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Signal input for a chemotactic response in the cellular slime mold *Dictyostelium discoideum*

(3′:5′-cyclic AMP/receptors/amoebae/cell aggregation)

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ABSTRACT Drops with different concentrations of 3′:5′-cyclic AMP were deposited at various distances from small populations of *Dictyostelium discoideum* amoebae, and the distances over which 50% of the amoebae drops reacted positively were determined. The linear regression analysis of a double logarithmic plot of distance against concentration gives a straight line with a slope of 1/4.25, which value suggests that the amoebae respond to a spatial gradient of cyclic AMP concentration. The threshold value for the signal is 3.6×10^{-9} M/mm, with a sensitivity of measurement of about 1%. These findings are discussed in relation to our present knowledge of cyclic AMP receptors.

Triggered by starvation, single amoebae of the cellular slime molds aggregate and develop a differentiated multicellular organism (1). In the larger species of *Dictyostelium* cell aggregation is mediated by 3′:5′-cyclic AMP (2); amoebae move towards the aggregation center periodically (3), probably due to a periodic release of cyclic AMP from the center. Cyclic AMP signals are recognized by a cell-membrane-bound cyclic AMP receptor (4, 5), only present in those species that use cyclic AMP as the chemotactic molecule (5). Some of the most interesting questions about how cyclic AMP signals can be translated into directed movement are: (*1*) What precisely is the macroscopic signal input? (*2*) How is the signal measured by an individual cell? and (*3*) How sensitive is the organism to this signal? In 1947 Bonner (6) proposed the hypothesis that the cells move towards an aggregate by measuring a spatial gradient of concentration over its total length. In the present work, evidence in favor of such a hypothesis is given by comparison of the mathematical parameters of a gradient formed by diffusion to the characteristics of the threshold amoeba response. Also a threshold value for this gradient has been obtained and the sensitivity of amoebae to cyclic AMP has been measured.

METHODS AND TECHNIQUES

Amoebae of *D. discoideum*, NC-4, were grown in association with *Escherichia coli* on a solid medium, harvested in the pre-aggregative stage, freed of bacteria, and adjusted to 5×10^6 cells per ml with a 1% saline solution (7). Small drops (0.1 μl) of this cell suspension were placed on hydrophobic agar, giving a final radius of 0.3 mm. The chemotactic activity of cyclic AMP (concentration ranging from 10^{-7} to 10^{-4} M) was tested when the amoebae were close to aggregation (8). Cyclic AMP drops (0.1 μl) were placed at different distances from the amoebae populations (40 per given distance). The distance—measured between the centers of both drops—at which 50% of the amoebae populations react positively for a given cyclic AMP concentration was taken as the threshold distance for chemotaxis. The chemotactic response was called positive when more than twice as many cells pressed against the margin closest to the attracting drop as against the opposite side. The chemotactic response was observed at different time intervals after the deposition of the cyclic AMP drop (from 15 to 30 min) to get the maximal response for a given distance and cyclic AMP concentration. At all but the largest distance used the maximal gradient was reached within 30 min after placing the cyclic AMP drops.

RESULTS

The chemotactic threshold distance obtained for various quantities of cyclic AMP is presented in Table 1. These data were transformed into a dimensionless form by dividing them by their minimal values, respectively, 0.95 mm and 10^{-14} mol of cyclic AMP, and then represented in a double logarithmic plot (Fig. 1). The linear regression analysis of the plot gives a straight line with a slope of 1/4.25. This value lies close to the theoretical value of 1/4 (see Table 2), which represents a system in which amoebae are responding to a spatial cyclic AMP gradient of concentration. To evalu-

Table 1. The threshold distances over which amoebae respond to different quantities of cyclic AMP

Cyclic AMP (mol)	Threshold distance (mm)	Cyclic AMP/ 10^{-14} mol	Threshold distance/ 0.95 mm
	5.55		5.84
10^{-11}	5.80	10^3	6.11
	6.40		6.74
	4.35		4.58
5×10^{-12}	4.25	5×10^2	4.47
	4.55		4.79
	3.65		3.84
10^{-12}	3.85	10^2	4.05
	3.70		3.90
10^{-13}	2.50	10^1	2.63
	2.30		2.42
	1.60		1.69
5×10^{-14}	1.70	5	1.79
	1.75		1.84
	1.20		1.26
10^{-14}	1.00	1	1.05
	0.95		1.00

Experiments were carried out in triplicate except at 10^{-13} mol of cyclic AMP.

