Dictyostelium Amebae Alter Motility Differently in Response to Increasing versus Decreasing Temporal Gradients of cAMP

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ABSTRACT Using a perfusion chamber, we examined the behavior of individual amebae in increasing and decreasing temporal gradients of cAMP. We demonstrated that amebae respond to increasing temporal gradients of cAMP with stimulated motility and to corresponding decreasing temporal gradients with depressed motility. Depressed motility observed in decreasing temporal gradients corresponded to the inhibited levels observed when cAMP was applied at constant concentrations. These results were consistent with a simple model for the motile behavior of amebae in an early aggregation territory in which nondissipating waves of cAMP originate at the aggregation center and travel outward periodically. We conclude that chemotactically responsive amebae can assess whether a temporal gradient of chemoattractant is increasing or decreasing in the absence of a spatial gradient, and can adjust their motility accordingly.

When multiplying amebae of the cellular slime mold *Dictyostelium discoideum* are washed free of nutrients and dispersed as a cell carpet on a proper substratum, they will begin to aggregate after a period of 7 h (1), and then, as multicellular units, progress through a sequence of morphogenetic stages culminating in the formation of a fruiting body (2, 3). Under proper conditions, *Dictyostelium* amebae initially move towards aggregation centers in response to outward-traveling, nondissipating waves of the chemoattractant cAMP (4–6). With each wave, an ameba first encounters a positive spatial and increasing temporal gradient of cAMP (the front, or positive phase, of the wave), then a negative spatial and decreasing temporal gradient (the back, or negative phase, of the wave). Changes in the rate of motility have been observed in response to these natural waves in aggregation territories (7), but it has not been demonstrated which aspect of the wave is responsible. In this study, we have used a simple perfusion chamber that allows one to treat individual amebae with repeated temporal waves of cAMP in the absence of a spatial gradient, and to continuously video-monitor subsequent cell behavior. Our results demonstrate that in the absence of a spatial gradient, amebae respond to an increasing temporal gradient with stimulated movement, and to a decreasing temporal gradient with decreased movement. In the latter case, decreased movement corresponds to the previously demonstrated inhibitory effects of cAMP at constant concentrations above 10^{-8} M (8). These results demonstrate that chemotactically responsive amebae can assess the sign of a temporal gradient of a chemoattractant and alter their motility accordingly. This behavior is incorporated into a simple model for the motile responses of cells to nondissipating, outward moving waves of cAMP in an early aggregation territory.

MATERIALS AND METHODS

Growth and Development of Dictyostelium: Amebae of strain Ax-3, clone RC-3, were cloned once a month from-desiccated stocks (9) to ensure genotypic and phenotypic homogeneity. Amebae were grown in the axenic medium HL-5 (10) at 22°C according to methods previously described (11). Mid-log phase amebae were used in all experiments. To induce development, amebae were washed free of nutrient medium, dispersed on a black Whatman No. 29 filter (Whatman Laboratory Products Inc., Clifton, NJ), 4 cm in diameter, supported by two Millipore prefilters (AP10037) (Millipore Continental Water Systems, Bedford, MA) saturated with buffered salts solution (12). The cell density was between 4 and 8×10^6 per cm^2. The filter and supporting pads were placed in a petri dish which was in turn incubated in a humidity chamber at 22°C with incandescent lighting. When cells began to aggregate at 7 h, the ripple stage (1), they were washed from the filter pad in buffered salts solution, and vortexed into suspension. These experiments were performed exclusively with 7-h developing cells at the onset of aggregation, because cell motility has been found to be rigidly regulated in the developmental program (Varnum, B., K. B. Edwards, and D. R. Soll, manuscript in preparation).

