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ROLE OF GUANINE NUCLEOTIDES IN THE STIMULATION OF THYROID ADENYLATE CYCLASE BY PROSTAGLANDIN E_1 AND CHOLERA TOXIN *

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Summary

Cholera toxin in the presence of GTP increased adenylate cyclase activity in a purified bovine thyroid plasma membrane preparation, whereas, in the presence of guanosine 5′-(β, γ-imido)-triphosphate (Gpp(NH)p), cholera toxin had no stimulatory effect. Similarly, prostaglandin E_1 enhanced the adenylate cyclase activity induced by GTP but not by Gpp(NH)p.

Gpp(NH)p-stimulated adenylate cyclase activity, assayed with hydrolysis-resistant adenosine 5′-(β, γ-imido)-[^{32}P]triphosphate as substrate and no ATP-regenerating system was inhibited by GDP in a competitive fashion. Furthermore, prostaglandin E_1, but not cholera toxin, influenced the GDP inhibition of Gpp(NH)p-stimulated activity by increasing the concentration of GDP resulting in 50% inhibition approx. 2-fold.

Inosyl nucleotides mimicked the effects of guanyl nucleotides on thyroid adenylate cyclase in that ITP could substitute for GTP in enhancing cholera toxin- and prostaglandin E_1-induced activities and that inosine 5′-(β, γ-imido)-triphosphate [Ipp(NH)p] was also a potent stimulator per se.

Conclusions. (1) Cholera Toxin and prostaglandin E_1 enhance thyroid adenylate cyclase activation by GTP (or ITP), but have no stimulatory effect on the Gpp(NH)p (or Ipp(NH)p) response; (2) the stimulatory effect of prostaglandin E_1 on adenylate cyclase may result from decreased affinity for GDP at the guanine nucleotide regulatory site; (3) the data regarding cholera toxin stimu-

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lation of thyroid adenylate cyclase are consistent with the hypothesis that cholera toxin exerts its effect by inhibiting an endogenous GTPase.

Introduction

While the effects of hormones, prostaglandins and cholera toxin on adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) have been studied in a variety of animal tissues, the mechanism(s) by which these agents stimulate the enzyme are not understood. In recent years, it has been demonstrated that hormone stimulation of adenylate cyclase is dependent on GTP binding at a regulatory site on the enzyme [1]. In a series of elegant studies, Cassel and Selinger [2], as well as other workers [3,4], have shown that cholera toxin requires GTP for optimal cyclase stimulation and suggested that cholera toxin activates adenylate cyclase by inhibiting a specific GTPase, associated with the guanyl nucleotide regulatory site, which controls the 'off-rate' of the activated state of adenylate cyclase enzyme. Involvement of a GTPase in terminating the activated cyclase could, therefore, account for the fact that non-hydrolyzable GTP analogs such as guanosine 5′-(β, γ-imido)triphosphate [Gpp(NH)p], are potent and persistent activators of adenylate cyclase [5]. While it has been shown that prostaglandin E also requires GTP for optimal adenylate cyclase stimulation [6,7], very little is known about its mode of stimulation, although it has been suggested that it acts as a secondary enzyme regulator by reducing the ability of GDP to inhibit the activated enzyme [8]. Prostaglandin E has been shown to enhance various parameters of thyroid function [9,10] presumably via stimulation of adenylate cyclase [9]. Similarly, cholera toxin has also been shown to stimulate the adenylate cyclase-cyclic AMP system in thyroid [11,12] as well as other parameters of thyroid function [13]. The studies detailed herein were performed to compare and contrast the mechanism(s) of action of these compounds on thyroid adenylate cyclase. The results obtained show that GTP plays an obligatory role in the expression of thyroid adenylate cyclase activation by both cholera toxin and prostaglandin E and are consistent with the suggestion that these agents exert their effects at the guanyl nucleotide regulatory site.

