

UNIFIED CONTROL OF CHEMOTAXIS AND cAMP MEDIATED cGMP
ACCUMULATION BY cAMP IN DICTYOSTELIUM DISCOIDEUMJosé M. Mato, Frans A. Krens, Peter J.M. van Haastert
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SUMMARY

Guanosine 3'5' cyclic monophosphate (cGMP) accumulation in response to adenosine 3'5' cyclic monophosphate (cAMP) in the aggregateless Dictyostelium discoideum mutant, Agip 55, has been investigated in view of cGMP's proposed role during chemotaxis. This mutant was deficient in chemotaxis to cAMP and also showed a deficient cGMP accumulation in response to this attractant. A deficient chemotactic response can be reverted in Agip 55 cells into a normal chemotactic response by pulsating with cAMP. cAMP pulsed cells showed a higher cAMP binding capacity and also gave a normal cGMP accumulation in response to cAMP. Therefore it is concluded that the same cAMP receptor controls chemotaxis and cGMP accumulation. These results show also that cAMP controls the development of cAMP receptors and cAMP mediated cGMP accumulation and that the chemotactic response involves changes in the cGMP levels.

cAMP acts as chemoattractant for aggregative cells of D. discoideum (1). Evidence is accumulating that the processing of a cAMP signal involves changes in the cGMP levels (2-5). Suspensions of cAMP sensitive cells of D. discoideum respond to cAMP with increased cGMP levels (2-5). Sensitivity, specificity and developmental regulation of cAMP mediated cGMP accumulation are in agreement with a cGMP role in chemotaxis (2,4). These results suggest that the same cAMP receptor controls chemotaxis and cGMP accumulation, but are not definitive. The aggregateless D. discoideum mutant, Agip 55, is deficient in cAMP chemotaxis (6,5) and a normal chemotactic response can be obtained by cAMP when applied in pulses although no aggregation competence is induced (6). In the present study we examined in this mutant the cGMP content in response to cAMP and the effect of cAMP pulses on the development of cAMP mediated cGMP accumulation and cAMP binding.

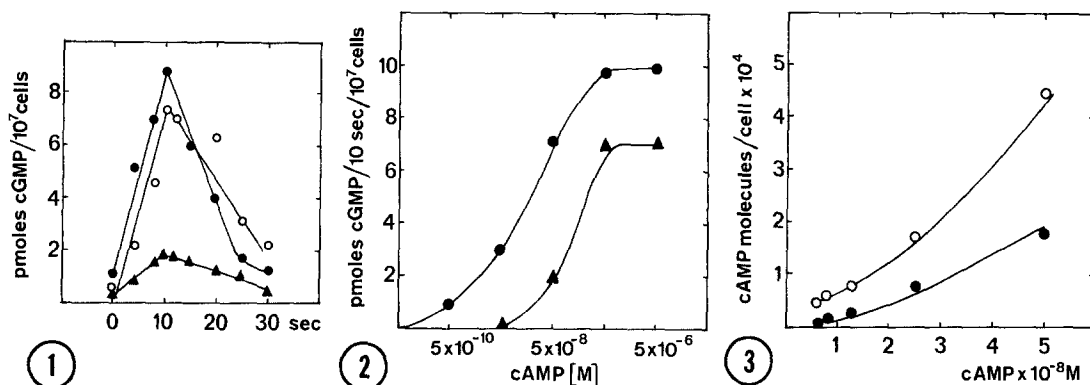


Fig. 1. cGMP levels in NC-4(H) (●) and Agip 55 (○,▲) cells in response to cAMP (5×10^{-8} M final concentration). Cells were starved for 5 hours. Samples of 100 μ l containing 2×10^7 cells were stimulated at time zero with cAMP. At different times 200 μ l ethanol-HCl were added and cGMP content measured by radioimmunoassay as previously described (2). ○, cells were pulsed during the last 3 hours of starvation with 10^{-7} M cAMP at 5 min. intervals (6). ▲, cells were pulsed with phosphate buffer.

Fig. 2. cAMP concentration dependence of cAMP mediated cGMP formation. Cells were starved for 5 hours and stimulated with various cAMP concentrations as described in Figure 1. Samples were taken at 0, 4, 6, 8, 10, 15, 20 and 30 seconds after cAMP stimulation. cGMP peaks were obtained at 10 seconds. The maximal cGMP increase with respect to basal concentration was then calculated and plotted versus the cAMP concentration used for stimulation. ●, NC-4(H) cells; ▲, Agip 55 cells. A dose-response curve for NC-4(H) cells has been previously published (2).