Video Analysis of Amebae Perfused With Temporal Gradients of cAMP: To monitor the response of individual amebae to temporal gradients of cAMP, 1 ml of a dilute suspension of 7-h developing cells (at the onset of aggregation) was inoculated into a Sykes-Moore chamber (Bellco Glass, Inc., Vineland, NJ). This chamber consisted of a rubber 0-ring sandwiched between two glass chamber walls held together by a stainless steel

clamp. Immediately after inoculation, the chamber was closed. Cells were allowed to settle and attach to the bottom chamber wall, a process which took less than 5 min. The chamber was then inverted, and placed on the stage of a phase-contrast microscope fitted with a long range condensor. The chamber had one inlet and one outlet tube, at opposite sides of the metal ring wall. The inlet tube was connected to a reservoir containing the proper perfusion solution, and the outlet tube connected to a peristaltic pump. The flow rate, which was regulated by the peristaltic pump, was 4 ml per minute, and resulted in a replacement of chamber fluid every 15 s. This rapid flow rate had no adverse effect on the rate of cell motility (data not shown), and was used to insure that the microenvironment was not conditioned. The motile path of an ameba was continuously recorded in real time through a video monitoring system. Video tapes were then replayed and the position of the estimated centroid for each cell was traced at 21-s intervals onto a clear plastic sheet overlayed on the video monitor screen (see Fig. 2, *A–D*). The distance moved between centroids for each 21-s interval was measured with the aid of a MICROPLAN II digitizer (Laboratory Computer Systems, Inc., Cambridge, MA) attached to an IBM personal computer programmed for data storage and calculation of motion parameters.

To simulate temporal waves of cAMP, amebae were perfused four times with increasing, then decreasing, temporal step-gradients of cAMP. (To exemplify this procedure, four consecutive waves in the concentration range of 7.8 $\times$ 10^{-9} to 10^{-6} M cAMP are diagrammed in the upper portion of Fig. 3.) In each experiment, amebae were first perfused with 5 ml of buffer solution lacking cAMP (buffer solution contained 40 mM PBS, pH 6.2), then with 2 ml of buffer solution containing 7.8 $\times$ 10^{-9} M cAMP. Every 30 s, 2 ml of a new solution was perfused which contained twice the cAMP concentration of the preceding solution. After perfusion of 2 ml of buffer solution containing 10^{-6} M cAMP (the peak concentration), 2 ml of a new solution was perfused every 30 s; this solution contained half the cAMP concentration of the preceding solution. After perfusion of 2 ml of buffer solution containing 7.8 $\times$ 10^{-9} M cAMP (the trough concentration), the second, third, and fourth wave was created in a similar fashion (see Fig. 3). Each 2-ml increment was added to the inlet reservoir manually when the preceding 2-ml solution had decreased in the reservoir to less than 0.2 ml. Temporal waves in the concentration ranges 7.8 $\times$ 10^{-11} to 10^{-8} M cAMP and 7.8 $\times$ 10^{-8} to 10^{-5} M cAMP were generated in a similar fashion. Control experiments were also performed in which the same experimental regime for generating temporal gradients of cAMP was used to examine the behavior of amebae in buffer lacking cAMP and containing a constant concentration of cAMP at 10^{-10}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M.

RESULTS

Motility of Individual Amebae Perfused With Constant Concentration of cAMP

In a previous study, we examined the behavior of individual amebae incubated in buffered salts solution containing different concentrations of cAMP in unperturbed cultures (8). We found that constant concentrations of cAMP above 10^{-8} M inhibited the mobility of individual amebae obtained from aggregating cultures, but we could not be sure of the exact concentration in the ameba's microenvironment because of (*a*) the presence of membrane-bound phosphodiesterase (13, 14), and (*b*) the capacity of aggregating cells to secrete cAMP (15, 16). We therefore re-examined the effects of constant concentrations of cAMP in a perfusion chamber in which the medium is replaced four times per minute (see Materials and Methods). The results were similar (Fig. 1). Concentrations of cAMP above 10^{-8} M inhibited the average rate of motility over a 20-min period in a dose-dependent fashion.

