Excitation, adaption, and deadaptation of the cAMP-mediated cGMP Response in dictyostelium discoideum
van Haastert, Peter J. M.; van der Heijden, Paul R.

Published in:
The Journal of Cell Biology

DOI:
10.1083/jcb.96.2.347

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1983

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Excitation, Adaptation, and Deadaptation of the cAMP-Mediated cGMP Response in *Dictyostelium discoideum*

PETER J. M. VAN HAASTERT and PAUL R. VAN DER HEIJDEN

Cell Biology and Morphogenesis Unit, Zoological Laboratory, University of Leiden, Kaiserstraat 63, 2311 GP Leiden, The Netherlands

**ABSTRACT** Extracellular cAMP induces chemotaxis and cell aggregation in *Dictyostelium discoideum* cells. cAMP added to a cell suspension is rapidly hydrolyzed (half-life of 10 s) and induces a rapid increase of intracellular cGMP levels, which reach a peak at 10 s and recover prestimulated levels at about 30 s. This recovery is not due to removal of the stimulus because the nonhydrolyzable analogue adenosine 3',5'-monophosphorothioate-Sp-stereoisomer (cAMPS) induced a comparable cGMP response, which peaked at 10 s, even at subsaturating cAMPS concentrations.

When cells were stimulated twice with the same cAMP concentration at a 30-s interval, only the first stimulus produced a cGMP response. Cells did respond to the second stimulus when the concentration of the second stimulus was higher than that of the first stimulus. By increasing the interval between two identical stimuli, the response to the second stimulus gradually increased. Recovery from the first stimulus showed first-order kinetics with a half-life of 1-2 min.

The stimulation period was shortened by adding phosphodiesterase to the cell suspension. The cGMP response was unaltered if the half-life of cAMP was reduced to 2 s. The peak of the transient cGMP accumulation still appeared at 10 s even when the half-life of cAMP was 0.4 s; however, the height of the cGMP peak was reduced. The cGMP response at 10 s after stimulation was diminished by 50% when the half-life of 10^{-7} M cAMP was 0.5 s or when the half-life of 10^{-8} M cAMP was 3.0 s.

These results show that the cAMP signal is transduced to two opposing processes: excitation and adaptation. Within 10 s after addition of cAMP to a cell suspension the level of adaptation reaches the level of excitation, which causes the extinction of the transduction of the signal. Deadaptation starts as soon as the signal is removed, and it has first-order kinetics with a half-life of 1-2 min.

The cellular slime mold *Dictyostelium discoideum* lives in the soil where it feeds on bacteria. After exhaustion of the food supply the amoebae pass an interphase, followed by cell aggregation and the formation of a fruiting body consisting of dead stalk cells, and spores embedded in a slime droplet, on top of them. Cell aggregation is mediated by chemotaxis to cAMP (8). Aggregating cells probably detect cAMP by cell surface receptors (9).

Addition of cAMP to aggregative cells induces several responses such as the entrance of calcium, an increase of cGMP levels, the methylation of proteins and phospholipids, the accumulation of dephosphorylated myosin heavy chains, the excretion of protons, and the production and excretion of cAMP (for a review, see reference 26).

The detection and analysis of chemotactic signals by the slime molds is poorly understood. Investigations of the cAMP-mediated production and excretion of cAMP revealed that this relay response is controlled by an adaptation process (4-6); cells react to an increase of the cAMP concentration, but accommodate to constant concentrations by extinguishing the relay response. Adaptation of the relay response is essential during cell aggregation in *D. discoideum* (23). However, the...
properties of the relay response cannot be simply transferred to the detection of chemotactic signals since (a) many species do not have a relay mechanism although they react chemotactically (e.g. *D. lacteum* and *D. minutum*) and (b) adaptation of the relay response is a relatively slow process (several minutes), whereas directed pseudopod formation is very fast (about 5 s [7]).

Chemotactic stimulation induces an increase of cGMP levels within 2 s; cGMP levels reach a peak at 10 s and prestimulated levels are recovered at about 30 s after stimulation (12). Several other chemotaxant substances such as folic acid (19), pterin (20), and partially purified active extracts that attract specifically *Dictyostelium lacteum* (15), or *Polysphondylium violaceum* (28), induce similar transient elevations of cGMP levels in sensitive cells (10, 16, 27). The involvement of cGMP during chemosenory transduction is further suggested by mutants that have altered cGMP metabolism and altered chemotactic behavior (11, 21).

