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Effects of Cholera Toxin on the Coupling of Thyrotropin-Releasing Hormone to a Guanine Nucleotide-Binding Protein in Cultured GH_3 cells

YUKIKO YAJIMA, YOSHIKO AKITA, and TOSHIKAZU SAITO

The Tokyo Metropolitan Institute of Medical Science, 3-18, Honkomagome, Bunkyo-ku, Tokyo 113, Japan (Y.Y., Y.A.), and the Division of Endocrinology and Metabolism, Jichi Medical School, 3311-1 Yakushiji, Minamikawachimachi, Kawachigun, Tochigi-ken 329-04, Japan (T.S.)

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SUMMARY

The effects of cholera toxin on the coupling of the thyrotropin-releasing hormone (TRH) receptor to a guanine nucleotide-binding (G) protein were examined in a GH_3 clonal strain of rat pituitary tumor cells. Incubation of the cells with cholera toxin (50 ng/ml) for 16 hr caused a decrease in [^{3}H]methyl-TRH binding to 59% of the control level and in TRH-stimulated low K_m GTPase activity from 143 to 107% of the control level in the membrane-containing fraction. The effects of cholera toxin were time dependent; TRH-stimulated GTPase activity was reduced after a 3-hr incubation, whereas cholera toxin decreased [^{3}H]methyl-TRH binding in the membrane-containing fraction after a 5-hr incubation. These results suggest that the inhibition of TRH-stimulated GTPase activity by cholera toxin treatment is not due to the decrease of receptor binding caused by this toxin. On the other hand, incubation of GH_3 cell membranes with preactivated cholera toxin and NAD^+ did not substantially alter the binding of [^{3}H]methyl-TRH. In contrast, the cholera toxin-treated membranes demonstrated a partial reduction in the activity of TRH-induced low K_m GTPase activity and a 10-fold increase in the concentration of guanine nucleotide required for a half-maximal effect in regulating the TRH receptor affinity for [^{3}H]methyl-TRH. These data suggest that cholera toxin may act directly on a G protein that is associated with TRH-receptors.

The hypothalamic tripeptide TRH binds to specific high affinity receptors on target cells of the pituitary and the central nervous system and, within seconds, stimulates the degradation of polyphosphoinositides by phospholipase C and the concomitant formation of inositol phosphates and diacylglycerol. These are thought to be key events in the TRH-induced stimulation of hormone release (1).

Hinkle and Kinsella (2) reported that guanine nucleotides decrease the affinity of the membrane receptor for TRH to as much as 70% and that TRH stimulates the hydrolysis of GTP by a low K_m GTPase only in membranes from TRH-responsive clones (3). These data strongly suggest that the TRH receptor is coupled to G protein in the membranes. A number of guanine nucleotide-binding regulatory membrane proteins have been described, such as G_s, G_i, G_o, and transducin. Bacterial toxins and cholera and pertussis toxin have provided valuable insights into the functions of G_s, G_i, and transducin. Cholera toxin can ADP-ribosylate the α subunits of G_s and transducin, whereas pertussis toxin selectively catalyzes the ADP-ribosylation of the α subunits of G_i, G_o, and transducin (4). Recently, G protein has also been proposed to couple cell-surface receptors to phospholipase C-mediated polyphosphoinositide breakdown (5). Reports in the past 2 years have indicated that there are at least two classes of G proteins involved in receptor-phospholipase C coupling, which are sensitive and insensitive to pertussis toxin in several cell types (6–9). Recent evidence concerning TRH indicates that pertussis toxin does not inhibit TRH action (10, 11). Therefore, it is not known whether the TRH receptor is coupled to one of these proteins. To determine whether the TRH receptor is functionally coupled to G_s, or some other G proteins, we have examined [^{3}H]methyl-TRH binding and TRH-induced low K_m GTPase activity in cholera toxin-treated cells and membranes and have demonstrated that cholera toxin affects the coupling of TRH receptors to a G protein in GH_3 cells.

Materials and Methods

Materials. Drugs and chemicals were obtained from the following sources: TRH, Tanabe Pharmaceutical Co. (Osaka, Japan); cholera toxin and pertussis toxin, Kaken Chemical Co. (Kumamoto, Japan); Gpp[NH]$_p$ and GTP, Calbiochem-Bohring (La Jolla, CA); [^{32}P]NAD (19.19 Ci/mmol), [L-*histidyl*-4-^{3}H, L-prolyl-3,4-^{3}H]3-methyl histidine-

ABBREVIATIONS: TRH, thyrotropin-releasing hormone; G protein, guanine nucleotide-binding protein; G_s, the stimulatory guanine nucleotide-regulatory protein of adenylate cyclase; G_i, the inhibitory guanine nucleotide-regulatory protein of adenylate cyclase; G_o, 39,000-Da pertussis toxin substrate; Gpp[NH]p, guanine 5′-[$\beta\gamma$-imido]triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate.