The Motility of Amebae Treated With Consecutive Increasing and Decreasing Temporal Gradients of cAMP in a Range which Simulates the Natural Wave

In Fig. 2, we have plotted the tracks of four amebae treated with four consecutive waves of cAMP which simulate the calculated parameters of an average natural wave (7, 17). The concentration range of cAMP was 7.8 $\times$ 10^{-9} to 10^{-6} M, and

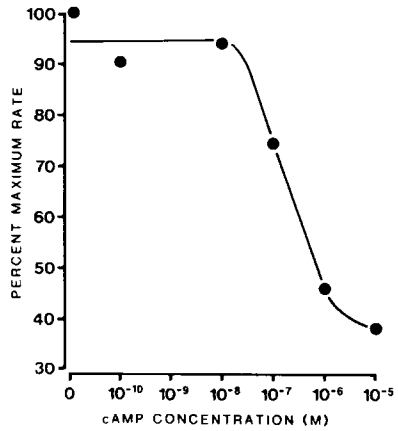

FIGURE 1 The effects of constant concentrations of cAMP on the average rate of motility. 7-h developing cells, at the onset of aggregation, were inoculated into a perfusion chamber and perfused with buffered salts solution containing a single concentration of cAMP. Each point represents the mean for 30 cells. Each cell was monitored for 20 min and the mean rate was calculated. The data is presented as percent maximum rate, which was taken as the average rate of motility in buffered salts solution lacking cAMP. The maximum rate between experiments varied between 9 and 11 μm per minute, but the percent inhibition caused by cAMP between 10^{-7} M and 10^{-5} M was relatively invariant.

the period of each consecutive wave was 7 min (see upper portion of Fig. 3 for a diagram of simulated consecutive waves in this range). The centroids of cells during the increasing phase of each wave are represented by filled circles, and the centroids during the decreasing phase by unfilled circles. Consecutive waves are numbered in order. Tracks were selected that did not cross back on themselves. In all cases, the length of the track during the entire increasing phase and half of the decreasing phase of the first wave was significantly less than that of the corresponding portions of waves 2 to 4. During waves 2 to 4, the tracks during the increasing phases were significantly longer on average than during the decreasing phases. For the most part, motility was continuous even though changes in rate were evident.

To demonstrate this pattern of behavior in a more quantitative fashion, we first plotted (lower portion of Fig. 3) the rate (μm/min) for each 21-s interval in the track of a single ameba perfused with four waves of cAMP in the range 7.8 $\times$ 10^{-9} to 10^{-6} M. During the first wave, rates were depressed to below 5 μm/min except for a short burst of activity in the latter portion of the decreasing phase. With the increasing phase of the second wave, the rate increased to roughly 14 μm/min. During the decreasing phase, the rate remained depressed below 5 μm/min. At the very end of the decreasing phase, the rate of motility increased. With the increasing phase of the third wave, the rate remained constant at roughly 14 μm/min, then again decreased during the latter third as the concentration approached peak value. During the decreasing phase, the rate again remained depressed below 5 μm/min. The pattern of motility during the fourth wave was similar to that observed during the third wave.

In Fig. 4*B*, we have averaged the mean rates of motility in each phase of the four waves for 10 individual amebae. It should be noted that the pattern of averaged data was similar to the results presented in Figs. 2 and 3 for individual amebae. In addition, the average rate of motility during the increasing phases of wave 2 was less than that of waves three and four,