To investigate the way cells detect chemotactic signals we measured the cGMP response of aggregative *D. discoideum* cells to cAMP under a variety of dynamic conditions of the cAMP stimulus. The results show that the transduction of the signal is rapidly (within 10 s) terminated by an adaptation process. Cells remain adapted as long as the stimulus is present, and they immediately start to deadapt after removal of the stimulus. Deadaptation is relatively slow and shows first-order kinetics with a half-life of 1–2 min.

**MATERIALS AND METHODS**

**Chemicals:** cAMP was purchased from Boehringer Mannheim Biochemicals (Mannheim, W. Germany); (8-all) cAMP (0.9 TBq/mmol) and the cGMP radio-immunoassay were obtained from Radiochemical Centre (Amersham, England). Snake venom (Ophiophagus hannah) was obtained from Sigma Chemical Co. (St. Louis, MO). Adenosine 3',5'-monophosphorothioate-Sp-stereoisomer (cAMPS) (2) was a generous gift of Drs. Baraniak and Stec.

**Culture Conditions:** *D. discoideum* NC-4 (H) was grown in association with *Escherichia coli* B/r (13) on a solid medium containing 3.3 g peptone, 3.3 g glucose, 4.5 g KH2PO4, 1.5 g Na2HPO4·H2O, and 15 g agar per liter. Late log phase cells were harvested in 10 mM Na2HPO4/KH2PO4, pH 6.0 (PB) and freed from bacteria by centrifuging three times at 150 g for 4 min. Cells were starved on nonnutrient agar (1.5% agar in PB) at a density of 1.5 × 105 cells/ml. After 4.5 h, cells were collected, washed twice with PB and, unless mentioned otherwise, suspended in PB at a density of 107 cells/ml. Air was bubbled through the suspension (15 strokes/min) for at least 10 min.

**Cell Stimulation and cGMP Assay:** Cells were stimulated under a variety of conditions; the exact protocols are given in the legends of the figures. Cells were lysed by 100 μl cold perchloric acid (3.5% vol/vol). The lysates were neutralized by the addition of 50 μl K2CO3 (50% saturated at 20°C), and centrifuged for 2 min at 8,000 g. The supernatant was analyzed by high performance liquid chromatography on the anion exchanger Partisol 10-SAX (Whatman Inc., Clifton, NJ) with 50 mM KH2PO4, 15% propanol-1, 15% methanol, pH 5.0, as the mobile phase liquid.

**Calculations on cAMP Receptor Occupancy:** Extracellular cAMP may bind to cell surface receptors or to cell surface phosphodiesterase, which results in its degradation. The fraction of receptors occupied by cAMP (b) is given by

\[ \frac{db}{dt} = -k_2[b] (1 - b) - k_1 b. \]  

(1)

The free cAMP concentration, [cAMP], is given by

\[ \frac{dcAMP}{dt} = -J_{max} \frac{[cAMP]}{[cAMP] + K_a} - \frac{db}{dt} \]  

(2a)

Assuming that [cAMP] ≪ Km this equation is simplified to

\[ \frac{dcAMP}{dt} = -J_{max} \frac{[cAMP]}{[cAMP] + K_a} \]  

(2b)

In these equations, k1 is the rate constant of dissociation, k−1 is the rate constant of dissociation, [R] is the receptor concentration (6 × 106 receptors/cell and 107 cells/ml yields [R] = 105 M), and k2 is the half-life of cAMP.

The occupancy of the receptor (b) was calculated by numerical solution of Eqs. 1 and 2b. Integration was performed in steps of 1 ms. Equilibrium binding of cAMP to living cells shows nonlinear Scatchard plots (17), which can be interpreted in terms of two receptor sites with different affinity (Kd1 = 10 μM and Kd2 = 100 μM); binding data can also be explained by negative cooperativity. The rate constant of dissociation (k−1) at 0°C is approximately 0.2–0.3 s⁻¹ (17). At 22°C this value might be higher, up to 1 s⁻¹. The rate constant of association (k1) might have values at 22°C in the range of 2 × 10⁶ M⁻¹ s⁻¹ to 10⁷ M⁻¹ s⁻¹.