Materials and Methods

Guanosine 5′-(β, γ-imido)triphosphate [Gpp(NH)p], adenosine 5′-(β, γ-imido)triphosphate [App(NH)p], [α-^{32}P]ATP (tetra-triethylammonium salt, 10—25 Ci/mmol) and [α-^{32}P]App(NH)p (tetra-triethylammonium salt, 10—25 Ci/mmol) were purchased from ICN Pharmaceuticals, Inc. Phosphocreatine, creatine phosphokinase (160 U/mg), ATP (No. 2388), GTP, ITP, NAD^+ and dithiothreitol were purchased from Sigma Chemical Co. Cholera enterotoxin was purchased from Schwarz-Mann. (The concentrations of cholera toxin reported in the text were not corrected for the fact that the material supplied by Schwarz-Mann contains only 10—15% actual cholera toxin). The prostaglandins were kindly supplied by Dr. John Pike (Upjohn Co.).

Tissue preparation. Bovine thyroid plasma membrane was prepared by the method of Yamashita and Field [14]. The material at the interface between

densities 1.16 and 1.18 was used as the enzyme source for the adenylate cyclase assay.

Adenylate cyclase assay. Adenylate cyclase activity was assayed at 37°C for 10 min according to a modification of the method of Wolff and Jones [15]. The incubation mixture (final vol. 200 μl) contained: 40 mM Tris-HCl, (pH 7.5)/5 mM $MgCl_2$/0.6 mM dithiothreitol/10 mM theophylline/0.1% bovine serum albumin/10 mM creatine phosphate/35 μg creatine phosphokinase (160 U/mg) and 200 μM [α-^{32}P]ATP (approx. 1 μCi/tube). The reaction was initiated by the addition of plasma membrane (approx. 75 μg) and terminated by boiling the incubation mixture for 4 min. The assay for cyclic [^{32}P]AMP thus formed was performed by the combined methods of Ramachandran [16] and Krishna and Birnbaumer [17] as described by Sato et al. [7]. In assays involving cholera toxin or prostaglandin E_1, the appropriate amount of 'vehicle' (i.e., 0.05 M Tris-HCl/1 mM Na_2EDTA/3 mM NaN_3/0.2 M NaCl (pH 7.5) or (0.2 mg/ml) Na_2CO_3/ethanol (9 : 1, v/v) respectively) was added to the 'control' tubes.

As opposed to the stimulation by prostaglandin E_1 which was added directly to the adenylate cyclase assay mixture, cholera toxin stimulation involved pretreating the plasma membranes with cholera toxin prior to assay, as described by Lin et al. [18] except that intact cholera toxin was used as opposed to a purified A_1-subunit. Briefly, the preincubation mixture (150 μl) contained approx. 75 μg of plasma membrane/40 mM Tris-HCl, (pH 7.5)/2 mM NAD^+/0.6 mM dithiothreitol and cholera toxin. This mixture was incubated for 5 min at 37°C after which 1.5 ml 40 mM Tris-HCl, (pH 7.5) was added and the sample centrifuged at 2400 × *g* for 20 min. The pellet was resuspended in 40 mM Tris-HCl buffer and used for the assay of adenylate cyclase.

Protein was measured by the method of Lowry et al. [19]. All data were analyzed for statistical significance by Student's *t*-test (unpaired samples).

Results

Effects of GTP and Gpp(NH)p on basal, prostaglandin E_1- and cholera toxin-stimulated thyroid adenylate cyclase activity