TRH ([^{3}H]methyl-TRH, 57.8 Ci/mmol) and [γ-^{32}P]GTP (28.31 Ci/mmol), New England Nuclear Corp. (Boston, MA).

Treatment of GH_3 cells with cholera toxin and pertussis toxin. GH_3 cells were grown in a monolayer culture as previously described (12). The cells were maintained as a monolayer culture in the growth medium, which was composed of Hams' F10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum. The culture medium was removed from the dishes before the reactions and replaced with a fresh growth medium containing pertussis toxin (50 ng/ml) or cholera toxin (50 ng/ml). The cells were incubated at 37° in an atmosphere of 5% CO_2 for 16 hr. To prepare a membrane-containing fraction, the culture was washed twice with 0.15 M NaCl and the cells were suspended in Tris/Mg buffer (20 mM Tris·HCl, 2 mM $MgCl_2$, pH 7.6), allowed to swell for 10 min, and then homogenized in a Dounce glass homogenizer at 15 strokes with pestle B. The homogenate, after being freed of undisrupted cells and nuclei by a 5-min centrifugation at 100 × g at 4°, was centrifuged at 5000 × g for 10 min at 4°, and resuspended in Tris/Mg buffer at a concentration of 0.2–0.5 mg/ml. Binding reactions were carried out in a final volume of 100 μl containing [^{3}H]methyl-TRH, 41–85 μg membranes, and nucleotide as described in the text. After incubation at 22° for 30 min, the reaction mixture was diluted with 2 ml of ice-cold Tris/Mg buffer and filtered through a Whatman GF/C filter. The filter was washed three times with 5 ml of buffer, dried, and counted to determine receptor-bound [^{3}H]methyl-TRH. Nonspecific binding, determined in parallel tubes containing a 500-fold excess of unlabeled TRH, was usually less than 10% of total binding and was subtracted. In experiments measuring the rate of [^{3}H]methyl-TRH dissociation, GH_3 cell membranes (1–2 mg of protein/ml) were incubated with 20 nM [^{3}H]methyl-TRH for 60 min at 22°. At the end of the incubation period, TRH (10^{-5}M) was added to the membranes to stop the association of [^{3}H]methyl-TRH with TRH receptors and to prevent the rebinding of [^{3}H]methyl-TRH to the receptors during the dissociation experiments. The membranes were then diluted 5-fold in Tris/Mg buffer containing various concentrations of guanine nucleotide and incubated for 10 min at 37°. This incubation was terminated by the addition of cold Tris/Mg buffer and the diluted sample was filtered immediately through Whatman GF/C filters. The radioactivity retained on the filters was determined by scintillation counting. The protein content in the membranes was determined by the methods of Lowry *et al.* (13) using bovine serum albumin as a standard.

Measurement of GTPase activity. GTPase activity was determined by a modification of the procedure described by Hinkle and Phillip (3). Reaction mixtures contained, in a final volume of 100 μl, the following: 25 mM Tris·HCl, 6 mM $MgCl_2$, 1 mM ouabain, 10 mM creatine phosphate, 5 units of creatine kinase, 0.5 mM adenylylimidodiphosphate, 1 mM ATP, 2 mM dithiothreitol, 0.1 mM EDTA, 0.05 μM [γ-^{32}P]GTP, unlabeled GTP, and membranes (22–58 μg of protein) at pH 7.6. Reactions were initiated by the addition of cell membranes and proceeded for 20 min at 22°. Reactions were terminated by placing the tubes on ice and adding 0.9 ml of 5% charcoal (Norit) in 6.7 mM phosphoric acid, pH 2.3. Tubes were allowed to sit at 0° for 10 min and then centrifuged at 1500 × g for 5 min. A 500-μl aliquot containing the supernatant fluid and released $^{32}P_i$ was removed, and radioactivity was determined by liquid scintillation counting. High K_m GTPase activity, determined in the presence of 100 μM unlabeled GTP, averaged 70% of the total GTPase activity and was subtracted to yield low K_m activity. Statistical comparisons were performed with Student's t test.

