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RELATIONSHIP BETWEEN ADAPTATION OF THE FOLIC ACID AND THE cAMP MEDIATED cGMP RESPONSE IN *DICTYOSTELIUM*

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Chemotactic stimulation of post-vegetative *Dictyostelium* cells with folic acid or aggregative cells with cAMP results in a fast transient cGMP response which peaks at 10 s; basal levels are recovered in about 30-40 s. Stimulation with folic acid or cAMP rapidly desensitizes the cells for equal or lower concentrated stimuli. However, cells remain responsive for stimuli with higher concentration, which indicates that desensitization is caused by an adaptation process. Removal of the stimulus induces deadaptation, which for both cAMP and folic acid has first order kinetics with a half-life of 1.5 min.

Cells were prepared which are simultaneously sensitive to folic acid and to cAMP. The cGMP responses to saturated folic acid and cAMP stimuli are not additive, which suggests that the transduction pathways of these signals meet each other at or before the guanylate cyclase. Cells which are adapted to folic acid are not adapted to cAMP and *vice versa*. This demonstrates that adaptation of *Dictyostelium* cells to chemotactic stimuli is localized at a step in the transduction chain before the transduced folic acid and cAMP signals combine in one pathway.

Dictyostelium cells live in the soil and feed on bacteria. These vegetative cells are chemotactic to folic acid (1). Food deprivation induces an interphase which terminates when some cells start to secrete a compound called acrasin. Other cells react chemotactically to the source of acrasin resulting in an aggregative phase after which cells differentiate into stalk cells and spores. The acrasins of some species have been, sometimes partially, identified (2-5). *D.discoideum* and related species react specifically to cAMP (2,6).

Folic acid and cAMP are detected by separate cell surface receptors (7). Stimulation of slime mold cells with the appropriate chemoattractant induces a fast cGMP response (8-12).cGMP levels peak at 10 s after stimulation; prestimulated levels are recovered in about 30-40 s. The involvement of cGMP in chemotaxis is not only suggested by the co-existence of

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both processes, but especially by mutant cells (streamer F) which have very low intracellular cGMP-hydrolyzing activity. These cells have an extended cGMP response, and react chemotactically for longer periods than wild-type cells (13,14).

As in many sensing cells, constant stimuli induces desensitization. The reduced chemotactic and cGMP responsiveness of *D.discoideum* cells is due to adaptation (15,16). Adaptation of the cAMP mediated cGMP response is completed within a few seconds. Removal of the cAMP stimulus induces deadaptation which has first order kinetics with $t_{0.5}$ = 1.5 min (16).

In this report I describe the relationships between adaptation of the folic acid and the cAMP mediated cGMP response. Although the transduction pathways of these two stimuli start at separate cell surface receptors, they meet each other at or before the guanylate cyclase. Adaptation must be localized at the separate branches of the pathway, since cells do not show cross adaptation. These results are compatible with the properties of adaptation during chemotaxis (15).

Materials and Methods

D.discoideum, NC 4(H), and *D.mucoroides* were grown, harvested and starved on non-nutrient agar as described (16). *D.discoideum* cells were made equally sensitive to folic acid and cAMP by starving them for three hours in liquid. During the last hour 10 pulses of 1 µM folic acid were given at 6 min intervals.

After starvation, cells were washed twice and resuspended in 10 mM KH_2PO_4/Na_2HPO_4, pH 6.5, at a density of 10^8 cells/ml. 100-µl Aliquots were stimulated with 20 µl cAMP or folic acid, and cells were lysed by the addition of 100 µl perchloric acid (3,5%, vol/vol). cGMP levels were measured in the neutralized lyzates with a radioimmunoassay purchased from Amersham.

Results

Adaptation of the cAMP-mediated cGMP response is evident from the results presented in fig. 1. 100 nM cAMP induces a strong cGMP response which has disappeared at about 30-40 s (fig.1A). In this period cells became desensitized, since a newly applied 100 nM stimulus at 30 s does not induce a new cGMP response (fig.1A). The same result is observed if two 10 nM cAMP stimuli are given; cells only react to the first stimulus,

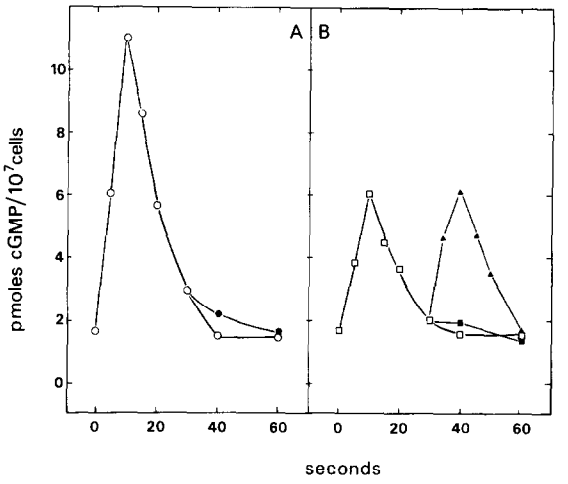

Figure 1. Adaptation to cAMP. Aggregative *D.discoideum* cells ($t_{4.5}$) were divided into 100-µl portions, stimulated with 20 µl cAMP, and lysed with 100 µl perchloric acid at the indicated times.
A. (o), 100 nM cAMP was given at 0 s. (●), 100 nM cAMP was given at 0 s and at 30 s.
B. (□), 10 nM cAMP was given at 0 s. (■), 10 nM cAMP was given at 0 s and at 30 s. (▲), 10 nM cAMP was given at 0 s and 100 nM cAMP was given at 30 s.