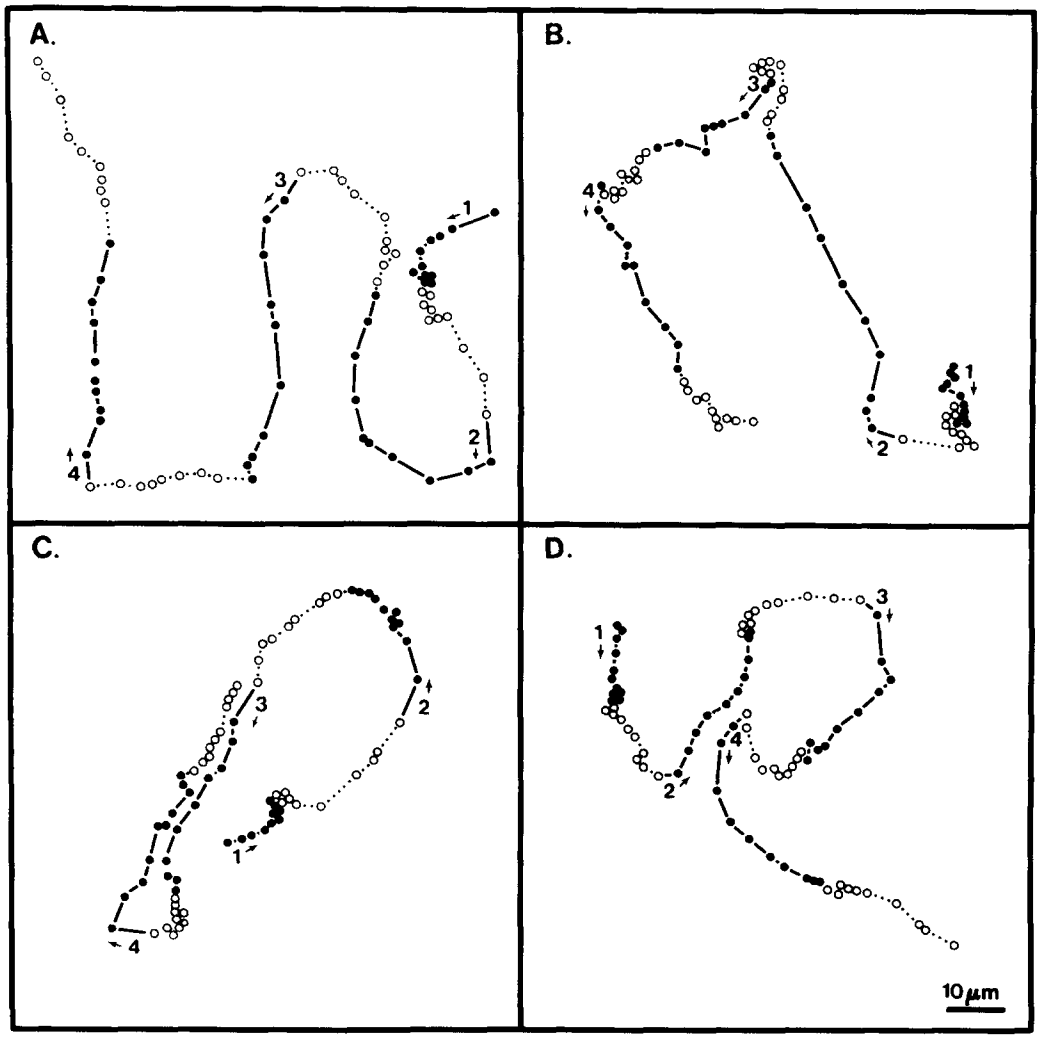

FIGURE 2 The tracks of individual amebae during four temporal waves of cAMP. The concentration of cAMP during the increasing and decreasing portion of each wave varied between 7.8×10^{-9} to 10^{-6} M as described in the text and diagrammed in the upper portion of Fig. 3. Each panel represents the track of an individual ameba. The centroid of each individual cell was determined at 21-s intervals during the increasing (●) and decreasing (○) phase of each wave. Consecutive waves are numbered 1 through 4 in each track. The arrows represent the direction of movement.