**RESULTS**

*D. discoideum* cells react chemotactically to cAMP, which is secreted periodically during cell aggregation. This oscillating activity is also observed in suspensions of aggregative cells and might complicate the investigation of the mechanisms involved in the cAMP-mediated cGMP response. Therefore, we have used cells that were starved on nonnutrient agar for 4.5 h. In suspension these cells are sensitive to cAMP, but do not yet oscillate autonomously; the cAMP signal is not yet relayed in an oscillatory way, and the cGMP response is monophasic (c.f. Fig. 4A).

The dynamics of the chemotaxant-mediated cGMP accumulation are investigated by applying two chemotaxants: cAMP and its slowly hydrolyzable agonist cAMPS (22). In aggregative *D. discoideum* cells, cAMPS is chemotactically about 100-fold less active than cAMP (25); it is also about 100-fold less active than cAMP for the production of a cGMP response.

**Demonstration of Adaptation**

Although cAMP and cAMPS are hydrolyzed at quite different rates, both of them induce similar transient cGMP accumulations, even at subsaturating concentrations of the stimuli (Fig. 1). Apparently, signal transduction is blocked shortly
after the addition of the stimulus. The mechanism by which signal transduction terminates was investigated by cell stimulation at 0 s and 30 s with different cAMP concentrations and detection of the cGMP levels during 60 s. In those cases in which cGMP levels changed, the peak was always reached at about 10 s after stimulation. A summary of the results has been shown in Fig. 2.

Cell stimulation with different cAMP concentrations at 0 s

![Figure 1](image1.png)

**Figure 1** Degradation of cAMP and cAMPS, and their induction of a cGMP response. (A) Aggregative *D. discoideum* cells were divided into 100-µl samples. At 0 s, 20 µl 3H-cAMP or 20 µl cAMPS were added, and at the times indicated 100 µl perchloric acid was added. The hydrolysis of 3H-cAMP and cAMPS was measured in the neutralized lysates. (■) Hydrolysis of 10⁻⁷ M cAMP; (□) hydrolysis of 10⁻⁴ M cAMPS. (B) Cells were divided into 1-ml portions and stimulated with 200 µl cAMP or cAMPS. At the times indicated, 100-µl samples were added to 100 µl perchloric acid. The cGMP concentrations were determined in the neutralized lysates. Response to 10⁻⁷ M cAMP; (■) response to 10⁻⁴ cAMPS; (▲) response to 10⁻⁸ M cAMP; (△) response to 10⁻⁸ M cAMPS. The results shown are from a typical experiment repeated two times.

![Figure 2](image2.png)

**Figure 2** Aggregative cells were divided into 100-µl portions. At 0 s, all cells were stimulated with 20 µl cAMP (concentrations are shown on the abscissa). One portion of cells (●) was lysed at 10 s by the addition of 100 µl perchloric acid. A second portion of cells (▲) was re-stimulated at 30 s with the same cAMP concentration and cells were lysed at 40 s. A third portion of cells (△) was re-stimulated at 30 s with 10⁻⁸ M cAMP and cells were lysed at 40 s. cGMP levels were determined in the neutralized lysates. The sum of (●) and (△) is indicated by (+). The increase of cGMP levels at 10 s after stimulation with 10⁻⁸ M cAMP was set at 100%. The increase of cGMP levels by the second stimuli at 30 s (▲ and △) was corrected for 16% recovery of responsiveness (see Fig. 4 C). The results shown are the mean of three experiments.

The Kinetics of Signal Transduction to Excitation and Adaptation

The rate of signal transduction was investigated by adding various amounts of phosphodiesterase activity to the cell suspension, followed by stimulation with 10⁻⁷ M cAMP. 30 s after the addition of cAMP, the slowly hydrolyzable analogue cAMPS was added. The cGMP responses to these two stimuli were measured. The rationale behind this experiment is that the first stimulus (cAMP) has different half-life periods; the cGMP response reveals the rate of entrance of the signal to excitation. The second stimulus (cAMPS) is slowly hydrolysable and saturating. The response to this stimulus reveals the...
level of adaptation caused by the first stimulus and thus shows the rate of entrance of the signal to adaptation.

