Cholera Toxin Induces cAMP-independent Degradation of $\alpha_s$.

(Received for publication, November 22, 1988)

Fu-Hsiung Chang and Henry R. Bourne‡
From the Departments of Pharmacology and Medicine and the Cardiovascular Research Institute, University of California, San Francisco, California 94143

Cholera toxin stimulates adenylyl cyclase by catalyzing ADP-ribosylation of the $\alpha$ chain ($\alpha_s$) of $G_s$, a guanine nucleotide binding regulatory protein. In a rat pituitary cell line, GH3, the toxin-induced increase in GTP-dependent adenylyl cyclase activity is maximal at 1 h; adenylyl cyclase remains elevated for at least 32 h. Surprisingly, cholera toxin also induces a 74–95% decrease in the amount of immunoreactive $\alpha_s$ in the same cells, as assessed on immunoblots probed with either of two antisera directed against separate $\alpha_s$ peptide sequences. The decrease in immunoreactive $\alpha_s$, which begins after 1 h of toxin treatment and is complete by 8 h, is accompanied by a comparable decrease in the amount of biochemically active $\alpha_s$, as assessed by its ability to complement the biochemical defect of $\alpha_s$-deficient S49 cyc- membranes. Cholera toxin induces similar decreases in $\alpha_s$ in wild type S49 lymphoma cells, in S49 $\text{kin}^-$ mutants, which lack cAMP-dependent protein kinase, and in S49 H21a mutants, in which $\alpha_s$ is unable to assume an active conformation upon binding GTP. The toxin-induced decrease in $\alpha_s$ is somewhat temperature-dependent, but is not blocked by agents that increase lysosomal pH or by colchicine, which promotes breakdown of microtubules. $\alpha_s$ in detergent-solubilized GH3 membranes is susceptible to proteolysis by an endogenous protease; this susceptibility is markedly increased in membranes from cells previously exposed to cholera toxin for 1 h. Taken together, these results suggest that cholera toxin-induced covalent modification of $\alpha_s$ marks the protein for accelerated degradation. In addition, the persistence of elevated GTP-dependent adenylyl cyclase activity despite loss of a substantial fraction of $\alpha_s$ suggests that the amount of $\alpha_s$ membranes is greater than the amount necessary for maximal activation of cAMP synthesis by cholera toxin.

The $G_s$ proteins, a family of membrane-bound guanine nucleotide binding proteins, play key roles in transducing hormonal and sensory signals (1–3). These proteins are heterotrimeric, composed of $\alpha$ (39–52 kDa), $\beta$ (35–36 kDa), and $\gamma$ (8–10 kDa) chains. The proteins are distinguished principally by their structurally distinct $\alpha$ chains, which bind and hydrolyze GTP; the more highly conserved $\beta$ and $\gamma$ chains serve to attach the $G_s$ proteins to the cytoplasmic face of the plasma membrane and to present the $\alpha$ chain to the receptor. The mechanism of signal transduction by these proteins is best characterized for $G_s$, the stimulatory regulator of adenylyl cyclase, and retinal transducin, which mediates stimulation of cAMP phosphodiesterase by photorhodopsin (1–3). In both cases, hormone- or light-activated receptors promote binding of GTP by the $G_s$ protein $\alpha$ chain, which in turn stimulates the appropriate effector enzyme; stimulation of the effector is terminated when the $\alpha$ chain hydrolyzes its bound GTP.

Bacterial toxins induce characteristic changes in the function of $G_s$ proteins by catalyzing covalent modification of their $\alpha$ chains (1–3). Cholera toxin catalyzes transfer of ADP-ribose from NAD$^+$ to the $\alpha$ chains of $G_s$ and transducin, while pertussis toxin attaches ADP-ribose to a different amino acid residue on the $\alpha$ chains of transducin, $G_s$, and $G_\alpha$. ADP-ribosylation of the $\alpha$ chain of $G_s$ ($\alpha_s$) by cholera toxin stabilizes the GTP-bound conformation of $\alpha_s$ and decreases its intrinsic GTPase activity, thereby producing increased stimulation of adenylyl cyclase and elevated intracellular cAMP (1–4). Most cells contain two distinguishable forms of $\alpha_s$, 52 and 45 kDa in apparent molecular weight, which are produced by alternative splicing of primary transcripts of a single $\alpha_s$ gene (5, 6).

