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PROSTAGLANDIN RECEPTOR-ADENYLATE CYCLASE SYSTEM IN PLASMA MEMBRANES OF RAT LIVER AND ASCITES HEPATOMAS, AND THE EFFECT OF GTP UPON IT

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Summary

1. Adenylate cyclase in plasma membranes from rat liver was stimulated by prostaglandin E_1, and to a lesser extent by prostaglandin E_2. Prostaglandin $F_{1\alpha}$, $F_{2\alpha}$ and A_1 did not stimulate the cyclase. The prostaglandin E_1-mediated activation was found to require GTP when the substrate ATP concentration was reduced from 3 mM to 0.3 mM in the reaction mixture. Adenylate cyclase of the plasma membranes from rat ascites hepatomas AH-130 and AH-7974 was not stimulated by prostaglandin E_1 in the presence or the absence of GTP, although the basal activity of adenylate cyclase as well as its stimulation by GTP alone were similar to normal liver plasma membranes.

2. Liver plasma membranes were found to have two specific binders for [^{3}H]prostaglandin E_1 with dissociation constants of $17.6 \cdot 10^{-9}$ M and $13.6 \cdot 10^{-8}$ M (37°C) and one specific binder for [^{3}H]prostaglandin $F_{2\alpha}$ with a dissociation constant of $2.31 \cdot 10^{-8}$ M (37°C). The specific binders for prostaglandin E_1 could not be detected in the hepatoma plasma membranes.

3. Binding of [^{3}H]prostaglandin E_1 to the liver plasma membranes was enhanced by GTP, dGTP, GDP, ATP and GMP-P(N)P, but not by GMP, cGMP, dTTP, UTP or CTP. The increase in the binding of [^{3}H]prostaglandin E_1 was found to be due to the increased affinity of the specific binders to prostaglandin E_1, but not to the increase in the quantity of binders. Binding of [^{3}H]prostaglandin $F_{2\alpha}$ was not affected by GTP.

4. GTP alone was found to increase V of adenylate cyclase of liver plasma membranes, while GTP plus prostaglandin E_1 was found to decrease K_m of adenylate cyclase in addition to the increase of V to a further extent.

Introduction

Prostaglandins appear to be involved in a number of physiological activities [1—9] and are known to increase the cyclic AMP level in various tissues including

the liver [10—14]. In fact, recent investigations have revealed that prostaglandins stimulate adenylate cyclase in plasma membranes isolated from tissues [15—18]. The inhibitory effect of some prostaglandins on cellular proliferation also seems to be ascribed to the elevated level of cyclic AMP [19—20]. In this regard, the recent investigations showing the impairment of prostaglandin E_1-mediated activation of adenylate cyclase in some tumors such as rat adrenal gland tumor [21] and virus-transformed kidney fibroblast cells [22] seem particularly interesting.

The presence of specific binding sites for prostaglandins in plasma membranes isolated from some tissues [23—26] has been reported. However, the interacting system of prostaglandin receptor and adenylate cyclase has not yet been fully understood even in the case of plasma membranes of normal tissues, and much less in the case of plasma membranes of tumors. In the present study, the interactions of various prostaglandins with plasma membranes from rat liver cells and rat ascites hepatoma cells have been investigated.

Materials and Methods

Preparation of plasma membranes

Plasma membranes of the liver were prepared from male Wistar rats weighing 120—160 g by the method of Ray [27]. Plasma membranes of ascites hepatomas were prepared by the method of Emmelot and Bos [28], as has been modified by Shimizu [29], from AH-7974 and AH-130 cells harvested from the peritoneal cavities of Donryu strain rats weighing 120—160 g several days after inoculation. Electron-microscopical observations on the plasma membrane preparations (liver and ascites hepatomas) revealed that the contamination of mitochondria or other subcellular organells appeared negligible.

Analysis of prostaglandin E_1- and prostaglandin $F_{2\alpha}$-binding sites in plasma membranes by Millipore filtration

Plasma membranes (about 1 mg protein/ml) suspended in 80 μl of 20 mM Tris · HCl (pH 7.4) containing [^{3}H]prostaglandin E_1 or [^{3}H]prostaglandin $F_{2\alpha}$ at various concentrations were incubated at 37°C for 40 min except where otherwise specified. After incubation, a 50-μl aliquot of it was quickly filtered on a small Millipore disc (HAWP304FO), and then washed with 10 ml of the ice-cold 20 mM Tris · HCl buffer (pH 7.4). The above procedures were accomplished within 15 s. [^{3}H]prostaglandin E_1 or [^{3}H]prostaglandin $F_{2\alpha}$ bound to plasma membranes was assayed by measuring radioactivity retained on the Millipore disc. K_d (M) (dissociation constant) and N (pmol/mg protein) (number of binding sites) were obtained by the least square method from the Scatchard plots of the binding. Under the present experimental conditions, counts of radioactivity retained on Millipore discs were 4—20% of the total counts in the assay mixture.

