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EFFECT OF GUANYL NUCLEOTIDES ON THE STIMULATION OF ADENYL CYCLASE ACTIVITY IN HUMAN THYROID PLASMA MEMBRANES BY THYROID-STIMULATING HORMONE AND PROSTAGLANDIN E_2

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SUMMARY

Thyroid homogenates and thyroid plasma membranes were prepared from human thyroid and the effects of thyroid-stimulating hormone (thyrotropin), NaF, and prostaglandins E_1 and E_2 on adenyl cyclase activity in these preparations were studied. The basal level of adenyl cyclase activity in plasma membranes was 5–8 times greater than that of the original homogenates. Adenyl cyclase activity in plasma membranes was stimulated 4.7-fold by 100 munits/ml of thyrotropin and 5-fold by 10 mM of NaF, but the activity in the homogenates was only stimulated 2-fold by either thyrotropin or NaF. Prostaglandin E_1 (10^{-6}–10^{-3} M) and prostaglandin E_2 (10^{-7}–10^{-4} M) failed to stimulate adenyl cyclase activity in plasma membranes, but they did stimulate adenyl cyclase activity in the homogenates. A marked stimulatory effect of prostaglandin E_2 (10^{-5} M) on adenyl cyclase activity in plasma membranes resumed in the presence of GTP (10^{-7}–10^{-4} M), although GTP itself only slightly stimulated enzyme activity. GDP and GMP were also effective in this respect, although their potencies varied from compound to compound. GTP potentiated slightly the action of thyrotropin on adenyl cyclase in plasma membranes, but it significantly depressed an increase of enzyme activity produced by NaF. Since GTP did not affect the ATP-regenerating system, it seems that GTP, GDP or GMP was required for the manifestation of prostaglandin E_2 action on adenyl cyclases of human thyroid plasma membranes.

INTRODUCTION

Onaya and Solomon [1] have shown that prostaglandin E_1 markedly enhanced glucose oxidation and colloid droplet formation in canine thyroids in vitro and colloid droplet formation and ^{131}I release in mice thyroids in vivo. Kaneko et al. [2] have shown that prostaglandin E_1 caused an increase in cyclic AMP concentration in canine thyroid slices. In addition to a high concentration of prostaglandin in the thyroid, these data strongly suggested that prostaglandin may play an important role in the regulation of thyroid function at least in experimental animals. Our previous studies [3,4] have also indicated that prostaglandins apparently acted on isolated bovine thyroid cells to augment adenyl cyclase activity. However, Wolff and Jones [5] have noted that thyroid stimulating hormone (thyrotropin) stimulated adenyl cyclase in bovine thyroid plasma membranes, but that prostaglandin E_1 failed to do so. Since a recent study by Krishna and Harwood [6] has indicated an important role for GTP in the manifestation of the prostaglandin E_1 effect on adenyl cyclase activity in platelet membranes, it would be useful to investigate if GTP, GDP and GMP similarly play a permissive role in the manifestation of prostaglandin action on adenyl cyclase activity in thyroid plasma membranes. Because of a possible physiological importance of prostaglandin in the regulation of human thyroid, thyroid plasma membranes were prepared from human thyroid, and the effects of thyrotropin, prostaglandin E_1 and E_2 and NaF on adenyl cyclase activity were studied in the presence or absence of GTP, GDP and GMP.