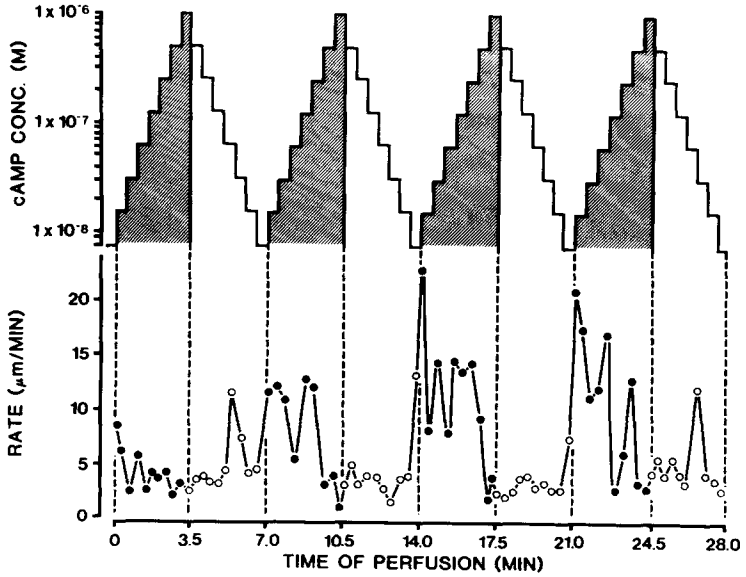

FIGURE 3 The changes in motility of a single ameba (lower portion of figure) during the increasing and decreasing phases of four consecutive temporal waves of cAMP (upper portion of figure). Each step of the increasing temporal gradient (shaded area) is at two times the concentration of the previous step, and each step of the decreasing temporal gradient is at one-half the concentration of the previous step. The rate of motility was calculated for each 21-s segment of a track of a single ameba, and plotted during the increasing (●) and decreasing (○) phases of the four waves.

which were comparable, indicating that the responsiveness of amebae to repeated temporal waves of cAMP increased to a maximum by wave 3. The average rates of motility during the increasing phases of the second and third waves were greater than the average rates of motility for amebae perfused with buffer solution lacking cAMP (dashed line, Fig. 4*B*) or

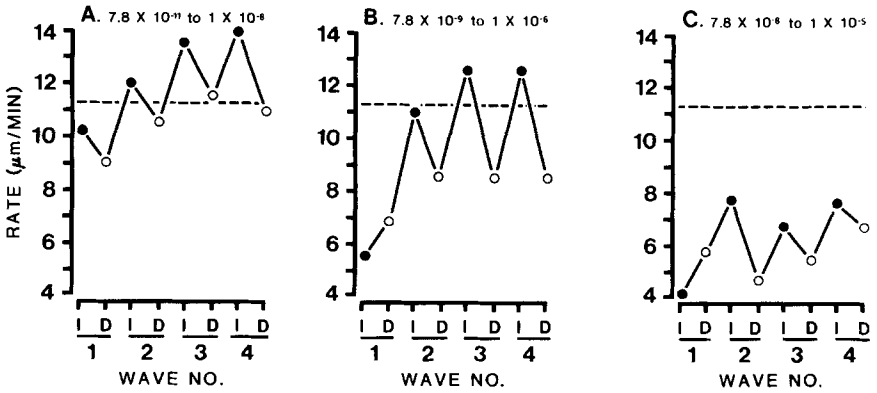

FIGURE 4 The average rate of motility during the increasing phase (*I*) and decreasing phase (*D*) of each wave (1 through 4) was calculated for 10 individual amebae from three different experiments. The mean rates for the 10 amebae are plotted during the increasing (●) and decreasing (○) phases. The dashed line represents the mean rates of motility of 10 individual amebae perfused with buffer solution lacking cAMP. The average rates of motility for amebae perfused at constant cAMP concentrations of 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-5} were 11, 11, 5, and 4.2 μm/min, respectively.

containing a constant concentration of cAMP, indicating that an increasing temporal gradient of cAMP actually stimulated motility.

The Motility of Amebae Treated With Consecutive Waves of cAMP in a Low Concentration Range