Bovine thyroid adenylate cyclase activity, assayed with a purified plasma membrane preparation is modestly but consistently stimulated by prostaglandin E_1 and cholera toxin (Table I). GTP alone is also modestly stimulatory, whereas the hydrolysis-resistant GTP analog, Gpp(NH)p is highly stimulatory. These results further show that after the thyroid plasma membranes are exposed to cholera toxin (or to prostaglandin E_1) the adenylate cyclase activity is rendered nearly as responsive to GTP as to Gpp(NH)p. Under these circumstances the enhanced ability of GTP to activate adenylate cyclase is a measure of the cholera toxin (or prostaglandin E_1) effect on the cyclase system. Accordingly, we have expressed the results of some experiments in terms of the ratio of adenylate cyclase activity in the presence of GTP to that in the presence of Gpp(NH)p. This ratio is a convenient measure of a stimulator's effect, normalized for any effect it may have on the Gpp(NH)p response [20]. Thus, prostaglandin E_1 and cholera toxin both significantly increase the GTP/Gpp(NH)p ratios from 0.22 ± 0.02 to 0.47 ± 0.06 ($P < 0.005$) and from 0.19 ± 0.02 to 0.73 ± 0.06 ($P < 0.005$), respectively (Table I). (Since fluoride-stimu-

TABLE I

EFFECTS OF GTP AND Gpp(NH)p ON BASAL, PROSTAGLANDIN E_1- AND CHOLERA TOXIN-STIMULATED THYROID ADENYLATE CYCLASE ACTIVITY

Results expressed as pmol cyclic AMP formed per min per mg protein ± S.E.

Treatment	None	Additions		
		GTP (100 μM)	Gpp(NH)p (100 μM)	GTP/Gpp(NH)p
Control	8.9 ± 0.4	13.9 ± 0.7	62 ± 5	0.22
Prostaglandin E_1 (50 μM)	12.1 ± 0.6 * (n = 35)	31.0 ± 4.1 * (n = 17)	66 ± 5 (n = 11)	0.47
Control	5.2 ± 0.3	9.0 ± 0.8	48 ± 4	0.19
Cholera Toxin (265 μg/ml)	7.7 ± 0.5 * (n = 29)	32.0 + 2.6 * (n = 8)	44 ± 3 (n = 11)	0.73

* Significantly ($P < 0.005$) greater than control.

lated adenylate cyclase activity is greater than the Gpp(NH)p-stimulated activity (i.e. Gpp(NH)p = 45 ± 7 (N = 8), F^- = 150 ± 14 (N = 8) pmol cyclic AMP formed per min per mg protein) and since TSH stimulates Gpp(NH)p-stimulated activity by approx. 50% (data not shown), findings consistent with previously reported results [5], it is clear that the Gpp(NH)p response is not the maximal attainable adenylate cyclase activity. Thus, the lack of effect by cholera toxin (or prostaglandin E_1) on Gpp(NH)p activity versus its effect on GTP activity clearly indicates that the difference is due to the intrinsic differences between the nucleotides). Adenylate cyclase activity was less in the cholera toxin experiments than in those with prostaglandin E_1, presumably because in the toxin experiments the plasma membranes were preincubated with the cholera toxin 'vehicle' at 37°C for 5 min, which may have caused a loss in cyclase activity.

It has been reported that in most systems Gpp(NH)p activates adenylate cyclase with a distinct lag ranging from 1 min to longer periods of time, whereas GTP does not [5,18,21,22]. Therefore, to preclude any major effects of cholera toxin or prostaglandin E_1 on such a lag period per se, time-course studies were done on GTP and Gpp(NH)p activities in the presence and absence of prostaglandin E_1 and cholera toxin. Fig. 1, a shows that using assay conditions for cholera toxin stimulation there was indeed a modest lag period of approx. 3 min in achieving steady state kinetics for Gpp(NH)p stimulation which was not affected by cholera toxin. Conversely, GTP showed no lag period and was linear both in the presence and absence of cholera toxin. Fig. 1, b shows that there was no appreciable lag period for either GTP or Gpp(NH)p when adenylate cyclase was assayed using the assay conditions for prostaglandin E_1 stimulation. Since the data in Table I were obtained when steady state conditions had already prevailed for 7—10 min and since the combination of GTP and stimulator was higher than GTP at all times, it may be concluded that neither cholera toxin nor prostaglandin E_1 are increasing the GTP/Gpp(NH)p ratio indirectly by affecting the lag period for Gpp(NH)p, but rather by a direct activation of GTP action.