ADP-ribosylation of GH_3 cell membranes. GH_3 cell plasma membranes were treated with cholera toxin that had been thiol-preincubated by a modification of the method of Akita *et al.* (14), as we previously described in some detail (12). Briefly, GH_3 cell membranes (1–2 mg/ml) were incubated at 30° for 10 min with 1 mM NAD in the presence of thiol-preactivated cholera toxin in a reaction mixture consisting of 100 mM potassium phosphate buffer (pH 8.0), 10 mM thymidine, 1 mM ATP, 1 mM $MgCl_2$, 0.5 mM EDTA, 5 mM HEPES, 20 mM dithiothreitol, 4 mM GTP, 10 mM potassium phosphoenolpyruvate, and 50 μg/ml of pyruvate kinase in a final volume of 100 μl. The reaction was terminated by a 5-fold dilution of ice-cold Tris/Mg buffer (pH 7.6) and centrifuged at 7500 × g for 10 min. The pellets were washed twice with the same buffer, and [^{3}H]methyl-TRH binding and low K_m GTPase activity were determined. For the study of radiolabeling of GH_3 cell membranes with [α-^{32}P]NAD, the membranes were ADP-ribosylated with preactivated cholera toxin and [α-^{32}P]NAD, using the same procedure described above. The pellets ADP-ribosylated by cholera toxin were dissolved in Laemmli's sample buffer (15) by heating at 100° for 3 min, and then subjected to electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. The gels were stained with Coomassie brilliant blue, destained, dried, and exposed to Kodak X-Omat film using two intensifying screens for 2 to 4 days at −80°.

Results

Effects of cholera toxin on intact GH_3 cells. First we attempted to test the effect of cholera toxin on intact GH_3 cells. Cells were incubated with cholera toxin (50 ng/ml) at 37° for 16 hr. The data in Fig. 1 (*left panel*) show the effect of cholera toxin treatment on the membrane binding of [^{3}H]methyl-TRH. In membranes from control cells, Gpp[NH]p, a nonhydrolyzable analog of GTP, reduced [^{3}H]methyl-TRH binding to 69%. In the membranes prepared from cholera toxin-treated cells, [^{3}H]methyl-TRH binding was reduced to 59% of the values of the control cells, whereas Gpp[NH]p reduced [^{3}H]methyl-TRH binding to 67% in membranes from these cells, the same degree as in the control cells. Those observations confirmed published results in which the decrease in [^{3}H]TRH binding caused by the cholera toxin has been shown previously to be due to a lowering of the number of available receptors (16). In addition, we examined low K_m GTPase activity in the membrane prepared from cholera toxin-treated cells (Fig. 1, *right panel*). In the control membranes, addition of 1 μM TRH resulted in a 43% stimulation of low K_m GTPase activity. By contrast, in the plasma membranes prepared from cholera toxin-treated cells, the TRH-stimulated low K_m GTPase activity was about 7%, lower than that of the control membranes. These observations are similar to those previously reported by Wojcikiewicy *et al.* (11) in GH_3 cells. The present data indicate that the decrease in [^{3}H]methyl-TRH binding in GH_3 cells caused by cholera toxin may be associated with parallel inhibition of TRH-induced low K_m GTPase activity. There was no effect of incubation with pertussis toxin on [^{3}H]methyl-TRH binding and the basal level or TRH-stimulated GTPase activity (Fig. 1).

Time courses of cholera toxin's action. The effects of cholera toxin were time dependent. A 3-hr exposure of GH_3 cells to cholera toxin was long enough to inhibit TRH receptor-mediated GTPase activity, while cholera toxin did not decrease [^{3}H]methyl-TRH binding to membranes at this time point (Fig. 2). When cells were incubated with cholera toxin for 5 hr, binding of [^{3}H] methyl-TRH to membrane fractions was shown to decrease significantly. These results suggested that the reduction of receptor-mediated GTPase activity by cholera toxin treatment was not implicated in the decrease of [^{3}H]methyl-TRH binding.

Effects of cholera toxin on plasma membranes. We next attempted to determine whether the treatment of membranes by cholera toxin directly affects the activity of TRH receptors. We found that [^{3}H]methyl-TRH binding on isolated GH_3 cell plasma membranes treated with an ADP-ribosylation mixture in the presence of thiol-preactivated cholera toxin and NAD^+ was unchanged as compared with that on the control

