The Cyclic AMP Stimulus Sensed by *Dictyostelium* Cells During Aggregation : A Theoretical Estimate

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SUMMARY

Using known experimental data, we have made a theoretical investigation of the strength of the cyclic AMP stimulus acting on single cells of Dictyostelium discoideum during argregation. This strength is assumed to be some function of the number of cyclic AMP receptors bound on the cell surface. Our basic finding is that this stimulus is at all times weak, and in the case of chemotaxis, very close to the threshold for response. Implications of this finding are discussed.

1. INTRODUCTION

Single amoebae of the cellular slime mold Dictyostelium discoideim aggregate after being starved of food; this aggregation is known to be due to chemotaxis (Bonner 1967), and the chemotactic agent, cyclic AMP (cAMP), is synthesised and released by the cells themselves (Konijn, van de Meene, Bonner and Barkley, 1967). Aggregation of Dictyostelium amoebae is probably the most primitive example of social behaviour in a eukaryote; this social behaviour is largely a consequence of the chemotactic response of the amoebae. Therefore Dictyostelium cells are ideal both for studying a simple sensory system as well as the implications of such a system for the development of cooperative behaviour in a group of cells. Experimentally measured profiles of intra- and extra-cellular cAMP levels show a temporal periodicity (Gerisch et al., 1977). This periodicity is thought to be the cause of the observed oscillatory pattern of aggregation. After its release by a cell, cAMP diffuses in the intercellular medium - 2 percent agar in phosphate buffer under standard conditions - and is sensed by another amoeba by means of specific receptors on its outer surface (Malchow and Gerisch, 1974; Green and Newell, 1975). In addition to diffusion, cAMP levels are depleted by cell-bound as well as extracellular phosphodiesterases. There are reasons for believing that the extracellular phosphodiesterase does not play a

significant role in modulating cAMP levels during aggregation (Malchow et al. 1975, Nanjundiah and Makhow, 1976). The physiological response of a cell to cAMP is thought to be mediated by the number of cell-surface receptors which are bound at any time. Since the cAMP released by a cell should also bind to its own surface receptors, it is clear that a degree of self-stimulation must occur. External cAMP is believed to affect a cell in at least two ways : (1) if present in a spatially non-uniform level, the cell moves towards increasing concentrations (chemotaxis); and (2) a time-varying level of external cAMP can stimulate a cell to synthesise and release cAMP on its own (relay).(Roos et al., 1975; Shaffer, 1975).

Because the release of cAMP by a cell is likely to be isotropic, self-stimulation can result in one or both of two things: (a) it can block receptors and thus decrease the sensitivity of a cell to external stimuli (refractoriness), or (b) enhance intracellular cAMP synthesis and subsequent release. In fact, such self-stimulation may even explain cAMP oscillations (unpublished calculations). Possibility (a) is interesting to investigate, since it is known that following a cAMP stimulus, some of the cell's responses, which are correlated with its oscillatory behaviour, are inhibited for a short time (Malchow et al., 1978a).

In this article, an attempt will be made to answer the following questions :

1. How much of the cAMP released by a cell is bound to its own receptors ?
2. Can this in any way account for the refractoriness mentioned above ?
3. What is the temporal profile of cAMP detected by a cell when the source is another cell a certain distance away ?
4. Are there circumstances in which inherent variability in binding (i.e. 'noise') affects the response of a cell to an external cAMP stimulus ?

The method of approach is a combination of analytical techniques and computer analysis, discussed below.

2. THE MODEL

(a) Self-stimulation

(i) Description of the model : Aggregation-competent cells of Dictyostelium discoideum are on a substratum of 2 percent agar ; each cell is a sphere. Cyclic AMP released by a cell diffuses within and on the agar. Dictyostelium cells have a class of high-affinity receptors for cAMP as well as a class of low-affinity receptors (Green and Newell, 1975; Mullens and Newell 1978). Binding of cAMP to each is a simple bimolecular reaction; dissociation of the cAMP-receptor complex is a first-order reaction.