Despite rapid recent advances in the molecular characterization of $G_s$ protein structure and function, we know remarkably little about the processes that determine their relative abundance in cells. In many cases, including that of hormone-stimulated adenylyl cyclase, the stoichiometric relation among concentrations of $G_s$ proteins, receptors, and effectors remains poorly defined. In the course of investigating effects of cholera toxin in a rat pituitary cell line, GH3, we came upon a surprising observation that sheds light on these issues: Several hours of exposure to cholera toxin caused a 74–95% decrease in the amount of immunoreactive $\alpha_s$ in GH3 cells, while GTP-dependent adenylyl cyclase activity remained elevated. Here we report experiments that explore the biochemical mechanism and implications of this phenomenon.

EXPERIMENTAL PROCEDURES

Materials—Cholera toxin was obtained from List Biological (Campbell, CA). Radioactive ATP, NAD$^+$, and cAMP were obtained from Du Pont–New England Nuclear. [3H]-Protein A was supplied by Amersham. All other reagents were purchased from Sigma.

Cell Culture—GH3 cells, obtained from the Cell Culture Facility, University of California (San Francisco, CA), were propagated in Dulbecco's modified Eagle's-H21 medium with 10% fetal calf serum, as described (7). Wild type and mutant S49 mouse lymphoma cells were propagated in the same medium, supplemented with 10% horse serum, as described (8).

Membrane Preparation—Both GH3 and S49 cells were allowed to swell for 10 min at 0°C in 50 mM Tris-HCl (pH 7.5) containing 2 mM MgCl$_2$ and 1 mM phenylmethylsulfonyl fluoride (PMSF). After Dounce homogenization, nuclei were removed by centrifugation at

$^*$ This work was supported in part by Grants GM-27800 and GM-28310 from the National Institutes of Health and grants from the March of Dimes. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence and reprint requests should be addressed.

The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Gpp(NH)p, guanyl-5'-yl imidodiposphate.
500 × g for 5 min, and the supernatant fraction (hereafter called the postnuclear supernatant fraction) was then centrifuged at 16,000 × g for 10 min. The resulting pellet (membrane particulate fraction) was resuspended in 50 mM Tris-HCl (pH 7.5) containing 1 mM PMSF. PMSF was omitted from the preparation in experiments designed to test effects of endogenous proteases. Aliquots of membranes were stored at -70 °C before use. Protein content was measured by the method of Lowry et al. (9).

Adenylyl Cyclase and G, Activities—Adenylyl cyclase was measured by the method of Salomon et al. (10), slightly modified as described (7). For assays of G, activity, GH3 membranes (0.1 mg/ml) were extracted with 0.2% (w/v) Lubrol (11), and G, activity in the extract was assessed by its ability to complement the G, deficiency of S49 mouse lymphoma cycl- membranes in vitro, exactly as described (7, 12, 13). Briefly, the Lubrol extract (0-20 μl) from GH3 membranes (donor extract) was added to cycl- membranes (200 μg) and incubated at 30 °C for 40 min in the presence of GTP or Gpp(NH)p (each at 100 μM). Then, [32P]ATP was added, and adenylyl cyclase activity was measured for 20 additional min in a total volume of 100 μl. In order to maintain constant concentrations of detergent, protein, and lipid in reactions containing less than 20 μl of donor extract, these reactions were supplemented with appropriate volumes (20-0 μl) of donor extract that had been inactivated by heating at 90 °C for 15 min. In these experiments, unless otherwise stated, the following protease inhibitors were used in combination to retard degradation of α, in the extract: PMSF (1 mM) and 1 μM concentration of each of the following: leupeptin, antipain, pepstatin A, and chymostatin.

Toxin-catalyzed Radiolabeling—Particulate fractions from control cells or cholera toxin-treated varying times with cholera toxin were subjected to cholera toxin-catalyzed ADP-ribosylation, using radioactive NAD*, exactly as described (14). After gel electrophoresis, autoradiograms were quantitated by scanning with a Hoffer densitometer. Relative intensities of the radioactive bands were assessed by comparison to an autoradiogram of serially diluted control GH3 membranes. Control membranes (i.e., [32P]ADP-ribosylated membranes from cells not previously exposed to cholera toxin).

