>$K_m$ of activated cGMP-specific PDE</td>
<td>[5]</td>
</tr>
<tr>
<td>$K_{aH}$</td>
<td>M</td>
<td>$2.4 \times 10^{-5}$</td>
<td>$K_m$ of basal cGMP-specific PDE</td>
<td>[5]</td>
</tr>
<tr>
<td>$K_{a'}$</td>
<td>M</td>
<td>$2.5 \times 10^{-6}$</td>
<td>$K_m$ of nonspecific PDE</td>
<td>[6]</td>
</tr>
<tr>
<td>$k_2$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$1.4 \times 10^{-5}$</td>
<td>Rate constant of activation of cGMP-specific PDE</td>
<td>[5]</td>
</tr>
<tr>
<td>$k_4$</td>
<td>s$^{-1}$</td>
<td>$2.0 \times 10^{-2}$</td>
<td>Rate constant of deactivation of cGMP-specific PDE</td>
<td>[5]</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$7.5 \times 10^{-8}$</td>
<td>Basal activity of PLC</td>
<td>[7]</td>
</tr>
<tr>
<td>$\beta$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$7.5 \times 10^{-7}$</td>
<td>Activity of stimulated PLC</td>
<td>[7]</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>s$^{-1}$</td>
<td>$7.0 \times 10^{-1}$</td>
<td>Rate of degradation of IP$_3$</td>
<td>[8]</td>
</tr>
<tr>
<td>$V_L$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$4.8 \times 10^{-6}$</td>
<td>$V_{\text{max}}$ of unstimulated $\text{Ca}^{2+}$ channel</td>
<td>[9]</td>
</tr>
<tr>
<td>$V_H$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$1.04 \times 10^{-5}$</td>
<td>$V_{\text{max}}$ of stimulated $\text{Ca}^{2+}$ channel</td>
<td>[9]</td>
</tr>
<tr>
<td>$K_{P_{el}}$</td>
<td>M</td>
<td>$1.15 \times 10^{-4}$</td>
<td>$K_m$ of unstimulated $\text{Ca}^{2+}$ channel</td>
<td>[9]</td>
</tr>
<tr>
<td>$K_{P_{hi}}$</td>
<td>M</td>
<td>$1.85 \times 10^{-5}$</td>
<td>$K_m$ of stimulated $\text{Ca}^{2+}$ channel</td>
<td>[9]</td>
</tr>
<tr>
<td>$C$</td>
<td>s$^{-1}$</td>
<td>$3 \times 10^{-4}$</td>
<td>Basal $\text{Ca}^{2+}$ release from IP$_3$-sensitive store</td>
<td>[10]</td>
</tr>
<tr>
<td>$D$</td>
<td>s$^{-1}$</td>
<td>$3 \times 10^{-3}$</td>
<td>As C, activated by IP$_3$</td>
<td>[10]</td>
</tr>
<tr>
<td>$E$</td>
<td></td>
<td>6</td>
<td>Rate of $\text{Ca}^{2+}$ pump from cytosol to extracellular</td>
<td>[10]</td>
</tr>
<tr>
<td>$F$</td>
<td>s$^{-1}$</td>
<td>6</td>
<td>Rate of $\text{Ca}^{2+}$ pump from cytosol to store</td>
<td>[10]</td>
</tr>
<tr>
<td>$M$</td>
<td></td>
<td>2</td>
<td>Hill-coefficient of $\text{Ca}^{2+}$ channel for IP$_3$</td>
<td>[10]</td>
</tr>
<tr>
<td>$q$</td>
<td>M</td>
<td>$7 \times 10^{-7}$</td>
<td>$K_m$ of $\text{Ca}^{2+}$ channel for IP$_3$</td>
<td>[10]</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{out}}$</td>
<td>M</td>
<td>$10^{-5}$</td>
<td>$[\text{Ca}^{2+}]$ outside the cell</td>
<td>[11]</td>
</tr>
<tr>
<td>$[\text{Ca}^{2+}]_{\text{in}}$</td>
<td>M</td>
<td>$10^{-6}$</td>
<td>$[\text{Ca}^{2+}]$ inside the store</td>
<td>[12]</td>
</tr>
<tr>
<td>$k_{i_1}$</td>
<td>M$^{-1}$ s$^{-1}$</td>
<td>$2 \times 10^{7}$</td>
<td>Rate constant of association of cAMP to receptor R</td>
<td>[12]</td>
</tr>
<tr>
<td>$k_{i_2}$</td>
<td>s$^{-1}$</td>
<td>$7 \times 10^{-1}$</td>
<td>Rate constant of dissociation of cAMP receptor complex</td>
<td>[14]</td>
</tr>
<tr>
<td>$k_3$</td>
<td>s$^{-1}$</td>
<td>$1.7 \times 10^{-1}$</td>
<td>Converting rate constant from R<em>L to R</em>L</td>
<td>[12]</td>
</tr>
<tr>
<td>$k_2$</td>
<td>s$^{-1}$</td>
<td>$7.3 \times 10^{-3}$</td>
<td>Converting rate constant from R<em>L to R</em>L</td>
<td>[12]</td>
</tr>
<tr>
<td>$k_5$</td>
<td>s$^{-1}$</td>
<td>$5.78 \times 10^{-4}$</td>
<td>Converting rate constant of R<em>L to R</em>L</td>
<td>[13]</td>
</tr>
<tr>
<td>$k_3$</td>
<td>s$^{-1}$</td>
<td>$5.2 \times 10^{-3}$</td>
<td>Converting rate constant of R<em>L to R</em>L</td>
<td>[13]</td>
</tr>
<tr>
<td>$k_4$</td>
<td>s$^{-1}$</td>
<td>$1.6 \times 10^{-1}$</td>
<td>Converting rate constant of R<em>L to R</em>L</td>
<td>[13]</td>
</tr>
<tr>
<td>$k_{a_4}$</td>
<td>s$^{-1}$</td>
<td>$1.73 \times 10^{-2}$</td>
<td>Converting rate constant of R<em>L to R</em>L</td>
<td>[13]</td>
</tr>
<tr>
<td>$a_1$</td>
<td>s$^{-1}$</td>
<td>$1.0 \times 10^{-3}$</td>
<td>Specific activity of R$^L$</td>
<td>[13]</td>
</tr>
<tr>
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<td>s$^{-1}$</td>
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<td>[13]</td>
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<tr>
<td>$a_3$</td>
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<td>$1.9 \times 10^{-4}$</td>
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<tr>
<td>$a_4$</td>
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<tr>
<td>$K_R$</td>
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<td>$1.5 \times 10^{-1}$</td>
<td>Dissociation constant of R*L</td>
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</tr>
<tr>
<td>$K_D$</td>
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<tr>
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<td>[14]</td>
</tr>
<tr>
<td>$k_4$</td>
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<td>$7.3 \times 10^{-3}$</td>
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<td>[14]</td>
</tr>
<tr>
<td>$k_5$</td>
<td>s$^{-1}$</td>
<td>$1.7 \times 10^{-3}$</td>
<td>Converting rate constant from R*L to RL</td>
<td>[14]</td>
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</tbody>
</table>