Cyclic AMP is hydrolysed by the cell-bound phosphodiesterase. If one assumes that all the cells in a well-shaken population release cAMP in phase with one another, it can be shown that the release profile is approximately sinusoidal (Unpublished calculations, based on results given in Gerisch and Wick, 1975). Recent results of Devreotes et al. (1979a, 1979b) involving measurements on continuously perfused cells resting on a substratum lead support to the view that the release of cAMP is indeed gradual. On the other hand, if in such an experiment the release occurs in cascades, the individual profile can be much sharper. Our model makes allowance for this by investigating the consequence of both a simple sinusoidal profile as well as sharper (but still symmetric) ones.

The analytical scheme is as follows:

$$\frac{\partial c}{\partial t} = D \nabla^2 C \,, \quad \text{for} \quad |\bar{r}| > a$$

$$-\frac{\partial c}{\partial r} \cdot 4\pi a^2 = \dot{n} - \lambda c - k_1 c N_f + k_{-1} N_b, \quad \text{at} \quad |\bar{r}| = a$$

and

$$\frac{dN_b}{dt} = +k_1 C N_f - k_{-1} N_b \,, \text{ also at } \quad |\bar{r}| = a \qquad (1)$$

Here $c(r,t)$ is the concentration of cAMP at a time t and position r (measured from the centre of the cell). The other parameters in equation (1) are explained in Table 1; the same table also gives their numerical values. The aim of this model is to obtain an expression for the time-dependence of the number of bound receptors N_b, given the manner in which the release profile $\dot{n}$ varies in time.

(ii) Simplifying assumptions

There are two useful approximations that one can make in attempting to solve equations (1), and both of them give upper limits to N_b. The first approximation is to assume that $N_f \gg N_b$, implying that at any time the number of bound receptors is a small fraction of the total number. The motivation for this assumption is as follows. With the values used by us, a typical time for the equilibration of cAMP binding to the receptors is of the order of 3.4 sec. (from $k_{-1} = 0.29$ sec^{-1} for the high affinity receptor). There are two other time constants in the problem: one is set by the rate of cAMP release from a cell, and the other by diffusion of cAMP into the extracellular medium. The first time-constant is of the order of 75 sec. for a pure sinusoidal release profile to 28 sec. for the sharpest profile considered by us. The time-constant due to diffusion is of the order of 4 msec,

and is significantly smaller than the others. The point is that within the parameters of this problem, a significant amount of released cAMP diffuses away before it can bind to the cell-surface receptors (Nanjundiah, 1978), and therefore at any given time, only a small fraction of the receptors is bound. The other approximation that can be made is to independently work out the distribution of cAMP by diffusion, and having done so, to estimate the number of bound receptors from the relation

$$k_1 N_f C = k_{-1} N_b \qquad (2)$$

The basis for this assumption is also the existence of widely differing time-scales mentioned earlier. Crudely speaking, within a very short time diffusion determines the spatial distribution of cAMP in the neighbourhood of a cell, and over longer times, this distribution determines how much cAMP is bound to the receptors. Over a yet longer time, the spatial profile itself changes on account of changes in release rate.

It must be emphasised that both the assumptions lead to overestimates for the number of bound receptors, and therefore between the two estimates, we choose the lower one as being more reliable.

(b) cAMP Profile due to a Distant Source

(i) Description of the model

A point source centred at the origin is assumed to

release cAMP at a rate of $\dot{n}$ molecules per unit time. This cAMP diffuses within and on a substrate. The receiver is a cell whose centre is situated at a distance r from the source. The equation for the variation of the cAMP concentration is

$$\frac{\partial c}{\partial t} = D\nabla^2 c + \dot{n}(t)\,\delta(r) \qquad (3)$$

where $\delta(r)$ is the Dirac delta function (Lighthill, 1970) and the other symbols have the same meaning as before. Solving this equation for c and using the second of the approximate methods mentioned in the previous section, we estimate the number of receptors of each type which bind cAMP on the second cell.

(ii) Simplifying assumptions

Our values for binding will once again be over-estimates, since we ignore extracellular phosphodiesterase. The effect of enzyme hydrolysis at the source is taken into account by scaling the result obtained by this method (when r = the cell radius) with the result obtained by the previous method.

