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Mechanism of Action of Cholera Toxin and the Mobile Receptor Theory of Hormone Receptor–Adenylate Cyclase Interactions

(membrane fluidity/solubilized adenylate cyclase/glucagon receptors/spare receptors/affinity chromatography/immunoprecipitation)

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ABSTRACT **Rat liver membrane adenylate cyclase (EC 4.6.1.1) that has been stimulated more than 10-fold by cholera toxin (choleragen) has a 3-fold greater sensitivity to stimulation by glucagon. Choleragen similarly increases the sensitivity of cyclase to other peptide (ACTH, vasoactive intestinal polypeptide) and nonpeptide (catecholamines) hormones in this and other tissues. The rate of ^{125}I-labeled glucagon–membrane dissociation is decreased about 2-fold in toxin-treated liver membranes. Toxin-activated cyclase activity of fat cell membranes is retained upon solubilization with Lubrol PX. Provided ^{125}I-labeled choleragen is first incubated with cells under conditions resulting in enzyme activation, the solubilized cyclase activity migrates with a component of ^{125}I-labeled choleragen on gel filtration chromatography. Agarose derivatives containing the "active" subunit (molecular weight 36,000) of the toxin can specifically adsorb solubilized adenylate cyclase. Toxin-stimulated cyclase can be immunoprecipitated with antitoxin or anti-"active" subunit antibodies. There is a large excess of membrane receptors (ganglioside G_{M1}) which, with the use of choleragenoid, can be shown to be functionally equivalent with respect to cyclase activation. Choleragenoid, an inactive competitive antagonist of toxin binding, can occupy and block a large proportion of toxin receptors without affecting toxin activity. A scheme of toxin action is proposed that involves lateral membrane diffusion of the initially inactive toxin–receptor complex with subsequent direct interaction with and modulation of adenylate cyclase. The basic features of this scheme may be pertinent to the mechanisms by which hormone receptors normally modulate adenylate cyclase.**

Cholera toxin (choleragen) stimulates ubiquitously membrane-found adenylate cyclase (1) [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] and binds specifically cell surface receptor glycolipids (G_{M1} gangliosides) (2–7). The properties of the toxin-activated adenylate cyclase resemble those of cyclase activated by catecholamines and polypeptide hormones*. Choleragen most likely does not interact directly with receptors for hormones since choleragenoid (8–11), a biologically inactive toxin derivative which binds to the same choleragen receptors, thereby acting as a toxin antagonist (2), does not affect the sensitivity of cyclase toward other hormones*. Further, toxin action exhibits an absolute, temperature-dependent "lag" period (2–7) during which the properties of adenylate cyclase with or without hormones are unaltered. On the basis of the extreme persistence of the toxin's biological effects (*, 12, 13), the hydrophobic nature of the 36,000 molecular weight "active" subunit, the irreversible binding of ^{125}I-labeled toxin to cell membranes (2), and the nature of the kinetics of cyclase activation*, it is proposed* that the toxin molecule (or a portion thereof) possibly becomes incorporated into the phospholipid bilayer as an "integral" (14) membrane protein so that it may complex directly with adenylate cyclase.

Choleragen action may provide a model for the interaction of hormone receptors with adenylate cyclase. Here we suggest that choleragen action involves mobility of the initial toxin–ganglioside complex in the plane of the membrane, with the ultimate formation of stable complexes between the toxin and the cyclase. It is suggested that hormone receptors may normally be relatively free to diffuse within the plane of the membrane, and that when complexed with hormone, they encounter and modify membrane enzymes by analogy with protein–protein interactions in solution (15–17).

MATERIALS AND METHODS

Cholera toxin was obtained from Dr. C. E. Miller, SEATO Research Program. Antiserum against cholera toxin was provided by Dr. S. Craig, and antisera against the active subunit (molecular weight 36,000) of the toxin was a gift of Dr. R. Finkelstein. Choleragenoid and the "active" subunit (molecular weight 36,000; refs 8 and 9) were purified to homogeneity (sodium dodecyl sulfate disc gel electrophoresis) from crude culture filtrates on ganglioside–agarose columns (ref. 8 and unpublished). Choleragenoid was repurified four times on affinity columns to remove all traces of native toxin; this derivative could compete for ^{125}I-labeled toxin binding (2) as effectively as the native toxin, but at concentrations as high as 2 μg/ml it failed to stimulate adenylate cyclase or inhibit DNA synthesis in cultured fibroblasts (18), effects that are maximally affected by 1 ng/ml and 10 pg/ml of choleragen, respectively. [α-^{32}P]ATP (10–20 Ci/mmole) was synthesized by the method of Symons (*, 19). Preparation of ^{125}I-labeled choleragen (10–20 μCi/μg) (2) and glucagon (1.2 Ci/μmole) (20) is described elsewhere.