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### The Box-adaptation Model

The model is based on experimental observations of cAMP-binding to surface receptors that are supposed to interact with adenyl cyclase in *Dictyostelium* (Knox et al., 1986). cAMP can interact with two interconvertible forms of the receptor; each of the occupied and unoccupied receptor forms possesses different activity. Simulation of the box-adaptation model reveals complete adaptation of the cGMP response at each stimulus concentration (Figure 2D). The model predicts that the kinetics of the cGMP

$$\text{CAMP} + R \overset{k_{-1}}{\underset{k_1}{\rightleftharpoons}} R^L$$

Scheme 1.

$$\text{CAMP} + R \overset{k_{-2}}{\underset{k_2}{\rightleftharpoons}} R^L$$

Scheme 2.
response alters at different concentrations of the cAMP stimulus: at higher stimulus concentrations the response increases and returns to basal levels faster than at lower stimulus concentrations. This has not been observed for the cAMP-stimulation of guanylyl cyclase (see Figure 2A). Furthermore, the model predicts that the rate of cGMP increase is maximal immediately after cAMP addition (Figure 2D), whereas experimental observations reveal a 1-s lag period between cAMP addition and the increase of cGMP levels (see Figure 2A, inset) (Van Haastert, 1987a). Although the box-adaptation model predicts perfect adaptation, several properties of the predicted response are not in agreement with experimental observations for the cGMP response.