3. RESULTS

(a) Self-stimulation

The analytical solution of equations (1), after making

the simplifying assumptions, is

$$N_b(t) = \frac{2\gamma\, k_1 N_o}{\beta^2 + k_{-1}} \int_0^t d\tau\, \dot{n}\,(t-\tau)\, f(\tau), \qquad (4)$$

with N_o = the total number of receptors on the cell surface,

$$f(\tau) = \exp(-k_{-1}\,\tau)\,[\beta - i\sqrt{k_{-1}}\,\operatorname{erf}(i\sqrt{k_{-1}\tau})]$$

$$- \beta \exp(\beta^2\tau)\ \operatorname{erfc}(\beta\sqrt{\tau}), \qquad (5)$$

$$\gamma = \frac{1}{4\pi a^2\sqrt{D}} \quad \text{and} \quad \beta = \gamma\,(\lambda + 4\pi a D).$$

The source release profile $\dot{n}$ is taken to be

$$\dot{n}(t) = \text{constant}\ \sin^n\left[\frac{\pi t}{T}\right] \qquad (6)$$

with $n = 2, 4, 8, 16$, and the value of the constant determined by using the result $\eta = \int_0^T \dot{n}\, dt$, where η is the total number of molecules released in one period. Using the higher powers of the sine effectively makes a single pulse more and more sharp, as illustrated in Figure 1. The integral involved in equation (4) was evaluated numerically on a DEC-1090 computer by using a 32-point Gaussian quadrature (Krylov, 1962). The alternative method of

estimating N_b involved computing the cAMP concentration at the cell surface by

$$C(a,t) = 2\gamma \int_0^t \dot{n}(t-\tau)\left[\frac{1}{\sqrt{(\pi\tau)}} - \beta \exp(\beta^2\tau)\,\mathrm{erfc}(\beta\sqrt{\tau})\right] d\tau, \tag{7}$$

a result which also follows from equations (1). Having computed C, N_b is estimated on the basis of equation (2) as

$$N_b(t) = \frac{N_o\, C(a,t)}{C(a,t) + (k_{-1}/k_1)} \tag{8}$$

Figure 2 illustrates binding as a function of time for the broadest and sharpest source profiles considered by us and for binding to each of the receptor classes. Table 2 summarises our results by giving the peak values for binding and cAMP concentration at the surface for all the source profiles, both the receptor classes, and the two methods of estimation. In Table 2, figures marked with an asterisk indicate the more reliable of the two estimates.

(b) Stimulus from a distant source

The solution of equations (3) is

$$C(r,t) = \frac{1}{Dr\,\pi^{3/2}} \int_{r/\sqrt{4Dt}}^{\infty} \dot{n}\left(t - \frac{r^2}{4Dx^2}\right) \exp(-x^2)\, dx. \tag{9}$$

Since the source $\dot{n}$ is periodic, we can ignore initial

transients in computing integral (9)and get the steady-state concentration as

$$C(r,t) \simeq \frac{1}{D\, r\, \pi^{3/2}} \int_0^\infty \dot{n}\left(t - \frac{r^2}{4Dx^2}\right) \exp(-x^2)\, dx \qquad (10)$$

For all the release profiles considered by us, this integral can be evaluated exactly; in particular, if

$$\dot{n}(t) = \frac{\eta}{T}(1 - \cos \omega t), \qquad (11)$$

then

$$C(r,t) = \frac{\eta}{T} \frac{1}{2\pi Dr} [1 - \exp(-qr) \cos(\omega t - qr)], \qquad (12)$$

where $q = \sqrt{\pi/(DT)}$.

The result is similar for the other cases as well. The concentration of cAMP oscillates with the same frequency as the source, but the amplitude of oscillations gets damped exponentially with increasing distance from the source. The higher the frequency of oscillations at the source, the closerto the source does its effect get damped out.

Figure 3 gives the temporal profiles of cAMP concentrations and values for its binding to receptors at a cell 50 μm from the source. This is done both for a simple sinusoidal rate of release as well as a rate $\sin^{16}(\pi t/T)$

(Figure 1). Table 3 lists the mean and peak values of concentration as well as the maximum extent of its variation at different distances from the source.