Since it was previously demonstrated that cAMP at constant concentrations above 10^{-8} M depressed the average rate of single cell motility in a concentration-dependent fashion (see Fig. 1 and reference 8), it could be argued that the pattern of motility described in Fig. 3 for a single ameba administered consecutive waves of cAMP between 7.8×10^{-9} and 10^{-6} M may reflect the following cycle: (*a*) increased motility during the first three-fourths of the increasing phase due to stimulation by the temporal gradient; (*b*) depressed motility during the last fourth of the increasing phase of each wave due to the inhibitory effect of the very high concentration of cAMP; (*c*) depressed motility through the first three-fourths of the decreasing phase due to the high cAMP concentrations and the decreasing temporal gradient; and (*d*) increased motility during the last fourth of the decreasing phase due to the low concentrations of cAMP and subsequent recovery from inhibition. To investigate the validity of this interpretation, we first monitored motility in consecutive temporal waves of cAMP in the low concentration range of 7.8×10^{-11} to 10^{-8} M, which for the most part included concentrations of cAMP which are non-inhibitory when maintained at a constant level (see Fig. 1 and reference 8). In Fig. 4*A*, we have averaged the mean rates of motility in each phase of the four waves for 10 individual amebae. It is immediately clear that the pattern of increased motility during the increasing phase and decreased motility during the decreasing phase of waves 2 to 4 is similar to that observed for waves in the concentration range of 7.8×10^{-9} to 10^{-6} M cAMP (compare Fig. 4, *A* and *B*). Again, the average rates during the increasing phases of waves 2 to 4 are higher than the average rates of cells perfused with buffer solution lacking cAMP (dashed line in Fig. 4*A*), indicating positive chemokinesis. Interestingly, the average rates during the decreasing phases of waves 2 to 4 are very close to those of cells perfused with buffer solution lacking cAMP, indicating that motility is not inhibited by a negative temporal gradient.

These experiments do not exclude the possibility that amebic motility may be inhibited by a negative gradient if it does not follow a positive gradient. To test this possibility, we treated amebae for 4 min with a constant concentration of cAMP at 10^{-8} M, and then with a decreasing temporal gradient of cAMP in the range 10^{-8} to 7.8×10^{-11} M. The concentration was then increased to 10^{-8} M in a single step, maintained for 4 min, and the gradient repeated. This procedure was performed a total of four times for 10 individual cells. In every case, the average rate of motility during the decreasing gradient was approximately equal to the rate observed during the preceding constant concentration of 10^{-8} M, demonstrating that a decreasing temporal gradient per se is not inhibitory.

The Motility of Amebae Treated with Consecutive Waves of cAMP in a High Concentration Range

If we are correct in concluding that amebae respond to an increasing temporal gradient of cAMP with stimulated motility and to a decreasing temporal gradient with motilities correlating to the inhibited levels observed at constant concentration (see Fig. 1 and reference 8), then one would expect to observe this pattern even in a very high concentration range in which even the lowest concentration is inhibitory when maintained at a constant level. We therefore monitored motility in consecutive temporal waves of cAMP in the high concentration range of 7.8×10^{-8} to 10^{-5} M. In Fig. 4*C*, we have averaged the mean rates of motility in each phase of the four waves for seven individual amebae. Again we see the same pattern of higher average rates in the increasing phases and lower average rates in the decreasing phases of waves 2 to 4. However, when these results (Fig. 4*C*) are compared to those for the concentration range which most closely simulates a normal wave, 7.8×10^{-9} to 10^{-6} M (Fig. 4*B*), and to those for the very low concentration range of 7.8×10^{-11} to 10^{-8} M (Fig. 4*A*), we find that the entire pattern is depressed to much lower rate values. These results indicate that even though motility is suppressed throughout the entire concentration range of 7.8×10^{-8} to 10^{-5} M, the increasing phase of the wave still stimulates motility when compared to average rates during the decreasing phase.

DISCUSSION

The results obtained in this study demonstrate that cell motility is affected quite differently by increasing and decreasing temporal gradients of cAMP. In three overlapping concentration ranges (7.8×10^{-11} M to 10^{-8}, 7.8×10^{-9} M to 10^{-6}, and 7.8×10^{-8} to 10^{-5} M cAMP), the average rate of motility in an increasing temporal gradient of cAMP was greater than in a corresponding, decreasing temporal gradient. We have subjected cells to repeated temporal waves of cAMP with a periodicity which simulates the average natural wave experienced by an ameba in an early aggregation territory (17). We

have found that for the majority of amebae tested, motility was depressed during the first simulated wave and no discrimination was made by amebae between the increasing and decreasing phases. However, during the second, third, and fourth waves, the average rate of motility increased and was significantly higher during the increasing phase than during the decreasing phase. In addition, the level of stimulation during the increasing phase seemed to reach a maximum by the third wave. It is not immediately obvious why cells did not respond to the first wave, but it seems reasonable to suggest that cells acquire competence to respond to a temporal wave only after they have experienced an initial wave.