Adenylate cyclase activity was determined* in 0.1 ml containing [α-^{32}P]ATP (62 μM, 300–700 cpm/pmole), 5'-GTP (5 μM), $MgCl_2$ (6.2 mM), aminophylline (5 mM), and an ATP-regenerating system (5 mM phospho*enol*pyruvate and 50 μg/ml of pyruvate kinase), Tris·HCl, (50 mM, pH 8), and 10–50 μg of membrane protein. The assays were stopped by boiling (1 min), and cAMP was isolated from neutral alumina columns (21, 22). Production of cAMP by the Lubrol-solubilized enzyme (untreated and toxin-activated) was linear for at least 60 min at 15° and below.

* Bennett, V. & Cuatrecasas, P. (1974) *J. Membrane Biol.* submitted.

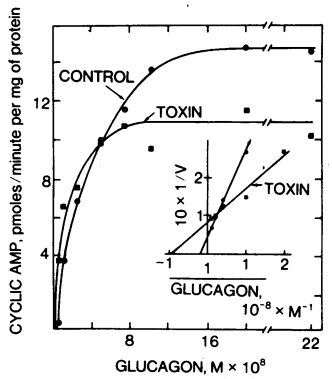

FIG. 1. Glucagon stimulation of adenylate cyclase of control (●) and cholera toxin-treated (■) rat liver membranes, showing a change in the apparent affinity for the hormone. Male rats (130 g) were injected intracardially with 0.4 ml of Krebs–Ringer bicarbonate containing 100 μg of cholera toxin. Twelve hours later the livers were homogenized (Brinkman Polytron) in 40 ml of ice-cold 0.25 M sucrose, 10 mM Tris·HCl (pH 7.7). The 900 × *g* supernatant was centrifuged at 0° for 20 min at 12,000 × *g*, and the upper pellet layer (23) was suspended in 50 mM Tris (pH 7.7), centrifuged again, and suspended in 50 mM Tris, 1 mg/ml of bacitracin (19) (pH 7.7) at 10 mg/ml of membrane protein. Cyclase activity of 20-μl samples, determined at 30° for 10 min, is expressed as stimulated activity (stimulated minus basal). The basal activities of the control and toxin-treated membranes were 2.2 pmole/min per mg and 25.7 pmoles/min per mg, respectively. Similar effects were reproduced in at least five different consecutive experiments.

