Cholera toxin inhibits prostaglandin E_1 but not adrenaline-induced stimulation of GTP hydrolysis in human platelet membranes

Klaus Aktories, Günter Schultz and Karl H. Jakobs

Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, FRG

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1. INTRODUCTION

Hydrolysis of GTP at the guanine nucleotide-binding regulatory component (N-protein) appears to terminate hormone-induced adenylate cyclase stimulation [1,2]. Hormonal factors which stimulate adenylate cyclase increase the activity of a high affinity GTPase, which appears to be an integrated part of the N-protein [3–5]. In several systems, cholera toxin has been shown to inhibit this hormone-induced GTPase stimulation [5–7]. This GTPase inhibition, which appears to be due to a toxin-induced ADP-ribosylation of the N-protein [8], results in a persistent activation of the adenylate cyclase. Hormonal factors, which inhibit adenylate cyclase, such as α-adrenergic agonists in human platelets [9], prostaglandins and adenosine receptor agonists in hamster adipocytes [10,11] and opiates in neuroblastoma × glioma hybrid cells [12], also stimulate a high affinity GTPase in membrane preparations. Here, we report that in human platelet membranes cholera toxin inhibits the high affinity GTPase stimulation by prostaglandin E_1 (PGE_1), which stimulates adenylate cyclase, whereas the toxin does not impair GTPase stimulation by adrenaline, which inhibits adenylate cyclase in this membrane system.

2. MATERIALS AND METHODS

Materials used were as in [13]. Human platelet membranes, prepared as in [13], were pretreated in a medium containing, if not otherwise stated, 0.1 mM ATP, 0.2 mM GTP, 5 mM $MgCl_2$, 2 mM NAD, 2 mM dithiothreitol (DTT), 1 mM EDTA, 5 mM creatine phosphate, 0.4 mg creatine kinase/ml, 0.2% bovine serum albumin and 50 mM sodium phosphate buffer (pH 7.1) or 50 mM triethanolamine–HCl (pH 7.4) for 10 min at 30°C without and with cholera toxin at the indicated concentrations. The toxin was preactivated for 10 min at 37°C with 20 mM DTT. After treatment, the membranes were pelleted by centrifugation (5 min, 30 000 × *g*) and resuspended in 10 mM triethanolamine–HCl (pH 7.4) (fig.1). In the other experiments shown, the membranes were washed twice with an excess of triethanolamine–HCl (10 mM, pH 7.4)/EDTA (0.1 mM) and were then used for enzyme assays or treatment with *N*-ethylmaleimide (NEM). For this treatment, the washed platelet membranes were incubated for 30 min at 0°C with NEM (5 or 10 mM) and were then washed with an excess of β-mercaptoethanol, essentially as in [3,5]. After centrifugation for 10 min at 30 000 × *g*, the final pellet was suspended in 10 mM triethanolamine–HCl (pH 7.4) for enzyme assays.

Adenylate cyclase activity was determined as in [14] with 0.1 mM [α-^{32}P]ATP (~0.4 μCi/tube), 2 or 30 mM $MgCl_2$, 5 mM creatine phosphate, 1.2 mg creatine kinase/ml, 0.2% bovine serum albumin, 30 μM D,L-propanolol, 1 mM DTT, 0.1 mM EDTA, 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM cyclic AMP and 0.3 μM GTP in 50 mM triethanolamine–HCl (pH 7.4) in 100 μl total vol. Reactions were initiated by addition of platelet membranes (50–80 μg protein/tube) and conducted for 5 min at 30°C. Cyclic AMP formed was isolated as in [14]. GTPase activity was determined according to [3] with essentially the same

reaction mixture as used for adenylate cyclase assay, with the exception that 0.3 μM [γ-^{32}P]GTP (~0.2 μCi/tube) and 0.1 mM unlabeled ATP were present. Reactions were started by addition of platelet membranes (3–10 μg protein/tube) and conducted for 5 or 10 min at 30°C. Determination of the released $^{32}P_i$ was performed by the charcoal method exactly as in [11].