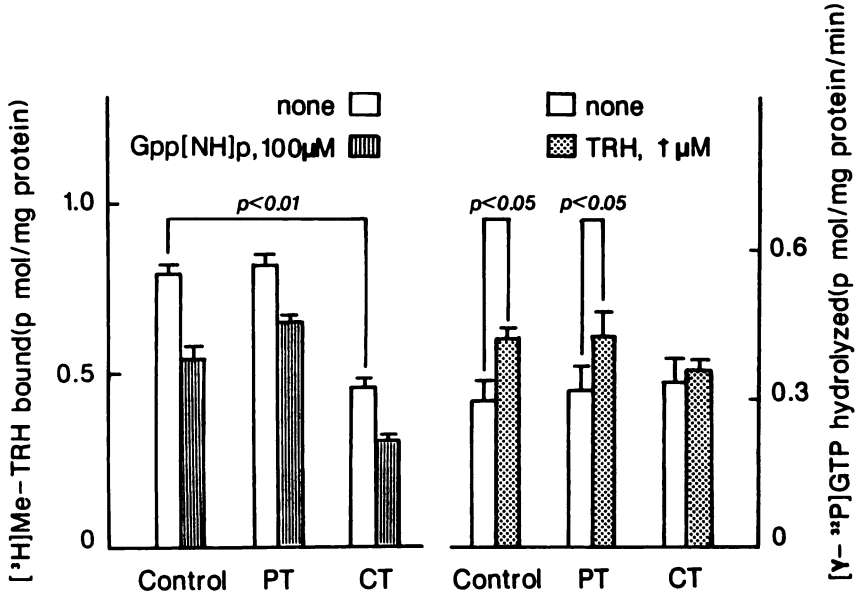

Fig. 1. Effects of chronic exposure to pertussis and cholera toxins on TRH receptors and TRH-stimulated GTPase activity in GH_3 cells. Cells were incubated for 16 hr with 50 ng/ml of pertussis (*PT*) or cholera toxin (*CT*). Membrane-containing fractions were prepared as described in Materials and Methods. *Left panel*, saturable binding of 20 nM [^{3}H]methyl-TRH was determined in membranes prepared from the pretreated cells. The binding reaction was carried out either in the presence or absence of 100 μM Gpp[NH]p for 30 min at 22°. *Right panel*, low K_m GTPase activity in membranes prepared from the pretreated cells was determined after 20-min incubation with 0.05 μM [γ-^{32}P]GTP in the presence or absence of 1 μM TRH, as described in Materials and Methods. Each *bar* represents the mean ± standard error of triplicate determinations of five experiments. The significance (*p* values) of the differences in [^{3}H]methyl-TRH binding between cells with cholera toxin treatment and those without, and in low K_m GTPase activity between membranes exposed to 1 μM TRH and those not exposed to TRH are given.

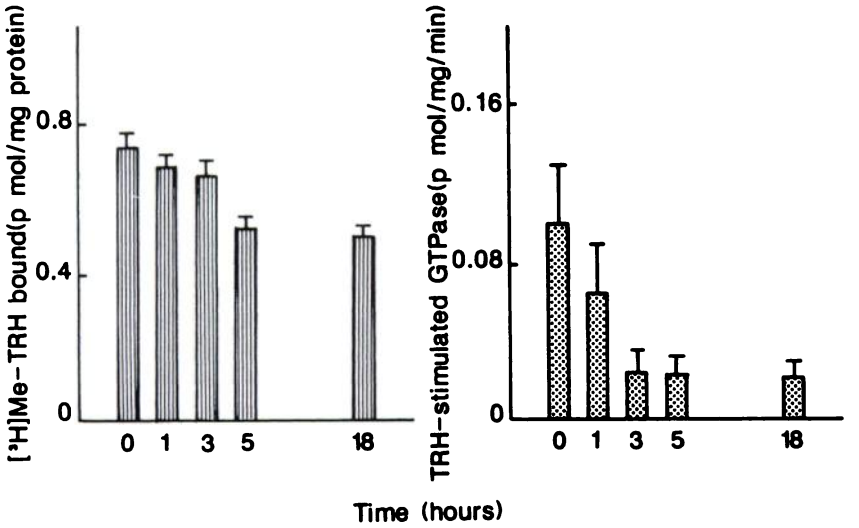

Fig. 2. Effects of cholera toxin after various pretreatment periods on [^{3}H]methyl-TRH binding and TRH-stimulated low K_m GTPase activity in membrane fractions. Confluent cultures of GH_3 cells were preincubated with cholera toxin (50 ng/ml) for the indicated time. Membrane-containing fractions were prepared as described in Materials and Methods. Saturable binding of 20 nM [^{3}H]methyl-TRH (*left panel*) and low K_m GTPase activity (*right panel*) in membranes were determined as described in the legend to Fig. 1. The increment in GTPase activity stimulated by TRH is shown on the *ordinate*. The basal GTPase activity of membranes was 0.461 ± 0.053 pmol/ml/min. Each *bar* represents the mean of three determinations. The results are representative of three separate experiments.

