Effects of cAMP on Single Cell Motility in *Dictyostelium*

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ABSTRACT The motility of individual, aggregation-competent amebae of *Dictyostelium* has been analyzed at different concentrations of cAMP under both nongradient and gradient conditions. The following is demonstrated: (*a*) concentrations of cAMP $>10^{-8}$ M inhibit motility in a concentration-dependent fashion, decrease the frequency but not the degree of turning, and cause rounding in cell shape; (*b*) no concentration of cAMP stimulates motility, or positive chemokinesis; (*c*) concentrations of cAMP that stimulate a maximal chemotactic response do not affect motility and concentrations of cAMP that maximally inhibit motility do not stimulate chemotaxis under gradient conditions; and (*d*) the concentrations of cAMP that inhibit motility are identical under gradient and nongradient conditions.

Cyclic AMP functions as the chemoattractant during the aggregation phase of morphogenesis in the cellular slime mold *Dictyostelium discoideum* (1, 2). In a number of different studies, it has been noticed that cAMP may affect the rate of motility of aggregation-competent amebae (3–5). However, no rigorous analysis has been made of the effects of different concentrations of cAMP on single cell motility under gradient and nongradient conditions. In the present study, we employed a simple chamber (6) to monitor continuously the behavior of single amebae during 20-min periods either in solutions containing constant concentrations of cAMP or in gradients of cAMP. In the latter case, the average concentration of cAMP at the cell body during the period of analysis was calculated by the diffusion equation. The results obtained demonstrate that cAMP does not stimulate the rate of single cell motility at concentrations ranging from 10^{-10} to 10^{-3} M. Rather, cAMP depresses the rate of motility in a concentration-dependent fashion at concentrations $>10^{-8}$ M in a similar manner under gradient and nongradient conditions. Interestingly, a maximum chemotactic response was elicited at cAMP concentrations (10^{-9} and 10^{-8} M) that have no effect on the rate of single cell motility. However, no significant chemotactic response was elicited at cAMP concentrations ($>10^{-7}$ M) that depress the rate of motility by >50%. The inhibition of cell motility by cAMP was accompanied by a decrease in the frequency but not in the degree of turning, and by a rounding in cell shape. Here our results will be discussed briefly in relationship to the aggregation process and contrasted to previous observations suggesting that cAMP stimulates the rate of motility.

MATERIALS AND METHODS

Growth and Development: Amebae of strain AX-3, clone RC-3, were grown in axenic medium in suspension as previously described (7). To induce development, we washed amebae free of nutrient medium, dispersed them on a development filter saturated with buffered salts solution (8) and incubated them at 22°C in a humidity chamber (9). Under these conditions, aggregation began at 7 h and loose aggregates formed uniformly in the cell carpet by 8 h (10). 8-h cells were capable of rapidly recapitulating the loose aggregate stage in 40 min when disaggregated and dispersed on a fresh filter pad (11, 12) and had acquired all aggregation-associated functions (D. R. Soll, R. Finney, B. Varnum, and B. Slutsky, unpublished observations). These cells were deemed aggregation-competent and were employed in all experiments described in this report.

Monitoring Cell Motility: Cell motility was monitored in an apparatus fashioned after the one developed by Sally Zigmond (6) for monitoring leukocyte chemotaxis. The apparatus consisted of a 2-mm Plexiglas bridge bordered on either side by parallel troughs 2 mm wide and 1 mm deep. A droplet of aggregation-competent amebae was placed on a coverslip that was in turn inverted and placed over the bridge and troughs. Amebae dropped to the bridge surface at a final density of 10 to 20 per mm^2. The troughs were immediately filled with buffer with or without cAMP. *Under nongradient conditions*, both troughs were filled with the same solution. *Under gradient conditions*, one trough was filled with buffer solution containing the test concentration of cAMP (source) and the other trough was filled with buffer solution only (sink). Buffer solution contained 40 mM phosphate buffer, pH 6.2.

Cells were continuously monitored with either a Wild dissection microscope fitted with a 1.6× magnifying lens, or with a Leitz compound microscope fitted with a long distance condensor. In both cases, continuous movement was videorecorded for 21 min and the tapes analyzed at a later time. To analyze motility, turning, and cell shape, a plastic sheet was placed on the screen of the video monitor. At 1–3-min intervals, the position of the center of the cell was marked by a dot and the perimeter of the cell was traced. The dots were then connected to develop a track of cell movement. Examples of drawn overlays for amebae in 10^{-8} M and 10^{-5} M cAMP are presented in Fig. 1, *a* and *b*,

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respectively.

