Excitation, Adaptation, and Deadaptation of the cAMP-Mediated cGMP Response in *Dictyostelium discoideum*

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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.
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 chemotactic signals such as follic acid (19), pterin (20), and partially purified active extracts that attract specifically *Dictyostelium lacteum* (15), or Polyphemus violaceum (28), induce similar transient elevations of cGMP levels in sensitive cells (10, 16, 27). The involvement of cGMP during chemotaxis 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-H) 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 (Mannheim, W. Germany); (8-all) cAMP (0.9 TBq/mmol) and the cGMP radio-immunoassay were obtained from Radiochemical Centre (Amersham, England)

**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 NaH2PO4·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 × 107 cells/cm3.

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 (150 ml/min) for at least 10 min. Cells were lysed by 100 μl cold perchloric acid (3.5% vol/vol). The lysates were neutralized with 50 μl KHCO3 (50% saturated at 20°C). After centrifugation for 2 min at 8,000 g, 150 μl of the supernatant was incubated with 100 μl snake venom (50 μg/ml) for 30 min. Nonhydrolyzed cAMP was removed by the addition of 1 ml of anion exchanger (1 part Dowex AG1X2 and 2 parts water, pH 5). After shaking for 2 min, the slurry was centrifuged for 2 min at 8,000 g and the radioactivity in 500 μl of the supernatant was determined.

For the determination of the hydrolysis of cAMPS, 100-μl cell suspensions were stimulated with 20 μl of cAMPS (10−4 M final concentration). At the times indicated in Fig. 1, perchorlic acid (100 μl, 3.5% vol/vol) was added. The lysates were neutralized with KHCO3 as described before. After centrifugation, 30 μl of the supernatant was analyzed by high performance liquid chromatography on the anion exchanger Partisil 10-SAX (Whatman Inc., Clifton, NJ) with 50 mM KH2PO4, 0.15% propanol-1, 1.5% 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

\[
\frac{db}{dt} = -k_c[cAMP](1 - b) - k_l b.
\]

(1)

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

\[
\frac{dcAMP}{dt} = -\frac{db}{dt}[cAMP] + \frac{dc}{dt}[R].
\]

(2a)

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

\[
\frac{dcAMP}{dt} = -\frac{K_m}{K_m + [cAMP]} \frac{db}{dt}.
\]

(2b)

In these equations *k* is the rate constant of association, *k*_ − 1 is the rate constant of dissociation, [R] is the receptor concentration (6 × 104 receptors/cell and 10 s 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 (k+) might have values at 22°C in the range of 2 × 105 M−1 s−1 to 106 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 chemoattractant-mediated cGMP accumulation are investigated by applying two chemoattractants: 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 stimu-
lization 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

![Graph](https://via.placeholder.com/150)

**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 concen-
trations 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.

![Graph](https://via.placeholder.com/150)

**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^{-6} 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^{-6} M cAMP
was set at 100%. The increase of cGMP levels by the second stimu-
lation at 30 s (▲ and △) was corrected for 16% recovery of responsiveness
(see Fig. 4C). The results shown are the mean of three experiments.

A summary of the results has been shown in Fig. 2.

To explain these observations at a molecular level, we may
consider the following mechanisms: (a) absolute refractoriness;
the chemoattractant induces a cGMP response and subse-
sequently all molecules of one species (e.g. guanylate cyclase)
become insensitive to further stimulation; (b) relative refracto-
riness; the chemoattractant induces a cGMP response and
subsequently the molecules which have been active become
insensitive to further stimulation; (c) adaptation; the chemo-
trattractant induces a fast excitation response and a slow adapta-
 tion response; the cGMP accumulation depends on the balance
of excitation and adaptation; the cGMP accumulation is ter-
m inated as soon as the level of adaptation exceeds the level of
excitation (4–6). The fact that cells never did react to an
immediately following stimulus when the concentration was
the same as that of the first stimulus, and that cells always did
respond to the second stimulus when the concentration was
higher than that of the first stimulus (Fig. 2), excluded relative
and absolute refractoriness. The observations are compatible
with adaptation. This is confirmed by an experiment in which
standardized with cAMP concentration at different times after
the addition of cAMPS induce cGMP accumulations with a
magnitude independent of the time period that cAMPS was
present previously (data not shown). This indicates that cells
rapidly adapt to cAMPS, and that the level of adaptation
remains constant if the stimulus is not removed.

Although the previous experiments point to an adaptation
process, they do not exclude the possibility of a short absolute
refractory period that lasts less than 30 s. This is important to
investigate, because cells extrude a pseudopod to a capillary
filled with cAMP within 5 s (7). Therefore, cells were stimulated
with a saturating cAMP concentration (10^{-9} M) at 0 s, followed by a saturating cAMP concentration (10^{-7} M) at 5 s.
The subsaturating concentration had no effect on the response
to the saturating concentration, which makes the existence of
a short absolute refractory period unlikely (data not shown).