membranes (Fig. 3). The binding of [^{3}H]methyl-TRH was saturable in both the control and the cholera toxin-ADP ribosylated membranes; the effect of Gpp[NH]p in them was almost the same. The results of the Scatchard plots showed that there was thus little or no effect of ADP-ribosylation on the apparent affinity of [^{3}H]methyl-TRH for its receptors in the presence or absence of Gpp[NH]p. Consequently, the decrease in [^{3}H] methyl-TRH binding caused by the intact cells incubated with cholera toxin is not due to the ADP-ribosylation by cholera toxin that precedes an increase of intracellular cAMP concentrations. Fig. 4, however, shows that cholera toxin treatment of membranes brought about an approximately 10-fold shift toward a lower apparent affinity in the potency of Gpp[NH]p in promoting the dissociation of [^{3}H]methyl-TRH. These experiments indicated that cholera toxin treatment of GH_3 cell membranes markedly decreased the potency of guanine nucleotides for the regulation of receptor affinity for agonists with no direct effect on the hormonal binding sites of the receptors. We also found that cholera toxin treatment of cell membranes decreased TRH-induced low K_m GTPase activity (Fig. 5). The response of low K_m GTPase activity measured at 32 min after the addition of TRH was only 10% in cholera toxin-treated membranes, whereas it was 32% in the control. There was no significant effect of incubation with cholera toxin on the basal level of low K_m GTPase activity. The effect of cholera toxin on TRH-induced low K_m GTPase activity was dose dependent (Fig. 6). In the absence of cholera toxin, TRH increased GTPase activity by 0.272 pmol/ml/min (36% stimulation). The stimulatory effect of TRH was reduced to 0.220 pmol/mg/min when the membranes were treated with 10 μg of cholera toxin/ml (10% of stimulation). Treatment of membranes with cholera toxin is thought to lead to a persistent activation of adenylate cyclase. To determine whether cAMP was mediating the effects of cholera toxin on the potency of guanine nucleotides for the regulation of receptor affinity for TRH and TRH-simulated low K_m GTPase activity, membranes of GH_3 cells were incubated with forskolin (100 μM) or cAMP (1 mM) in the presence of isobutyl methylxanthine (0.2 mM). This treatment failed to reproduce the effects of cholera toxin in such a way that the potencies of Gpp[NH]p for the regulation of TRH receptor affinity and TRH-stimulated enzyme activity were decreased (data not shown). When membranes from GH_3 cells were

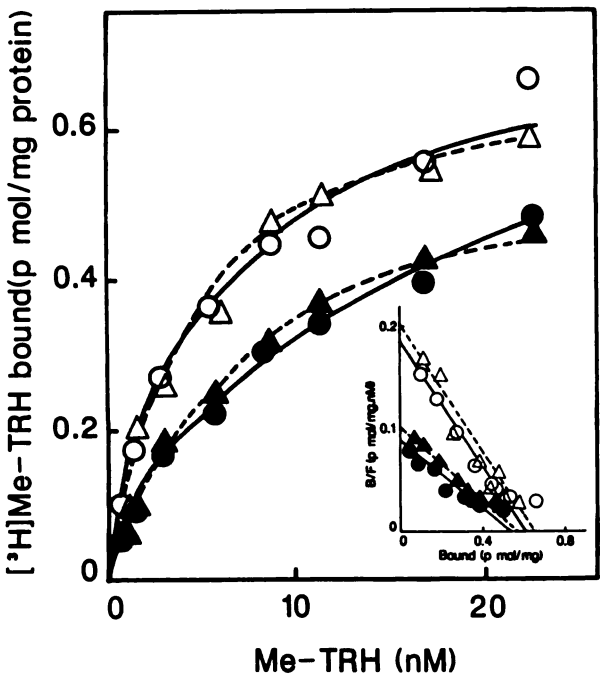

Fig. 3. Effect of cholera toxin treatment on TRH receptors in GH_3 cell membranes. GH_3 cell membranes, treated (△ and ▲) or not treated (○ and ●) with 50 μg/ml of preactivated cholera toxin and NAD^+, were incubated with the indicated concentration of [³H]methyl-TRH in the presence (▲ and ●) or absence (△ and ○) of 100 μM Gpp[NH]p for 30 min at 22°. The specific binding of the labeled ligand to membranes was measured as described in Materials and Methods. Each *point* represents the mean of two determinations. The results represent three separate experiments. The *inset* shows a Scatchard plot of the data from which equilibrium dissociation constants (K_d) of 3.3 nM for control and 3.1 nM for toxin-treated membranes in the absence of nucleotide and of 5.9 nM for control and 5.7 nM for toxin-treated membranes in the presence of Gpp[NH]p were calculated.