which induces about half-maximal response, but do not react to the second stimulus (fig.1B). Cells are not refractory (impaired to respond), because a 100 nM stimulus added after a 10 nM stimulus induces a cGMP response (fig.1B). This has always been observed with coupled stimuli: cells never respond to an equal or lower stimulus concentration applied at 30 s after a first stimulus, but always respond to a higher stimulus concentration (until about 1 µM at which concentration the cAMP receptor saturates). The cAMP mediated cGMP response in aggregative *D.discoideum* cells is controlled by an adaptation process, which has two quantitative properties (16). Firstly, the sum of the responses to two cAMP stimuli applied at 30 s intervals equals the response to a single stimulus with the higher concentration (e.g. see fig.1; the sum of the responses to 10 nM at 0 s and 100 nM at 30 s in 1B equals the response to 100 nM at 0 s in 1A). Secondly, removal of the stimulus induces deadaptation. This process follows first-order kinetics with a half-life of 1.5 min (16).

The folic acid mediated cGMP response in post-vegetative *D.discoideum* cells is also controlled by an adaptation process. Also for folic acid the

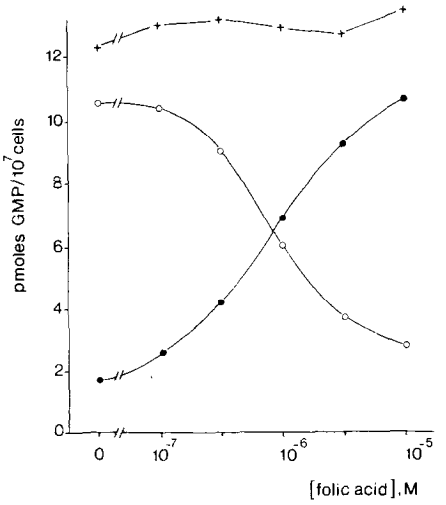

Figure 2. Adaptation to folic acid. Post-vegetative *D.discoideum* cells (t_2) were stimulated at t = 0 s with the indicated folic acid concentrations. Perchloric acid was added at t = 10 s to half of the suspensions. The other half of the suspensions were all stimulated again at 30 s with 10 μM folic acid, and lysed at 40 s. (●), cGMP levels at 10 s. (o), cGMP levels at 40 s. (+), the sum of ● and o.

responses to two stimuli at 30 s interval are additive as if only the strongest stimulus was given (fig.2). Furthermore, deadaptation of the folic acid mediated cGMP response also follows first-order kinetics with a half-life of 1.5 min (fig.3). This suggests that the process of adaptation to folic acid and to cAMP stimuli has identical molecular characteristics. If this is the case we face the exciting question whether cells are adapted to cAMP when they have been stimulated by folic acid or not.

Unfortunately, the folic acid induced cGMP response disappears during interphase (at about t_3), before cAMP induces a strong cGMP response (at about t_4, data not shown). cAMP and folic acid reduce the length of the interphase and induce the earlier appearance of cAMP receptors (17-19). Therefore, cells were starved for two hours, and then pulsed during one hour with folic acid. Folic acid receptors may remain present in these cells while cAMP receptors are induced. The results of fig.4 indeed show that these cells are simultaneously sensitive to cAMP and to folic acid. Folic acid desensitizes the response to folic acid, but not to cAMP. cAMP desensitizes the cells for cAMP, but not for folic acid. Clearly