The half-life periods of the cAMP stimuli (10^{-7} M) were determined from the hydrolysis of 10^{-7} and 10^{-9} M 3H-cAMP at 3 s after their addition (Fig. 3 A); for calculations, we assume Michaelis-Menten kinetics with a $K_m$ equal to 1 $\mu$M (24). Fig. 3 B shows that addition of phosphodiesterase to the cell suspension reduces the cGMP response to 10^{-7} M cAMP. Two controls show that this is due to the increased hydrolysis velocity of cAMP, and not to other components of the phosphodiesterase preparation; boiled phosphodiesterase does not reduce the response to cAMP, and active phosphodiesterase does not reduce the response to the slowly hydrolyzable analogue cAMPS. Fig. 3 B also reveals that the response to the second slowly hydrolyzable stimulus (cAMPS) increases if the first stimulus (cAMP) is hydrolyzed faster. Apparently, by the addition of phosphodiesterase, cAMP is not present for sufficient time to achieve complete transduction of the signal to excitation and to adaptation. In the experiment of Fig. 3 B the cGMP levels were determined only at 10 s after stimulation. The results of Fig. 3 C show that this is justified; cGMP levels still peak at 10 s after stimulation even if 50% of the first stimulus is hydrolyzed after 0.4 s.

The results of the experiments as shown in Figs. 3 A and 3 B are combined in Fig. 3 D. This reveals that cAMP has to be present for only a few seconds to achieve complete transduction of the signal. The cGMP response is still half-maximal when the half-life of cAMP is reduced to 0.5 s. Interestingly, the level of adaptation and the magnitude of the response depend on the half-life of cAMP in a similar way. Apparently, the entrance of the signal to excitation and to adaptation has the same velocity. Detection of the cGMP response to 10^{-9} M cAMP with different half-lives is also shown in this figure. A cell detects 10^{-7} M cAMP more slowly than 10^{-5} M cAMP, half-maximal transduction requires a half-life of ~3 s.

Because we detect the result of excitation at 10 s after addition of cAMP, and we measure the level of adaptation at 30 s after addition of cAMP, Fig. 4 D does not inform us of the kinetics of excitation and adaptation themselves, but only of the kinetics of the entrance of the signal to excitation and adaptation.

The Kinetics of Deadaptation

The rate of recovery from adaptation (deadaptation) was investigated by variation of the time interval between two cAMP stimuli and detection of the cGMP response to the second stimulus (Fig. 4). The first stimulus (10^{-8} M or 10^{-7} M) was calculated from the increase of cGMP levels at 40 s (y) using the equation:

$$\text{Adaptation} = \frac{(1 - y/x)}{0.84}. $$

The factor 0.84 is derived from a 16% deadaptation during a 30 s period (see Fig. 4 C). By a procedure similar to that in A and B, the response to 10^{-8} M cAMP with different half-lives was determined. The response to 10^{-7} M cAMP, (+) adaptation to 10^{-7} M cAMP, (C) response to 10^{-8} M cAMP.

![Figure 3](https://www.jcb.org)
will induce adaptation. We define the level of adaptation at time \( t \) by \( A(t) \), and the level of responsiveness by \( R(t) \); \( A(t) + R(t) = 1 \). We assume that the response to the second saturating stimulus \( 10^{-7} \) M represents the level of responsiveness \( R(t) \). We further assume that in the absence of cAMP the transition \( A \rightarrow R \) takes place (cells deadapt with first-order kinetics), that this transition starts at \( t = a \) s after the first stimulus, and that at that moment the level of adaptation equals \( a \) \( (A(a) = a) \). A mathematical description of these assumptions is

\[
\ln \frac{1 - R(t)}{a} = k(t - a).
\]

The magnitude of \( a \) is derived from Fig. 2; \( 10^{-7} \) M cAMP induces complete adaptation to \( 10^{-7} \) M \((a = 1)\), whereas \( 10^{-8} \) M cAMP induces only half-maximal adaptation to \( 10^{-7} \) M \((a = 0.5)\). The magnitude of the responsiveness to the second stimulus, \( R(t) \), is calculated from the data of Fig. 4A and B by applying

\[
R(t) = \Delta[cGMP]_{i0(t)}/\Delta[cGMP]_{i0(\infty)},
\]

where \( \Delta[cGMP]_{i0(t)} \) is the increase of cGMP levels at \( 10 \) s after stimulation with the second stimulus; the second stimulus is added at \( t \) seconds after the first stimulus.