4. DISCUSSION

(a) General remarks

We have made an estimate of the amount of cyclic AMP bound to cell-surface receptors of Dictyostelium amoebae at the stage of aggregation. All the figures that we have relate to an assumed release of 10^7 molecules of cAMP per cell within one period, a value inferred from the literature (Roos, et al., 1975; Gerisch and Wick, 1975; Devreotes, et al. 1979a,b). If a very much more liberal estimate is taken of the release, say 10^8 molecules, all the concentrations and binding values will have to be revised upwards. However, the change in these is unlikely to be by as much as a factor of 10; as already stated, the calculations in this paper are overestimates. Another factor related to the release of cAMP by a single cell, about which we have no direct information, is the temporal profile. The simplest possible assumption is that this profile is identical to the summed profile as measured in a synchronised population of oscillating cells; and the latter is sinusoidal, with a time-dependence $\sin^2(\pi t/T)$ for the rate of release or $\int_0^t dx \sin^2(\pi x/T)$ for the total number of molecules released. If one makes the plausible

assumption that the profiles of spontaneous and induced release are identical, the experiments of Devreotes et al. (1979, a and b) provide strong evidence for a broad profile. Their results indicate clearly that induced cAMP release is not an all-or-none event, and that it can continue for some minutes if the stimulus is maintained. However, the possibility is still open that the release of cAMP by a single cell can be much sharper in time (though at the same frequency) than the release by a population. Therefore we have also investigated this by considering a $\sin^{16}(\pi t/T)$ profile. The difference in the profiles is illustrated in Figure 1. Table 2 summarises some of the consequences of systematically sharpening the source profile while keeping the total number of molecules released in one 'pulse' fixed.

(b) Concentration profiles

Since the release of cAMP by the source is periodic, the time-dependence of cAMP concentration at any distance from the centre is also periodic; Figure 3 illustrates this for a distance of 50 μm. This periodicity is attained following an initial transient rise in concentration from zero. At a distance of 50 μm from the centre, the error made by neglecting transients is less than 6 percent at a time 100 sec. after signalling begins and less than 5 percent beyond 300 secs. Our estimates of cAMP concentration as well as binding ignore transients

and refer to times beyond 300 secs., that is, from the beginning of the second period. Apart from the fact that the cAMP signal at any point is periodic in time, its relative amplitude decreases exponentially with distance (equation (12), Table 3 and Figure 4). The scale of length over which this decrease is significant is of the order of $1/q = \sqrt{DT/\pi}$, which is about 300 μm. A point of interest to note is that other things being the same, the higher the frequency of signalling by the source, the smaller its 'range' (as measured by $1/q$).

At a given distance, the maximum concentration goes up and the minimum goes down with increasing sharpness of the source profile. The mean concentration at a distance stays the same, since it depends only on the total output from the source and the period of oscillations (Table 3). The critical cell density for aggregation to occur is 5.10^4 /cm^2, which implies a mean cell-cell separation of about 50 μm (Konijn and Raper 1961; Cohen and Robertson, 1971). Even at this separation, the effective stimulus is sufficient to bind, at best, only about 8 percent of the high-affinity receptors and 0.8 percent of the low-affinity receptors (Figure 3, $\sin^{16}(\pi t/T)$ release profile). The number of receptors bound to a cell works out to 130 and 88 respectively. Both of these are extremely small numbers, and, in fact, very closeto the estimated binding at the threshold for a chemotactic response (Mato, et al. 1975). Another way of making the

same point is to note that at a cell-cell separation of 50 μm , the peak concentration is only 8.4×10^{-10} M at at 200 μm , 1.2×10^{-10} M. Both figures are very close to the smallest concentrations of cAMP that elicit either a light-scattering response or a pH response (Gerisch and Hess, 1974; Malchow et al. 1978b). The implications of this for measurement of cAMP by the cells will be taken up in the accompanying article.