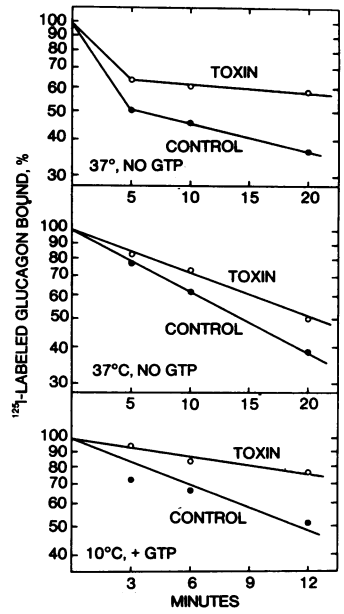

FIG. 2. Effect of cholera toxin treatment on the rate of dissociation of liver membrane–glucagon complexes. Heavy (ref. 23; middle and lower panels) and microsomal (ref. 24; upper panel) membranes from livers of toxin-treated rats (Fig. 1) were suspended (about 1.5 mg of protein per ml) in 50 mM Tris·HCl (pH 7.4), 0.1% (w/v) bovine serum albumin, 0.1% (w/v) bacitracin (19). Native glucagon (1 μg/ml) was added to some samples (to correct for nonspecific binding; ref. 19) and all the samples were incubated for 10 min at 24° with ^{125}I-labeled glucagon (1.5 to 3 × 10^6 cpm, 1.2 Ci/μmole) before they were placed in ice. Zero time samples were assayed, and native glucagon (1 μg/ml) was added to all and 0.2 mM GTP to some (lower panel only) of the samples. Binding was determined by the oil flotation (Beckman microfuge) technique (ref. 25; dibutyl phthalate–dinonyl·phthalate, 2:1) after incubation at 37° (upper and middle panels) or 10° (lower panel); with GTP, dissociation is too fast to measure accurately at 24° or 37°. Binding did not differ significantly in the control and treated membranes.

RESULTS

Toxin-Induced Changes in Hormone Affinity. Liver membranes, whose basal activity of adenylate cyclase is stimulated about 12-fold by treatment *in vivo* with choleragen, demonstrate (Fig. 1) a 3-fold decrease in the concentration of glucagon required for half-maximal activation (about 3 × 10^{-8} M to 10^{-8} M). Choleragenoid has no effect on the K_a for hormone activation. The liver-glucagon system was chosen since binding of ^{125}I-labeled glucagon can be measured readily. The apparent affinity of binding of ^{125}I-labeled glucagon to liver membranes is increased about 2-fold after cholera toxin treatment (not shown). This is due primarily to a fall in the rate of dissociation of the ^{125}I-labeled glucagon–membrane complex (Fig. 2). The decreased rate of dissociation is also observed in the presence of GTP, even though GTP itself greatly accelerates the rate of glucagon dissociation.

A similar increase in the apparent affinity for hormonal activation of adenylate cyclase after choleragen treatment has been detected for (−)-isoproterenol in rat and toad erythrocytes* and liver, and for (−)-isoproterenol, glucagon, vasoactive intestinal polypeptide, and ACTH in fat cell membranes (unpublished).

Solubilization of Cyclase and Gel Filtration. The choleragen-induced activation of fat cell adenylate cyclase is retained (Table 1) after dissolution of the membrane with the nonionic detergent, Lubrol PX, and the same concentration dependence for toxin is observed as for the particulate enzyme (not shown). In the "solubilized" state, the stimulation by isoproterenol is lost while the effect of NaF remains. The toxin-treated enzyme is not stimulated but rather is inhibited by NaF in the absence* or presence of detergents (Table 1). The stimulated state of the dispersed toxin-treated enzyme gradually decreases above 15° or after prolonged (4 hr) exposure to detergent.

Gel filtration of the detergent-solubilized native and toxin-activated fat cell enzyme reveals elution in 1.5 and 2.3 of the void volumes (V_0) on Sepharose 6B and 4B, respectively (Figs. 3 and 4); the activity is present in the V_0 of Sephadex G-50. The recovery of activity on Sepharose 4B is nearly the same for control and toxin-treated enzyme, and the toxin-stimulated state is retained (Fig. 3).

Solubilization of Membrane-Bound ^{125}I-Labeled Toxin. The chromatographic patterns of adenylate cyclase activity and of membrane-bound ^{125}I-labeled cholera toxin from solubilized membranes were determined after exposing fat cells to fully active ^{125}I-labeled toxin for either 3 hr or 10 min at 37° (Fig. 4). In 10 min at 37° choleragen binding to fat cells is complete, but there is no effect on adenylate cyclase activity (unpublished). On Sepharose 6B a major portion of the membrane-bound ^{125}I-labeled toxin appears in the V_0, and this is relatively independent of the time or temperatures of preincubation with choleragen. A small radioactive peak is found associated with the adenylate cyclase activity provided the cells have been incubated at 37° for 3 hr with ^{125}I-labeled toxin. This small peak, which corresponds to about 1000

TABLE 1. *Retention of cholera toxin-activated adenylate cyclase activity after solubilization of fat cell membranes with Lubrol PX*

Lubrol PX (v/v)	Adenylate cyclase activity (nmol of cAMP/hr per mg) Basal	NaF (10 mM)	(−)-isoproterenol (10^{-5} M)	% Activity solubilized*
None, Control	0.5	0.8	0.9	2
Toxin-treated	1.6	1.2	1.8	1
0.2%, Control	0.3	0.9	0.3	71
Toxin-treated	1.2	1.1	1.2	68
1%, Control	0.2	0.6	0.2	84
Toxin-treated	0.9	0.8	1.0	77
2%, Control	0.2	0.6	0.2	79
Toxin-treated	0.8	0.7	0.8	74

* Determined for each condition by sonication (5 sec) and centrifugation at 250,000 × *g* for 25 min at 0°.