Under both gradient and nongradient conditions, the rate of motility for an individual ameba was determined by dividing total distance of the track by 21 min. Under gradient conditions, the chemotactic index (C.I.) was calculated by dividing directional distance (net distance towards source) by total distance (13). By this method, a cell moving directly towards the source will exhibit a C.I. of +1.0 and, conversely, a cell moving directly away from the source will exhibit a C.I. of −1.0.

To determine the frequency and degree of turning, we drew a straight line between two consecutive dots in a track, and determined the angle of the subsequent dot in relation to the line (see the methods described for Fig. 1 *c*). Changes of <10° were not considered significant.

Monitoring Cell Shape: Both the length and width of an individual ameba were measured at 4-min intervals during the 21-min period of analysis. The length was considered the cell diameter in the direction of movement, and the width was considered the diameter perpendicular to the direction of movement. A shape index was calculated by dividing cell width by length. This value is 1.0 for a spherical cell and is proportionately lower for more elongate cells.

RESULTS

Cyclic AMP Reduces Motility under Nongradient Conditions

Motility of aggregation-competent amebae was first monitored under nongradient conditions in solutions containing cAMP at concentrations ranging from 10^{-10} to 10^{-3} M. Motility was monitored for each ameba over a 21-min period and the rate calculated by dividing total distance traveled by total time. The average rate of motility for 50 individually monitored amebae at each concentration is presented as the filled circles in Fig. 2 *a*. The average rate in 0, 10^{-10}, 10^{-9}, and 10^{-8} M cAMP was roughly 9.25 μm/min. The distribution of rates within each population of 50 cells was similar at these concentrations, ranging from 0 to 25 μm/min. However, at a cAMP concentration of 10^{-7} M, the average rate decreased to 5.5 μm/min, and at 10^{-4} M, it decreased to 3.8 μm/min. At 10^{-3} M, the average rate was 3.5 μm/min, representing a decrease of >60%, and the distribution of rates was dramatically compressed towards lower values. It should be noted that no stimulated motility, or positive chemokinesis, was observed at any concentration of cAMP tested in the range of 10^{-10} to 10^{-3} M.

In Fig. 1, we have presented the rates at each cAMP concentration averaged over a 21-min period. To be sure that no transient stimulation of motility occurred immediately after cells were exposed to solutions of cAMP, we initiated video recordings before addition of the cAMP solutions and continued them for 20 min after addition. Rates were calculated for each 1-min interval during a 6-min period preceding addition of cAMP solution and for each 1-min interval during the 20-min period following addition. The most careful measurements were made during the 4-min period immediately following addition. Solutions of 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M cAMP were tested. In no case was transient stimulation

