CHOLERA TOXIN CAN RECOUPLE HORMONE RECEPTORS THAT ARE UNCOUPLED FROM ADENYLATE CYCLASE

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1. Introduction

The mechanism whereby occupancy of cell surface receptors for hormones and neurotransmitters is coupled to intracellular events is of much interest. Because occupancy by agonists of many such receptors activates adenylate cyclase, events involved in receptor–cyclase coupling have been emphasized. In characterizing components of the cyclase system much work has involved wild-type S49 lymphoma cells and variant S49 clones having lesions in the pathway for generation of adenosine 3′:5′-monophosphate (cAMP) [1,2]. One of these S49 variants was termed UNC because it possessed adenylate cyclase and hormone receptors for β-adrenergic amines and prostaglandin E_1 (PGE_1), but occupation of these receptors was unable to stimulate cAMP generation in these cells [3]. Studies in S49 and other cells indicate that GTP is an obligatory co-factor for hormonal activation of adenylate cyclase and that hydrolysis of GTP may 'turn off' activated adenylate cyclase [2,4,5]. In addition, a non-hormonal activator of adenylate cyclase, cholera toxin (choleragen, the enterotoxin derived from the bacterium *Vibrio cholerae*), enhances cAMP synthesis, apparently by inhibiting GTP hydrolysis [6,7]. Here, we show that as a possible consequence of this inhibition of GTP hydrolysis, cholera toxin treatment of intact wild-type S49 cells enhances coupling of hormone receptors to adenylate cyclase. Moreover, we find that incubation of the UNC variant S49 cells with cholera toxin allows expression of catecholamine and PGE_1-stimulated cAMP generation. These results suggest that cholera toxin may be a useful tool for enhancing hormone-stimulated cAMP generation even in cells in which receptors are functionally uncoupled from adenylate cyclase.

2. Experimental

S49 cells were grown in suspension culture in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% heat-inactivated horse serum and 3 g glucose/l (final conc.). Cells growing logarithmically ($1–2.5 \times 10^6$ cells/ml) were centrifuged and then resuspended in either DME medium or medium in which 20 mM Na-Hepes [4(-2-hydroxyethyl)-1-piperazine-ethanesulfonate] replaced $NaHCO_3$ and 0.1% bovine serum albumin replaced the horse serum. Cells were then incubated in the presence or absence of 100 ng cholera toxin/ml (Schwarz Mann) for 1 h at 37°C. At the completion of this incubation, aliquots of cells were used to measure cAMP accumulation or [^{3}H]dihydroalprenolol binding. For cAMP accumulation, cells were added to tubes containing freshly prepared 1 mM ascorbic acid (final conc.) and variable concentrations of (−)-isoproterenol (kindly donated by Sterling-Winthrop Research Labs). Tubes were incubated for 10 min at 37°C and the incubations were terminated by centrifuging samples in a Beckman microfuge for 20–30 s, immediate removal of the extracellular medium and addition of 50 mM sodium acetate (pH 4.0) and 0.2 mM isobutylmethylxanthine. The resuspended cells were immediately boiled for ⩾3 min and cAMP was determined in the resultant solution using a competitive binding protein assay method [10]. [^{3}H]Dihydroalprenolol (New England Nuclear, 48.6 Ci/mmol) binding was determined in samples incubated for 5 min at 37°C as in [6,10]. Non-specific binding, i.e., [^{3}H]dihydroalprenolol bound in the presence of 0.3 μM (−)-alprenolol, was subtracted from bound radioactivity to determine specific binding.

3. Results

3.1. *Effects of cholera toxin pretreatment of beta-adrenergic receptors and cAMP accumulation of wild-type S49 cells*

Wild-type S49 cells grow in suspension culture and generate cAMP in response to β-adrenergic catecholamines, PGE_1, and cholera toxin; membranes prepared from these cells also produce cAMP in response to Mn^{2+}, NaF, and non-hydrolyzable GTP derivatives (such as Gpp(NH)p) [1,2,8,9]. Radioligand binding assays have been used to show that these cells have receptors for β-adrenergic catecholamines and PGE_1 [8–12]. When intact wild-type S49 cells were incubated with cholera toxin prior to treatment with the β-adrenergic agonist isoproterenol, the cells had enhanced stimulation of cellular cAMP accumulation (fig.1, top); this result is similar to data observed in a variety of cell types [14–18]. The primary effect in S49 cells was an enhanced maximal response to isoproterenol. In addition, there was a 2–4-fold increase in potency of the β agonist after cholera toxin treatment. Cholera toxin also enhanced maximal responses of wild-type S49 cells to PGE_1 several-fold (not shown).