Adipocytes were incubated for 3 hr at 37° ± 0.2 μg/ml of toxin. Isolated membranes (Fig. 3) were suspended (2 mg of protein per ml) in $MgCl_2$ (5 mM), bovine serum albumin (0.15%, w/v), GTP (1 μM), and 50 mM Tris·HCl (pH 8). Cyclase activity was measured for 30 min at 15° (see *Materials and Methods*).

molecules of toxin per cell, appears in the same position after rechromatography, although heating in 1% sodium dodecyl sulfate shifts the peak from 1.5 V_0 to about 2 V_0. If ^{125}I-labeled toxin is added directly to the solubilized membranes before chromatography, some radioactivity appears in the V_0 but no peak is observed in the position of adenylate cyclase.

TABLE 2. *Specific immunoprecipitation of detergent-solubilized adenylate cyclase from cholera toxin-treated fat cells with antitoxin antibodies*

Precipitating conditions	Enzyme	Immunoprecipitated activity*
Goat anti-rabbit IgG + rabbit serum	Control	0.4 ± 0.03
	Toxin-stimulated	0.4 ± 0.16
Rabbit anti-cholera toxin + cholera toxin	Control	1.5 ± 0.06
	Toxin-stimulated	4.8 ± 0.58
Rabbit anti-36,000 MW subunit + 36,000 MW subunit	Control	0.5 ± 0.10
	Toxin-stimulated	3.5 ± 0.19

* pmoles of cAMP per hr ± standard error of the mean.

Adenylate cyclase was extracted in 0.7% Lubrol PX from control and toxin-treated fat cells (0.1 μg/ml, 3 hr at 37°, as in legend of Fig. 3). Samples (0.15 ml) of the 250,000 × *g* supernatants were incubated with goat antiserum against rabbit IgG (50 μl), rabbit antiserum against cholera toxin (0.1 ml), or rabbit antiserum against the 36,000 molecular weight subunit (0.1 ml) in a volume of 0.5 ml in 50 mM Tris·HCl, pH 8. After 2 hr at 0°, 0.1 ml of rabbit serum, 44 μg of cholera toxin, and 20 μg of purified 36,000 molecular weight subunit were added as indicated. After 60 min the precipitates were washed and assayed for cyclase activity (90 min, 13°). The total activities before centrifugation were: 78 pmole/hr (control), 236 pmole/hr (toxin-treated) in the presence of antibody against rabbit IgG; 323 pmole/hr (control), 740 pmoles/hr (toxin-treated) in the presence of antitoxin; 344 pmoles/hr (control), 850 pmoles/hr (toxin-treated) in the presence of antibody against active subunit.