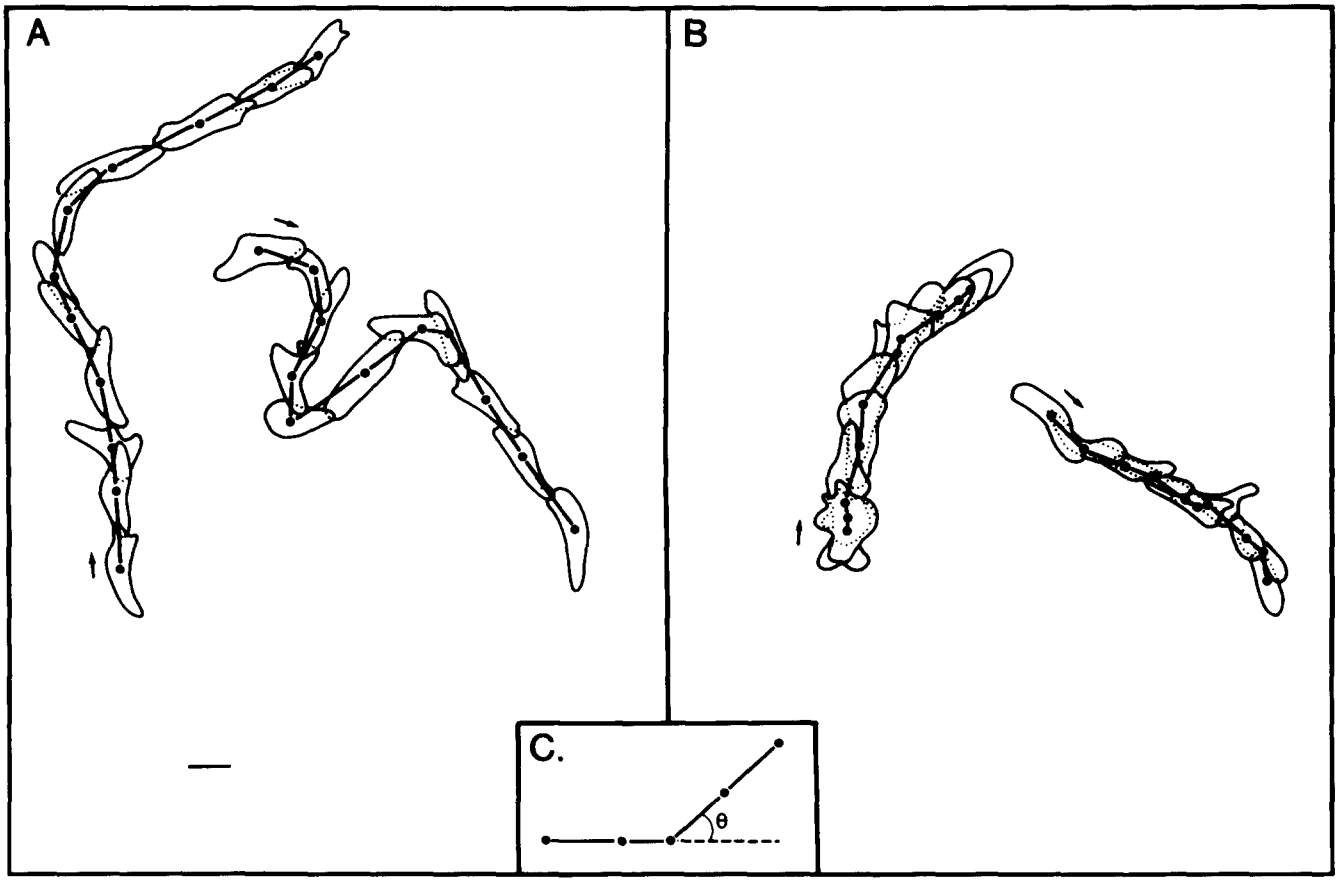

FIGURE 1 Examples of amebae migrating in 10^{-8} (*A*)and 10^{-5} M (*B*) cAMP. Amebae migrating on a Plexiglas bridge in homogeneous solutions of cAMP (nongradient conditions) at the respective concentrations were monitored for 10 min. Tracings of cell shape were made at 1-min intervals, and the center of each ameba was marked by a dot. Dots were connected to produce "tracks" of cell movement. Arrows represent the original position of the cell and the original direction of migration. In C, an example is given of a track in which the degree of turning (θ) is measured. Note that the tracks presented in *A* and *B* represent average migration patterns at 10^{-8} and 10^{-5} M cAMP, respectively. Bar, 10 μm.

observed. In the case of 10^{-8} M cAMP, cells moved at exactly the same rates immediately after addition as they did before addition or 20 min after addition. In the case of 10^{-7} to 10^{-5} M cAMP, cells reduced their rates of movement within 30 s after addition to the constant, depressed levels that are presented in Fig. 2.

Cyclic AMP Reduces Motility under Gradient Conditions

To test the effects of different concentrations of cAMP on cell motility under gradient conditions and to compare the effects of different concentrations of cAMP on cell motility and chemotaxis under gradient conditions, we dispersed aggregation-competent amebae on the bridge of a chemotaxis chamber (6) that contained a test solution of cAMP in one trough ("source") and a solution of buffered salts lacking cAMP in the opposing trough ("sink"). Test solutions were varied between 10^{-9} and 10^{-5} M cAMP, and the approximate concentration of cAMP at the position of an ameba halfway through the period of analysis was calculated by the diffusion equation, assuming that in the short period during which cell motility was monitored, the trough with test solution functioned as an infinite source, and the trough with buffer alone functioned as an infinite sink. Both the rate of motility and the chemotactic index were calculated for each of 50 cells analyzed at each test concentration of cAMP. The average rates of motility and the average chemotactic indices are plotted as unfilled circles in Fig. 2, *a* and *b*, respectively. Just as in the case of nongradient conditions (filled circles in Fig. 2*a*), the average rates of cell motility at calculated cAMP concentrations of 10^{-10} to 10^{-8} M were roughly the same as those of cells in buffered solution lacking cAMP. At calculated concentrations of cAMP $>10^{-8}$ M, motility was depressed in roughly the same concentration-dependent fashion as under nongradient conditions. The highest average chemotactic index was observed at 10^{-9} and 10^{-8} M cAMP, concentrations that did not depress the rate of motility. At 10^{-7} M cAMP, the average chemotactic index was ~75% of peak value, and the average rate of cell motility was ~66% of the maximum value. At a cAMP concentration of 10^{-6} M, the average chemotactic index approached zero and the average rate of motility was ~50% of the maximum value. These results demonstrate that the sensitivity of single cell motility to concentrations of cAMP $>10^{-8}$ M are similar under nongradient and gradient conditions, and indicate that the assumptions employed to calculate the concentration of cAMP at the position of the cell body are valid.