The enhanced potency and maximal response to isoproterenol after cholera toxin treatment was not attributable to an alteration in β-adrenergic receptors of intact wild-type S49 cells (fig.1, bottom). When β-adrenergic receptors were characterized in intact cells following cholera toxin treatment, we found that the treatment altered neither the no. [^{3}H]dihydroalprenolol binding sites/cell (1550) nor the affinity of these sites for the radioligand itself (0.65 μM) or for isoproterenol (~1.6 μM) (fig.1, bottom). Similar results were obtained using plasma membranes isolated from toxin-treated wild-type S49 cells [8,9]. Thus, the enhanced response to isoproterenol produced by cholera toxin derives from an event distal to the receptor binding sites for the catecholamine. Moreover, addition of the toxin increases even further the prominent discrepancy between the low concentrations of isoproterenol required for stimulation of cAMP accumulation and the much higher concentrations required to compete for radioligand binding sites in S49 cells [11,12].

3.2. *Effect of cholera toxin pretreatment on cAMP accumulation in UNC S49 cells*

The S49 variant cells called UNC are characterized

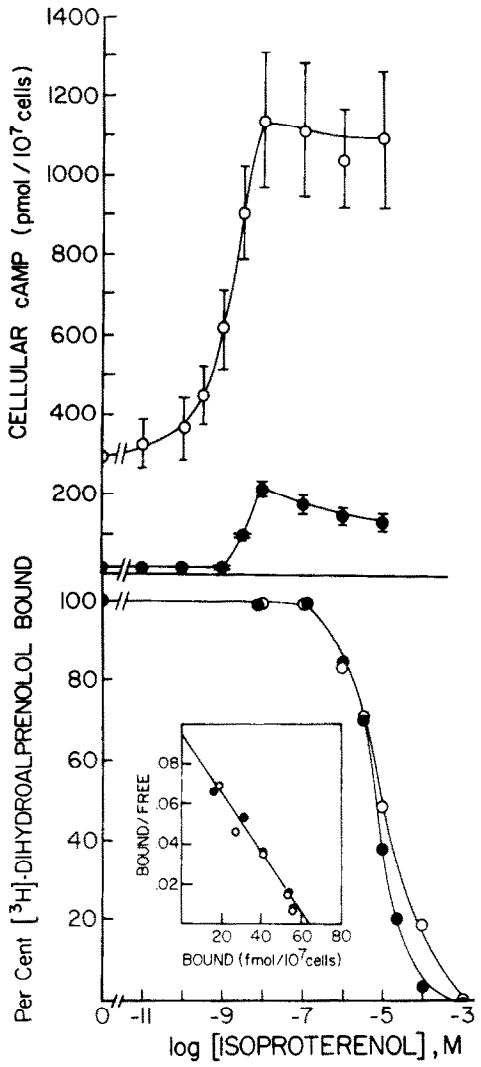

Fig.1. Effect of cholera toxin on the stimulation of cAMP accumulation by isoproterenol (top) and on the binding of [^{3}H]dihydroalprenolol (bottom) of wild-type S49 cells. Cells were incubated in the presence (○) or absence (●) of 100 ng cholera toxin/ml for 1 h and then cAMP accumulation and [^{3}H]dihydroalprenolol binding were determined as described. (top) Data shown are the mean ± SEM of values (duplicate determinations) from 3 separate cAMP accumulation studies. (bottom, inset) A Scatchard plot of binding data in which variable concentrations of [^{3}H]dihydroalprenolol were incubated with cells. (bottom) The larger part illustrates mean data derived from 2–3 separate experiments in which 3–4 nM [^{3}H]dihydroalprenolol was incubated with cells in the presence of varying concentrations of (−)-isoproterenol and 1 mM ascorbic acid. The experiments shown were performed in the absence of a phosphodiesterase inhibitor. Addition of an inhibitor such as Ro 20-1724 produces a 2–3-fold increase in cAMP accumulation in either the absence or presence of cholera toxin.

Table 1
Effects of cholera toxin on isoproterenol- and PGE_1-stimulated cyclic AMP accumulation in UNC S49 cells

Expt	Basal	ISO	PGE_1	CT	CT + ISO	CT + PGE_1
1	235	202	224	1569	2478	3044
2	9	7	5	380	734	854
3	129	154	154	1041	2076	2648

UNC S49 cells were incubated in the absence or presence of cholera toxin (CT) (100 ng/ml) for 2 h. Aliquots of cells were then incubated with 1 μM isoproterenol (ISO), 1 μM prostaglandin E_1 (PGE_1), or neither for an additional 6 min prior to termination of incubation and assay of cyclic AMP as in fig.1. The data shown are the mean values of duplicate cyclic AMP (pmol/10^7 cells) determinations in separate studies

by the presence of hormone receptors that are unable to generate cAMP in response to either PGE_1 or β-adrenergic agonists ([3] and table 1). β-Adrenergic receptors in intact UNC cells are similar in number and affinity to receptors in wild-type S49 cells [12]. In spite of their unresponsiveness under basal conditions, UNC cells that were incubated with cholera toxin demonstrated PGE_1- and isoproterenol-stimulated cAMP accumulation (fig.2, table 1). Toxin-treated UNC cells had a similar dose–response pattern (EC_{50} value of ~5 nM) for isoproterenol-stimulated cAMP accumulation as did wild-type S49 (fig.2) and also had no change in the ability of isoproterenol to compete for [^{3}H]dihydroalprenolol binding sites. As table 1 and fig.2 show, in a series of experiments we observed much variability between absolute levels of cAMP determined under basal and stimulated conditions. Nevertheless, cholera toxin enhances hormonal response by roughly similar percentages (50–100% for isoproterenol and 90–150% for PGE_1) in all the studies. The variability in results between these experiments makes it difficult for us to calculate the extent to which recoupling of UNC with cholera toxin yields hormonal responses that are quantitatively similar to those seen with wild-type cells; on a percentage basis, the enhancement of response in wild-type cells appears to be ~3-fold greater than for UNC cells.