Assay of adenylate cyclase

Plasma membranes equivalent to about 1 mg protein were suspended in 1 ml of the standard reaction mixture, containing 3 mM ATP, 10 mM theophylline, 5 mM phosphoenolpyruvate, 10 μg pyruvate kinase, 5 mM $MgCl_2$, 10 mM KCl,

1 mM EDTA, 20 mM Tris · HCl buffer (pH 7.4) according to the method of Rall and Sutherland [30]. Prostaglandins and/or other chemicals were added to the above reaction mixture at concentrations as indicated in the text. The whole reaction mixture was incubated at 37°C for 15 min except when otherwise specified, heated in a boiling-water bath for 3 min to stop the reaction, and then centrifuged at 10 000 rev./min for 15 min. A 50-μl aliquot of the supernatant was used for the assay of cyclic adenosine 3′,5′-monophosphate by the method of Gilman [31], using the cyclic AMP-binding protein (the cyclic AMP binding capacity of 0.3 pmol cyclic AMP per μg protein) partially purified from bovine heart muscle according to the method of Miyamoto et al. [32].

Assay of radioactivity and protein

The radioactivity trapped on a Millipore disc was counted in a liquid scintillation spectrometer after the disc had been dissolved in 1 ml of ethylcelllcsolve by heating at 70°C for 2 h, using PPO/POPOP in toluene/ethylcellosolve as a scintillation fluid. Protein was assayed by the method of Lowry et al. [3] with bovine serum albumin as a standard.

Chemicals and biochemicals

Cyclic AMP, cyclic GMP, 5′-GMP, 5′-GDP, GTP, dGTP, 5′-guanylylimidodiphosphate (GMP-P(N)P), ATP, UTP, CTP, dTTP, phosphoenolpyruvate and pyruvate kinase were purchased from the Boehringer-Mannheim, G.F.R. Prostaglandin E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, and A_1, as well as [^{3}H]prostaglandin E_1 (87 Ci/mmol) and [^{3}H]prostaglandin $F_{2\alpha}$ (9.7 Ci/mmol) were donated by the Ono Pharmaceutical Co., Japan. [^{3}H]cyclic AMP (27.5 Ci/mmol) was purchased from the Radiochemical Centre, U.K.

Results

(1) Effects of various prostaglandins on adenylate cyclase of plasma membranes from rat liver

As summarized in Table I, prostaglandin E_1, and to a lesser extent prosta-

TABLE I

EFFECTS OF PROSTAGLANDINS ON ADENYLATE CYCLASE OF PLASMA MEMBRANES FROM RAT LIVER

Rat liver plasma membranes were assayed for adenylate cyclase under the standard assay conditions in the presence or the absence of various prostaglandin derivatives (each at 10^{-5} M) as described in Materials and Methods. Plasma membranes equivalent to 1.10 mg protein were used in each assay.

Prostaglandin	Adenylate cyclase activity * (nmol of cyclic AMP formed/mg protein per h)	%
None	1.91 ± 0.04	100
Prostaglandin E_1	2.88 ± 0.18	151
Prostaglandin E_2	2.17 ± 0.09	114
Prostaglandin $F_{1\alpha}$	1.59 ± 0.28	84
Prostaglandin $F_{2\alpha}$	1.87 ± 0.15	98
Prostaglandin A_1	1.68 ± 0.30	88

* Means of duplicate determinations ± deviation.

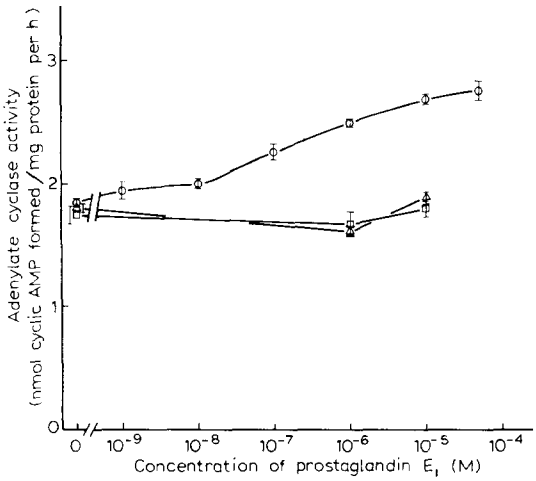

Fig. 1. Comparison of the prostaglandin E_1-mediated activation of adenylate cyclase of plasma membranes from liver and ascites hepatomas. Plasma membranes prepared from rat liver and ascites hepatomas AH-130 and AH-7974 were incubated at 37°C for 15 min with the standard reaction mixture containing prostaglandin E_1 at various concentrations for assaying the adenylate cyclase activity as described in Materials and Methods. The abscissa indicates the concentrations of prostaglandin E_1 added to reaction mixture and the ordinate indicates the adenylate cyclase activity. Each point in the figure is a mean of two experiments, each with duplicate determinations with ± average deviation. ○, liver plasma membranes; △, AH-130 plasma membranes; □, AH-7974 plasma membranes.

glandin E_2, stimulated the adenylate cyclase of liver plasma membranes at 10^{-5} M. The other prostaglandins such as prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$ and A_1 showed no stimulation when they were added at the same concentration (Table I).

(2) Effect of prostaglandin E_1 at various concentrations on adenylate cyclase of plasma membranes from rat liver and ascites hepatomas

As shown in Fig. 1, the adenylate cyclase activity of plasma membranes from rat liver was stimulated by prostaglandin E_1 dose-dependently, while that of plasma membranes from AH-130 or AH-7974 was not stimulated to any significant extent by prostaglandin E_1 even at concentrations as high as 10^{-5} M (Fig. 1).