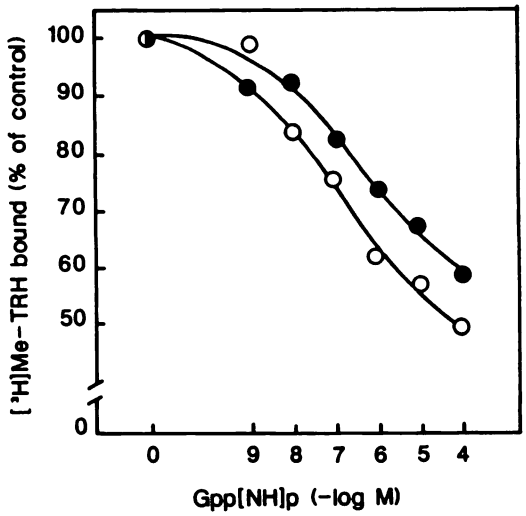

Fig. 4. Effect of cholera toxin treatment of membranes on the potency of Gpp[NH]p for promoting the dissociation of [³H]methyl-TRH. GH_3 cell membranes were treated (●) or not treated (○) with preactivated cholera toxin (50 μg/ml) and NAD^+ as described in Materials and Methods. The membranes were incubated with [³H]methyl-TRH (20 nM) for 60 min at 22°. At the end of this incubation period TRH (10 μM final) was added. The [³H]methyl-TRH was released from TRH receptors by the addition of Gpp[NH]p as described under Materials and Methods. Control binding refers to the [³H]methyl-TRH that remained bound in the absence of added guanine nucleotides. The binding data are expressed in percentage of total [³H]methyl-TRH binding. The *points* represent the mean of duplicate determinations. Results represent four separate experiments.

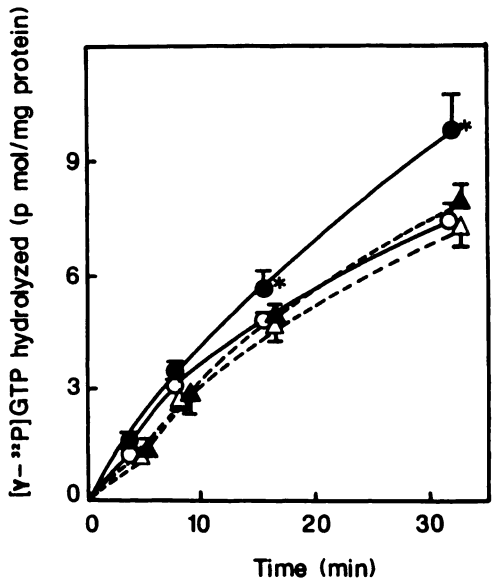

Fig. 5. Time courses for GTP hydrolysis in cholera toxin-treated or nontreated GH_3 cell membranes. Membranes were treated (△ and ▲) or untreated (○ and ●) with preactivated cholera toxin (50 μg/ml) and NAD^+. They were assayed for a low K_m GTPase activity in the presence (▲ and ●) or absence (△ and ○) of 1 μM TRH as described in Materials and Methods. Low K_m GTPase activity is expressed on the *ordinate* as GTP hydrolysis (pmol/mg of protein). Each point is the mean ± standard error of triplicate incubations. *, $p < 0.05$ versus the respective controls.

incubated with [^{32}P]NAD and different concentrations of cholera toxin, we observed an increasing amount of radioactive bands for proteins of M_r 41,000 and a triplet of M_r 46,000–50,000 with increasing amount of cholera toxin (Fig. 7). At this time, however, we do not know which protein bands are responsive to TRH receptors.

Discussion

Data obtained from the present experiments indicate that cholera toxin may act directly on a G protein associated with TRH receptors in GH_3 cells. It is known that the effects of cholera toxin may be mediated by cAMP, because cholera toxin treatment of intact cells leads to a persistent activation of adenylate cyclase activity. Imai and Gershengorn (16) reported that a similar decrease in [³H]TRH binding was found in cells incubated with 8-bromo-cAMP and dibutyryl cAMP as well as cholera toxin. It has also been shown that the treatment of GH_3 cells with forskolin or dibutyryl cAMP did reduce the TRH-stimulated GTPase activity (11). It seemed that cAMP analog and cAMP stimulant mimicked the effects of cholera toxin on the activities of TRH receptor and TRH-sensitive G protein. However, reports concerning the time course of the action of cholera toxin indicated that [³H]TRH binding was not decreased after 4 hr of exposure (16). In addition, Wojcikiewicz *et al.* (11) reported that TRH-stimulated GTPase activity was already decreased after 3 hr of exposure to cholera toxin. These results were consistent with our present data of cholera toxin time dependency, in which the decrease of TRH-stimulated GTPase activity occurred earlier, before the appearance of the decrease of [³H]methyl-TRH binding (Fig. 2). These results suggested that the reduction of receptor-mediated GTPase activity by cholera toxin treatment was not due to the