**The Cycle-adaptation Model.** This model is based on experimental observations on the binding of cAMP to a subpopulation of surface cAMP receptors that are supposed to be involved in the activation of guanylyl cyclase (Van Haastert, 1987b). cAMP binds reversibly to the inactive receptor R, which sequentially converts to an active form R* and to a desensitized form R0, which slowly recovers to the inactive receptor R. The rate constants of these interconversions have been measured (Van Haastert, 1987b). The cycle-model predicts a response, which shows perfect adaptation (Figure 2E). Furthermore, the kinetics of the response is independent of the cAMP stimulus concentration. Finally, the predicted response exhibits a short delay before the cGMP concentration rises rapidly to a peak at 8–10 s; basal levels are recovered at 30 s after stimulation.

Considering these data we conclude that the cycle-adaptation model fits best with experimental observations. Therefore this cycle-model was used to perform the following experiments on the role of cGMP-phosphodiesterase and intracellular Ca2+.

cGMP Degradation

Intracellular cGMP is hydrolyzed by two cyclic nucleotide phosphodiesterases: a nonspecific phosphodiesterase with low activity and a cGMP-specific cGMP phosphodiesterase with high activity (Van Haastert et al., 1983). The latter enzyme is stimulated about threefold by cGMP with a half-time of ~20 s (Van Haastert and Van Lookeren Campagne, 1984). The role of the cGMP-specific phosphodiesterase for the receptor-stimulated cGMP response was studied by simulating the absence of cGMP specific enzyme activity (Vc = 0) or by simulating an enzyme that can not be activated by cGMP (kx = 0). The results (Figure 3A) reveal in both cases that the cGMP response is increased and prolonged. When cGMP can not activate the enzyme, cGMP peak levels are increased with a factor 1.7 relative to the response with normal phosphodiesterase; the cGMP peak is reached at 14 s and basal levels are recovered after 50 s. When the enzyme is absent, the cGMP response is enhanced with a factor of 3.5 relative to the control response; the peak is reached after 22 s, and basal levels do not recover within 100 s.

A Dictyostelium mutant *stmF* has been isolated that lacks the cGMP-specific phosphodiesterase (Ross and Newell, 1981; Van Haastert et al., 1982). The cAMP-mediated cGMP response in this mutant (Figure 3B) closely resembles the calculated cGMP levels: a prolonged and increased response, with recovery of the basal cGMP levels at 100–120 s after stimulation.

**Intracellular Ca2+ Levels**

Guanylyl cyclase in *Dictyostelium* is strongly inhibited by intracellular Ca2+ ions with half-maximal inhibition at 200 nM and a Hill coefficient of 2.3 (Van Valkema and Van Haastert, 1992). Cytosolic Ca2+ concentrations are regulated in a complex manner that are not completely understood in *Dictyostelium*. In the model we have incorporated experimental data on the cAMP surface receptor-mediated uptake of Ca2+ and on the release of Ca2+ from intracellular stores by IP3 that is produced by receptor stimulated phospholipase C. The role of Ca2+ was investigated by simulating the absence of phospholipase C activity and modifying Ca2+ concentrations in the extracellular medium or in the intracellular stores.