(3) Effect of GTP on the prostaglandin-mediated activation of adenylate cyclase of plasma membranes from liver and ascites hepatomas

As shown in Fig. 2a, prostaglandin E_1 did not activate the adenylate cyclase of liver plasma membranes when the ATP concentration was reduced to 0.3 mM from 3.0 mM. Addition of GTP at 10^{-5} M restored the prostaglandin E_1-dependent activation of adenylate cyclase. In contrast, prostaglandin $F_{2\alpha}$ was unable to activate the adenylate cyclase of liver membranes not only in the absence of GTP but also in the presence of it. Fig. 2b shows that the adenylate cyclase of hepatoma plasma membranes was not activated by prostaglandin E_1 even in the presence of 10^{-5} M GTP. It should be noted that the basal adenylate cyclase activity as well as a slight activation of it by GTP alone were similar among liver and hepatoma plasma membranes (Fig. 2a,b).

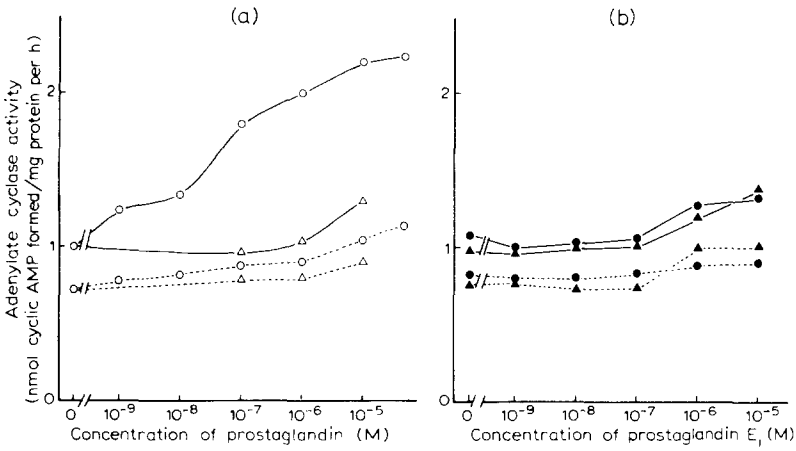

Fig. 2. Effect of GTP on prostaglandin-mediated activation of adenylate cyclase of plasma membranes from liver and ascites hepatomas. Plasma membranes (equivalent to 0.8—1.1 mg protein) from (a) liver and (b) hepatomas such as AH-130 and AH-7974 were incubated at 37°C for 15 min with 1 ml of the reaction mixture containing 0.3 mM ATP and prostaglandin E_1 or $F_{2\alpha}$ of various concentrations in the presence of the absence of 10^{-5} M GTP. Solid lines correspond to the presence of 10^{-5} M GTP and dotted lines correspond to the absence of it. ○ and △ in (a) indicate prostaglandin E_1 and $F_{2\alpha}$, respectively. ● and ▲ in (b) indicate plasma membranes of AH-130 and AH-7974, respectively. Each point in the figures is a mean of duplicate determinations.

(4) Kinetics of the binding of [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin $F_{2\alpha}$ to plasma membranes from rat liver and the effect of GTP upon it

As shown in Fig. 3a and b, amounts of these prostaglandins bound to the plasma membranes increased with time during the first 20 min of incubation, but then levelled off, reaching equilibrium by 30 min after the start of incu-

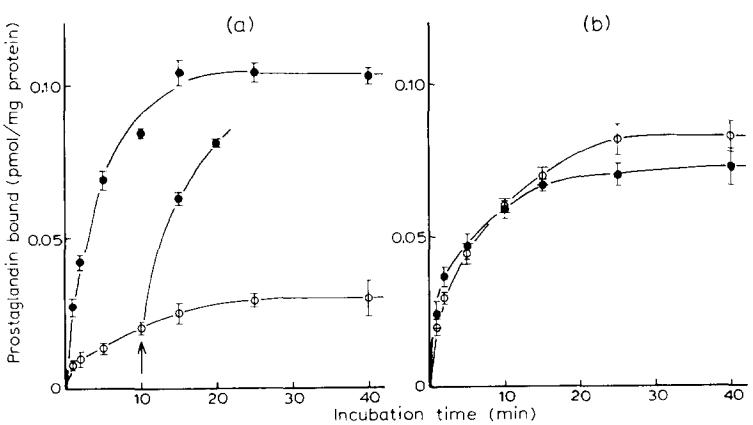

Fig. 3. Effect of GTP on the kinetics of binding of [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin $F_{2\alpha}$ to plasma membranes of rat liver. Plasma membranes equivalent to 75 μg protein were incubated with 80 μl of 20 mM Tris · HCl buffer (pH 7.4) containing (a) [^{3}H]prostaglandin E_1 (87 Ci/mmol) at $1.1 \cdot 10^{-9}$ M or (b) [^{3}H]prostaglandin $F_{2\alpha}$ (9.7 Ci/mmol) at $5 \cdot 10^{-9}$ M, at 37°C for various intervals of time. In the case of investigating the effect of GTP upon the binding kinetics, GTP was added to the incubation mixture at $3.5 \cdot 10^{-4}$ M at the onset of the incubation or at 10 min (as indicated by an arrow in the (a). Each point is a mean of two experiments ± average deviation, each with a single determination. ○ and ● correspond to the absence and presence of GTP, respectively.

bation. The addition of GTP did not affect the general kinetic feature of the binding, but increased the level of [^{3}H]prostaglandin E_1 bound to the membranes considerably (3—4-fold) (Fig. 3a). On the other hand, the binding of [^{3}H]prostaglandin $F_{2\alpha}$ to the membranes was not affected by GTP (Fig. 3b).