MATERIALS AND METHODS

Human thyroid tissues were obtained from hyperthyroid patients. The patients received methimazole (30–40 mg/day) and excess iodide (10–20 mg/day) for about four weeks before the operation. After removal of the thyroid glands, plasma membranes were prepared by using the method of Emmelot et al. [7] as modified by Yamashita and Field [8]. The plasma membranes thus obtained were suspended in 1 ml $NaHCO_3$ (pH 7.5) and stored at -20 °C until use. Adenyl cyclase activities of thyroid plasma membranes were measured by the technique originally reported by Krishna et al. [9]. Unless otherwise stated, the incubation medium was a mixture of the following substances: 40 mM Tris–HCl (pH 7.8) containing 1 μCi [α-^{32}P]ATP, 1 mM ATP, 4 mM cyclic AMP, 3.5 mM $MgCl_2 \cdot 6H_2O$, 10 mM theophylline, 0.1% crystalline bovine serum albumin, 20 mM phosphoenol pyruvate, 250 μg/ml pyruvate kinase and 250 μg/ml myokinase. 50 μl of incubation medium and 50 μl of plasma membranes (50–100 μg protein) were mixed and incubated for 10 min, at 37 °C. After incubation, 300 μl of the recovery solution containing 10 mM of each nucleotide (ATP, ADP, 5′-AMP, cyclic AMP) and 0.05 μCi of ^{3}H-labeled cyclic AMP were added to the incubation medium, and this mixture was boiled for 3 min to terminate the enzyme reaction. After centrifugation ^{32}P-labeled cyclic AMP newly produced was purified by the combined methods of an alumina column (0.5 cm$\times$2 cm) and a resin column (0.7 cm$\times$3.5 cm) as reported previously [10]. The sample was first purified by the alumina column, the method being essentially similar to the method reported by Ramachandran ((1971) Anal. Biochem. 43, 227). The sample was then repurified by the resin column, by using the technique reported by Krishna et al. [9]. Protein concentration was measured by the method of Lowry et al. [11], using a standard solution newly prepared each occasion from the stock solution of bovine serum albumin. GTP, GDP and GMP were obtained from Miles Laboratories Inc. (Kankakee, Ill.) and thyrotropin was obtained from Armour Pharmaceutical Co. Prostaglandins E_1 and E_2 were generally supplied by Ono Pharmaceutical Co., Ltd. 1 mg of prostaglandin E_1 and prostaglandin E_2 were each dissolved in 0.1 ml absolute ethanol and 0.9 ml Na_2CO_3 (0.2 mg/ml, pH 7.0) was further added. This solution was diluted with buffer to obtain a desired concentration of prostaglandin E. ^{32}P[ATP] and ^{3}H-labeled cyclic AMP were obtained from the Radiochemical Center (R.C.C., England). Pyruvate kinase (150 units/mg) and myokinase (360 units/mg) were obtained from Boehringer Mannheim Biochemicals. AG 50-WX2(H^+) form, 100–200 mesh, was purchased from Bio-Rad Laboratories and activated Al_2O_3 for chromatography was obtained from Kanto Chemical Co. The other reagents were of highest purity available from commercial sources. The analysis of nucleotides after incubation was performed by using thin-layer chromatography [12]. The reaction mixture and carrier nucleotides were applied to recoated polyethylene–imine-impregnated cellulose sheet (Baker Chemical Co.) and developed for 40 min in a solvent solution of 0.3 or 1.0 M LiCl. Each nucleotide was visualized by ultraviolet light, cut out and counted in 10 mM of toluene scintillator fluid.

RESULTS

Effect of thyrotropin, prostaglandin E and NaF on adenyl cyclase activity in thyroid homogenates

Addition of 50 munits/ml thyrotropin apparently stimulated adenyl cyclase activity in thyroid homogenates as evidenced by a marked increase of ^{32}P-labeled cyclic AMP newly produced (Fig. 1). 100 munits/ml thyrotropin were less effective than 50 munits/ml thyrotropin in this respect. Postaglandin E_1 (10^{-6} M) and prostaglandin E_2 (10^{-7} M) also stimulated adenyl cyclase activity, the magnitude of stimulation being roughly comparable to that produced by 50 or 100 munits/ml thyrotropin. NaF (10 mM) stimulated adenyl cyclase activity to an extent comparable to that produced by thyrotropin and prostaglandin

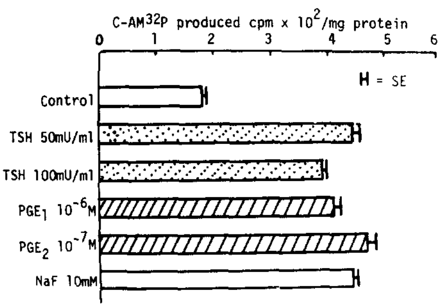


Fig. 1. Effect of thyrotropin (TSH), prostaglandin E (PGE) and NaF on the adenyl cyclase activity in human thyroid homogenates. Each value represents the mean ±S.E. of triplicate determinations. 1000 cpm corresponded to 236 pmoles/mg protein.