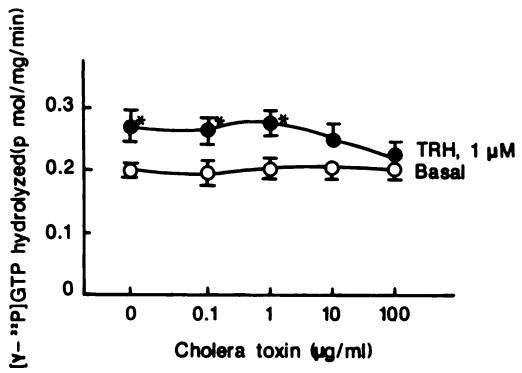

Fig. 6. Concentration dependence of cholera toxin inhibition of TRH-induced low K_m GTPase activity. GH_3 cell membranes were treated or untreated with preactivated cholera toxin at the indicated concentrations. After the washing procedure described under Materials and Methods, low K_m GTPase activity was determined in the presence (●) and absence (○) of 1 μM TRH. Incubation was for 20 min at 22°. Values represent mean ± standard error of four experiments done in triplicate. *, $p < 0.05$ versus the respective controls.

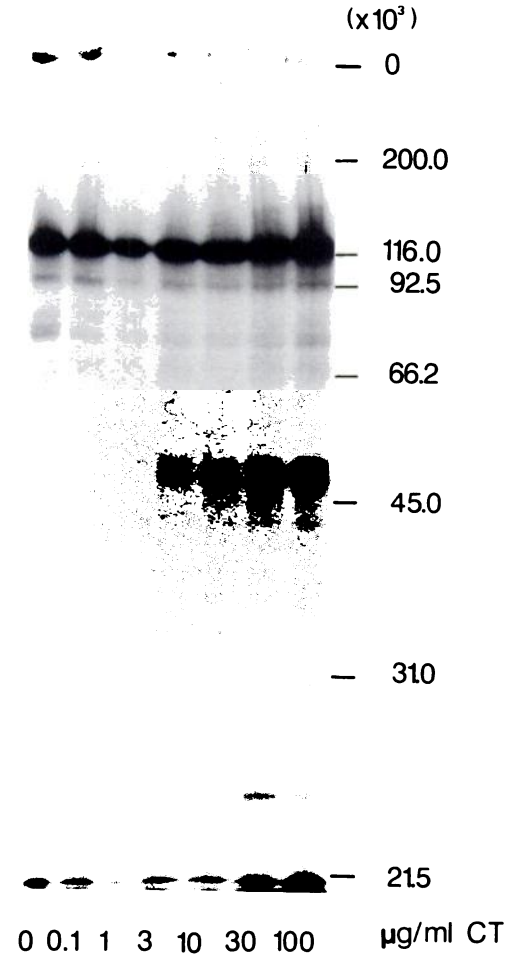

Fig. 7. ADP-ribosylation of GH_3 cell membranes by cholera toxin. GH_3 cell membranes (120 μg) were incubated with the reaction mixture for ADP-ribosylation, which contained 10 μM [^{32}P]NAD and various concentrations of preactivated cholera toxin for 10 min at 30°, as described in Materials and Methods. The samples were applied to a 10% acrylamide gel, and autoradiograms of the dried gel were developed as described in Materials and Methods. Molecular masses (in kilodaltons) in the electrophoresis dimension are indicated.

decrease of [^{3}H]methyl-TRH binding. On the other hand, plasma membranes that were ADP-ribosylated directly by cholera toxin in the presence of NAD also showed decreased TRH-stimulated GTPase activity, although [^{3}H]methyl-TRH binding was unaltered in those membranes in the present experiment. It seems that the slight evidence that the decrease in TRH-stimulated GTPase activity induced by cholera toxin was not due to the down-regulation of the affinity of TRH receptor in intact cells was somewhat strengthened by the plasma membrane experiment. Furthermore, unlike the results obtained with cholera toxin, treatment of membranes with cAMP or forskolin did not suppress TRH-stimulated GTPase activity (data not shown). Thus, it is suggested that effects of cholera toxin on GTPase are the result of ADP-ribosylation per se. And it appears that the decrease in [^{3}H]methyl-TRH binding caused by the intact cells incubated with cholera toxin could be due to the increase in intracellular cAMP, which may induce phosphorylation of TRH receptors (17).