The dose-relationships between prostaglandin E_1 and GTP in the activation

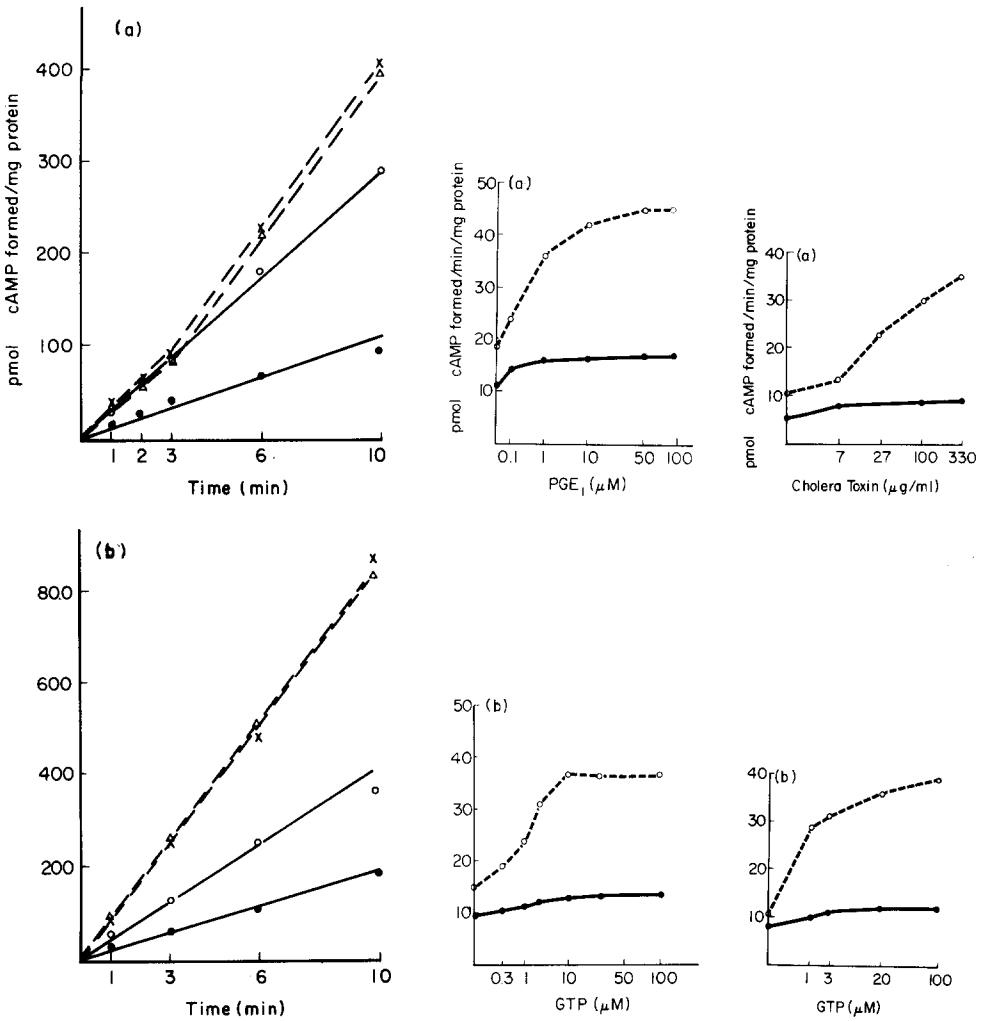


Fig. 1. (Left). (a) Effect of cholera toxin or prostaglandin E_1 on the time-course of adenylate cyclase activity after stimulation by GTP or Gpp(NH)p. Tubes containing GTP (100 μM) or Gpp(NH)p (100 μM) were treated with cholera toxin (265 μg/ml) or 'vehicle' and then incubated at 37°C for various incubation times. GTP (●———●); GTP + cholera toxin (○———○); Gpp(NH)p (X- - - - -X); and Gpp (NH)p + cholera toxin (△ - - - - - -△). (b) The effect of prostaglandin E_1 (50 μM) on the time-course of GTP (100 μM) and Gpp(NH)p (100 μM) stimulated activities at 37°C are depicted in part b. GTP (●———●); GTP + prostaglandin E_1 (○———○); Gpp(NH)p (X - - - - - -X); and Gpp(NH)p + prostaglandin E_1 (△ - - - - - -△). The data are from a representative experiment (2 experiments) which gave similar results.

Fig. 2. (Centre). Dose-relationships between prostaglandin E_1 (PGE_1) and GTP in the activation of adenylate cyclase. The effect of prostaglandin E_1 on adenylate cyclase was tested in the absence and in the presence of GTP (100 μM) (a, ○ - - - - - - ○, GTP 100 μM); additionally, the effect of GTP was tested in the absence and in the presence of prostaglandin E_1 (50 μM) (b, ○ - - - - - - ○, PGE_1, 50 μM). The data are from a representative experiment (3 experiments) which gave entirely similar results.