Effect of thyrotropin, prostaglandin E and NaF on adenyl cyclase activity in thyroid plasma membranes

When the plasma membrane preparation was assayed for adenyl cyclase activity, its specific activity was about 5–8 times greater than that of the original thyroid homogenate. The enzyme activity in plasma membranes was stimulated 3.7-fold by 50 munits/ml thyrotropin and 4.7-fold by 100 munits/ml thyrotropin as compared to the basal level (Fig. 2). Also, the enzyme activity was stimulated 5-fold by 10 mM NaF. In contrast, low and high concentrations of prostaglandin E_1 (10^{-6} and 10^{-3} M) and prostaglandin E_2 (10^{-7} and 10^{-4} M) failed to stimulate adenyl cyclase activity in plasma membranes.

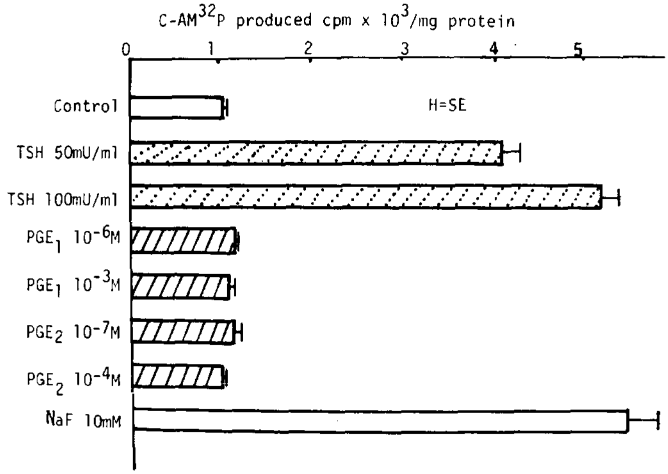


Fig. 2. Effect of thyrotropin (TSH), prostaglandin E (PGE) and NaF on the adenyl cyclase activity in human thyroid plasma membranes. Each value represents the mean ±S.E. of triplicate determinations. ATP concentration was 10^{-3} M. 1000 cpm corresponded to 236 pmoles/mg protein.

Effect of thyrotropin, prostaglandin E_2 and NaF on adenyl cyclase activity in thyroid plasma membranes in the presence of GTP

As shown in Fig. 3, thyrotropin (50 munits/ml) again stimulated adenyl cyclase activity in the thyroid plasma membranes. NaF (10 mM) also stimulated adenyl cyclase activity in the thyroid plasma membranes, but prostaglandin E_2 (10^{-5} M)

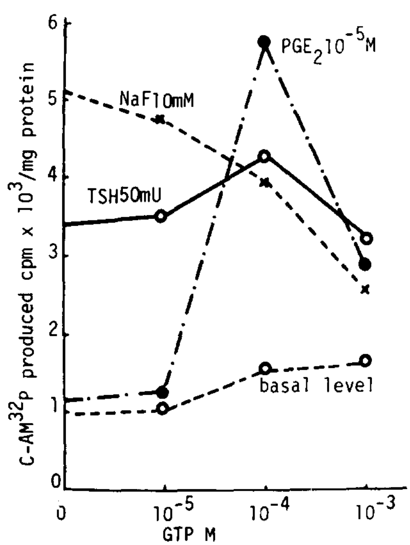


Fig. 3. Effects of GTP on the adenyl cyclase activity in human thyroid plasma membranes in the presence of thyrotropin (TSH) (50 munits/ml), prostaglandin E_2 (PGE_2) (10^{-5} M) and NaF (10 mM). The ATP concentration was 10^{-3} M. Each value represents the mean of duplicate determinations. 1000 cpm corresponded to 254 pmoles/mg protein.

failed to stimulate it. Addition of graded doses of GTP produced a progressive increase of adenyl cyclase activity (basal level), but the magnitude of the increase was quite small. However, when 10^{-4} M of GTP and 10^{-5} M of prostaglandin E_2 were present, adenyl cyclase activity increased 5-fold as compared to the basal level. A higher dose of GTP (10^{-3} M) was less effective in this respect. Thyrotropin action on adenyl cyclase activity was slightly augmented in the presence of 10^{-4} M of GTP. On the other hand, NaF action on adenyl cyclase activity decreased progressively with increasing doses of GTP.