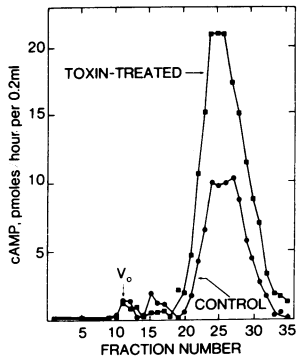

FIG. 3. Sepharose 4B chromatography of Lubrol PX-solubilized adenylate cyclase of untreated (●) and cholera toxin-activated (■) fat cell membranes. Cells from fat pads of 27 rats (150–170 g) were divided into two portions (about 2 × 10^7 cells each) and incubated for 3 hr at 37° in 50 ml of Krebs–Ringer bicarbonate, bovine serum albumin 2% (w/v), pH 7.4, with or without cholera toxin (1 nM). The cells were suspended in 50 mM Tris·HCl, 0.5 mM Na EDTA (pH 7.7), homogenized, and centrifuged (0°) for 30 min at 35,000 × *g*. The pellets were suspended (22.5 mg of protein per ml) in 1.1 ml of 50 mM Tris·HCl, 2.7% (v/v) Lubrol PX (pH 8.0) and sonicated three times for 1 sec each. The suspensions were centrifuged (30 min at 250,000 × *g*) after addition of 50 μl of 0.1 M $MgCl_2$, 3% bovine serum albumin (w/v), 2 mM GTP, and 50 mM Tris·HCl (pH 8.0). The supernatant (0.75 ml) was applied to Sepharose 4B columns (1 × 57 cm; flow rate, 10 ml/hr; 1-ml fractions) equilibrated at 4° with $MgCl_2$ (4 mM), bovine serum albumin (0.15%, w/v), 0.2% Lubrol PX, and Tris·HCl (50 mM, pH 8.0). Cyclase activity was measured at 14° for 90 min. The total activity recovered was 419 pmoles/hr for the control membranes, and 849 pmoles/hr for the toxin-treated sample; the total activity added was 1130 and 2180 pmoles/hr, respectively.

^{125}I-labeled toxin alone elutes in a complicated pattern in Lubrol PX, but the largest molecular weight species appears at about 2 V_0. If native toxin is added to the membranes or solubilized proteins before ^{125}I-labeled toxin, the radioactive peaks I and II disappear almost completely.

Adsorption of Cyclase to Toxin Affinity Columns. Agarose affinity columns containing the 36,000 molecular weight "active" subunit (8–11) of cholera toxin (26) can specifically adsorb solubilized adenylate cyclase activity (Fig. 5). The loss of activity in the column eluate is not due to inactivation since the activity can be detected by directly assaying the washed agarose beads (Fig. 5). Albumin–agarose columns do not adsorb the enzyme. Adsorption of adenylate cyclase to the insolubilized subunit is still observed after activation with cholera toxin. The adsorption is relatively specific, however, since the recoveries of membrane protein from the "active" subunit and albumin columns are the same.

Specific Immunoprecipitation of Toxin-Activated Cyclase. Specific immunoprecipitation to toxin-stimulated, solubilized adenylate cyclase activity can be obtained with antisera to choleragen or to its "active" subunit (Table 2). The precipitation is specific since little activity is obtained with unactivated adenylate cyclase, and since nonspecific precipitins (goat antibody to rabbit IgG plus rabbit serum) have little activity in either control or toxin-activated samples. The specific precipitins contain a very small fraction (about 0.5%) of the total enzyme activity, even though 80–85% of the ^{125}I-labeled toxin can be precipitated.

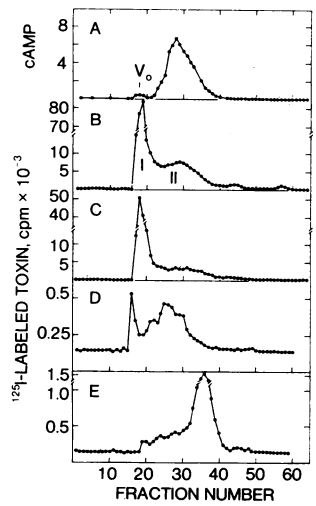

FIG. 4. Sepharose 6B chromatography of Lubrol PX-solubilized adenylate cyclase from fat cells incubated with ^{125}I-labeled cholera toxin. Cells from 25 rats (200 g) were incubated at 37° for 3 hr as in Fig. 3. ^{125}I-labeled cholera toxin (2.4 μg, 9.4 μCi/μg) was added either at the beginning of the incubation or for the final 10 min. Samples (0.75 ml) of the 250,000 × *g* membrane detergent extracts (Fig. 3) were applied to Sepharose 6B columns (1 × 57 cm; flow rate 10 ml/hr; 0.6-ml fractions) and cyclase activity (panel A) was determined as in Fig. 3. Panels A and B refer to cells exposed to toxin for 3 hr, while panel C is for the 10-min exposure to ^{125}I-labeled toxin. The peak of cyclase in panel A (fractions 28–30) was rechromatographed with (panel E) or without (panel D) heating the sample in 1% sodium dodecyl sulfate (60 min at 40°).