3. RESULTS

Pretreatment of human platelet membranes with cholera toxin (1–100 μg/ml) increased basal adenylate cyclase activity measured with 2 mM $MgCl_2$ up to ~30-fold (fig.1). At 100 μg/ml, the cholera toxin-induced activation was identical to that induced by PGE_1 (10 μM). At this maximally effective concentration of PGE_1, cholera toxin caused no further increase in activity. Adrenaline (300 μM) decreased basal and PGE_1-stimulated adenylate cyclase activities. After preactivation with cholera toxin, the adrenaline-induced inhibition was largely increased in the absence of PGE_1 and not affected in the presence of PGE_1. At 100 μg cholera toxin/ml, inhibitions of control and PGE_1-stimulated activities were identical. Under

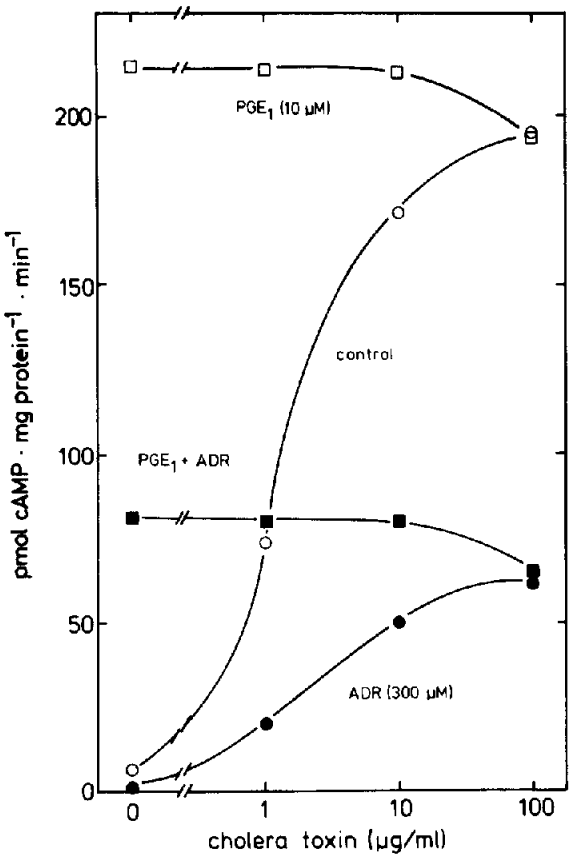

Fig.1. Influence of cholera toxin on human platelet adenylate cyclase. Human platelet membranes were pretreated without and with cholera toxin at the indicated concentrations for 5 min at 37°C with 30 μM GTP in the preactivation medium. Adenylate cyclase activity was determined with 2 mM $MgCl_2$ in the absence (○) and presence of PGE_1 (□, 10 μM), adrenaline (●, 300 μM) or PGE_1 plus adrenaline (■).