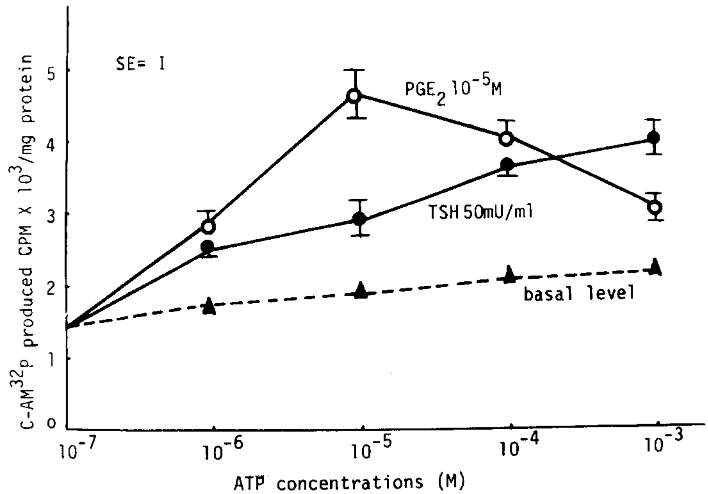


Fig. 4. Effect of various ATP concentrations on the activation of adenyl cyclase by thyrotropin (TSH) and prostaglandin E_2 (PGE_2) in human thyroid plasma membranes in the presence of GTP. GTP concentration was 10^{-4} M. Each value represents the mean ±S.E. of triplicate determinations. Basal value represents the mean of duplicate determinations. 1000 cpm corresponded to 254 pmoles/mg protein.

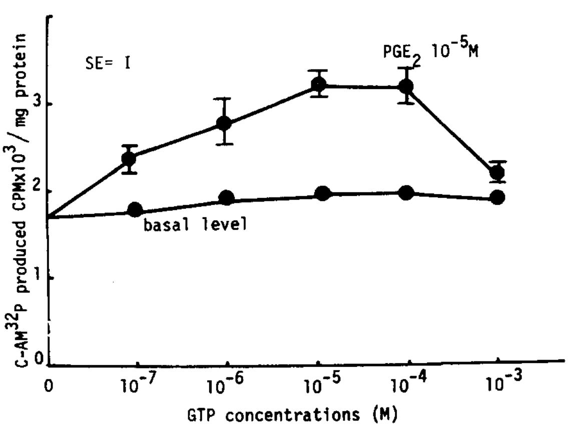


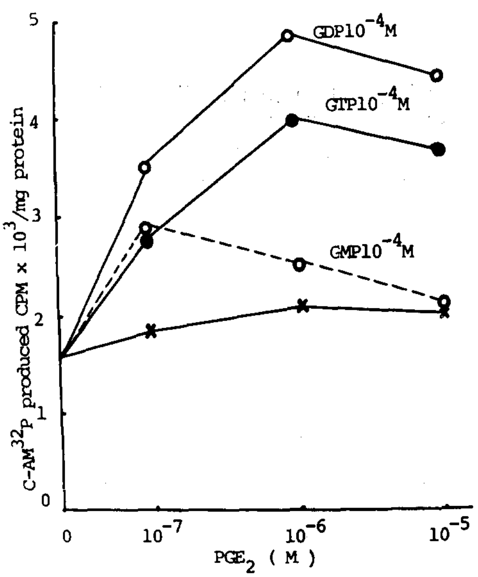
Fig. 5. Effect of GTP on the prostaglandin E_2 (PGE_2) stimulation of adenyl cyclase in human thyroid plasma membranes. 10^{-5} M ATP was used as the substrate. Each value represents the mean ±S.E. of triplicate determinations. The basal value represents the mean of duplicate determinations. 1000 cpm corresponded to 236 pmoles/mg protein.

