THE STIMULATION OF RAT LIVER ADENYLATE CYCLASE BY PROSTAGLANDINS E_1 AND E_2*

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SUMMARY: Adenylate cyclase associated with particulate fractions from rat liver is stimulated by prostaglandin E_1 (PGE_1)[1] and prostaglandin E_2 (PGE_2), but not by prostaglandins $F_{1\alpha}$ or $F_{2\alpha}$. Significant stimulation by prostaglandins is dependent on the presence of GTP using substrate ATP concentrations from 0.2 to 2.0 mM. Guanosine-5'-(ß,γ-imino)-triphosphate (GMPPNP) stimulated adenylate cyclase itself, but PGE_1 did not stimulate in the presence of optimal or suboptimal concentrations of this GTP analog. In contrast, glucagon caused additional stimulation when optimal concentrations of GMPPNP were included.

The diverse physiological effects of prostaglandins may depend on the influence these ubiquitous compounds have on cyclic nucleotide metabolism (1). Earlier studies showed prostaglandins of the E series to inhibit the stimulation of adipocyte adenylate cyclase by hormones (2). Contrary to the case with adipocytes, subsequent investigations have demonstrated that prostaglandins act as stimulators of adenylate cyclase from many other tissues (1,3).

Our studies showed that stimulation of rat liver adenylate cyclase by prostaglandins is dependent on GTP and that optimal concentrations of PGE_1 plus GTP stimulate rat liver adenylate cyclase more than glucagon alone but less than glucagon plus GTP (4). The dependency on GTP for prostaglandin stimulation has since been observed with adenylate cyclase from pancreas (5), thyroid (6), and kidney (7). The results reported

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[1]Abbreviations: Cyclic AMP, adenosine-3',5'-monophosphate; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; GMPPNP, guanosine-5'-(ß,γ-imino)-triphosphate.

in this paper show that PGE_1 does not stimulate liver adenylate cyclase in the presence of the nucleotide analog GMPPNP; whereas, glucagon causes additional stimulation in the presence of GMPPNP.

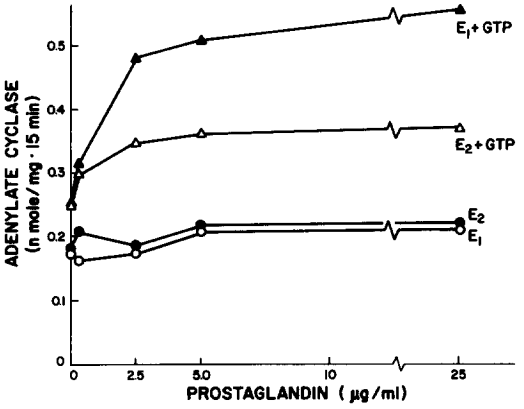
MATERIALS AND METHODS

Rats used in these experiments were albino males (200 to 250 g) from the Holtzman Co., Madison, WI. Prostaglandins were donated by Dr. John Pike, The Upjohn Co., Kalamazoo, MI. Radioisotopes were purchases from New England Nuclear and were used as received. Unlabeled ATP, GTP, cyclic AMP, phosphoenolpyruvate, pyruvate kinase (Type II), glucagon, and other reagents were obtained from Sigma Chemical Co., St. Louis, MO. GMP-PNP was purchased from International Chemical and Nuclear Corporation, Irvine, CA.

Plasma membrane enriched fractions were prepared by centrifugation of rat liver homogenates 1200 x g as described elsewhere (8). Adenylate cyclase incubations contained the following in a total volume of 0.1 ml: 50 to 100 µg of the plasma membrane enriched fraction, 22 mM Tris-HCl, pH 7.65; 10 mM $MgCl_2$, 0.6 mM theophylline; 0.15 mg/ml bovine serum albumin; 9 mM phosphoenolpyruvate; 35 units/ml pyruvate kinase; 1 mM disodium cyclic AMP; 5 mM KCl; 22 mM ammonium sulfate, added with pyruvate kinase; 0.25 mM dithioerythritol added with enzyme; 1.0 mM ATP; and [α-^{32}P] ATP (2-4 x 10^6 cpm). Incubations were for 15 min at 37° and were terminated by immersing the samples in boiling water for 2 min. Tracer amounts of [^{3}H] cyclic AMP were added to monitor the recovery of biosynthetic [α-^{32}P] cyclic AMP which was isolated by sequential chromatography of the samples of Dowex 50W-X4 (Biorad Laboratories) and alumina columns as described elsewhere (9). Cyclic AMP recoveries were 70 to 80 percent using this assay procedure. When enzyme was omitted from incubations, levels of [^{32}P] radioactivity recovered as [^{32}P] cyclic AMP were 80-100 cpm using 2-4 x 10^6 cpm of substrate. Protein was measured by the method of Lowry et al. (17)