Fig. 3. (Right). Dose-relationship between cholera toxin and GTP in the activation of adenylate cyclase. The effect of cholera toxin on adenylate cyclase was tested in the absence and in the presence of GTP (100 μM) ○ - - - - - - ○, (a); additionally, the effect of GTP was tested in the absence and in the presence of cholera toxin (265 μg/ml) ○ - - - - - - ○, (b). The data are from a representative experiment (3 experiments) which gave similar results.

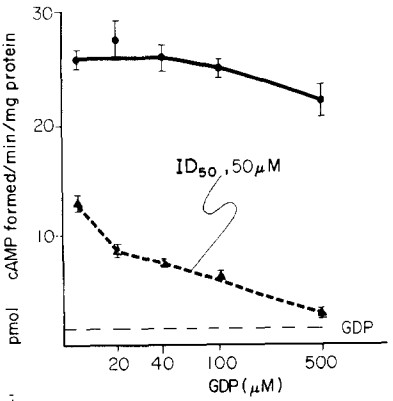
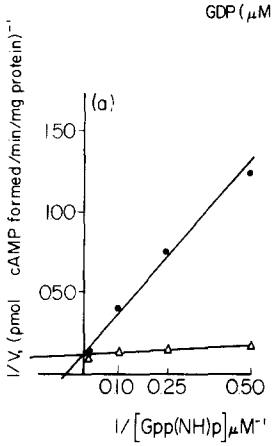
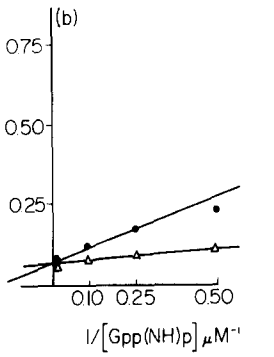
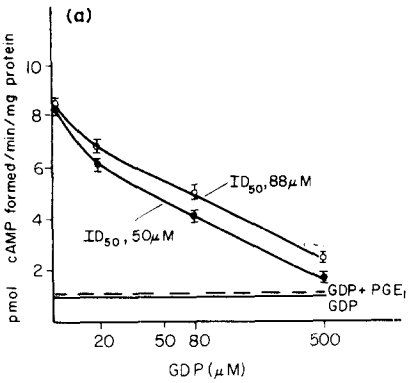


Fig. 4. (Left). Effect of GDP on activation of thyroid adenylate cyclase by Gpp(NH)p and fluoride. Adenylate cyclase was assayed with [^{32}P]App(NH)p (100 μM) as substrate with no ATP-regenerating system. Adenylate cyclase was stimulated by Gpp(NH)p (100 μM) and fluoride (10 mM) and the effects of increasing doses of GDP on these activities were tested. I_{50} (ID_{50}) = 50% inhibition of the Gpp(NH)p response. ●———●, fluoride stimulation; ▲- - - - -▲, Gpp(NH)p stimulation.