Substitution of \( a \) and \( R(t) \) in Eq. 3, and expression of the left part of this equation versus \( t \), yields a straight line (Fig. 4C), which affirms the assumption that deadaptation has first-order kinetics. The slope of this line equals \( k \), the rate constant of deadaptation. Cells deadapt from \( 10^{-8} \) M to \( 10^{-7} \) M cAMP with comparable rate constants \((k = 7.5 \times 10^{-3} \text{ s}^{-1}; t_{0.5} = 1.5 \text{ min})\), which confirms that deadaptation has first-order kinetics. Furthermore, both lines intersect the x-axis close to 0 s. Taking into account that hydrolysis of the first stimulus requires only a few s, we conclude that deadaptation starts immediately after removal of the stimulus. The rate of deadaptation was also measured in the presence of added phosphodiesterase (half-life of \( 10^{-7} \) M cAMP was 2 s); the same rate constant was observed \((k = 7 \times 10^{-3} \text{ s}^{-1}; \text{data not shown})\). Fig. 4A reveals that cells do not completely recover the responsiveness of the first stimulus. This has also been observed for deadaptation of the relay response in \( D. \) discoideum (5).

Although the results of Fig. 4A–C clearly suggest that deadaptation has first-order kinetics, we have observed repeatedly an oscillatory like responsiveness as is shown in Fig. 4D. Such cells do not oscillate autonomously before addition of the first stimulus (cAMP levels, cGMP levels, or cAMP response to \( 10^{-7} \) M cAMP). The cGMP or cAMP levels also do not oscillate after addition of the first stimulus; only the cGMP responses to further stimulations with cAMP show oscillatory behavior. These results might be explained by two events: deadaptation as shown in Fig. 4A and B in combination with an intracellular oscillation of responsiveness to extracellular cAMP. These observations are the subject of further investigations.

### Calculations on the Kinetics of Cell Surface cAMP-Receptors

Aggregative \( D. \) discoideum cells contain cell surface receptors for cAMP (9). These receptors might be involved in the transduction of the extracellular cAMP signal to an intracellular accumulation of cGMP. Calculations on the cAMP-receptor interaction under nonequilibrium conditions might be helpful to understand signal destruction, signal transduction, adaptation, and deadaptation. Occupation of the receptor by \( 10^{-7} \) M cAMP is very fast (Fig. 5A). Maximal occupancy is reached...
after about 2 s; thereafter occupancy declines due to hydrolysis of cAMP and dissociation of the cAMP-receptor complex. Addition of a small amount of phosphodiesterase activity (to\_n = 2 s, Fig. 5 B) has minor effects on the maximum of receptor occupancy by $10^{-7}$ M, but a pronounced effect on the rate of decline of receptor occupancy. Addition of a high amount of phosphodiesterase (to\_n = 0.2 s, Fig. 5 C) also has a pronounced effect on the maximum of receptor occupancy. Occupation of the receptor by $10^{-8}$ M cAMP is less fast than by $10^{-7}$ M cAMP (maximum after about 4 s, Fig. 5 A), and fewer receptors get occupied. Addition of phosphodiesterase activity has a more pronounced effect on the maximum of receptor occupancy by $10^{-8}$ M cAMP than by $10^{-7}$ M cAMP (Fig. 5 B and C). This is in agreement with experimental observations (Fig. 3 D).

Summarizing the results, we have shown that the cAMP-mediated cGMP response is controlled by an adaptation process. The entrance of the signal to excitation and adaptation is probably a cAMP-receptor determined process; the entrance of the signal is completed within a few seconds. Although the kinetics of excitation and adaptation are not precisely known, these processes are completed within 10 s. In contrast to these fast processes, deadaptation is slow, with a half-life of 1–2 min.