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Table 2. Theoretical values when N molecules of cyclic AMP applied in a small drop at a distance d from an amoeba are just sufficient to stimulate it chemotactically

Input signal chemotactic response	Characteristic of a log d–log N plot
Concentration (C)	Linear, slope = 1/3
Spatial gradient of concentration (ΔC)	Linear, slope = 1/4
Relative spatial gradient ($\Delta C/C$)	log N uncorrelated with log d
Temporal gradient (dC/dt)	Linear, slope = 1/5
"Dose" measured over a finite time τ ($\int_0^\tau C\,dt$)	Varies from linear with slope = 1 to strongly nonlinear

ate the threshold gradient the following formula has been used (see *Appendix*):

$$\frac{1}{4}\log 0.64\frac{N_{\min}}{d_{\min}{}^4 \times (\nabla C^*)} = 0.084$$

where $N_{\min}$ is the minimal amount of cyclic AMP used (10^{-14} mol), $d_{\min}$ is the minimal chemotactic threshold distance obtained (0.95 mm), ∇C^* is the threshold gradient, and 0.084 is the intercept point in the double logarithmic plot ($\log N/N_{\min} = 0$). By substituting these values in the above-mentioned formula a threshold gradient of 3.6×10^{-9} M/mm is obtained.

DISCUSSION

The double logarithmic plot of the dose-response data gives a slope of 1/4.25 (Fig. 1). This result comes close to one of the theoretical alternatives we have considered (Table 2). Granted that the actual mechanism of chemotactic response could well involve a combination of such inputs, we shall make the simplest possible interpretation of our data, namely, that the macroscopic signal for a directed movement of *D. discoideum* amoebae is a spatial concentration gradient. As a possible mechanism, the amoeba may detect this macroscopic signal by measuring concentration differences over its total length. This mechanism would be similar to that which has been reported for chemotaxis in leukocytes (9). The threshold value for the macroscopic signal is 3.6×10^{-9} M/mm; therefore the concentration difference between one end of an amoeba and the other (taking 10 μm as a reasonable length of an amoeba) would be $\Delta C = 3.6 \times 10^{-11}$ M. This difference has to be detected by an amoeba while a certain concentration of cyclic AMP (C) may be present in the background; the ratio $\Delta C/C$ is a measure of the sensitivity of the amoeba. In our experiments this sensitivity has a threshold value of 0.9% at the farthest distance (5.9 mm; background of cyclic AMP: 4.3×10^{-9} M); in other words, the concentration difference between the two ends of an amoeba 5.9 mm from the attracting point would be about 0.9% of the total concentration (see *Appendix* for the calculation of C at a given distance). Since this percentage increases at shorter distances, the estimation by Bonner [and L. J. Savage (6)] of about 3% sensitivity at 0.8 mm fits well with the present results.

Under our experimental conditions, the cyclic AMP signal—and therefore its equilibrium binding to cell-surface receptors—is diffusion limited. This being the case, it is probably more relevant to regard the difference in cyclic AMP molecules bound to the receptors between the two ends of the cell at a given time, rather than the external concentration as such, as the critical variable. When the mean cyclic AMP concentration in the background is 4.3×10^{-9} M and one amoeba measures a concentration gradient of 3.6×10^{-11} M between its two ends, the difference in occupied cyclic AMP receptors between both ends would be about 0.9% of the total number of occupied receptors. Then, taking a value of 10^6 receptors per cell[5] and a dissociation constant of 10^{-8} M[5], the total number of cyclic AMP receptors occupied at 4.3×10^{-9} M cyclic AMP is about 1.3×10^3 (assuming a cyclic AMP–receptor equilibration time of 1 msec, which implies a reaction volume of 5×10^{-10} ml) and a difference of about 12 occupied receptors between the two ends of the amoeba is obtained.