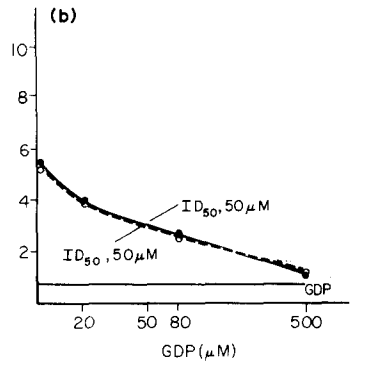


Fig. 5. (Left). Lineweaver-Burk plot of the inhibitory effects of GDP (a) and IDP (b) on Gpp(NH)p-stimulated adenylate cyclase. Adenylate cyclase was assayed with [^{32}P]App(NH)p (100 μM) as substrate with no ATP-regenerating system. The effects of varying doses of Gpp(NH)p were tested in the absence △———△, and in the presence of GDP (20 μM) a, ●———●, and IDP (150 μM) b, ●———●. The data are from a representative experiment (2 experiments) which gave entirely similar results.

Fig. 6. (Right). Effects of prostaglandin E_1 and cholera toxin on the GDP inhibition to activation of thyroid adenylate cyclase by Gpp(NH)p. Adenylate cyclase was assayed with [^{32}P]App(NH)p (100 μM) as substrate with no ATP-regenerating system. Increasing doses of GDP were tested in inhibiting the Gpp(NH)p (100 μM) response in the absence and in the presence of prostaglandin E_1 (PGE_1) (50 μM) and cholera toxin (265 μg/ml). ID_{50} = 50% inhibition of the Gpp(NH)p response.

The data are pooled from 3 separate experiments which gave entirely similar results. PGE_1, ○———○; Cholera toxin, ○- - - - -○; ●———●, absence of test materials.

of adenylate cyclase are presented in Fig. 2. Prostaglandin E_1 in the presence of GTP (100 μM) increased the cyclase activity in a dose-related manner, with half-activation at 0.6 μM. Similarly, the enhancement of the prostaglandin E_1 (50 μM) response by GTP was also dose-related with half-activation at approx. 2 μM.

The relative potencies of the various prostaglandins in the activation of thyroid adenylate cyclase were tested. It was observed that in the presence of GTP (100 μM), prostaglandin E_1 was the most potent stimulator. Although active, prostaglandin E_2 and prostaglandin A_1, were less potent whereas prostaglandin F_{2a} and prostaglandin B_1 were relatively ineffective. 15-keto prostaglandin E_1, the metabolic degradatory product of prostaglandin E_1 was ineffective in increasing the activity.

Fig. 3 depicts the dose-relationships between cholera toxin and GTP in the activation of adenylate cyclase. Cholera toxin in the presence of GTP (100 μM) increased the activity in a dose-related manner, with the activity still raising even at the highest dose tested (330 μg/ml). The enhancement of the cholera toxin (265 μg/ml) response by GTP was also dose-related.

Although not detailed here, cholera toxin inhibited fluoride-stimulated adenylate cyclase activity in a dose-related manner, with 50% inhibition at approx. 75 μg/ml, thus, confirming similar findings in other tissues [2,18,23]. Neither Gpp(NH)p (1—100 μM), GTP (1—500 μM), nor prostaglandin E_1 (1—50 μM) inhibited the fluoride response.