DISCUSSION

cAMP is a chemoattractant in aggregative D. discoideum cells. The input signal for chemotaxis might be a temporal gradient of cAMP (7) (as in bacterial chemotaxis [3]) or a spatial gradient of cAMP (13) (as in chemotaxis of leukocytes [30]); a combination of these input signals can not be excluded. If cells react to a spatial gradient, then they might detect the concentration difference between the two ends of the cell. The difference of concentration over the cell length is only 1% of the mean concentration around the cell (13), which implies a very unfavorable signal-to-noise ratio. Adaptation to the mean concentration around the cell will greatly improve the signal-to-noise ratio. If cells react to a temporal gradient, then they detect the concentration difference at different times. As a cell moves up a gradient the background concentration will increase, thus complicating the measurement of still higher concentrations. Again, adaptation, which sets the background concentration to zero, will facilitate the detection of temporal gradients. Cells make a pseudopod in the direction of a capillary filled with cAMP within 5–10 s (7). This suggests that, whenever adaptation is involved in the detection of chemotactic signals, it will be a fast process. Direct evidence for an adaptation process during chemotaxis has not been presented. Indirect evidence has been derived from observations on the dynamics of chemotaxis and signal relay which reveal that an absolute refractory period, whenever present, does not last longer than 12 s (1), and that cells do not react chemotactically to a gradient of cAMP that is lower in magnitude and has a polarity opposite that of a previous gradient to which the cells have reacted chemotactically (23).

All chemoattractants studied so far induce a similar elevation of cGMP levels (10, 12, 16, 27, 29). Besides its general occurrence, the cGMP-response is also a fast reaction. Furthermore, mutants with altered cGMP behavior have altered chemotactic behavior (11, 21). This may suggest the involvement of cGMP during a chemotactic reaction; however, direct evidence has not been presented. In this study we investigated the cAMP-mediated cGMP response, which appears to be controlled by an adaptation process.

Adaptation of the cAMP-mediated cGMP response has two characteristics in common with the cAMP-mediated excretion of cAMP (relay) in D. discoideum (5, 6). For both reactions the magnitude of the response and the level of adaptation are additive, and both have similar rate constants of deadaptation. The main difference is the rate of adaptation, which is completed within a few seconds for the cGMP response, and which lasts several minutes for the relay response.

The entrance of the stimulus for a cGMP response is very fast (Fig. 3). The observation that transduction of $10^{-8}$ M cAMP requires a longer presence of the stimulus than the transduction of $10^{-7}$ M cAMP suggests that the entrance of the signal is a second-order process. Calculations on the occupancy of the cAMP cell-surface receptor under nonequilibrium conditions with different cAMP half-lives reveals that this receptor has the required rate constants to detect these fast changes of the cAMP concentration. The entrance of the stimulus to excitation and to adaptation has the same rate (Fig. 3 D), which may indicate that the signal for excitation and for adaptation enter the cell via the same receptor.

After removal of the signal cells recover from adaptation. The rate of deadaptation remains the same for different magnitudes and duration of the stimulus. This suggests that deadaptation is initiated by dissociation of cAMP from the receptors, but that the rate of deadaptation is cAMP-independent. The cGMP response always reaches a peak at 10 s after stimulation and prestimulated levels are recovered at about 30 s. Also, this is independent of the magnitude and duration of the stimulus. This may indicate that the alterations in the cGMP metabolism are initiated by cAMP, but that the pace of the alterations are stimulus independent.

Mato and Malchow (14) have shown that the cGMP response is produced by activation of the guanylate cyclase, rather than by inhibition of an intracellular phosphodiesterase. We may, therefore, describe the following processes as occurring after addition of cAMP to a cell suspension. In the extracellular space, cAMP is hydrolyzed or is bound to cAMP receptors localized on the cell surface. Occupation of the cAMP receptors activates the guanylate cyclase, producing an increase of cGMP levels. Occupation of the cAMP receptors also activates an adaptation process that rapidly (within 10 s) terminates the increase of cGMP levels, and causes the cells not to respond with an increase of cGMP levels after a second stimulation with the same cAMP concentration. Hydrolysis of cAMP and dissociation of cAMP from the receptors initiates deadaptation. Cells gradually reacquire responsiveness to cAMP in a first-order manner with a half-life of about 1.5 min.

We greatly acknowledge Theo Konijn for encouragement and stimulating discussions.

This work was supported by the foundation for Fundamental Biological Research (BION), which is subsidized by The Netherlands Organization for the Advancement of Pure Research.

Received for publication 8 February 1982, and in revised form 27 September 1982.

REFERENCES

5. Dinse, M. C., T. L. Steck, and P. N. Devreotes. 1980. Cyclic 3',5'-AMP relay in...