Immunoblot Analysis—Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed as described by Laemmli (15), using 12.5% acrylamide gels. Samples were denatured by boiling for 5 min in a solution containing 2% SDS, 2.5% 2-mercaptoethanol, 50 mM Tris-HCl (pH 8), and 7.5% glycerol. Gel slices containing separated proteins were electrophoretically transferred to nitrocellulose paper (Schleicher & Schuell). Proteins transferred to nitrocellulose paper were visualized by pre-staining in 0.2% (v/v) Ponceau S (16). The nitrocellulose paper was then incubated in blocking solution (containing 5% nonfat dry milk, 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 0.01% NaN₃, and 0.05% Tween 20) for 1 h at room temperature with constant shaking, followed by incubation in antibody to peptide antibody in blocking solution for 2-3 h. After subsequent rinses and incubation in 10 ml of blocking solution containing 1% labeled protein A (5 × 10⁶ cpm, Amersham), the nitrocellulose membranes were washed and subjected to autoradiography.

Antiserum—Antibody U10 was raised (17) against a synthetic peptide corresponding to a sequence near the carboxyl terminus of α, (residues 323-338, DATPEPGEQPRVKTR). Antiserum A569, generously provided by Susan Mumby and Alfred G. Gilman, is directed against a conserved sequence located near the amino terminus of G protein α chains (residues 47-61 of α, GAGESGKSTIKRKQRK). It detects a broad spectrum of G protein α chains (8).

RESULTS

After exposure to cholera toxin (1 μg/ml) for 8 h, GH3 cells were homogenized, and fractions separated by centrifugation were analyzed by immunoblotting with antibodies against G protein α chains (Fig. 1). Cholera toxin caused a marked reduction in the 52-kDa form of α, as assessed by an antisera and (U10), directed against a sequence near the carboxyl terminus of α, (Fig. 1, lane 2 versus lane 1); the effect of cholera toxin was less marked on the 45-kDa form of α, which is much less prominent in GH3 membranes than the 52-kDa form (7). The possibility that cholera toxin treatment induced transfer of α, from membranes to cytosol is ruled out by the observation that the toxin-induced decrease was also seen in postnuclear supernatant fractions (Fig. 1, lane 4 versus lane 3), which contain both membranes and cytosol.

![Fig. 1. Effect of cholera toxin on immunoreactive α,](https://example.com/fig1.png)

**Fig. 1.** Effect of cholera toxin on immunoreactive α, GH3 cells were treated with or without cholera toxin (CT) (1 μg per ml) for 8 h, as indicated. The cells were then homogenized, and particulate fractions (100 μg of protein, lanes 1, 2, 5, and 6) or postnuclear supernatant fractions (200 μg of protein; lanes 3, 4, 7, and 8) were subjected to electrophoresis in 12.5% SDS-polyacrylamide gels, transferred to nitrocellulose, and probed with antisemirum U10 (directed against a peptide unique to α,; lanes 1-4) or antisemirum A569 (directed against a sequence common to G protein α chains; lanes 5-8). Numbers at the right margin are molecular weight markers (X 10⁶). Arrows on the left indicate the positions of the 52- and 45-kDa forms of α,.

Parallel decreases in immunoreactive α, in both membranes and postnuclear supernatant fractions, were also seen with an antibody (A569) directed against a peptide sequence located near the amino terminus of G protein α chains (Fig. 1, lanes 5-8). The parallel results with two antibodies directed against spatially separate portions of the α, polypeptide make it quite unlikely that toxin-catalyzed ADP-ribosylation somehow prevented these antibodies from detecting α, in addition, the A569 antibody showed that cholera toxin caused no change in the amount of a broad band migrating at approximately 40 kDa, which corresponds to the α chains of G and Gβ. Antibody U10 recognized a 58-kDa band in postnuclear supernatant fractions (Fig. 1, lanes 3 and 4); this band was unaffected by cholera toxin and was not detected by antibody A569; it is almost certainly not a G protein α chain.