RESULTS

Stimulation of liver adenylate cyclase is seen with PGE_1 and PGE_2 concentrations of 0.25 to 0.4 µg/ml (1.13 µM), and is optimal with concentrations of 5 µg/ml (14 µM) (Fig. 1). PGE_1 causes greater stimulation than PGE_2, but the concentration of each prostaglandin causing optimal stimulation is the same. As noted in Figure 1, significant stimulation by PGE_1 or PGE_2 is dependent upon the inclusion of GTP. With about half of the enzyme preparations tested, PGE_1 alone caused 10 to 20% inhibition of basal activity. With the other preparations, PGE_1 alone stimulated basal activity 10 to 20%. The factors responsible for this variation between enzyme preparations were not determined, but PGE_1 and PGE_2 always stimulated when GTP was included in incubations.



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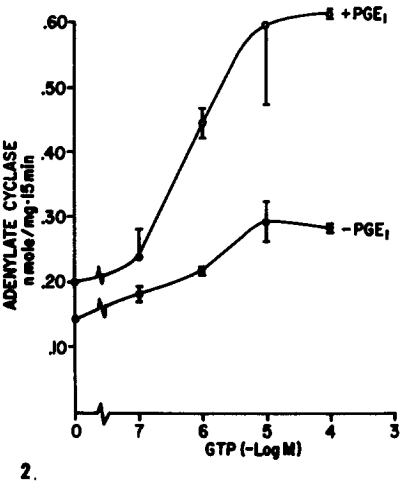

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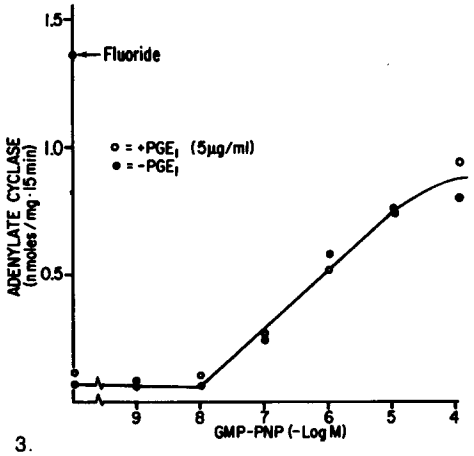
Figure 1. Dose response for PGE_1 and PGE_2 stimulations of liver adenylate cyclase. Besides the incubation components described in Methods, each sample contained PGE_1 or PGE_2 at the concentration indicated, with or without GTP = 10^{-4} and 0.5 to 0.7 mg/ml of protein. The values given are the means of duplicate determinations performed on four separate preparations of plasma membrane enriched fraction prepared as described in Methods.

Figure 2. Dose response for GTP stimulation of liver adenylate cyclase with and without PGE_1. Samples contained GTP at the concentrations noted with (+) or without (-) PGE_1 = 25 µg/ml and plasma membrane enriched fraction (0.5 to 0.7 mg/ml). Values are the mean of duplicate determinations performed on four separate liver preparations with the range indicated.

Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ had no effect on adenylate cyclase activity with or without the inclusion of GTP. Prostaglandin $F_{1\alpha}$ in concentrations of 5 to 50 µg/ml did not affect the stimulation caused by 5 µg/ml of PGE_1. The beta-adrenergic blocker propranolol did not influence stimulation by PGE_1, and there was no change in stimulations by PGE_1 when enzyme from adrenalectomized rats was used.

Figure 2 shows the effect of different GTP concentrations in the absence and presence of PGE_1. GTP alone caused less than 1-fold increase in basal activity. When PGE_1 was included, GTP stimulated basal activity 3- to 5-fold. The lowest concentrations of GTP (10^{-7} M) causing significant stimulation and the concentration (10^{-5} M) causing optimal stimulation were the same in the presence and absence of PGE_1.



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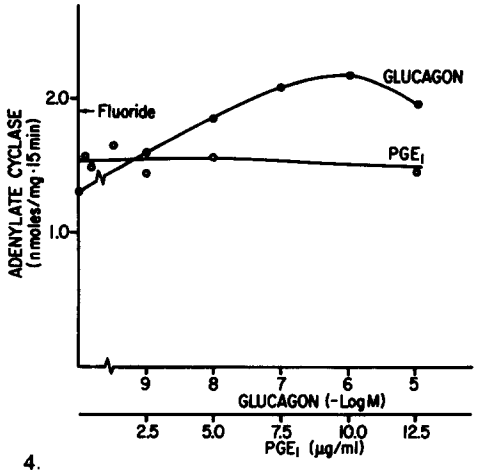

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Figure 3. GMPPNP dose curves measured with and without PGE_1. Samples contained the indicated concentration of GMPPNP with (O) and without (●) PGE_1 = 25 µg/ml. Plasma membrane enriched fraction (0.65 mg/ml) was used as an enzyme source. Values given are the mean of duplicate determinations. The specific activity measured in the presence of 10 mM fluoride is indicated.