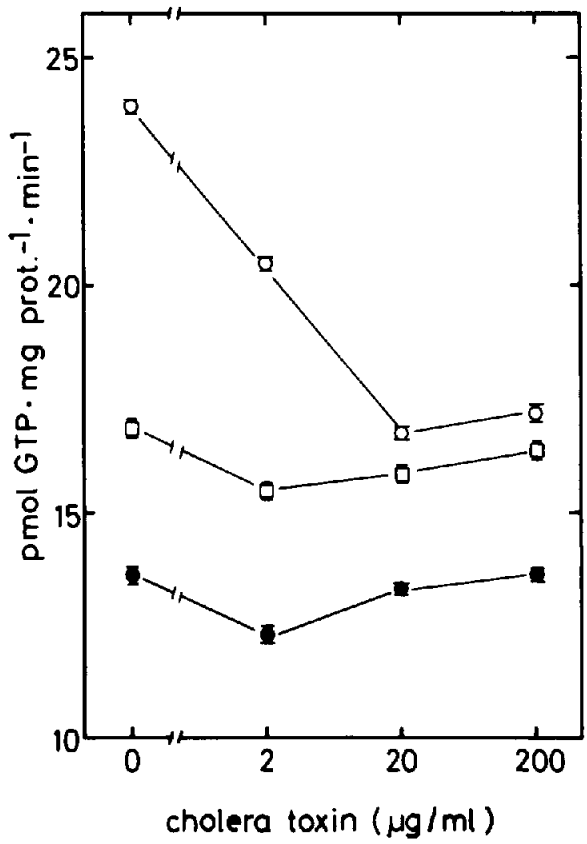

Fig.2. Influence of cholera toxin on human platelet membrane GTPase. Human platelet membranes were pretreated without and with cholera toxin at the indicated concentrations, and after the washing procedure with NEM (10 mM), as in section 2. GTPase activity was determined in the absence (●) and presence of adrenaline (□, 300 μM) or PGE_1 (○, 10 μM). Incubation was for 5 min in the presence of 30 mM $MgCl_2$. Means ± SEM are given.

Table 1

Influence of cholera toxin on adrenaline-induced increase in GTPase activity in human platelet membranes

Additions	Control	Cholera toxin
	(pmol GTP hydrolyzed . mg protein^{-1} . min^{-1})	
None	75.4 ± 2.2	70.8 ± 1.1
Adrenaline (100 μM)	103 ± 3.0 (36)	96.1 ± 1.5 (35)

Human platelet membranes were treated without and with cholera toxin (200 μg/ml). GTPase activity was determined in the presence of 2 mM $MgCl_2$ without and with adrenaline (100 μM). Means ± SEM are given. Numbers in parentheses indicate % stimulation

these conditions, which were optimal for demonstration of adrenaline-induced adenylate cyclase inhibition, the influence of cholera toxin on adrenaline-induced GTPase stimulation was studied. In the absence of cholera toxin, adrenaline (100 μM) increased GTP hydrolysis ~35% (table 1). Treatment of platelet membranes with cholera toxin (200 μg/ml) had absolutely no effect on GTPase stimulation by the α-adrenergic agonist. The small decrease in basal GTPase activity was not observed in other experiments.

Stimulation of GTP hydrolysis in human platelet membranes by PGE_1 was very small under these conditions and was hampered by the high background basal GTPase activity [5]. In order to study the effect of cholera toxin on PGE_1-induced GTPase stimulation, human platelet membranes were treated with the SH reagent, NEM, which has been shown to decrease basal but not PGE_1-stimulated GTP hydrolysis [3,5]. Pretreatment of platelet membranes with 10 mM NEM (30 min, 0°C) markedly decreased basal GTP hydrolysis (~5-fold). Under this condition, cholera toxin concentration-dependently reduced stimulation of the GTPase by PGE_1 (fig.2). In the absence of the toxin, PGE_1 (10 μM) increased GTPase activity by ~10 pmol . mg^{-1} . min^{-1} (80% stimulation). After treatment with 20 μg cholera toxin/ml, the