(5) Specificity of the binding sites for prostaglandin E_1 and $F_{2\alpha}$ in liver plasma membranes

In order to examine the specificity of the binding sites for prostaglandin E_1 and $F_{2\alpha}$ in the liver membranes, binding of [^{3}H]prostaglandin E_1 or [^{3}H]prostaglandin $F_{2\alpha}$ to liver plasma membranes was assayed in the presence of prostaglandin E_1, E_2, $F_{2\alpha}$ and A_1 added simultaneously at various concentrations.

As shown in Fig. 4a, the binding of [^{3}H]prostaglandin E_1 was inhibited most sensitively by unlabeled prostaglandin E_1, followed by prostaglandin E_2, $F_{1\alpha}$, $F_{2\alpha}$ and A_1 in the order of decreasing effect. On the other hand, as shown in Fig. 4b, the binding of [^{3}H]prostaglandin $F_{2\alpha}$ was inhibited most sensitively by unlabelled prostaglandin $F_{2\alpha}$, followed by prostaglandin $F_{1\alpha}$, E_2, E_1, and A_1. It should be noted that the binding of [^{3}H]prostaglandin E_1 was inhibited biphasically by prostaglandin E_1 and E_2, but monophasically by the other prostaglandins, while the binding of [^{3}H]prostaglandin $F_{2\alpha}$ was inhibited biphasically by prostaglandin $F_{2\alpha}$ and $F_{1\alpha}$, but monophasically by the other prostaglandins. These results seem to suggest the specificity of each prostaglandin receptor, although the crossreaction with other prostaglandins may occur to some extents depending on the structural closeness (Fig. 4).

(6) Binding of [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin $F_{2\alpha}$ to plasma membranes of liver and hepatomas and the effect of GTP upon it

Fig. 5a shows the binding of [^{3}H]prostaglandin E_1 to plasma membranes of

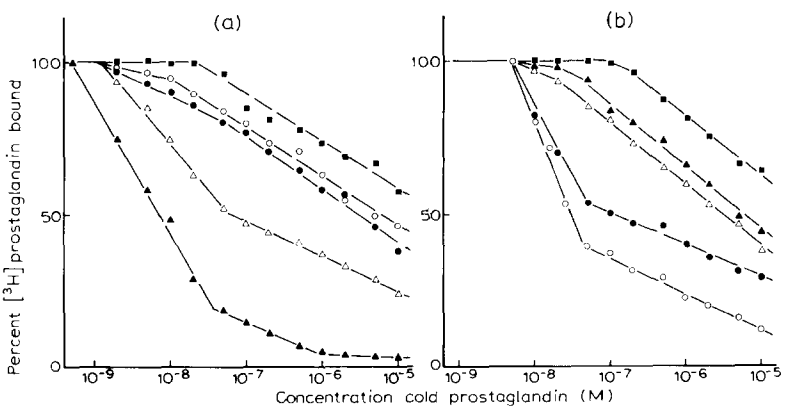

Fig. 4. Effects of unlabelled prostaglandins upon the binding of [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin $F_{2\alpha}$ to plasma membranes of rat liver. Plasma membranes equivalent to 75 μg protein were incubated with 80 μl of 20 mM Tris · HCl buffer (pH 7.4) containing (a) $5 \cdot 10^{-10}$ M [^{3}H]prostaglandin E_1 (87 Ci/mmol) or (b) $5 \cdot 10^{-9}$ M [^{3}H]prostaglandin $F_{2\alpha}$ (9.7 Ci/mmol) at 37°C for 40 min. To investigate the effects of various prostaglandins upon the binding of [^{3}H]prostaglandin E_1 or [^{3}H]prostaglandin $F_{2\alpha}$, prostaglandin E_1 (▲); E_2 (△); $F_{1\alpha}$ (●); $F_{2\alpha}$ (○) and A_1 (■) were added to the incubation mixtures at various concentrations (abscissa) simultaneously with the labelled E_1 or $F_{2\alpha}$. Each point is a mean of duplicate determinations.