In the next step, a further experiment was performed to see if the ATP concentration of the incubation medium would alter the effects of thyrotropin (50 munits/ml) +GTP (10^{-4} M) and prostaglandin E_2 (10^{-5} M)+GTP (10^{-4} M) on adenyl cyclase activity. Thus, concentrations of ATP from 10^{-7} to 10^{-3} M were tested. As shown in Fig. 4, the effects of prostaglandin E_2+GTP was maximal at the concentration of 10^{-5} M ATP. In contrast, the effect of thyrotropin+GTP on ^{32}P-labeled cyclic AMP synthesis increased progressively with increasing doses of ATP.

In the third step, the permissive role of GTP on prostaglandin E_2 action was studied by using 10^{-5} M ATP. As shown in Fig. 5, prostaglandin E_2 (10^{-5} M) effect on ^{32}P-labeled cyclic AMP synthesis was apparent within a wide dose range of GTP used (10^{-7}–10^{-4} M).

Effects of prostaglandin E_2 on adenyl cyclase activity in thyroid plasma membranes in the presence of GTP, GDP and GNP

The permissive role of three nucleotides on the manifestation of prostaglandin E_2 effect was studied by incubating thyroid plasma membranes for 10 min as shown



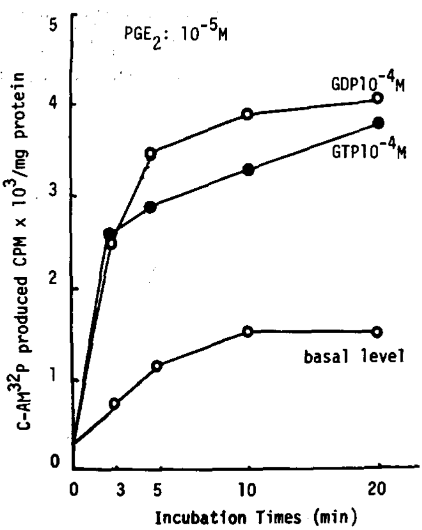


Fig. 6. Effects of guanyl nucleotides on the prostaglandin E_2 (PGE_2) dose-response curve of adenyl cyclase activation in human thyroid plasma membranes. Thyroid plasma membranes were incubated for 10 min in the presence of prostaglandin E_2 (10^{-7}–10^{-5} M) or prostaglandin E_2 +guanyl nucleotides (10^{-4} M). ×—×, prostaglandin E_2; GMP (10^{-4} M), prostaglandin E_2 +GMP (10^{-4} M); GDP (10^{-4} M), prostaglandin E_2+GDP (10^{-4} M); GTP (10^{-4} M), prostaglandin E_2+GTP (10^{-4} M). Each value represents the mean of duplicate determinations. The ATP concentration was 10^{-3} M. 1000 cpm corresponded to 236 pmoles/mg protein.

Fig. 7. Effect of prostaglandin E_2 (PGE_2) on adenyl cyclase activation in human thyroid plasma membranes in the presence of guanyl nucleotides. Basal level, thyroid plasma membranes were incubated for 3–20 min; GTP (10^{-4} M), thyroid plasma membranes were incubated in the presence of prostaglandin E_2 (10^{-5} M) and GTP (10^{-4} M) for 3–20 min; GDP (10^{-4} M), thyroid plasma membranes were incubated in the presence of prostaglandin E_2 (10^{-5} M) and GDP (10^{-4} M) for 3–20 min. ○, the mean value of duplicate determinations. ATP concentration was 10^{-3} M. 1000 cpm corresponded to 236 pmoles/mg protein.

in Fig. 6. In the absence of nucleotides, graded doses of prostaglandin E_2 (10^{-7}–10^{-5} M) failed to augment ^{32}P-labeled cyclic AMP synthesis in thyroid plasma membranes significantly. In the presence of guanyl nucleotides, however, prostaglandin E_2 apparently stimulated ^{32}P-labeled cyclic AMP synthesis. The maximal effect of prostaglandin E_2 was found at prostaglandin 10^{-7} M in the presence of GMP, whereas the maximal effects were found at 10^{-6} M in the presence of GDP and GTP. Furthermore, an increase of ^{32}P-labeled cyclic AMP was higher at all levels of prostaglandin E_2 concentration in the presence of GDP.