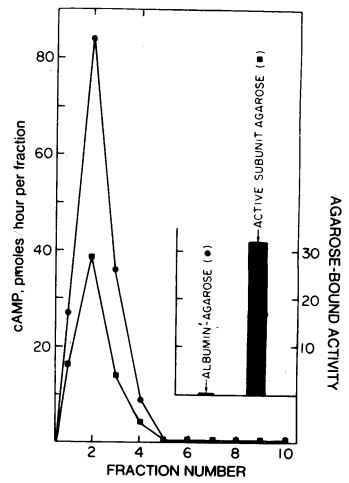

FIG. 5. Specific adsorption of detergent-solubilized fat cell adenylate cyclase to agarose columns containing "active" subunit (36,000 molecular weight) of cholera toxin. Cyclase activity was extracted with 0.5% Lubrol PX as described in the legend of Fig. 3 (without bovine serum albumin), and 0.2 ml of the 250,000 × *g* supernatant (1.1 mg of protein per ml) was added to 1-ml columns of either albumin–Sepharose 2B (●) (5 mg of albumin per ml of gel) or "active" subunit–Sepharose 2B (■) (5 mg of subunit per ml of gel) equilibrated (4°) with $MgCl_2$ (4 mM), Lubrol PX (0.05%), and Tris·HCl (50 mM, pH 8). After 1 hr the columns were washed (0.5-ml fractions) with the buffer. The agarose-bound activity was measured under the same conditions (90 min, 14°) with aliquots of the gels.

DISCUSSION

Activation of adenylate cyclase by choleragen is fully retained upon conversion (with Lubrol PX) to a nonsedimentable form (30 min at 250,000 × *g*) (Table 1 and ref. 27) and after gel filtration (Fig. 3), consistent with the formation of stable complexes between adenylate cyclase and choleragen. Furthermore, a peak of ^{125}I-labeled toxin is associated with adenylate cyclase activity on gel filtration (Fig. 4) provided the enzyme has been previously activated by toxin; it is not observed after brief exposure of intact cells to ^{125}I-labeled toxin or when ^{125}I-labeled toxin is added after the detergent, and it can be suppressed by prior addition of native toxin. The specific immunoprecipitation of toxin-stimulated cyclase, the adsorption of the enzyme to agarose derivatives containing the "active" toxin subunit (8–11), and the detailed kinetics of enzymatic activation* provide further evidence for direct toxin–cyclase interactions.

About 90% of the membrane-bound choleragen is not associated with activated enzyme (Fig. 4). Similar results were observed in erythrocytes, melanoma, and other cells in culture (*, 12, 27), where the apparent K_m for cyclase stimulation occurred with 2000 molecules of toxin per cell whereas specific binding was half-maximal at about 40,000 (or more) molecules per cell. These discrepancies between binding and activation could be explained either by a large number of equivalent "spare" receptors or by a small subpopulation of unique sites. Extensive binding studies (* 12, 18, 28) have not suggested "special" sites.

To further explore these two possibilities, studies were performed (Fig. 6) with choleragenoid, a totally inactive (see *Materials and Methods*) toxin analog that binds indistinguishably (2) and which can, therefore, be used to experimentally "decrease" the number of toxin-binding sites. Cultured fibroblasts were used since they contain an extraordinary number (5 × 10^5 to 10^6) of binding sites for toxin and, thus, provide an exaggerated example of "excess" receptors. A small functionally unique group of receptors is virtually excluded by the fact that choleragenoid has little influence on choleragen action even at concentrations 50-fold in excess of those required for maximal activation by toxin (Fig. 6). A very large number of the toxin receptors can be blocked without affecting the potential for toxin activation provided that a given number of toxin molecules can still bind. Choleragenoid can block activation in these cells, but only at concentrations 1000-fold in excess of the toxin.

The unique finding that a competitive antagonist with unmodified affinity for binding is relatively ineffective in preventing activation strongly suggests that the true receptors for cholera toxin are in large excess and that they are functionally equivalent and independent. This could explain why only a small percent of the receptors need to be occupied for maximal cyclase activation (*, 12, 18, 27).