Cholera toxin is known to activate adenylyl cyclase in a time- and concentration-dependent manner. In our experiments, cholera toxin in culture medium at concentrations ranging from 100 ng to 3 μg per ml caused quantitatively similar elevations of adenylyl cyclase and decreases in immunoreactive α, at 8-16 h (data not shown). Fig. 2 shows the time course of changes induced by a single concentration of toxin (1 μg per ml). The toxin caused a progressive decrease in immunoreactive α, beginning after the first hour of incubation and complete by 8 h (Fig. 2, open squares). By this time, densitometric analysis of immunoblots showed a 92% reduction in the 52-kDa form of α, Visual inspection of the autoradiograms (Fig. 2, bottom) shows that the 45-kDa form was also decreased; the small size of the 45-kDa peak prevented its reliable quantitation by densitometry. In other experiments, exposure of GH3 cells to cholera toxin for 8 h led to decreases in immunoreactive α, that ranged from 74 to 95%.

In contrast to the decrease in immunoreactive α, cholera toxin induced a 4- to 5-fold increase in GTP-stimulated adenylyl cyclase activity, which reached a maximum at about 1 h and persisted for 32 h (Fig. 2, solid squares).

To assess the extent of ADP-ribosylation of α, in intact cells exposed to cholera toxin, we measured the ability of the toxin to transfer radiolabel from [32P]NAD* to the 52-kDa α, polypeptide in particulate fractions prepared from cells ex-
posed for various times to cholera toxin in culture; only molecules that were not ADP-ribosylated by the toxin in particulate fractions from cells exposed to cholera toxin (52 kDa) band was reduced by 74% at 8 h of toxin treatment, as compared to 1 h of toxin treatment.

The cholera toxin-induced decrease in immunoreactive \( \alpha \) is not limited to GH3 cells. Exposure of wild type and three mutant S49 mouse lymphoma cell lines to the toxin for 8 h resulted in a substantial decrease in membrane \( \alpha \), as assessed with the U10 antiserum (Fig. 4A). The mutants included cells with altered \( \alpha \) (unc and H21a) (8) and a cell line lacking cAMP-dependent protein kinase (kin-). In addition, agents that increase or mimic cAMP (Fig. 4B, lanes 3 and 4) did not reproduce the action of cholera toxin in GH3 cells. These results, along with those in S49 H21a and kin- cells, conclusively rule out cAMP as a mediator of the toxin-induced decrease in immunoreactive \( \alpha \).

The cholera toxin-induced decrease in \( \alpha \) was partially temperature-dependent (Fig. 4C). Immunoreactive 52-kDa \( \alpha \) was measured in GH3 cells that were exposed to toxin for 1 h
performed with antiserum U10 in all cases.

**Fig. 4. Effects of mutations and drugs on cholera toxin-induced decreases in immunoreactive α.** Immunoblot analysis of membranes (150 μg per lane) of S49 cells treated with or without (+ and −) cholera toxin (1 μg per ml) for 8 h. Cell types include wild type (lanes 1 and 2), H21a (lanes 3 and 4), kin− (lanes 5 and 6), and unc (lanes 7 and 8). B, immunoblot analysis of membranes (150 μg) from GH3 cells treated with the following reagents for 8 h: no treatment (lane 1); cholera toxin, 1 μg per ml (lane 2); 8-Br-cAMP, 1 mM (lane 3); forskolin, 1 μM, plus 0.5 mM isobutylmethylxanthine (lane 4). C, immunoblot analysis of postnuclear supernatant fractions (150 μg per lane) from GH3 cells treated as follows: no treatment (lane 1); exposure to cholera toxin (1 μg per ml) for 1 h at 37 °C (lane 2); exposure to cholera toxin for 1 h at 37 °C, followed by continued exposure to the toxin for 7 h at 37 °C (lane 3), 4 °C (lane 4), or 18 °C (lane 5); cholera toxin for 1 h at 37 °C, followed by continued exposure to the toxin at 37 °C in the presence of 500 μM chloroquine (lane 6), 10 mM NH₄Cl (lane 7), or 8 μM colchicine (lane 8). Immunoblots were performed with antisem U10 in all cases. Arrows indicate the positions of the 52- and 45-kDa forms of α.  