4. Discussion

These results demonstrate that β-adrenergic and PGE_1 receptors are not permanently uncoupled from adenylate cyclase in the UNC variants. Based on

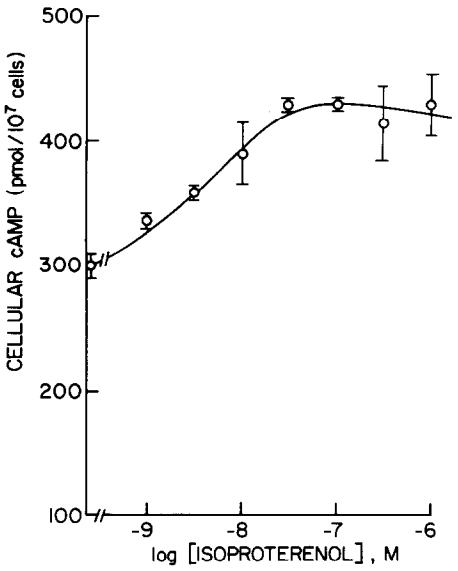

Fig.2. Effect of isoproterenol and cholera toxin on cyclic AMP levels in UNC S49 cells. Logarithmically growing UNC cells were handled essentially as in fig.1 with the exception that the phosphodiesterase inhibitor Ro 20-1724 at a final concentration of 0.1 mM was added to cells and cells were incubated in the presence of 100 ng cholera toxin/ml. Samples were incubated with toxin for 2 h and then varying concentrations of (−)-isoproterenol were added for an additional 6 min prior to termination of incubation and assay as described. The data shown are mean ± SEM for triplicate determinations at each concentration except for data obtained in the absence of isoproterenol in which 7 determinations were performed.

results in several systems, including S49 cells, cholera toxin appears to act by catalyzing the reversible ADP ribosylation of a nucleotide (probably GTP) binding component which is involved in hormone-stimulated adenylate cyclase activity [20,22]. In turkey erythrocyte membranes, this covalent modification of the GTP binding unit correlates with inhibition of a catecholamine-stimulated GTPase activity [20,21]. In plasma membranes from UNC cells, isoproterenol was able to stimulate a small amount of adenylate cyclase activity in the presence of the poorly hydrolyzed GTP derivative guanylylimidodiphosphate (Gpp(NH)p), but not in the presence of GTP [3]. The enhanced hormonal response that we find in intact UNC cells in response to cholera toxin may thus result from inhibition of GTP hydrolysis by the toxin in these cells. We conclude that the findings on the mechanism of toxin in isolated membranes are likely to be operative in intact cells [6].

UNC membranes contain the same cholera toxin [^{32}P]NAD labelled substrates as do wild-type membranes [22] and cholera toxin-labelled substrates in UNC have more acidic isoelectric points than do substrates from wild-type S49 cells [23]. Our results suggest that these altered cholera toxin substrates are still able to permit the 'recoupling' of receptors and adenylate cyclase. Nevertheless, considering the apparent quantitative differences that we observed in cholera toxin-promoted enhancement of hormonal responses in wild-type and UNC S49 cells, the more acidic toxin substrates may be functionally defective. Alternatively, we cannot exclude the possibility that cholera toxin may alter proteins in addition to the nucleotide binding unit [24].

The wider implication of these results involves the potential utility of cholera toxin to enhance receptor–cyclase coupling. Although increased hormonal response in the presence of cholera toxin has been noted [13–17,25], our finding that the toxin can confer hormonal response on cells containing receptors but otherwise lacking response suggests that the toxin may be useful in other systems as well in helping to unmask 'latent' or non-functional hormone receptors [25,26].

The prominent diarrhea associated with the disease cholera is produced by the increased cAMP levels in intestinal epithelial cells mediating fluid and electrolyte transport [26]. Although cholera toxin itself is able to stimulate cAMP levels, our findings raise the possibility that the toxin sensitizes the adenylate cyclase of those cells to local and circulating agonists for which the cells bear receptors, but which are functionally uncoupled in the absence of the toxin. Because we can only speculate about the possible existence of such receptors, further studies will be required to show whether this postulated effect of cholera toxin occurs in intestinal and other cells. It will also be of interest to determine whether recoupling of receptors occurs with other bacterial toxins that are thought to act in a manner similar to cholera toxin (e.g., the heat-labile toxin of *Escherichia coli* [28]).

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