There are a few reports about the effects of cholera toxin treatment of plasma membranes on agonist-receptor binding (18–20). Stadel and Lefkowitz (18) reported that agonist-induced association of β receptors with G_s did not interfere with cholera toxin-catalyzed ADP-ribosylation of the G_s subunit. They showed, however, that the action of cholera toxin appeared to prevent the high affinity complex consisting of hormone, receptor, and G protein from being cleaved in the presence of guanine nucleotides. For example, cholera toxin treatment of frog erythrocyte membranes markedly decreased the potency of guanine nucleotides toward lower apparent affinity for an agonist. These results are similar to those of the present experiment on the guanine nucleotide regulation of TRH receptor affinity for [^{3}H]methyl-TRH binding in GH_3 cell membranes (Figs. 3 and 4). On the other hand, β-adrenergic agonists and prostaglandin E_1, which stimulated the GTPase activity of G_s, have been shown to prevent any further receptor-mediated GTPase stimulation by treatment of membranes with cholera toxin (21, 22). These results also are similar to our finding of decreased TRH-stimulated GTPase activity after cholera toxin treatment (Figs. 5 and 6). Taken together, these results suggest that cholera toxin modulates the coupling of TRH receptors to the G protein as well as the coupling of β-adrenergic receptors or prostaglandin E_1 receptors to the G protein, G_s. The relation of the TRH receptor to G_s may not be positive, inasmuch as TRH does not activate adenylate cyclase in the plasma membrane. It is supposed that the TRH receptor may be coupled to a putative G protein. It has been reported that cholera toxin catalyses the ribosylation of a number of plasma membrane proteins (23). We have shown here four different proteins that are ADP-ribosylated by cholera toxin in GH_3 cell membranes (Fig. 7). We do not know, however, which protein bands are responsive to TRH action.

Recently, several observations have shown that cholera toxin modulates agonist response in cAMP-independent mechanisms (24, 25). There have also been several reports that cholera toxin inhibits the receptor-mediated increase in the hydrolysis of polyphosphoinositide by a cAMP-independent mechanism (26–28). A novel cholera toxin-sensitive G protein may also be involved in mediating receptor-phospholipase C coupling. A recent report concerning TRH indicates that cholera toxin had little effect on the TRH-induced phosphoinositide breakdown in GH_3 cells, although cholera toxin treatment of GH_3 cells

reduced the stimulation of TRH-induced low K_m GTPase activity (11). However, unlike the effects of pertussis toxin, which appears to block interactions between G protein and receptors (4), the pattern of the effects of cholera toxin observed in the previous studies was not simple stimulation of the effector systems, namely adenylate cyclase and cGMP phosphodiesterase systems. The ADP-ribosylation of the α subunit of transducin by cholera toxin (under conditions that led to the inhibition of GTPase activity) had little effect on the cGMP phosphodiesterase activity of rod outer segments (29). However, Jelsema (30) found that cholera toxin inhibits transducin-mediated light activation of both cGMP phosphodiesterase and phospholipase A_2 in rod outer segments. Concerning adenlyate cyclase, Cassel and Selinger (21) have shown that treatment of turkey erythrocyte membranes with cholera toxin and NAD^+ enhances the GTP stimulation and suppresses the F^- activation of adenylate cyclase. However, it has been reported that cholera toxin treatment has either no effect or only a slight potentiating effect on guanine nucleotide-mediated adenylate cyclase activity in rat liver membranes and frog erythrocyte membranes (18, 31). These results suggested that the action of cholera toxin on G protein functions, whether inhibitory, stimulatory, or ineffective, varies with the G protein being modified. Therefore, it is possible that cholera toxin is, in some way, able to modulate the coupling of the TRH receptor to a G protein without the appearance of any effect on TRH-stimulating phospholipase C activity.

The present study is the first examination of the effects of cholera toxin on the TRH-receptor system in GH_3 cell membranes. Cholera toxin treatment has so far been performed only on the intact GH cells, rather than on membranes (10, 11), but we found that cholera toxin modifies the coupling of the TRH receptor to a G protein in these membranes. This finding raises the possibility that a G protein that is sensitive to cholera toxin couples the TRH receptor to phosholipase C. Further studies are needed to determine the G protein involved in the TRH action.

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Send reprint requests to: Dr. Yukiko Yajima, Tokyo Metropolitan Institute of Medical Science, 3-18 Honkomagome Bunnkyo-ku Tokyo 113, Japan.