at 37 °C and then incubated in the same medium for a further 7 h at 37, 4, or 18 °C (Fig. 4C, lanes 3, 4, and 5, respectively). The toxin-induced decrease in α was diminished at 4 °C (30% decrease) and 18 °C (70% decrease), as compared to 37 °C (>90% decrease). This difference was not due to ineffective ADP-ribosylation, because, regardless of the temperature during the last 7 h of incubation, most of the α in the membranes was in the ADP-ribosylated form, as assessed by its unavailable for radiolabeling in vitro by cholera toxin and [3H]NAD⁺ (results not shown). The relatively weak temperature dependence of the cholera toxin-induced decrease in α suggests that endocytosis may not be involved. Because lysosomes are involved in ligand-induced down-regulation of cell surface receptors (21, 22), we asked whether lysosomotrophic agents could block the cholera toxin-induced decrease of α. Neither ammonium chloride (10 mM) nor chloroquine (500 μM) altered the effect of cholera toxin (Fig. 4C, lanes 6 and 7 versus lane 3). Similarly, colchicine (3 μM), which blocks assembly of microtubules and can prevent redistribution of organelles (23), also had no effect (Fig. 4C, lane 8 versus lane 3).  

Taken together, these experiments provide no evidence for possible roles of movement and fusion of organelles, including lysosomes, in the cholera toxin effect. The experiments shown in Fig. 5, however, do provide a strong hint that cholera toxin treatment increases the susceptibility of α to cleavage by an endogenous protease and also suggest that accessibility of α to the protease in vitro requires the presence of a detergent. In the first experiment (Fig. 5A), membranes were prepared from GH3 cells that had been incubated with or without cholera toxin for 1 h. The membranes were then incubated with 0.2% Lubrol, a nonionic detergent, for 8 h at 4 or 37 °C in the presence or absence of a mixture of protease inhibitors. The presence of Lubrol caused the 52- and 45-kDa α bands to migrate as a fuzzy broad band in SDS, but did not obscure the key result: α from cholera toxin-treated cells was cleaved to a 40-kDa immunoreactive species at 4 °C in the absence of protease inhibitors (lane 4); under identical conditions, α from control cells (not exposed to cholera toxin) was not detectably cleaved (lane 3). The conversion of α from 52 to 40 kDa was prevented by protease inhibitors (lane 2). The addition of 100 μM Gpp(NH)p to extracts from control cells did not accelerate degradation of α (data not shown), indicating that the degradation seen in extracts from toxin-treated cells was not due to an increased susceptibility to proteases that might be induced by activation of C.  

Incubation of detergent extracts for 8 h at 37 °C led to the appearance of a ∼38–40-kDa immunoreactive band, even in extracts of cells that had not been exposed to cholera toxin (Fig. 5A, lanes 5 and 6). The degradation of α at 37 °C was more extensive, however, in extracts from cholera toxin-treated cells (lane 6 versus lane 5). These observations suggest that modification of α by cholera toxin increases the protein’s susceptibility to a protease that can also act, although less effectively, on unmodified α. Although the identity of the protease is unknown, it is probably a serine or cysteine protease, because chymostatin, antipain, leupeptin, and PMSF each was able individually to block degradation of α in detergent extracts of toxin-treated cells (result not shown).  

In membranes, immunoreactive α is remarkably stable, even in the absence of protease inhibitors, as long as detergent is not present. After an 8-h incubation at 4 or even 37 °C (Fig. 5B), α was not cleaved in broken cell preparations derived from either control or toxin-treated cells. This experiment (Fig. 5B) utilized postnuclear supernatant fractions, rather than particulate fractions, in order to provide optimal accessibility of membrane-bound α to potential degradative enzymes in the cytoplasm. Addition of ATP and an ATP-regenerating system in such incubations did not affect the results (not shown).  

Because ADP-ribose attached to α appears to trigger its disappearance, it is reasonable to ask whether ADP-ribose
attached to α₁ chains (α subunits of G₄) causes these proteins to disappear also. This was not the case. Treatment of GH₃ cells with pertussis toxin had no effect on the amount of immunoreactive α₁ detected with the A569 antiserum (result not shown).

**DISCUSSION**

The mechanism by which cholera toxin activates adenyl cyclase is well established (see Refs. 1–3 for review). The toxin catalyzes ADP-ribosylation of the α chain of G₄, thereby stabilizing the protein in its active form; the resulting increase in active α₁ leads to increased adenyl cyclase activity. Here we describe a previously unsuspected additional effect. Cholera toxin induces a delayed, progressive disappearance of α₁ from toxin-treated cells. The new finding raises two questions. 1) What mechanism is responsible for the toxin-induced loss of α₁? 2) How can the disappearance of α₁ be reconciled with continued cholera toxin-induced elevation of adenyl cyclase?