In the next step, thyroid plasma membranes were incubated in the presence of GTP (10^{-4} M)+prostaglandin E_2 (10^{-5} M), GDP (10^{-4} M)+prostaglandin E_2 (10^{-5} M) or prostaglandin E_2 (10^{-5} M) for 3–20 min to see the time course of ^{32}P-labeled cyclic AMP synthesis. Fig. 7 indicates the mean value of duplicate determinations. As compared to prostaglandin E_2 alone (basal level), combined use of prostaglandin E_2+GTP apparently augmented adenyl cyclase activity of thyroid plasma membranes throughout the experimental period. Also combined use of prostaglandin+GDP augmented adenyl cyclase activity of thyroid plasma membranes similarly to prostaglandin E_2+GTP.

Effect of GTP on ATP-regenerating system

The effect of GTP in the ATP-regenerating system was studied under experimental conditions similar to those mentioned above. The labeled compounds were identified by comparison with the carriers, and the distribution of ^{32}P among the nucleotides was calculated by using the data of polyethylene–imine sheet thin-layer chromatography. As shown in Table I, addition of GTP did not influence the distribution patterns of ^{32}P-labeled adenosine nucleotides.

TABLE I

EFFECT OF GTP ON THE ATP-REGENERATING SYSTEM IN HUMAN THYROID PLASMA MEMBRANES

Nucleotides were isolated as described in Materials and Methods.

Reaction mixture		Distribution of radioactive adenosine nucleotides (%)			
Human thyroid plasma membranes	GTP (10^{-4} M)	^{32}P[ATP]	^{32}P[ADP]	^{32}P[5′-AMP]	Others
–	–	96.7	2.9	0.2	0.2
+	–	88.6	9.3	1.9	0.2
+	+	88.6	9.0	2.0	0.4

DISCUSSION

Our present study indicated that physiological concentrations of prostaglandins E_1 and E_2 can apparently stimulate adenyl cyclase activity in the homogenate of human thyroid obtained from hyperthyroid patients. Although the data are not included here, a physiological concentration of prostaglandin E_2 can also stimulate adenyl cyclase activity in normal thyroid homogenates [13]. These findings strongly suggested that prostaglandin E plays an important physiological role in the regulation of thyroid function in man.

As reported previously by Wolff and Jones [5] and by us [4], prostaglandins E_1 and E_2 failed to stimulate adenyl cyclase activity in bovine thyroid plasma membranes. In agreement with these findings, our present study indicates that prostaglandins E_1 and E_2 fail to stimulate adenyl cyclase activity in human thyroid plasma membranes. These findings are of considerable interest in assessing the mode of action of several thyroid stimulators for adenyl cyclase, since prostaglandin E apparently stimulates adenyl cyclase activity in human as well as bovine thyroid homogenates [14], and since thyrotropin and NaF stimulate adenyl cyclase activity in thyroid plasma membranes from both sources. A possible explanation would be that some factor(s) necessary for the manifestation of prostaglandin E action is lost during preparative processes of plasma membranes. The study by Krishna and Harwood [6] reported that prostaglandin E_1 apparently resumes its stimulatory action on adenyl cyclase of platelet plasma membranes in the presence of GTP. A recent study by Rodbell et al. [15] also showed that GTP greatly enhanced glucagon-activated adenyl cyclase activity of liver plasma membranes. In agreement with these findings, our present study clearly indicates that prostaglandin E_2 can resume its stimulatory effect on adenyl cyclase of human thyroid plasma membranes in the presence of 10^{-4} M GTP (Fig. 3). However, since the GTP concentration required here was 10- to 100-fold greater than those needed in the study by Rodbell et al. [15], the GTP effect was examined with a concentration of 10^{-5} M ATP rather than with the concentration of ATP generally used (10^{-3} M). It was shown that the GTP effect on prostaglandin E_2 was manifest in a wide dose range of GTP used (10^{-7}–10^{-4} M) when 10^{-5} M ATP was used as the substrate (Fig. 5). It was further shown that the effect of prostaglandin E_2 + GTP (10^{-4} M) on ^{32}P-labeled cyclic AMP synthesis was maximal at the concentration of 10^{-5} M but the effect of thyrotropin+GTP (10^{-4} M) on ^{32}P-labeled cyclic AMP synthesis increased progressively with increasing concentration of ATP. These findings indicated that thyrotropin and prostaglandin E_2 have their different optimal concentrations to manifest their maximal effect on ^{32}P-labeled cyclic AMP synthesis in human thyroid plasma membranes. Also our present study provided the information that prostaglandin E_2-stimulated adenyl cyclase astivity in human thyroid plasma membranes in the presence of GDP and GMP. While our study was in preparation, Wolff and Cook [16] reported a similar line of experiment. Although their findings on GTP effect were approximately similar to the one reported here, they were different from ours in two respects: (a) prostaglandin E_1 itself stimulated adenyl cyclase activity in beef thyroid plasma membranes, (b) the thyrotropin effect on beef plasma membranes was markedly enhanced by GTP, D-GTP and ITP. It is not known whether these differences were due to species specificity or due to a difference of plasma membranes used.