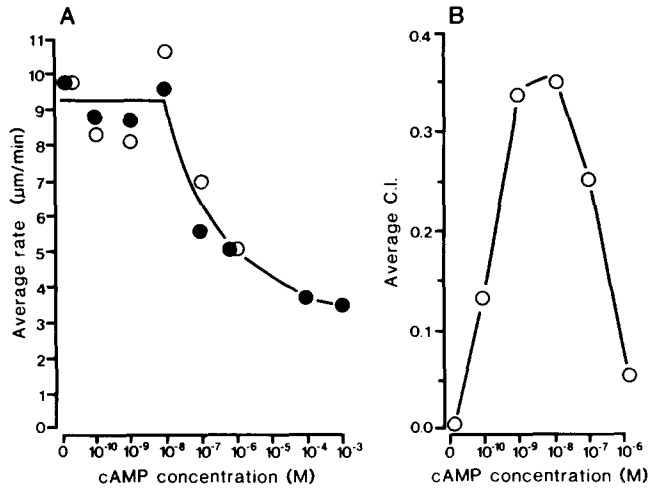

FIGURE 2 The average rate of cell motility (*A*) and the average chemotactic index (*B*) as a function of cAMP concentration. (*A*) Each circle represents the average rate of 50 individually analyzed amebae. The closed circles represent amebae in homogeneous solutions of cAMP (nongradient conditions) at the respective concentrations. The open circles represent amebae in gradients of cAMP. In the latter case, the average concentration of cAMP at the cell body during the period of analysis was calculated by the diffusion equation (see text for Discussion). (*B*) Each circle represents the average chemotactic index (C.I.) for 50 individually analyzed amebae. This value was calculated according to the procedure outlined in Materials and Methods. Note that the chemotactic indices were calculated for the same populations of amebae that were analyzed in *A* for single cell motility (open circles).

Cyclic AMP Reduces the Frequency of Turning

To test whether turning is also affected by concentrations of cAMP that suppress the rate of motility, we measured both the frequency and degree of turning over a 21-min migration period of aggregation-competent amebae in 10^{-8} and 10^{-5} M cAMP under nongradient conditions. The averaged results for 27 and 19 individual amebae, respectively, are presented in Table I. At 10^{-8} M cAMP, the average cell turned 2.8 times per 10 min and at 10^{-5} M, the average cell turned 1.3 times per 10 min. Therefore, the frequency of turns was reduced 54% by a concentration of cAMP that reduced the average rate of motility 57%. When the number of turns was calculated as a function of distance traveled (average number of turns per 10 μm), no difference was observed at noninhibitory (10^{-8} M) and inhibitory (10^{-5} M) concentrations of cAMP. In the former case, the number of turns per 10 μm was 0.27, and in the latter case 0.29. No significant difference was observed in the average degree of turning for cells in 10^{-8} M cAMP and in 10^{-5} M cAMP (Table I).

TABLE I

A Comparison of Turning and Cell Shape for Amebae Migrating in 10^{-8} and 10^{-5} M cAMP under Nongradient Conditions

cAMP concentration	No. of individual cells measured	Average rate of motility ± SD	Average frequency of turning ± SD	Average degree of turning ± SD	Average cell length ± SD	Average shape index ± SD
M		*(μm/min)*	*(turns/10 min)*		*(μm)*	
10^{-8}	27	10.37 ± 6.2	2.8 ± 1.4	67.8 ± 27.9	22.5 ± 8.3	0.42 ± 0.15
10^{-5}	19	4.44 ± 2.3	1.3 ± 1.3	78.4 ± 47.6	15.1 ± 3.4	0.59 ± 0.20
P value		<0.001	<0.005	NS	<0.001	<0.005

NS, not significant.

Cyclic AMP Affects Cell Shape

To test whether concentrations of cAMP that inhibit motility affect cell shape, we monitored the length and width of aggregation-competent amebae during 20 min of migration in a solution containing 10^{-8} M cAMP (noninhibitory) or 10^{-5} M cAMP (inhibitory). Measurements were made every 4 min, and the mean length and shape index (width divided by length) was calculated for each ameba. In Table I, the average mean length and mean shape index are presented for 27 and 19 individual amebae at 10^{-8} and 10^{-5} M cAMP, respectively. It is clear that amebae migrating in 10^{-5} M cAMP were significantly shorter than amebae migrating in 10^{-8} M cAMP. In addition, the former, less motile amebae exhibited a significantly larger shape index, indicating a rounder shape, at least in the plane that parallels the substratum.