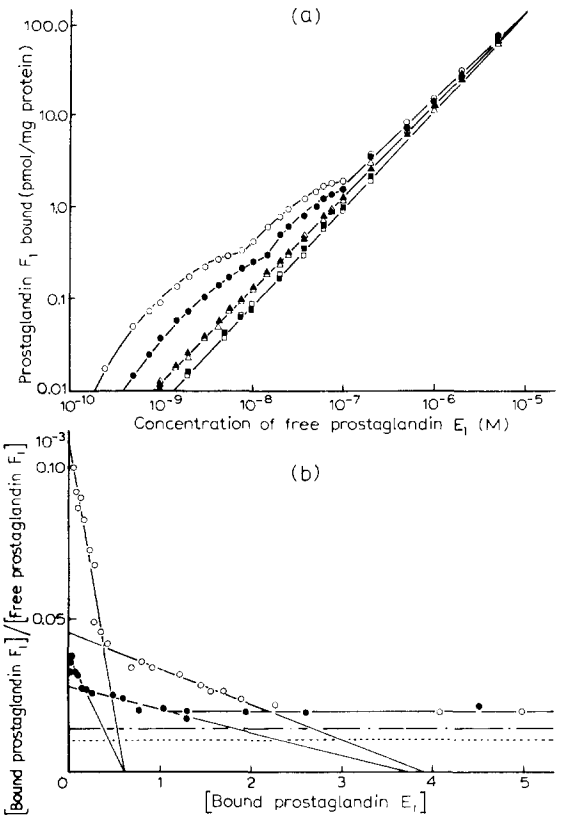

Fig. 5. Binding of prostaglandin E_1 at various concentrations to plasma membranes from liver and ascites hepatomas in the presence or the absence of $3.5 \cdot 10^{-4}$ M GTP. (a) Plasma membranes equivalent to 70—95 μg protein (liver, AH-7974 and AH-130) were incubated with 80 μl of 20 mM Tris · HCl buffer (pH 7.4) containing E_1 of various concentrations in the presence or absence of $3.5 \cdot 10^{-4}$ M GTP at 37°C for 40 min. Amounts of prostaglandin E_1 bound to plasma membranes (ordinate) and concentrations of free (unbound) prostaglandin E_1 in the incubation medium (abscissa) were measured as described in Materials and Methods. Throughout these experiments, the concentration of [^{3}H]prostaglandin E_1 (87 Ci/mmol) added to the incubation mixtures was kept constant ($5 \cdot 10^{-10}$ M) although the net concentration of prostaglandin E_1 was varied by adding unlabelled prostaglandin E_1. ●, ▲, and ■ correspond to plasma membranes of liver, AH-7974 and AH-130, respectively, in the absence of GTP, while ○, △, and □ correspond to plasma membranes of liver, AH-7974 and AH0130, respectively, in the presence of GTP. (b) The same data as shown in Fig. 5a were expressed according to the Scatchard plotting. Solid lines with ● and ○ symbols correspond to liver plasma membranes in the absence and the presence of GTP, respectively. The broken and dotted lines without symbols correspond to plasma membranes of AH-7974 and AH-130, respectively in both the presence and the absence of GTP.

liver and ascites hepatomas in the presence and the absence of GTP, and Fig. 5b shows the Scatchard plots of the [^{3}H]prostaglandin E_1 binding. Binding of [^{3}H]prostaglandin E_1 to liver plasma membranes appears to reach saturation at two different concentrations of [^{3}H]prostaglandin E_1; one at about 10^{-8} M and another at 10^{-7} M (Fig. 5a). Upon increasing the concentration of [^{3}H]prostaglandin E_1, the binding tends to increase without saturation. The Scatchard plots in Fig. 5b clearly indicate that the binding of [^{3}H]prostaglandin E_1 at concentrations higher than 10^{-7} M is of the nonspecific nature. Addition of GTP was found to sensitize the specific binding sites for prostaglandin E_1 with-

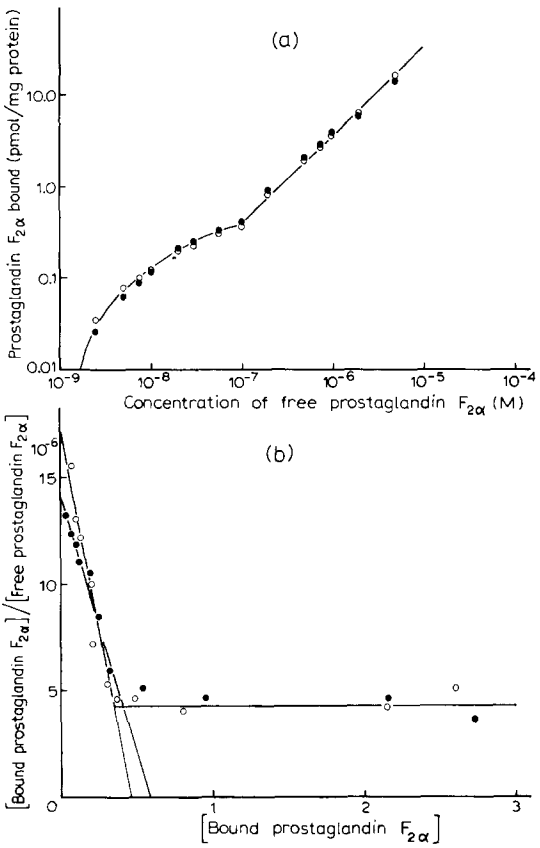

Fig. 6. Binding of prostaglandin $F_{2\alpha}$ to plasma membranes from liver. (a) Liver plasma membranes equivalent to 75 μg protein were incubated with 80 μl of 20 mM Tris · HCl buffer (pH 7.4) containing prostaglandin $F_{2\alpha}$ at various concentrations at 37°C for 40 min. Amounts of prostaglandin $F_{2\alpha}$ bound to the membranes (ordinate) and concentrations of free prostaglandin $F_{2\alpha}$ in the incubation medium (abscissa) were assayed as described in Materials and Methods. Throughout the experiments, the concentration of [^{3}H]prostaglandin $F_{2\alpha}$ (9.7 Ci/mmol) added to the incubation mixture was kept constant ($5 \cdot 10^{-9}$ M) although the net concentration of prostaglandin $F_{2\alpha}$ was varied by adding unlabelled prostaglandin $F_{2\alpha}$. • and ○ indicate the binding of prostaglandin $F_{2\alpha}$ in the absence of GTP and in the presence of $3.5 \cdot 10^{-4}$ M GTP, respectively. (b) The same data as in (a) were expressed according to the Scatchard plotting.