We have already mentioned that increasing the frequency of oscillations decreases the range of the source, in the sense that in the exponential term in equation (12),

$$q = \sqrt{\pi/DT}$$

increases with increasing period T or increasing frequency. Related to this is the observation that as aggregation proceeds and mean cell-cell separation decreases, the frequency of oscillations increases (Nanjundiah, 1976). One can show that at a given intercellular separation r , there is an optimal frequency at which the amplitude (= maximum - minimum) of oscillations is maximal. For a simple sinusoidal profile, the concentration varies as in equation (12), and the amplitude is

$$\frac{1}{\pi DT} \cdot \frac{\eta}{T} \cdot \exp(-\sqrt{\pi/DT}\, r) \qquad (13)$$

At a given r , this has a maximum as a function of T at

$$T = \frac{r^2}{4} \cdot \frac{\pi}{D} \qquad (14)$$

The commonly assumed average value of 5 min for the period corresponds to a distance of 600 μm , implying that if (a) a cell wishes to stimulate a second cell at this distance, and (b) the strength of the stimulus is measured by its amplitude then a period of 5 min will be optimal. The reported decrease in signal period from 10 min to 2 min during aggregation (Durston, 1974) would correspond in such an analysis to a cell-cell separation ranging from 873 μm to 390 μm. It should be stresses that the signal amplitude always increases as a cell approaches the centre.

A final point worth mentioning concerns the effect of sharpening the source profile on the range of the signal (Nanjundiah, 1973) , keeping the frequency fixed. Consider at one end of the spectrum an impulsive release, and at the other, a broad profile, say sinusoidal ($\sin(2\pi t/T)$). It follows from our results that the peak concentration due to a broad release profile is always higher beyond about 400 μm, given a period of 5 min.

c) Self stimulation

From the values given in Table 2, it is apparent that even at the sharpest profile considered by us ($\sin^{16}\frac{\pi t}{T}$) a substantial fraction of the high-affinity receptors and essentially all the low-affinity receptors on the cell surface stay free.

Therefore, the blocking of all of a cell's surface

receptors by its own release of cAMP cannot account for the phenomenon of refractoriness. However, it cannot be ruled out that in the unlikely eventuality that the signal is still sharper, the cell does succeed in blocking most of its high-affinity receptors. In the signal processing pathway, if the high-affinity receptors are involved in signal relay while the low-affinity ones are involved in chemotaxis, our results indicate how a cell can be refractory to relay for some time after being stimulated but essentially never refractory in responding chemotactically to an external gradient (Gerisch et al., 1975).

Another possibility, raised by us in the introduction, is that self-stimulation might in fact be a significant step in generating spontaneous cAMP oscillations. Our results lend support to this conjecture. The reason for saying this is that, it is only the high-affinity receptors that get bound to a significant extent, and also stay bound long enough, thus shutting off effective stimulus beyond a reasonable time (work in preparation). The possibility that self-stimulation or autocatalytic feedback plays a role in spontaneous cAMP oscillations has been discounted by Devreotes et al. (1979a,b). This is on the grounds that very large cAMP stimuli should inhibit any such feedback loops (simply by boocking further stimulation); what they actually observe is an accelerating release in response to such stimuli. However, in our opinion, this does not rule

out the feasibility of autocatalytic feedback loops operating at low stimulus levels, resulting in periodic cAMP release and eliciting a single prolonged response at high stimulus levels.

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LEGENDS TO TABLES

Table 1 — Values assumed for various parameters

Table 2 — A comparison of different source profiles in terms of the maximum cAMP concentration as well as maximum relative binding to receptors of the source cell. The $\sin^2(\pi t/T)$ and $\sin^{16}(\pi t/T)$ profiles are shown in Figure 1.

Table 3 — Maximum concentrations of cAMP and the relative amplitude of cAMP levels (defined as (maximum - minimum)/mean) at various distances from the source and for the release profiles of figure 1.

TABLE 1

Symbol and Meaning	Value	Reference
$\underline{T}$: Period of oscillatory release of cAMP	300 sec	Gerisch (1968)
$\underline{\eta}$: Number of molecules of cAMP released by a cell in one pulse	$10^7 - 10^8$	Nanjundiah and Malchow (1976) Devreotes et al. (1979 a,b)
$\underline{\lambda}$: Rate of cAMP hydrolysis by membrane phosphodiesterase expressed as the number of molecules of cAMP hydrolysed per second by a single cell	1.1×10^8 ml sec^{-1}	Malchow and Gerisch (1974)
$\underline{N_o}$: Total number of cAMP receptors on surface of aggregating cell	1.62×10^4 (high-affinity site) 1.10×10^5 (low-affinity site)	Mullens and Newell (1978)
$\underline{k_1}$: Association rate-constant for cAMP-receptor binding	5.37×10^{-14} ml.sec^{-1} (high-affinity site) 9.67×10^{-15} ml.sec^{-1} (low-affinity site)	-do-