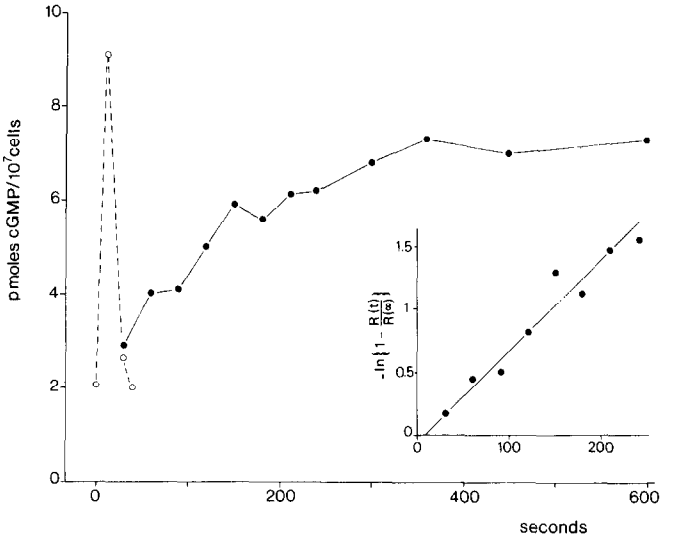

Figure 3. Deadaptation from folic acid. Post-vegetative *D.discoideum* cells (t_2) were stimulated at t = 0 s with 10 µM folic acid.
(o), cells were lysed at the indicated times. (●), cells were stimulated again with 10 µM folic acid at the indicated times, and cells were lysed 10 s later.
Inset: Linearization of the data of the main figure. R(t) is the responsiveness at time t, which is defined as $R(t) = \Delta(cGMP)_{10}(t) / \Delta(cGMP)_{10}(\infty)$, where $\Delta(cGMP)_{10}$ is the increase of cGMP levels at 10 s after stimulation (see ref.16).
The slope in this figure equals the rate constant of deadaptation, which yields $k = 7.4 \times 10^{-3} s^{-1}$ ($t_{0.5}$ = 1.54 min). The intercept on the abscissa equals the moment at which deadaptation starts, which yields t = 9 s.

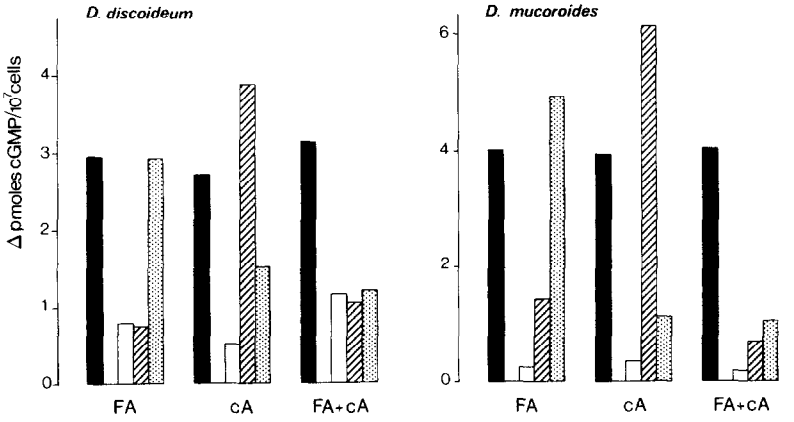

Figure 4. Cross adaptation. A. *D.discoideum* cells were starved in suspension and trained with folic acid pulses as described in Materials and Methods. B. *D.mucoroides* cells were starved for two hours.
For both species cells were stimulated at t = 0 s with either 100 µM folic acid, 1 µM cAMP, or 100 µM folic acid + 1 µM cAMP as is indicated below each set of 4 bars. The black bar represents the increase of cGMP levels at 10 s, while the other three bars at 40 s. For the open bars cells were only stimulated at 0 s. For the hatched bars cells were stimulated again at 30 s with 100 µM folic acid, and for the dotted bar at 30 s with 1 µM cAMP.

cells do not cross-adapt. Fig.4A also reveals that the cGMP increase in response to the simultaneously addition of folic acid and cAMP is identical to the increase as if only one stimulus was given. Thus the cGMP responses to saturated folic acid and cAMP stimuli are not additive. Whether the results of fig.4A are generally true was tested with another species, *D.mucoroides* which has a very short interphase. Post-vegetative cells (t_1) already respond to cAMP, while preaggregative cells (t_3) still respond to folic acid. The results with this species are essentially identical to those with *D.discoideum*: The cGMP-responses to folic acid and cAMP are not additive, and there is no cross adaptation (fig.4).

Discussion

Adaptation of the folic acid and cAMP mediated cGMP response have many properties in common (c.f. fig.2 and 3 with figs. 2 and 4 in ref. 16), which may suggest that the same molecular mechanism and molecular structures are responsible for these processes. The observation that the folic acid and cAMP mediated cGMP responses are not additive (fig.4) demonstrates that these stimuli use a structure of the transduction pathway in common. This structure is the guanylate cyclase or a component localized before this enzyme. In fig.4 it was also shown that cells adapted to folic acid are responsive to cAMP, and *vice versa*. Clearly, adaptation must be localized before the signals of the folic acid receptor and the cAMP receptor combine into one pathway before the guanylate cyclase. This can be either the receptors or a hypothetical transducer between receptors and guanylate cyclase.

It has been shown that also the chemotactic response is controlled by an adaptation process. Chemotactic signals did not show cross-adaptation (15). The results of the present report confirm the hypothesis for an important function of cGMP in slime mold chemotaxis. Further investigations on the chemoattractant induced cGMP response may shed light on the molecular mechanism of hormone-induced adaptation processes.

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