Figure 4. Glucagon and PGE_1 effects on liver adenylate cyclase in the presence of saturating GMPPNP. Plasma membrane enriched fraction (0.85 gm/ml) was used as an enzyme source. Samples contained GMPPNP (10^{-4} M) and the concentrations of glucagon or PGE_1 shown. Values given are the mean of duplicate determinations on two different preparations. Activity measured with 10 mM fluoride is indicated on the ordinate.

Xanthosine-5'-triphosphate and inosine-5'-triphosphate duplicated the effect of GTP seen in Figure 2; however, 10- to 20-fold higher concentrations of these nucleotides were required for comparable stimulations.

Figure 3 shows the result of replacing GTP with its relatively inert analog GMPPNP. In contrast to GTP, activities measured with different GMPPNP concentrations are not increased by the addition of PGE_1; however, GMPPNP stimulated adenylate cyclase to 64% of the activity expressed with 10 mM fluoride.

Figure 4 compares the effects of additions of PGE_1 and glucagon to samples also containing 10^{-4} M GMPPNP. Glucagon caused stimulation above that measured with optimal GMPPNP, but PGE_1 did not. The activity measured with the combination of optimal glucagon and GMPPNP exceeded that caused by 10 mM fluoride.

DISCUSSION

The *in vitro* stimulation of rat liver adenylate cyclase by prostaglandin E_1 has been observed in several laboratories (4, 10, 11, 12), and we have also reported this with liver enzyme from several other species (4).

Concentrations of prostaglandins stimulating liver adenylate cyclase (14 μM) *in vitro* (Fig. 1) are close to those causing optimal stimulation of adenylate cyclases from other tissues. Optimal PGE_1 concentrations with enzyme from pancreas were about 10 μM (5); from fibroblasts, 22.4 μM (7); and from platelets, 2 μM (13). Since the point at which prostaglandins act upon adenylate cyclase may be intra- and/or extracellular, the significance of the relatively high concentrations required for *in vitro* stimulation is uncertain. As observed with enzyme from other tissues, liver adenylate cyclase is also stimulated specifically by E and not by F prostaglandins (5, 7).

Prostaglandin stimulation is dependent on GTP with adenylate cyclases from several tissues, and this is demonstrated with liver adenylate cyclase by Figures 1 and 2. PGE_1 stimulation of kidney adenylate cyclase required GTP when crude washed membranes were used, but not when whole homogenates were used (7). Stimulation of pancreatic adenylate cyclase by PGE_1 required GTP over a wide range of ATP substrate concentrations (5). PGE_1 failed to stimulate adenylate cyclase of purified membranes from thyroid unless GTP was added, but no dependence on GTP was observed using less pure preparations (6). These observations show that stimulation of at least several adenylate cyclases by PGE_1 requires GTP.

The cooperative stimulation observed with GTP and PGE_1 was investigated further by using the GTP analog GMPPNP which is more resistant to hydrolysis than GTP. Our results parallel those reported by others showing that GMPPNP itself is a potent stimulator of adenylate cyclase (14), and GMPPNP alone caused stimulation of from 60 to 70% of that measured with optimal concentrations of fluoride. Glucagon added to samples with optimal concentrations of GMPPNP caused further enzyme stimulation, but PGE_1

did not cause stimulation greater than that caused by GMPPNP alone (Fig. 4).

Guanyl nucleotides stimulate liver adenylate cyclase by binding to relatively specific sites, and nucleotide binding also influences subsequent interactions of the enzyme system with hormones (15). The role of substrate ATP at the nucleotide binding site in situ is uncertain, but many hormone stimulations are measurable in vitro without the addition of guanyl nucleotides. In contrast, prostaglandin stimulation of liver adenylate cyclase was insignificant using substrate ATP concentrations from 0.2 to 2.0 mM unless GTP was added.

GTP taken up at specific binding sites is hydrolyzed to GDP which may act as an inhibitor of enzyme activity; whereas, GMPPNP does not undergo hydrolysis at the specific binding sites (16). Thus, PGE_1 may modify stimulation caused by GTP by inhibiting its hydrolysis. With this hypothesis, PGE_1 would predictably not stimulate adenylate cyclase in the presence of GMPPNP because of the inherent stability of this nucleotide.

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