$\underline{k_{-1}}$	Dissociation rate-constant of cAMP-receptor complex	$0.29\ sec^{-1}$ (high-affinity site) $0.58\ sec^{-1}$ (low-affinity site)	Mullens and Newell (1978)
$\underline{K_{0.5}}$	Equilibrium constant of cAMP-receptor binding, defined as k_{-1}/k_1	9.10^{-9} M (high-affinity site 1.10^{-7} M (low-affinity site)	-do-
$\underline{a}$	radius of a cell	5 µm	Own estimate
$\underline{D}$	Diffusion coefficient of cAMP in agar	$1x10^{-5}\ cm^2\ sec^{-1}$	Cohen et al. (1975)

TABLE 2

Source profile as a function of time (t)	Peak value of free cAMP concentration (M)	Peak relative binding (percentage)			
		High-affinity sites		Low-affinity sites	
		Estimate 1	Estimate 2	Estimate 1	Estimate 2
$\sin^2(\pi t/T)$	3.67×10^{-9}	33	29*	3.0*	3.6
$\sin^4(\pi t/T)$	4.83×10^{-9}	44	35*	4.0*	4.7
$\sin^8(\pi t/T)$	6.77×10^{-9}	60	43*	5.4*	6.3
$\sin^{16}(\pi t/T)$	9.33×10^{-9}	82	51*	7.5*	8.5
$\delta(t)$	1.87×10^{-5}				

TABLE 3

Distance from center (μm)	Maximum concentration (M) $\sin^2(\pi t/T)$	Maximum concentration (M) $\sin^{16}(\pi t/T)$	Relative variation in concentration $\sin^2(\pi t/T)$	Relative variation in concentration $\sin^{16}(?t/T)$
5	3.67×10^{-9}	9.33×10^{-9}	2.08	5.28
50	3.26×10^{-10}	7.39×10^{-10}	1.69	4.08
100	$o.52 \times 10^{-10}$	3.18×10^{-10}	1.44	3.42
200	6.73×10^{-11}	1.8×10^{-10}	1.05	2.34
400	2.81×10^{-11}	3.81×10^{-11}	0.55	1.14
600	1.68×10^{-11}	1.93×10^{-11}	0.29	0.56

LEGENDS TO FIGURES

Fig. 1 Spontaneous rates of cAMP release by a cell, assumed periodic with a period of 5 minutes. Release profiles: (- - - -) $\sin^2(\pi t/T)$ and (——) $\sin^{16}(\pi t/T)$, abscissa, time in seconds; ordinate, rate of release (molecules/sec.). With both profiles the total release in a pulse is the same, namely 10^7 molecules.

Fig. 2 Ordinate, fraction of cAMP receptors on a cell surface bound due to the cAMP released by the cell itself; abscissa, time in seconds. Release profiles, (- - - -) $\sin^2(\pi t/T)$, (——) $\sin^{16}(\pi t/T)$. The two upper curves (a and b) refer to the high-affinity receptors, and the two lower curves (c and d) to the low-affinity receptors.

Fig. 3 (a) cAMP concentration in moles/liter, and (b) relative binding of receptors, at a cell situated 50 μm away from the source. Abscissa, time in seconds. Release profiles (- - - -) $\sin^2(\pi t/T)$, (——) $\sin^{16}(\pi t/T)$. The relative binding in (b) refers only to high-affinity receptors; the values for the low-affinity receptors are consistently smaller by a factor of 11.1.

Fig. 4 cAMP concentrations in moles/liter as a function of the distance from the source of Fig. 1. (a) for the $\sin^2(\pi t/T)$ profile and (b) for the $\sin^{16}(\pi t/T)$ profile. Abscissa, distances in μm. The concentrations are given for two different times; 75 secs. and 150 secs. from the beginning of release.