Fig. 3. Binding of [3 H] cAMP to living cells of Agip 55. Cells were starved for 5 hours. During the last 3 hours of starvation cells were pulsed with 10^{-7} M cAMP (○) or phosphate buffer (●) as described in Figure 1. After pulsation cells were washed 3 times in cold phosphate buffer, adjusted to 5×10^7 cells per ml and aerated for 10 min. After aeration cAMP binding was measured in 100 μ l samples as described in Methods.

METHODS

D. discoideum NC-4(H) and Agip 55 (6) were used for all experiments. Cells were grown on a solid medium and harvested (7) and the chemotactic response of cells to cAMP was determined as described (8). Cell movement towards the cAMP source and radial response, that is, cells move radially away from their original confinement in response to cAMP, were both monitored (8). After harvesting, cells were resuspended in cold 10 mM phosphate buffer (pH 6.0) and washed twice in the same buffer. The cell suspension was adjusted to 10^7 cells per ml and starved by shaking (9). Agip 55 cells were pulsed with 10^{-7} M cAMP (6) and cAMP mediated cGMP accumulation was measured as previously described (2). Binding of cAMP to living cells was determined according to Malchow and Gerisch (10). Samples of 100 μ l (5×10^7 cells per ml) were shaken at 22°C with 20 μ l

[8-³H] cAMP (25 Ci/mmol, Amersham) in the presence of 2 mM dithiothreitol, a cAMP phosphodiesterase inhibitor in D.discoideum (11). After 5 to 10 seconds the cell suspension was pipetted into a tube that contained 3 drops of immersion oil (Merck) and centrifuged for 6 seconds in an Eppendorf microcentrifuge. After centrifugation 50 μ l of the supernatant was counted. cAMP binding to cells was calculated as molecules of cAMP bound per cell. These data were calculated taking into account a specific cAMP binding, about 4%, by adding a large excess of non-labelled cAMP. cAMP binding was maximal between 5 and 10 seconds decaying afterwards. A similar assay for binding has been recently described (12).

RESULTS

Agip 55 cells did not react to cAMP by moving towards the attractant source in the range 10^{-9} - 10^{-3} M but reacted radially with a threshold concentration of 10^{-7} - 10^{-6} M cAMP. Cells of the wild type react to cAMP by moving into the direction of the attractant source with a threshold concentration of 10^{-9} - 10^{-8} M and radial response is observed at 10^{-6} - 10^{-5} M cAMP (8). This deficient chemotaxis to cAMP in Agip 55 cells was accompanied by a decreased cGMP accumulation in response to cAMP when compared with wild type cells (Fig.1). Deficient cGMP response was observed in the range 10^{-9} - 10^{-6} M cAMP (Fig.2). As shown by Darmon et al. (6) after cAMP pulsation Agip 55 cells showed a normal chemotactic response, that is, moved towards the attractant source. Cells pulsed with cAMP gave a normal cGMP accumulation in response to cAMP (Fig.1), and showed an enhanced cAMP binding capacity in the range 5×10^{-9} - 5×10^{-8} M cAMP (Fig.3). No aggregation competence was induced by cAMP pulsation.

DISCUSSION

In D.discoideum a normal chemotactic response to cAMP comprehends cell movement into the direction of the attractant source in the range 10^{-9} - 10^{-7} M cAMP and radial response at 10^{-6} - 10^{-5} M cAMP (8,13). The signal that guides cells towards the attractant source is a spatial gradient of cAMP concentration (14) and the input signal for radial response seems to be a change of concentration over time (5). Agip 55 cells did not move towards a cAMP source which indicates a deficient analysis of the spatial signal. This mutant also showed a deficient cGMP accumulation in response to cAMP. Although these results strongly indicate a cGMP role in chemotaxis they are not definitive. It is possible that two independent mutations occur; one responsible for the loss in cGMP accumulation

and the other for the defective chemotaxis. To exclude this possibility reversion to a normal cAMP chemotaxis was obtained in this mutant by pulsating with cAMP and the pulsated cells showed a normal cGMP accumulation in response to cAMP and also a higher cAMP binding capacity. Therefore there seems no doubt that chemotaxis and cGMP accumulation are controlled by the same cAMP receptor. These results also show that cAMP controls the development of cAMP receptors and cAMP mediated cGMP response and that the chemotactic response to cAMP involves changes in the levels of cGMP.

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