Comparative effects of GTP and ITP on basal and stimulated adenylate cyclase activity

Table II summarizes the effects of ITP and Ipp(NH)p on basal as well as on prostaglandin E_1- and cholera toxin-stimulated cyclase activities. As was the case for GTP, cholera toxin and prostaglandin E_1 increased the response to ITP while having no effect on the Ipp(NH)p response, which can readily be seen by the increases in the ITP/Ipp(NH)p ratios (Table II). The concentration of ITP required to give half-maximal activation of the cholera toxin or prostaglandin

TABLE II

EFFECTS OF ITP AND Ipp(NH)p ON BASAL, PROSTAGLANDIN E_1- AND CHOLERA TOXIN-STIMULATED THYROID ADENYLATE CYCLASE ACTIVITY

Results expressed as pmol cyclic AMP formed per min per mg protein ± S.E.

Treatment	None	Additions		
		ITP (100 μM)	Ipp(NH)p (100 μM)	ITP/Ipp(NH)p
Control	6.1 ± 0.7	11.2 ± 1.5	39 ± 5	0.28
Prostaglandin E_1 (50 μM)	8.1 ± 1.1 * (n = 8)	19.2 ± 4.1 * (n = 8)	38 ± 5 * (n = 8)	0.50
Control	4.2 ± 0.3	7.6 ± 0.6	30 ± 1	0.25
Cholera toxin (265 μg/ml)	5.7 ± 0.2 * (n = 4)	24.3 ± 1.3 * (n = 4)	28 ± 2 * (n = 4)	0.86

* Significantly ($P < 0.05$) greater than control.

E_1 response was approx. 20 μM (versus approx. 2 μM for GTP). Ipp(NH)p (100 μM) per se was highly stimulatory giving 70% of the Gpp(NH)p (100 μM) response (Tables I and II). Additionally, Ipp(NH)p gave half-activation at approx. 15 μM (versus approx. 1 μM for Gpp(NH)p). Combinations of maximally effective doses of Ipp(NH)p and Gpp(NH)p were not additive, but were equal to the higher effect of Gpp(NH)p.

Inhibition of Gpp(NH)p-stimulated adenylate cyclase activity by GDP and IDP

GDP has been reported to act at the guanine nucleotide regulatory site as a negative enzyme effector [3,8,21]. Fig. 4 shows that this inhibition is also evident in thyroid, in that GDP inhibited the Gpp(NH)p-stimulated adenylate cyclase activity in a dose-related manner, the GDP ID_{50} being 50 μM whereas GDP had no inhibitory effect on fluoride-stimulated activity. These studies were performed using [^{32}P]App(NH)p as substrate and no ATP regenerating system to preclude any conversion of GDP to GTP.

Fig. 5 shows a Lineweaver-Burk plot of the effects of IDP and GDP on Gpp(NH)p-stimulated adenylated cyclase activity using [^{32}P]App(NH)p as substrate and no regenerating system. The data show competitive inhibition by both IDP and GDP of the stimulation by Gpp(NH)p. These data suggest that inosine and guanosine nucleotides are competing for the same site on the enzyme.

Effects of prostaglandin E_1 and cholera toxin on GDP inhibition of Gpp(NH)p-stimulated adenylate cyclase activity

Prostaglandin E_1 and cholera toxin were tested to see if they had any effect on the GDP inhibition of activation of thyroid adenylate cyclase by Gpp(NH)p.

These studies were done using non-hydrolyzable [^{32}P]App(NH)p as substrate with no ATP regenerating system. Fig. 6 shows the effects of prostaglandin E_1 and cholera toxin on GDP inhibition of Gpp(NH)p-stimulated activity. The results show that cholera toxin had no effect on the GDP ID_{50}, whereas, prostaglandin E_1 raised the GDP ID_{50} approx. 2-fold (88 μM versus 50 μM, $P < 0.005$), thus confirming similar effects in other tissues [8].