With respect to the first question, our experiments do not establish a precise mechanism by which cholera toxin induces disappearance of α₁, but do conclusively rule out several possibilities. For example, the decrease in α₁ cannot be attributed to masking of a specific α₁ epitope, because: (a) loss of immunoreactive protein was confirmed by two antisera, directed against widely separated peptide sequences in α₁ (Fig. 1); in addition, (b) the biochemical activity of α₁ decreased in parallel with immunoreactive protein (Fig. 3). Moreover, failure of forskolin and 8-bromo-cAMP to reproduce the effect of cholera toxin indicates that elevated cAMP is not responsible for proteolytic degradation. Toxin-induced degradations is susceptible to an endogenous protease only in the presence of added detergent, and this protease degrades the protein in extracts from cholera toxin-treated cells to a greater extent than in control extracts (Fig. 5). The requirement for detergent makes it attractive to imagine that toxin-catalyzed ADP-ribosylation in intact cells marks α₁ for degradation by a protease from which the protein is normally separated by a membrane. This notion is neither supported nor conclusively refuted by other data. Indeed, the 40-kDa α₁ degradation product seen in detergent extracts is not seen in intact cells treated with cholera toxin; thus, the endogenous protease that cleaved α₁ in detergent extracts is not necessarily responsible for toxin-induced degradation of α₁ in intact cells. Furthermore, the weak temperature dependence of the toxin effect (Fig. 4C) and the lack of effect of lysosomotropic agents argue, albeit weakly, that neither endocytosis nor maintenance of an acidic pH in lysosomes is required for degradation of α₁.

In summary, the data suggest that attachment of ADP-ribose to α₁ somehow increases the protein’s susceptibility to proteolytic degradation, but do not identify the degradative pathway involved.

The elevation of adenyl cyclase induced by cholera toxin persists for at least 32 h, despite a substantial decrease in α₁, which is completed much earlier, by 8 h (Fig. 2). The demonstration that toxin reduces the amount of biochemically active α₁ (Fig. 3) rules out the possibility that the toxin-induced decrease in immunoreactive protein was selective for a subpopulation of inactive α₁ molecules. Taken together, the evidence suggests that α₁ in toxin-treated cells is rare, at least early in the response (e.g. at 1 h), i.e. that the concentration of α₁ is substantially higher than is required for maximal activation of adenyl cyclase. Consequently, if we assume that GTP-dependent activation of the enzyme requires a one-to-one complex of α₁ and adenyl cyclase, the concentration of α₁ in GH₃ membranes must be severalfold greater than that of the catalytic protein, adenyl cyclase. To our knowledge the relative concentrations of α₁ and adenyl cyclase have not been defined in any mammalian cell.

The inference that α₁ is present in excess of adenyl cyclase appears to contradict a number of observations indicating that G₄ limits the maximal activity of adenyl cyclase. Thus, glucocorticoids induced an increase in G₄ activity and α₁ mRNA in GH₃ cells, changes which were associated with a parallel increase in adenyl cyclase activity (7). In ethanol-treated neuroblastoma cells, a decrease in α₁ protein and mRNA was associated with decreased hormone-sensitive adenyl cyclase (27). In addition, inherited deficiency of α₁, caused by loss of an autosomal allele, produces the characteristic resistance to parathyroid hormone and other hormones that is seen in pseudohypoparathyroidism, Type I (28, 29). Taken together, the evidence suggests that both increases and decreases in the expression of α₁ produce parallel changes in adenyl cyclase activity and implies that the amount of available G₄ is rate-limiting for stimulation of cAMP synthesis.

This implication can be reconciled with our present results if we take account of the possibility that ADP-ribosylated α₁ may be more sparse than the unmodified protein. In membranes of control cells, i.e. cells not exposed to cholera toxin, the transience and reversibility of each α₁-adenyl cyclase interaction could allow the concentration of α₁-adenyl cyclase complexes to depend on the concentration of free (uncomplexed) α₁; thus, in control cells, the amount of α₁ can be in molar excess, relative to adenyl cyclase, but may still limit maximal stimulation of cAMP synthesis. In membranes from toxin-treated cells, however, each α₁-adenyl cyclase complex would be expected to persist in the active (cAMP-synthesizing) form for minutes rather than seconds, because cholera toxin-catalyzed ADP-ribosylation stabilizes the active GTP-bound form of α₁ (1–4); the relative irreversibility of the
\(\alpha\)-adenylyl cyclase interaction would make toxin-modified \(\alpha\), a much more potent stimulator of adenylyl cyclase.