out affecting the nonspecific binding. In contrast to the liver, plasma membranes of ascites hepatomas appeared to lack the specific binding sites for [^{3}H]-prostaglandin E_1, showing only the nonspecific binding which was not affected by GTP.

As shown in Fig. 6, the binding of [^{3}H]prostaglandin $F_{2\alpha}$ to liver plasma membranes showed only one binding saturation at about 10^{-7} M (Fig. 6a). The Scatchard plots of the binding of [^{3}H]prostaglandin $F_{2\alpha}$ (Fig. 6b) showed the binding at concentrations higher than 10^{-7} M is of nonspecific nature. Contrary to prostaglandin E_1, the specific binding of [^{3}H]prostaglandin $F_{2\alpha}$ was not affected by GTP. Table II summarizes the apparent binding parameters of liver plasma membranes for prostaglandin E_1 and $F_{2\alpha}$; i.e. K_d and N. It seems clear that GTP increases the affinity of the specific binding sites (both higher and

TABLE II

APPARENT BINDING PARAMETERS OF LIVER PLASMA MEMBRANES FOR [^{3}H]PROSTAGLANDIN E_1 AND [^{3}H]PROSTAGLANDIN $F_{2\alpha}$

The binding of [^{3}H]prostaglandin E_1 and [^{3}H]prostaglandin $F_{2\alpha}$ was assayed in the same way as described in the legend to Fig. 5. K_d apparent dissociation constants for the prostaglandin-ligand complexes; N, number of specific binding sites. The numerical figures in this table are means ± average deviation from 2—4 experiments (the number of experiments is shown in parentheses).

Labelled prostaglandin	Higher affinity sites		Lower affinity sites	
	K_d (10^{-9} M)	N (pmol/mg protein)	K_d (10^{-8} M)	N (pmol/mg protein)
[^{3}H]Prostaglandin E_1 without GTP	17.6 ± 1.3 (4)	0.68 ± 0.05 (4)	13.6 ± 0.4 (3)	3.66 ± 0.18 (3)
[^{3}H]Prostaglandin E_1 with $3.5 \cdot 10^{-4}$ M GTP	6.25 ± 0.40 (4)	0.67 ± 0.03 (4)	7.39 ± 0.70 (3)	3.63 ± 0.20 (3)
[^{3}H]Prostaglandin $F_{2\alpha}$ without GTP			2.31 ± 0.06 (2)	0.41 ± 0.01 (2)
[^{3}H]Prostaglandin $F_{2\alpha}$ with $3.5 \cdot 10^{-4}$ M GTP			3.53 ± 0.50 (2)	0.50 ± 0.07 (2)

lower affinity sites) to prostaglandin E_1 without altering the maximal levels of the binding sites ("sensitization").

(7) Comparison of the sensitization effects of GTP and ATP on the binding of [^{3}H]prostaglandin E_1 to liver plasma membranes

As shown in Fig. 7, the binding of [^{3}H]prostaglandin E_1 at a very low concentration to liver plasma membranes increased in a sigmoidal fashion as the concentration of GTP or ATP was increased. GTP seemed to be a more sensi-

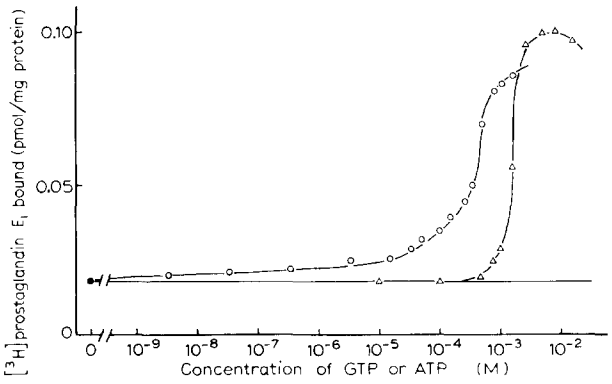

Fig. 7. Enhancement of the binding of [^{3}H]prostaglandin E_1 to liver plasma membranes by GTP and ATP at various concentrations. Liver plasma membranes (90 μg protein) were incubated with [^{3}H]prostaglandin E_1 (87 Ci/mmol; $5 \cdot 10^{-10}$ M) in 80 μl of Tris · HCl buffer (pH 7.4) containing ATP or GTP at various concentrations. Amounts of [^{3}H]prostaglandin E_1 bound to the membranes were measured as described in Materials and Methods. ○ and △ correspond to the presence of GTP and ATP, respectively. Each point is a mean from duplicate determinations in the case of added ATP or a mean from triplicate determinations in the case of added GTP.