Discussion

Both cholera toxin- and prostaglandin E_1-stimulated adenylate cyclase were highly stimulatable by GTP (Fig. 2 and 3) whereas no differences were seen between prostaglandin E_1, cholera toxin, or control membranes in response to Gpp(NH)p, findings which can readily be seen in the dramatic increase in the GTP/Gpp(NH)p ratios (Table I). These data show that cholera toxin and prostaglandin E_1 specifically enhance the GTP response relative to Gpp(NH)p and suggest that they either: (1) inhibit hydrolysis of GTP at the guanyl nucleotide regulatory site via inhibition of a specific GTPase; (2) enhance the GTP effect per se (possibly by modulating the inhibitory effect of GDP vide supra); or (3) promote GTP (and not Gpp(NH)p) action by altering the conformation of the nucleotide site.

GDP has been reported to be a potent inhibitor of Gpp(NH)p-stimulated adenylate cyclase activity in hepatic [21] and neuroblastoma [3,8] systems.

These findings were confirmed in thyroid and the inhibition was shown to be specific for Gpp(NH)p-stimulated activity suggesting the guanine nucleotide regulatory site as the locus of the inhibition. This was confirmed by showing that the inhibition by GDP of the Gpp(NH)p response was of the competitive type.

Blume and Foster [8] have postulated that prostaglandin E_1 may be acting as a secondary enzyme regulator in neuroblastoma cells (the primary one being the guanine nucleotide, GTP or Gpp(NH)p) by reducing the affinity of GDP for the nucleotide regulatory site. The studies outlined in Fig. 6 were done to ascertain if such a mechanism is operative in thyroid. The dose of GDP that gives 50% inhibition (ID_{50}) was used as a relative measure of the GDP effect on the enzyme [3,8]. The results obtained showed that the GDP-ID_{50} for the cholera toxin-treated membrane was the same as for the control, whereas prostaglandin E_1 increased the GDP-ID_{50} approx. 2-fold. These data are, therefore, in agreement with the findings of Levinson and Blume [3] in neuroblastoma cells wherein the amount of GDP required to inhibit 50% of the activation of control or cholera toxin-treated membrane by Gpp(NH)p was similar; and wherein prostaglandin E_1 decreased the affinities to GDP of control or cholera toxin-treated enzymes equally.

It has been reported that cholera toxin inhibits the fluoride-activated cyclase [2,18,23]. This finding was confirmed in thyroid and was shown to be specific for cholera toxin in that prostaglandin E_1, GTP and Gpp(NH)p (also thought to act at the guanine nucleotide regulatory site) had no inhibitory effect on the fluoride-stimulated activity. This lack of Gpp(NH)p effect differs from that found in liver or kidney [24,25] but is in agreement with the findings of Wolff and Cook [26] that GTP did not inhibit this activity in thyroid. In support of these data, it has recently been shown that cholera toxin can covalently label a 42 000-M_r protein (GTPase?) using [^{32}P]NAD^+, that is the GTP binding component of adenylate cyclase and that both the toxin enhancement of GTP stimulation and the suppression of fluoride stimulation are dependent on this modification [27].

ITP can substitute for GTP in activating both basal as well as thyrotropin- and prostaglandin E-stimulated adenylate cyclase in thyroid [26,28]. The results presented here extend these findings in that ITP could substitute for GTP in enhancing the cholera toxin-stimulated activity. Furthermore, it was shown that the non-hydrolyzable ITP analog, Ipp(NH)p was a very potent cyclase stimulator per se.

The results with cholera toxin confirm and extend the findings in other tissue systems to thyroid and clearly indicate a role for GTP in this stimulation. There is as yet, however, no unanimity in assuming that cholera toxin exerts its effect by inhibiting a GTPase [2—4] as can be seen from the findings of Lad et al. [22] who have recently postulated an alternate mechanism for cholera toxin action that does not implicate GTPase, but rather an effect on the nucleotide exchange reaction. However, in their system, cholera toxin also increased the response to Gpp(NH)p differing from the results reported herein. Thus, although we acknowledge that other mechanisms may obtain in thyroid, the 'circumstantial' evidence reported here is consistent with a cholera toxin effect on

GTPase. Clearly the final proof would be the demonstration of a specific GTPase in thyroid that is inhibited by cholera toxin.

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