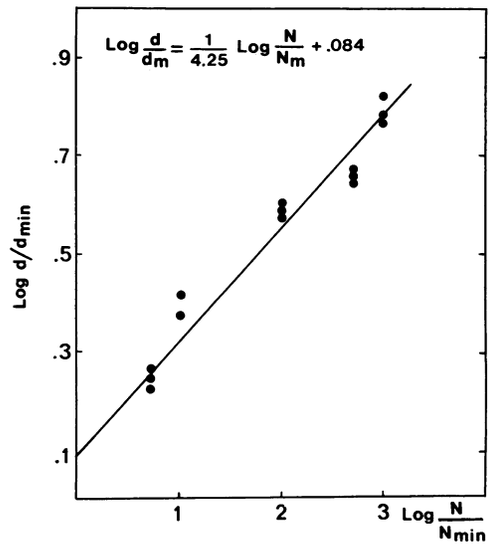

FIG. 1. Double logarithmic plot of the chemotactic threshold distance ($d/d_{\min}$) for various quantities of the cyclic AMP ($N/N_{\min}$). Data are from Table 1 in a dimensionless form. The linear regression analysis gives a correlation coefficient $r = 0.982$ (from 0.949 to 0.994) with $P < 0.1\%$. The slope is 1/4.25 (from 1/4.11 to 1/4.39; security coefficient: 95%) and the intercept point, 0.084 ($P > 97\%$).

Another mechanism involving conversion of a spatial gradient into a temporal gradient by rapid extension of pilot pseudopods in different directions and further extension of those receiving a positive input has been suggested (10). This mechanism would resemble the one involved in bacterial chemotaxis (11). This being the case, at the threshold value of the cyclic AMP signal (3.6×10^{-9} M/mm) the concentration difference during the extension of the pilot pseudopod (taking 1 μm as a reasonable length of this pseudopod) would be 3.6×10^{-12} M. Then the relative difference in concentration during this extension would be about 0.09% of the total cyclic AMP concentration (taking a mean cyclic AMP concentration in the background of 4.3×10^{-9} M) and (assuming that this pseudopod contains about 1% of the total number of receptors in the cell) a difference of only 0.01 occupied receptors along the length of the pseudopod is obtained. A further problem is that, at our estimated cyclic AMP concentration in the background, statistical fluctuations of the signal around the pilot pseudopod may be larger than the needed sensitivity (0.09%) independently of the number of cyclic AMP receptors that contains the pseudo-

pod. Therefore, to explain the present results, it seems more appropriate to consider a mechanism in which a signal is measured all over the amoeba instead of one that senses differences over the length of pilot pseudopods.

APPENDIX

The following assumptions are made in interpreting the experimental results:

(1) Point geometry. This assumes that the diameter of each amoebae drop is much smaller than the distance between the amoebae drop and the cyclic AMP drop. We have used the measurements at minimal distance to scale all the others, with the result that the shortest center distance used in the analysis is about 1.0 mm, compared with a mean drop radius of 0.3 mm.

(2) Measurement of the average chemotactic response of the population rather than the response of a single cell. Our thresholds, which depend on 50% response levels, are overestimates of an absolute threshold. We expect that our measurements reflect the cyclic AMP level at which the single amoeba has a 50% probability of responding. Such an interpretation makes sense, since the statistical fluctuations in signal input are fairly large at cyclic AMP levels of interest to us.