Critical tests of these notions will require measurements of adenylyl cyclase in lipid vesicles that incorporate known amounts of \(G\) components and adenylyl cyclase or experimental manipulations that alter the relative expression of \(\alpha\), adenylyl cyclase, and other components of the adenylyl cyclase system in intact cells.

Acknowledgments—We thank Janet Scott for antibody U10 and Alfred G. Gilman and Susan Mumby for the A569 antiserum. We also thank Judith M. White, Anthony L. DeFranco, Daniel J. Chin, Robert A. Nissenson, Ian C. Zachary, and Patrice M. Tremble for useful advice and criticism.

REFERENCES

1. Stryer, L., and Bourne, H. R. (1986) Annu. Rev. Cell Biol. 2, 391-419
2. Casey, P. J., and Gilman, A. G. (1988) J. Biol. Chem. 263, 2577-2580
3. Neer, E. J., and Clapham, D. E. (1988) Nature 333, 129-134
4. Cassel, D., and Selinger, Z. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3307-3311
5. Robishaw, J. D., Smigel, M. D., and Gilman, A. G. (1986) J. Biol. Chem. 261, 9587-9590
6. Bray, P., Carter, A., Simons, C., Guo, V., Puckett, C., Kamholz, J., Spiegel, A., and Nirenberg, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8885-8897
7. Chang, F.-H., and Bourne, H. R. (1987) Endocrinology 121, 1711-1715
8. Bourne, H. R., Beiderman, B., Steinberg, F., and Brothers, V. M. (1982) Mol. Pharmacol. 22, 204-210
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
10. Salomon, Y., Londos, C., and Rodbell, M. (1974) Anal. Biochem. 58, 547-548
11. Johnson, G. L., Kao, W. R., and Bourne, H. R. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3113-3117
12. Kao, W. R., Farfel, Z., Johnson, G. L., and Bourne, H. R. (1979) Mol. Pharmacol. 15, 472-483
13. Farfel, Z., Brickman, A. S., Kao, W. R., Brothers, V. M., and Bourne, H. R. (1980) N. Engl. J. Med. 303, 237-242
14. Goldman, D. W., Chang, F. H., Gifford, L. A., Goetzl, E. J., and Bourne, H. R. (1985) J. Exp. Med. 162, 145-156
15. Lasemli, U. K. (1970) Nature 227, 680-685
16. Salinovich, O., and Montelaro, R. C. (1986) Anal. Biochem. 156, 1-17
17. Masters, S. B., Sullivan, K. A., Miller, R. T., Beiderman, B., Lopez, N. G., Ramachandran, J., and Bourne, H. R. (1988) Science 241, 448-451
18. Mumby, S. M., Kahn, R. A., Manning, D. R., and Gilman, A. G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 265-269
19. Ross, E. M., and Gilman, A. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3715-3719
20. Insel, P. A., Bourne, H. R., Coffino, P., and Tomkins, G. M. (1975) Science 190, 896-898
21. King, A. C., Hernandez-Davis, L., and Custredcasas, P. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3283-3287
22. Zachary, I., and Rozenbarg, E. (1987) EMBO J. 6, 2233-2239
23. Wilson, L., and Bryan, J. (1974) Adv. Cell Mol. Biol. 21-72
24. Miller, R. T., Masters, S. B., Sullivan, K. A., Beiderman, B., and Bourne, H. R. (1988) Nature 334, 712-715
25. Sullivan, K. A., Miller, R. T., Masters, S. B., Beiderman, B., Heidenman, W., and Bourne, H. R. (1987) Nature 330, 758-760
26. Haga, T., Ross, E. M., Anderson, H. J., and Gilman, A. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2016-2020
27. Moehly-Rosen, D., Chang, F. H., Cheever, L., Kim, M., Diamond, I., and Gordon, A. S. (1988) Nature 333, 848-850
28. Van Dop, C., and Bourne, H. R. (1983) Annu. Rev. Med. 34, 259-266
29. Spiegel, A. M., Gierschik, P., Levine, M. A., and Downs, R. W., Jr. (1985) N. Engl. J. Med. 312, 26-33