Although it is premature to come to any definite conclusion as to the mechanism of action of GTP, GDP and GMP, a number of possibilities may be considered. It is possible that GTP, GDP and GMP augment the binding of prostaglandin E_2 to its receptor in the plasma membranes and thus facilitate the manifestation of the prostaglandin E_2 effect. However, this does not seem to be the case, since our data [14] indicated that GTP significantly depressed the binding of ^{3}H-labeled prostaglandin E_2

to human thyroid plasma membranes. Alternatively, it can be considered that GTP, GDP and GMP manifest the prostaglandin E_2 effect on adenyl cyclase activity by increasing the substrate, ATP. This hypothesis is not supported by our finding that GTP does not affect the ATP-regenerating system. Using an ATPase-resistant substrate (^{32}P[AMP]–*p*-nitrophenol), Krishna and Harwood [6] have also shown that GTP did not augment cyclic AMP concentration by depressing ATPase activity. Further experiments are required to clarify the exact mechanism of action of GTP, GDP and GMP on the prostaglandin E_2 effect in human thyroid plasma membranes.

REFERENCES

1 Onaya, T. and Solomon, D. H. (1970) Endocrinology 86, 423–426
2 Kaneko, T., Zor, U. and Field, J. B. (1969) Science 163, 1062–1063
3 Burke, G. and Sato, S. (1971) Life Sci. (II) 10, 969–981
4 Sato, S., Szebo, M., Kowalski, K. and Burke, G. (1972) Endocrinology 90, 343–356
5 Wolff, J. and Jones, A. B. (1971) J. Biol. Chem. 246, 3939–3947
6 Krishna, G. and Harwood, J. P. (1972) J. Biol. Chem. 247, 2253–2254
7 Emmelot, P., Bos, C. J., Bendetti, E. L. and Rümke, P. H. (1964) Biochim. Biophys. Acta 90, 126–145
8 Yamashita, K. and Field, J. B. (1970) Biochem. Biophys. Res. Commun. 40, 171–178
9 Krishna, G., Weiss, B. and Brodie, B. B. (1968) J. Pharmacol. Exp. Therap. 163, 379–385
10 Sato, S. (1973) Clin. Endocrinol. Tokyo 21, 73–78
11 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
12 Bra, H.-P. and Hechter, O. (1969) Anal. Biochem. 29, 476–489
13 Sato, S. and Yamada, T. (1973) Clin. Endocrinol. Tokyo 21, 725–729 (in Japanese)
14 Sato, S. (1973) Shinshu Med. J. 20, 233–243
15 Rodbell, M., Birnbaumer, L., Pohl, S. L. and Krans, H. M. J. (1971) J. Biol. Chem. 246, 1877–1822
16 Wolff, J. and Cook, G. H. (1973) J. Biol. Chem. 248, 350–355