Cell aggregation in vivo is a dynamic oscillating process with
periodicity of about 5 min (23), and with a fast detection of the
cAMP concentration (7). Therefore, the kinetics of signal
detection and recovery from stimulation were measured.

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 sus-
pension, 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 hydrolyz-
able and saturating. The response to this stimulus reveals the

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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 $10^{-5}$ M $^3$H-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 $10^{-7}$ 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) cells, phosphodiesterase was added after lysis of the cells; (iii) cGMP levels at 10 s, phosphodiesterase was added at 10 s and 3 $\times$ $10^{-7}$ M cAMPS at 0 s; (iv) cGMP levels at 10 s, boiled phosphodiesterase was added at $-10$ s and $10^{-7}$ M cAMP at 0 s. Each point is a determination. Data from the same experiment as shown in A. (C) Phosphodiesterase (20 $\mu$L amount indicated on the abscissa) was added, followed by the addition of 20 $\mu$L $^3$H-cAMP at 0 s ($10^{-7}$ or $10^{-5}$ M, final concentrations, both containing about 5 kBq). The degradation was terminated at 3 s by adding 100 $\mu$L perchloric acid. Hydrolysis of $^3$H-cAMP was determined in the neutralized lysates. (D) Hydrolysis of $10^{-7}$ M cAMP (C) hydrolysis of $10^{-8}$ M cAMP. From these data the half-life periods of $10^{-7}$ M cAMP (x) were calculated. The means of duplicate determinations of a single experiment are shown. (D) At about $-10$ s, 20 $\mu$L phosphodiesterase was added to 100-$\mu$L cell suspensions, and cells were stimulated at 0 s with 20 $\mu$L cAMP ($10^{-7}$ M, final concentrations). At 10 s, perchloric acid (100 $\mu$L) was added to half of the suspensions. The other suspensions were stimulated again at 30 s with 20 $\mu$L CAMPs ($3 \times 10^{-8}$ M, final concentration), and these cells were lysed at 40 s with 100 $\mu$L perchloric acid. cGMP was determined in the neutralized lysates. (ii) cGMP levels at 10 s; (iv) cGMP levels at 40 s. The following controls were taken: (C) cGMP levels of nonstimulated aggregative cells at $-10$ s. At 0 s, 200 $\mu$L cAMP was added (10 $^{-7}$ M, final concentration), and at 30 s, 140 $\mu$L cAMPS ($3 \times 10^{-8}$ M final concentration). The half-life of cAMP without adding phosphodiesterase to the cell suspension varied from 7 to 14 s. The response was calculated from the increase of cGMP levels at 10 s; the response to $10^{-7}$ M cAMP without adding phosphodiesterase (x) was set at 100%. The level of adaptation was calculated from the increase of cGMP levels at 40 s (y) using the equation:

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

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. (ii) Response to $10^{-7}$ M cAMP; (iv) adaptation to $10^{-7}$ M cAMP; (i) response to $10^{-8}$ M cAMP.
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 \( t = \alpha \) s after the first stimulus, and that at that moment the level of adaptation equals \( \alpha \) (\( A(\alpha) = \alpha \)). A mathematical description of these assumptions is

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

(3)

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

\[
R(t) = \Delta[cGMP]_{10(t)}/\Delta[cGMP]_{10(\infty)}.
\]

(4)

where \( \Delta[cGMP]_{10(t)} \) 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 \( \alpha \) 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 \( 10^{-8} M \) cAMP only after the first stimulus with comparable rate constants \( (k = 7.5 \times 10^{-3} s^{-1}; t_{0.5} = 1.5 \text { min}) \), which confirms that deadaptation has first-order kinetics. Furthermore, both lines intersect the abscissa 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 \( (k = 7 \times 10^{-3} s^{-1}; \text{data not shown}) \). 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 deadadaptation has first-order kinetics, we observed repeatedly an oscillatory oscillation of responsiveness as is shown in Fig. 4D. Such cells do not oscillate autonomously before addition of the first stimulus (cAMP levels, cGMP levels, or cGMP 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: deadadaptation 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 \( (t_{50} = 2 \text{ s}) \) has minor effects on the maximum of receptor occupancy by \( 10^{-7} \text{ M} \), but a pronounced effect on the rate of decline of receptor occupancy. Addition of a high amount of phosphodiesterase \( (t_{50} = 0.2 \text{ s}) \) also has a pronounced effect on the maximum of receptor occupancy. Occupation of the receptor by \( 10^{-8} \text{ M} \) cAMP is less fast than by \( 10^{-7} \text{ M} \) cAMP (maximum after about 4 s, Fig. 5A), and fewer receptors get occupied. Addition of phosphodiesterase activity has a more pronounced effect on the maximum of receptor occupancy by \( 10^{-8} \text{ M} \) cAMP than by \( 10^{-7} \text{ M} \) cAMP (Fig. 5B and C). This is in agreement with experimental observations (Fig. 3D).

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 cannot 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 gradients, 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 occupation of the cAMP receptors 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 adaptation has the same rate (Fig. 3D), 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 is 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.

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