Removal of phospholipase C activity from the model predicts a cGMP response that is only 1.2-fold higher than the response of cells that do possess phospholipase C activity (Figure 4A). This calculated response can be compared with experimental observations on strain HD10, which was obtained by disruption of the *Dictyostelium* phospholipase C gene; in this mutant cAMP...
Figure 2. Time course of cGMP formation upon stimulation with different cAMP concentrations. (A) Experimental observations, cAMP = 2 × 10^{-9} M (■), 10^{-8} M (▲), 10^{-7} M (▲), 10^{-6} M (○). Inset: Kinetics of excitation of cGMP response, cAMP = 10^{-7} M (redrawn from Van Haastert, 1987a). (B–E) Time course of cGMP formation in computer simulations according to different receptor models: simple-adaptation, (B); linear-adaptation, (C); box-adaptation, (D); circle-adaptation, (E). The concentrations of cAMP are (1) 10^{-8} M, (2) 10^{-7} M, (3) 10^{-6} M.
induces the nearly normal cGMP accumulation (Drayer et al., 1994). This suggests that the receptor-mediated activity of phospholipase C and subsequent expected release of Ca²⁺ does not significantly contribute to the cGMP response.

The removal of extracellular Ca²⁺ predicts a cGMP response that is 1.6-fold higher than the response of control cells (Figure 4A). Total depletion of Ca²⁺ inside and outside the cell gives a response that is 1.9-fold higher than the normal response. In both cases of changing the Ca²⁺ concentration, the kinetics of the response are unaltered; i.e., the peak is reached at the same time and cGMP levels recover with the same rate. When a constant intracellular Ca²⁺ concentration of 10⁻³ M is applied to the model, basal cGMP levels are reduced about fourfold and cAMP induces only a small cGMP response (~35% of the normal response; Figure 4A). Experimental observations with electroporated cells in Ca²⁺-free buffer (HEPES/5.9 mM EGTA) show a large cGMP increase upon stimulation with cAMP (Figure 4B). Electroporated cells in the presence of 1 μM or 1 mM Ca²⁺ have reduced basal cGMP levels and show only a slight increase in cGMP levels after cAMP stimulation (Figure 4B).

**DISCUSSION**

Extracellular cAMP is a chemoattractant for Dictyostelium cells, inducing cell aggregation and differentiation. Cells are stimulated by a wave of cAMP that is emitted from the aggregation center. As the wave approaches the cell, the cAMP gradient has two characteristics: the cAMP concentration increases with time and the gradient points towards the aggregation center, leading the
cell in this direction. When the maximal cAMP concentration of the wave passes the cell, both the spatial and temporal component of the cAMP gradient reverse: the direction of the gradient points away from the aggregation center and the cAMP concentration decreases with time. If cells would respond to this concentration gradient, they would move away from the aggregation center. Observations reveal that cells show directed movement on the rising flank of the cAMP wave and random movement after the wave has passed the cells (Alcantara and Monk, 1974). Dictyostelium cells extend pseudopods in the direction of the gradient within a few seconds upon stimulation with cAMP (Gerisch et al., 1975). Rapid excitation in combination with perfect and rapid adaptation of the signal transduction cascade could explain the observations on directed cell movement when a cAMP wave passes the cells (Van Haastert, 1983b).

Chemotaxis is a complex reaction combining temporal and spatial information of the cAMP gradient. Several experiments suggest that the second-messenger cGMP has an important function during chemotaxis. First, the kinetics of excitation and adaptation of the cGMP response are in good agreement with the kinetics of pseudopod formation during chemotaxis. Second, stmF mutants lacking a cGMP phosphodiesterase, show an enhanced cGMP response and prolonged chemotactic movement (Ross and Newell, 1981; Van Haastert et al., 1982). Third, nonchemotactic mutants have recently been isolated that do not respond to chemoattractants that are detected by different surface receptors; these Ki mutants have a defect in the central sensory transduction cascade shared by different chemoattractants (Kuwayama et al., 1993). Biochemical analysis reveals that most mutants show an altered cGMP response, varying from no guanylyl cyclase activity to an altered balance between excitation and adaptation of cGMP formation.

The cAMP-mediated cGMP response in Dictyostelium is composed of a network of activation and adaptation of surface cAMP receptor, activation of guanylyl cyclase, inhibition of this enzyme by Ca$^{2+}$ ions, and cGMP stimulation of a cGMP-specific phosphodiesterase. We have studied the enzymes that are involved in the formation of the cGMP response. To understand the function of each of the components that participate in the cGMP response in relation to chemotaxis, a computer model for simulation experiments was designed, which is based almost entirely on experimental data. Detailed kinetic analysis of the components provides the framework for the model.