If equivalent receptors exist in large excess over adenylate cyclase molecules, specific mechanisms for translocating the membrane-bound toxin to adenylate cyclase must exist. Otherwise, virtually all of the receptors (gangliosides) would have to exist in direct contiguity with adenylate cyclase in their free and toxin-bound forms, a situation that would pose serious geometric problems. Relocation would most likely occur by lateral diffusion of the toxin–ganglioside complex within the plane of the membrane, since rotation through the membrane bilayer to the inner membrane surface may be energetically unfeasible for larger molecules (14) and since direct measurements suggest negligible entry of toxin into the

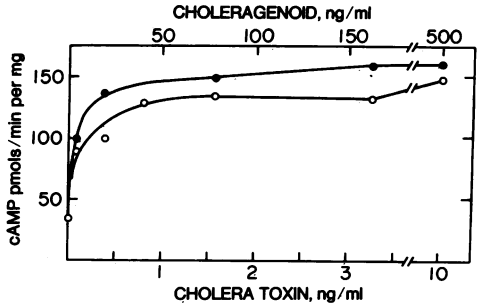

FIG. 6. Failure of purified choleragenoid to prevent activation of adenylate cyclase in cultured human fibroblasts. Fibroblasts derived from human foreskin, cultured (18) in Eagle's minimal essential medium with 10% fetal calf serum under 5% CO_2, were incubated at 24° for 40 min in serum-free medium containing cholera toxin with (○) and without (●) a premixed 50-fold excess of choleragenoid. The toxin-containing medium was removed and replaced with fresh medium; after 3.5 hr at 37°, the cells were harvested by scraping and homogenized (Polytron, 15 sec, 4°) in 5 mM Tris·HCl (pH 8.0). The pellet (40,000 × *g*, 30 min) was suspended in 50 mM Tris·HCl (pH 8.0) and incubated for 12 min at 33° with 0.5 mM ATP.

cytoplasm*. Surface redistributions have been detected with fluorescent toxin derivatives (unpublished). Furthermore, activation of cyclase occurs only after a "lag" phase, which exhibits a critical transition temperature (15–17° in toads, 26–30° in rats)*. It is proposed that an inactive toxin–ganglioside G_{M1} complex is formed, which, after lateral movement of the complex, leads ultimately to direct interaction of the "active" toxin subunit with adenylate cyclase.

This mechanism is similar to the general features of a model recently proposed (15–17) for modulation of adenylate cyclase by hormones, which emphasizes the fluidity (14, 29) of cell membranes. It is suggested that hormone receptors are relatively free to diffuse laterally along the membrane, and modulation of enzyme activity would depend on its direct encounter with receptors. Productive collisions would be favored when the receptors are complexed with hormone. The following equilibria should exist: $H + R \rightleftharpoons H\cdot R$; $H\cdot R + AC \rightleftharpoons H\cdot R\cdot AC \rightleftharpoons H + R\cdot AC$; $H\cdot R\cdot AC \rightleftharpoons H\cdot R + AC$; $R\cdot AC \rightleftharpoons AC + R$, where H, R, and AC refer to hormone, receptor, and adenylate cyclase, respectively. Choleragen may function as an "activated" (H·R) hormone receptor that can complex with and modify the enzyme and that dissociates very slowly or not at all.

Several unique features may follow from this scheme (15–17). For example, hormones may dissociate at different rates from free receptors than from the receptor–cyclase complexes, thus permitting changes in the hormone binding properties without directly perturbing the receptor binding site. Since enzyme activation involves two separate and sequential steps, the extent or apparent K_a of hormone activation may not be reflected in a simple way by the "binding" properties of the hormone.

These considerations could help to explain why cholera toxin increases the apparent affinity for glucagon and decreases the rate of hormone–receptor dissociation. Similar effects of toxin on the apparent K_a for hormone activation occur in various tissues with other hormones, such as catecholamines, vasoactive intestinal polypeptide, and ACTH (* and unpublished). This may reflect stabilization by choleragen of the complex between receptors and the cyclase (H·R·AC), thus resulting in both an increased efficiency of activation and an altered rate of dissociation of membrane-bound hormone. The ability of GTP to enhance hormone stimulation and decrease the apparent hormone affinity simultaneously may also be rationalized in this framework (15–17). A novel feature of the mobile receptor model is the concept of functionally important and dynamic collisions and associations between membrane proteins. This has been postulated previously on the basis of estimates of the collision frequency of rhodopsin molecules in rod outer segment membranes (30).

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