(3) Ignoring of cyclic AMP hydrolysis by phosphodiesterase. We justify this on the grounds that (*a*) the activity of the extracellular enzyme is extremely low at the stage of the cells used by us (12), (*b*) with our geometry, the membrane-bound enzyme affects the time course of an external cyclic AMP pulse significantly in its falling phase only, and to a very little extent during its rise (V. Nanjundiah, G. Gerisch, and D. Malchow, in preparation), and (*c*) in the present conditions the low pH and lack of magnesium salts in the hydrophobic agar (7), are conditions that antagonize phosphodiesterase activity.

Note, however, that any phosphodiesterase activity will imply that we have again overestimated the actual cyclic AMP level measured by the cell. In spite of these assumptions, the test between the various possibilities is fairly clear (see Table 2).

We will present the theory in detail only for the case of a mechanism that measures a spatial signal-gradient. The other predictions in Table 2 are derived in an identical way.

Suppose a point source of N molecules of cyclic AMP is applied on the agar surface at a distance d from the center of a drop of amoebae, at time $t = 0$. The cyclic AMP diffuses through the agar, and its concentration profile, if the agar is sufficiently deep (≥ 5 mm), is given by:

$$C(d,t) = [2N/(4\pi Dt)^{3/2}] \quad \exp(-d^2/4Dt) \qquad [1]$$

where D is the cyclic AMP diffusion coefficient (13). The spatial gradient is

$$\nabla C = (d/2Dt) \cdot C \qquad [2]$$

in magnitude. It reaches its maximum at a time

$$t_{max} = d^2/10D \qquad [3]$$

and the value of the maximum is given by

$$(\nabla C)_{max} = 0.64N/d^4 \qquad [4]$$

If d is the distance at which the amoebae show a 50% response, $(\nabla C)_{max}$ simply equals (∇C^*), the threshold gradient for a 50% response. Putting [4] in a dimensionless form by dividing by N_{min} and d_{min} (the measurements at lowest concentration and the shortest distance):

$$N/N_{min} = (d/d_{min})^4[(\nabla C^*)d_{min}^4/0.64N_{min}] \qquad [5]$$

or

$$d/d_{min} = (N/N_{min})^{1/4}(0.64N_{min}/\nabla C^* d_{min}^4)^{1/4} \qquad [6]$$

or in a logarithmic form:

$$\log \frac{d}{d_{min}} = \frac{1}{4} \log \frac{N}{N_{min}} + \frac{1}{4} \log \frac{0.64N_{min}}{(\nabla C^*)d_{min}^4} \qquad [7]$$

Thus a plot of $\log d/d_{min}$ against $\log N/N_{min}$ should be linear with a slope of 1/4; and the intercept should yield (∇C^*).

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1. Bonner, J. T. (1967) *The Cellular Slime Molds* (Princeton University Press, Princeton, N. J.).
2. Konijn, T. M., Barkley, D. S., Chang, Y. Y. & Bonner, J. T. (1968) *Am. Nat.* **102**, 225–234.
3. Arndt, A. (1937) *Roux' Arch. Entw. Mech.* **136**, 681–744.
4. Malchow, D. & Gerisch, G. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 2423–2427.
5. Mato, J. M. & Konijn, T. M. (1975) *Biochim. Biophys. Acta* **385**, 173–179.
6. Bonner, J. T. (1947) *J. Exp. Zool.* **106**, 1–26.
7. Konijn, T. M. & Raper, K. B. (1961) *Dev. Biol.* **3**, 725–756.
8. Konijn, T. M. (1970) *Experientia* **26**, 367–369.
9. Zigmond, S. H. (1974) *Nature* **249**, 450–452.
10. Gerisch, G., Malchow, D. & Hess, B. (1974) in *Biochemistry of Sensory Functions*, ed. Jaenicke, L. (Springer Verlag, Berlin-New York), pp. 279–298.
11. Macnab, R. & Koshland, D. E. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2509–2512.
12. Riedel, V. & Gerisch, G. (1971) *Biochem. Biophys. Res. Commun.* **42**, 119–123.
13. Crank, J. (1956) *The Mathematics of Diffusion* (Clarendon Press, Oxford).