TABLE III

EFFECTS OF VARIOUS NUCLEOTIDES ON THE BINDING OF [^{3}H]PROSTAGLANDIN E_1 TO LIVER PLASMA MEMBRANES AND THE PROSTAGLANDIN E_1-MEDIATED ACTIVATION OF ADENYLATE CYCLASE OF LIVER MEMBRANES

Binding of [^{3}H]prostaglandin E_1 ($5 \cdot 10^{-10}$ M) to liver plasma membranes was assayed under the same conditions as described in Fig. 7. Nucleotides were added to the incubation mixtures at $2.5 \cdot 10^{-4}$ M (A) and $1.5 \cdot 10^{-3}$ M (B). Adenylate cyclase was assayed under the same conditions as described in Materials and Methods except that the concentration of ATP was reduced to 0.3 mM, using plasma membranes equivalent to 1.2 mg protein. Nucleotides were added to the reaction mixtures containing 10^{-5} M prostaglandin E_1 only at $2.5 \cdot 10^{-4}$ M (A).

Nucleotides	[^{3}H]prostaglandin E_1 bound (A)		[^{3}H]prostaglandin E_1 bound (B)		Adenylate cyclase (A)	
	(pmol/mg protein)	(% stimulation)	(pmol/mg protein)	(% stimulation)	(nmol cyclic AMP/mg protein per h)	(% stimulation)
None	0.019	—	0.019	—	0.74	—
GTP	0.045	136	0.085	347	2.50	237
GDP	0.037	95	0.048	153	1.86	151
GMP	0.024	26	0.017	−10	1.05	42
cGMP	0.022	51	0.022	15	1.14	54
dGTP	0.042	121	0.061	221	2.22	200
GMP-P(N)P	0.040	110	0.055	189	2.42	230
ATP	0.025	32	0.056	194	—	—
CTP	0.025	32	0.042	121	0.93	25
UTP	0.022	15	0.027	42	0.87	17
dTTP	0.023	21	0.023	21	0.81	9

tive effector than ATP, although the maximal stimulation of the binding of [^{3}H]prostaglandin E_1 to the membranes was almost similar. A half maximal stimulation was observed at $3.5 \cdot 10^{-4}$ M GTP or at $1.5 \cdot 10^{-3}$ M ATP, which are nearly the physiological concentrations of these nucleotides (Fig. 7).

(8) Comparison of the effects of various nucleotides upon the binding of [^{3}H]-prostaglandin E_1 to liver plasma membranes and the prostaglandin E_1-mediated activation of adenylate cyclase

Table III summarizes the effects of various nucleotides on the binding of [^{3}H]prostaglandin E_1 to liver plasma membranes as well as on the prostaglandin E_1-mediated activation of adenylate cyclase of liver plasma membranes. It is interesting that GMP-P(N)P can stimulate not only the binding of [^{3}H]prostaglandin E_1 but also the prostaglandin E_1-mediated activation of adenylate cyclase similarly to GTP. Similar stimulatory effect was also observed with dGTP and GDP, but not with the other nucleotides tested.

(9) Effects of GTP, prostaglandin E_1 and GTP plus prostaglandin E_1 upon the kinetic parameters of adenylate cyclase of liver plasma membranes

Fig. 8 shows the initial velocity of adenylate cyclase reaction as a function of the substrate ATP concentration in the presence of GTP alone, prostaglandin E_1 alone, GTP plus prostaglandin E_1 and none of these effectors. The kinetic parameters such as K_m and V were obtained from the double reciprocal plots (velocity^{-1} vs. [ATP]$^{-1}$) according to Lineweaver and Burk, and are summarized in Table IV. It seems clear that the stimulatory effect of GTP alone is due to a partial increase of V. On the other hand, the addition of GTP plus prosta-

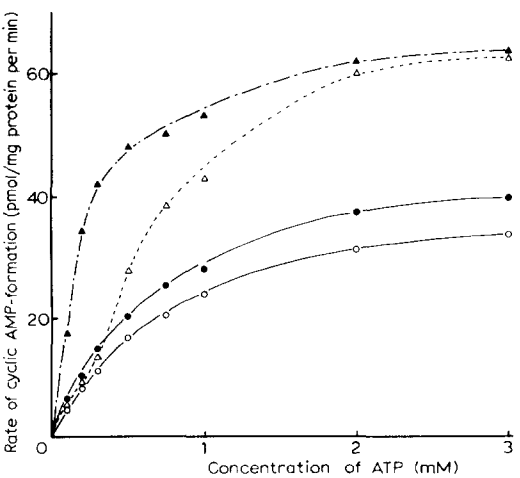

Fig. 8. Effects of GTP, prostaglandin E_1, and GTP plus prostaglandin E_1 upon the kinetic parameters of adenylate cyclase of liver plasma membranes. Liver plasma membranes equivalent to 0.7 mg protein were incubated at 37°C for 10 min in the standard reaction mixture containing ATP at various concentrations. GTP and/or prostaglandin E_1 were added at 10^{-4} M and $4 \cdot 10^{-6}$ M, respectively. ○, ●, △, and ▲ indicate the addition of none, GTP, prostaglandin E_1 and GTP plus prostaglandin E_1, respectively. Each point in the figure is a mean of triplicate determinations.