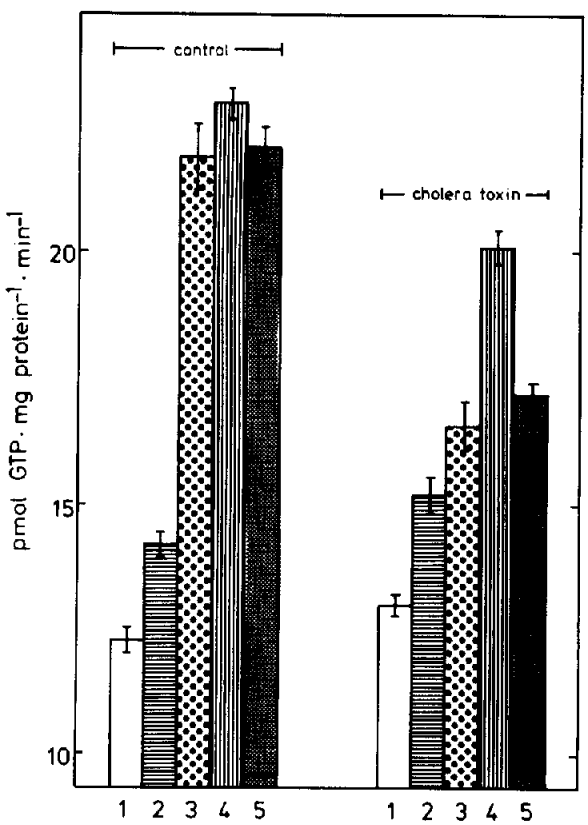

Fig.3. Influence of cholera toxin on GTPase stimulation by PGE_1, adrenaline and their combination. Platelet membranes were pretreated without and with cholera toxin (100 μg/ml) and with NEM (5 mM) as in fig.2. GTPase activity was determined in the presence of following additions: (1) none; (2) adrenaline (100 μM); (3) PGE_1 (1 μM); (4) adrenaline plus PGE_1; (5) adrenaline + PGE_1 + phentolamine (50 μM). Incubation was for 10 min in the presence of 30 mM $MgCl_2$. Means ± SEM are given.

stimulatory effect of PGE_1 was reduced to ~3 pmol · mg $^{-1}$ · min^{-1} (20% stimulation). Similar data were reported in [5]. In the NEM-pretreated membranes, GTPase stimulation by adrenaline was very small [13]. But this small stimulation was not affected by cholera toxin, even at the highest concentration used.

In human platelet membranes, stimulations of GTP hydrolysis by PGE_1 and adrenaline are largely additive, and only the adrenaline-induced stimulation is blocked by the α-adrenoceptor antagonist, phentolamine [9]. Similar data were obtained after treatment with cholera toxin (fig.3). Cholera toxin (100 μg/ml) largely reduced GTPase stimulation induced by PGE_1 (1 μM), whereas the small stimulation by adrenaline (100 μM) was not impaired but rather increased. In the presence of both hormonal factors, the stimulations were additive, and phentolamine (50 μM) specifically antagonized the adrenaline-induced GTPase activation.

4. DISCUSSION

In human platelet membranes, adrenaline via α_2-adrenoceptors inhibits adenylate cyclase and stimulates a high affinity GTPase [9]. In the same membrane system PGE_1, which stimulates adenylate cyclase, also increases GTP hydrolysis by a high affinity GTPase [5,9]. These data demonstrate that treatment of platelet membranes with cholera toxin enlarges adrenaline-induced adenylate cyclase inhibition [15] and does not attenuate GTPase stimulation by this α-adrenergic agonist. In contrast, cholera toxin markedly inhibited GTPase stimulation by PGE_1, as reported in [5]. These findings suggest that two functionally distinct GTPase enzymes are involved in adenylate cyclase regulation. One GTPase is activated by hormones that stimulate adenylate cyclase and appears to terminate the hormone-induced adenylate cyclase stimulation; this GTPase, which appears to be an integrated part of the stimulatory coupling protein, is inhibited by cholera toxin [1,2]. The other GTPase is stimulated by hormones that inhibit adenylate cyclase and appears to be closely related to this inhibitory process, possibly causing also termination of the hormone action; this GTPase is apparently not affected by cholera toxin. In hamster adipocyte membranes stimulation of a high affinity GTPase by an adenylate cyclase inhibitory hormone was not attenuated by cholera toxin [16]. This finding supports the view that two functionally distinct GTPases are involved in adenylate cyclase regulation by stimulatory and inhibitory hormones.

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