Four models on the adaptation of the receptor were investigated. Each model predicts different dynamics of the cGMP response, and only one model is in sufficient agreement with experimental data. This cyclic-adaptation model is based on observations on the interaction between cAMP and a subpopulation of receptors supposed to be coupled to the activation of guanylyl cyclase (Van Haastert et al., 1986; Van Haastert, 1987b). The less favorable box-adaptation model was proposed for the adaptation of adenyl cyclase predicts different kinetics of the cGMP response at different stimulus concentrations, which have not been observed experimentally. Two other more simple adaptation models, the simple-adaptation model and the linear-adaptation model, show insufficient adaptation. Although the cyclic-adaptation model predicts all aspects of the cGMP response, its biochemical background is not completely understood. The interconversions of receptor forms that are observed in vivo can be induced by guanine nucleotides in vitro, suggesting that they are related to the altered interaction of the activated receptors with G-proteins.

The role of activation of cGMP-phosphodiesterase by cGMP was investigated in the model as well as experimentally. The model predicts that cGMP-phosphodiesterase affects both the magnitude and especially the duration of the cGMP response. Previous experiments with mutant stmF, which lacks the cGMP-phosphodiesterase, support the conclusions of the model. This suggests that cGMP-phosphodiesterase functions by rapidly attenuating the cGMP response, even before guanylyl cyclase activity has completely recovered basal levels due to adaptation. During cell aggregation the cAMP concentration gradient directs movement to the aggregation centre for ~1.5 min, which is the period that the cAMP concentration increases with time. In mutant stmF the cAMP gradient has probably the same concentration profile, but cells respond to the gradient for nearly 3 min. This suggests that cells continue to move in the same direction as long as cGMP levels are elevated and that the function of the phosphodiesterase is to immediately erase the information contained in the cGMP response as soon as the cAMP concentration is no longer increasing with time.

Dictyostelium guanylyl cyclase is strongly regulated by nanomolar Ca$^{2+}$ concentrations. Because the occupied surface cAMP receptor stimulates both guanylyl cyclase and an increase of cytosolic Ca$^{2+}$ levels (Saran et al., 1994) (via Ca$^{2+}$ uptake and possibly via IP3-mediated release from internal stores), the exact regulation of cGMP levels upon stimulation are not easily understood. Experiments on the effect of Ca$^{2+}$ on cGMP levels in electropermeabilized cells reveal that Ca$^{2+}$ reduces both basal and receptor-stimulated cGMP levels, but has no strong effect on the duration of the response (Valkema and Van Haastert, 1992). The model predicts essentially this outcome, except that the effects of Ca$^{2+}$ are stronger in the model than in the experiment. This notion is especially valid for the effect of removing extracellular Ca$^{2+}$ and deletion of phospholipase C. In the model this will result in 1.7- and 1.3-fold increase of the response, but experiments reveal little effect of removing extracellular Ca$^{2+}$ (Valkema and Van Haastert,
In this study the dynamics of the cGMP response in time were investigated. Because chemotaxis combines temporal and spatial information of chemoattractant concentration, the next step will be to analyze the spatial distribution of cGMP during chemotactic movement. Unfortunately, cGMP levels can not be measured yet in single cells, leaving only calculations to provide some insight in this process. The present investigations suggest that the main components that affect the kinetics of cGMP response have been identified. This information is now to be combined with estimated values for the spatial distribution of receptors, guanylyl cyclase and phosphodiesterase, and with diffusion of cGMP, IP<sub>3</sub> and Ca<sup>2+</sup> inside the cell.

Summarizing we conclude that receptor adaptation is responsible for the kinetics of the cGMP response. The activity of cGMP-stimulated cGMP-specific phosphodiesterase controls the magnitude and especially the duration of the cGMP response. The regulation of the guanylyl cyclase activity by Ca<sup>2+</sup> ions gives Dictyostelium the opportunity for fine tuning of the cGMP response.

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