DISCUSSION

Here we have reported that the chemoattractant cAMP depresses the rate of cell motility at concentrations as low as 10^{-7} M. It has been observed elsewhere that chemoattractants of leukocytes depress motility (14), but in neither case is it clear why depression occurs. In *Dictyostelium*, the chemoattractant is periodically released by cells in an aggregation territory (15). This signal is relayed by the amebae in the territory, resulting in an outward-traveling wave of attractant. With each wave, amebae first encounter a positive spatial gradient, and as the wave passes, a negative spatial gradient. If the sensing mechanism is solely spatial, as has been suggested (3), a chemotactic response to the posterior portion of the wave would result in a reversal in the direction of ameboid movement, which has been demonstrated to be both possible and quite rapid (16, 17), and would clearly interfere with aggregation. Presumably, there is some process that prevents reversal. It has been estimated that the cAMP concentration (intra- and extracellular) at the peak of the wave is roughly 10^{-6} M (15), well within the range that depresses motility and above the range that stimulates chemotaxis (if the major portion of cAMP in the peak is extracellular). Possibly, suppression of movement at the peak of the wave may transiently inhibit chemotactic responsiveness to the negative spatial gradient that follows and may thus prevent reversal. Indeed, a pulse of cAMP, when released from a micropipette containing a very high concentration of attractant, causes a rapid, transient suppression of motility and cell rounding (3, 18) and may mimic the peak effect of a natural wave.

One must also consider the possibility that the effects of high concentrations of cAMP on cell motility and cell shape may reflect cell responses related to differentiation rather than to the mechanisms of chemotaxis and aggregation. Concentrations of cAMP that depress the rate of motility by >50% and that cause a rounding in cell shape, a morphological response previously reported by Ryter et al. (19), also have been reported to (*a*) stimulate stalk cell differentiation in single amebae in the absence of cell interaction of multicellular morphogenesis (20–22), (*b*) support the synthesis of a group of development-specific mRNAs and polypeptides in disaggregated cells (23), and (*c*) inhibit the dedifferentiation program (24). Changes in cell shape, and specifically the acquisition of a spherical shape, appear to be requisite to a number of cellular differentiations (e.g., reference 25), which include changes in gene expression (26). It may be no accident that the concentration range of cAMP that stimulates maximum chemotaxis does not stimulate cellular differentiation, and conversely that the concentration range of cAMP that affects cell differentiation, inhibits cell motility, and stimulates cell rounding, is not effective in stimulating chemotaxis.

The difference in the range of cAMP concentrations that elicits maximum chemotactic stimulation (27) and that depresses motility may simply be the result of independent processes (in this case, chemotaxis and motility) with different cAMP sensitivities. Alternatively, the difference may represent a cause-effect relationship in which the inhibition of cell motility in turn suppresses chemotaxis or the suppression of chemotaxis in turn suppresses motility. The results obtained in the present study do not distinguish between these interesting alternatives.

We have also found no indication that cAMP stimulates single cell motility, or positive chemotaxis, in the concentration range of 10^{-10} to 10^{-3} M. In contrast, Alcantara and Monk (5) observed that amebae in the vicinity of an aggregation stream move towards an opposing source of cAMP at an ever increasing rate. However, under the conditions that they employed, the cells may have been experiencing an increase in the slope of the cAMP gradient as they moved further away from the stream, which also releases a cAMP gradient laterally (28, 29). Futrelle et al. (3) also reported a transient increase in the rate of motility after a transient suppression of motility caused by a pulse of cAMP at relatively high concentration. Differences may exist between cells subjected to repeated pulses of attractant and cells continuously maintained in relatively constant concentrations of attractant. This possibility is now under investigation. Finally, we previously demonstrated that when a dense droplet of amebae was placed on agar containing cAMP under nongradient conditions, the droplet of cells spread rapidly in all directions (4, 24). This spreading response appeared to be lost later than the chemotactic response during the program of dedifferentiation and indicated dissociability of the two responses (29). One interpretation of the spreading response was that it represented a positive chemokinetic response (4). However, the lack of positive chemokinesis in individual amebae indicates either that positive chemokinesis can be stimulated only in groups of cells that are touching, or that a dense droplet of cells on agar containing cAMP generates a gradient of cAMP in the microenvironment through the action of the developmentally acquired phosphodiesterase that is membrane-bound (30). In the latter case, the spreading response (4) would in fact represent a chemotactic and not a chemokinetic response.

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