Increasing temporal gradients, which either completely (7.8×10^{-11} to 10^{-8} M) or partially (7.8×10^{-9} to 10^{-6} M) spanned the concentration range in which cAMP is non-inhibitory at constant concentrations, stimulated the rate of motility to levels above those observed for amebae constantly perfused with buffered salts solution lacking cAMP. We previously demonstrated that cAMP maintained at constant concentrations below 10^{-8} M neither stimulated nor depressed motility (when compared to motility in buffered salts solution lacking cAMP), and that cAMP maintained at constant concentrations above 10^{-8} M inhibited motility in a dose-dependent fashion (see Figure 1 and reference 8). Therefore, the stimulated levels of motility in an increasing temporal gradient represent positive chemokinesis. Stimulation was also observed in a range (7.8×10^{-8} to 10^{-5}) throughout which all concentrations were inhibitory when maintained at constant levels. In this case, the stimulated rates were still below those observed in constant buffered salts solution lacking cAMP and far below the maximum stimulated level in an increasing temporal gradient in a lower concentration range.

In contrast, a decreasing temporal gradient which spanned a concentration range in which cAMP is non-inhibitory if applied at constant concentrations (7.8×10^{-11} to 10^{-8} M) appeared to neither stimulate nor inhibit motility. The average rates in these particular decreasing gradients approximated the average rates of cells perfused with buffered salts solution lacking cAMP, indicating that increasing temporal gradients in this low range stimulate motility but decreasing temporal gradients in this range do not affect motility. Decreasing temporal gradients of cAMP which partially (7.8×10^{-9} to 10^{-6} M) or completely (7.8×10^{-8} to 10^{-5} M) spanned the concentration range in which cAMP is inhibitory at constant concentration did depress motility to levels below that of cells perfused with buffer lacking cAMP. The average levels of inhibition roughly approximated the levels predicted from inhibition at constant concentrations. It is therefore reasonable to conclude that motility is stimulated by a positive temporal gradient, but depressed during a negative temporal gradient to levels predicted by observed inhibition at constant concentration.

An analysis of the effects of rapid jumps in chemoattractant concentration on leukocyte morphology have also indicated that leukocytes respond differently to the direction of the concentration change (18). When the concentration of chemoattractant was rapidly changed to a higher one in the mid range of the dose-response curve, cells responded by transient generalized ruffling. In contrast, when the concentration was decreased from the mid range to a very low level, cells responded by transient blebbing across their surface. In both cases, there was a transient cessation of locomotion. Although these experiments were performed quite differently from those described in the present study, and morphology was the discriminating cellular characteristic, the results are consistent with the conclusion that chemotactically responsive amebae can distinguish between increasing and decreasing concentrations of chemoattractant, a capacity long known to exist in chemotaxing bacteria (19).

The results which we have described can be incorporated into a simple model for the motile behavior of cells in an early aggregation territory. When amebae first encounter each outwardly travelling, nondissipating wave of cAMP, they orient toward the aggregation center by virtue of the positive spatial gradient of cAMP at the front of the wave (6, 8). The increasing temporal gradient at the front of the wave stimulates increased motility, thus spurring cells toward the aggregation center. As the cAMP concentration approaches peak value, motility decreases and remains depressed through the major portion of the back of the wave. As the cAMP concentration approaches trough value, motility begins to increase, then is stimulated once again by the front of the subsequent wave. This description of rate kinetics still does not adequately explain why cells do not turn, albeit slowly, in the back of a wave in response to the negative spatial gradient. It is quite possible that the decreasing temporal gradient at the back of a wave inhibits turning, or orientation to a spatial gradient. This possibility is now being tested.

The authors are indebted to D. Morice and B. Salisbury for assistance with the manuscript.

This work was supported by grants GM25832 and HD18577 from the National Institutes of Health awarded to D. R. Soll. B-Varnum was supported in part by training grant NO. GM07228.

Received for publication 29 October 1984, and in revised form 19 February 1985.

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