TABLE IV

KINETIC PARAMETERS OF ADENYLATE CYCLASE OF LIVER PLASMA MEMBRANES IN THE PRESENCE OF GTP, PROSTAGLANDIN E_1 AND GTP PLUS PROSTAGLANDIN E_1

The reaction conditions are same as described in Fig. 8. Kinetic parameters were obtained graphically from the Lineweaver-Burk plots (V^{-1} vs. $[ATP]^{-1}$).

Addition	K_m (mM)	V (pmol cyclic AMP/mg protein per h)
None	0.72 ± 0.03 *	40.1 ± 1.1
GTP (10^{-4} M)	0.73 ± 0.03	53.2 ± 2.1
Prostaglandin E_1 ($4 \cdot 10^{-6}$ M)	—	(75.5 ± 2.8)
GTP plus prostaglandin E_1	0.29 ± 0.01	75.5 ± 3.0

* Mean ± average deviation (3 determinations).

glandin E_1 increased V to a further extent and moreover considerably reduced K_m. It seemed rather difficult to read the effect of prostaglandin E_1 alone because ATP at higher concentrations can function as an effector similarly to GTP. However, judging from the kinetic behavior under the lower concentrations of ATP, prostaglandin E_1 alone does not seem to affect the kinetic parameters of andenylate cyclase. As the concentration of ATP increases, prostaglandin E_1 alone seemed to increase the V as similarly as GTP plus prostaglandin E_1.

Discussion

In the present study, we have shown that plasma membranes of rat liver contains two specific binding sites for prostaglandin E_1 and one specific binding site for prostaglandin $F_{2\alpha}$ (Table II, Figs. 5 and 6). Among these binding sites, only the prostaglandin E_1-binding sites were found to be involved in the adenylate cyclase activation (Table I, Fig. 2). In parallel to the biphasic binding curves for prostaglandin E_1 (Fig. 5), the activation of adenylate cyclase by prostaglandin E_1 was also found to occur biphasically (Fig. 1 and Fig. 2a), suggesting that both the higher and the lower affinity binding sites may be responsible for the prostaglandin E_1-mediated activation of adenylate cyclase.

In contrast to the liver, plasma membranes from the ascites hepatomas were shown to be devoid of the specific binding sites for prostaglandin E_1 (Fig. 5). This fact may explain why the adenylate cyclase activation by prostaglandin E_1 can not be observed with the hepatoma plasma membranes (Figs. 1 and 2). As shown in the present paper as well as in the earlier papers [34—36], the basal adenylate cyclase activity and its activation by GTP alone or NaF are almost similar among the liver and hepatoma plasma membranes.

In the present study, the role of GTP (or ATP at higher concentrations) in the interacting system of prostaglandin E_1-receptor and adenylate cyclase has been investigated rather extensively. The increased binding of prostaglandin E_1 to the receptors in liver plasma membrane in the presence of GTP was shown to be ascribed to the increase in the affinity of receptors to prostaglandin E_1 (reduction of K_d) (Fig. 5, Table II), suggesting that GTP may exert its effect on the receptor moiety. On the other hand, GTP added alone was shown to increase the V of adenylate cyclase to a certain extent without altering K_m, while GTP

plus prostaglandin E_1 was found to affect both V and K_m: reducing K_m and at the same time increasing V to a further extent (Fig. 8, Table IV), suggesting that GTP may exert its effect on the adenylate cyclase moiety as well, especially in the presence of prostaglandin E_1. These findings seem to indicate that the coherently interacting system of receptor and adenylate cyclase may be established only in the presence of both hormone and GTP, thus exhibiting the maximal activation of adenylate cyclase. The action point of GTP has not yet been elucidated in the present study. Possibilities exist that GTP may be bound to the receptor moiety, to the cyclase moiety, to both the receptor and cyclase moieties, or to another hypothetical component, "coupling factor", which may link the receptor and the cyclase. Since GMP-P(N)P shows the same effect with GTP, the action mechanism of these nucleotides may not involve phosphorylation of some key components in the interacting system or decomposition of the nucleotides, suggesting an allosteric effect as the most plausible mechanism of action.

In the present study, rat liver plasma membranes were shown to contain only one specific binding site for prostaglandin $F_{2\alpha}$. The prostaglandin $F_{2\alpha}$-binding sites were, however, found to be irrelevant to the activation of adenylate cyclase. Moreover, GTP did not affect the binding of prostaglandin $F_{2\alpha}$ to the membranes. The biological role of the prostaglandin $F_{2\alpha}$-binding sites remains to be clarified. In the earlier papers [36,37], we have reported the presence of specific binding sites for the natural glucocorticoids in rat liver plasma membranes and their reduction in the hepatoma plasma membranes. Neither adenylate cyclase nor cyclic AMP phosphodiesterase of liver plasma membranes were activated by cortisol.

When we compare the results obtained in the present paper with those obtained for the epinephrine receptor-adenylate cyclase system as reported in the preceding paper, [34—36], the following two schemes may be presented for the insensitivity of tumor cells to the hormonal controls; one may be due to the lack of receptor itself as we have seen in the case of prostaglandin E_1, and another may be due the impaired coupling mechanism between receptor and cyclase as we have seen in the case of epinephrine. It is interesting that GTP or ATP at physiological concentrations can modulate the hormone receptor-adenylate cyclase system in the liver plasma membranes, but not in the hepatoma ones.

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