THE STIMULATION OF HEPATIC ADENYLATE CYCLASE BY PROSTAGLANDIN E_1

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SUMMARY

Adenylate cyclase activity associated with particulate preparations from rat, mouse, rabbit, and dog liver is stimulated 2- to 5-fold by prostaglandin E_1 (PGE_1). This stimulation is dependent upon the presence of guanosine-5'-triphosphate (GTP). Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ do not alter the enzymatic activity under these same conditions. Optimal concentrations of PGE_1 + GTP stimulate rat liver adenylate cyclase more than glucagon alone, but less than glucagon + GTP. Activity measured with glucagon + GTP is not affected by addition of PGE_1. Stimulation from PGE_1 + GTP is increased by glucagon to the same level measured with glucagon + GTP.

In liver, the glycogenolytic effects of glucagon and catecholamines depend on their ability to activate adenylate cyclase and thus increase cyclic AMP concentrations (1). The consequence of hormone action in many organs can also be reproduced by prostaglandins, and it has been established that prostaglandins influence cyclic AMP accumulation (2,3). Many of the prostaglandin studies have used intact tissue preparations, precluding assignment of the site of prostaglandin action. Furthermore, a prostaglandin effect on liver adenylate cyclase has not been described previously.

In this paper, we show the stimulation of adenylate cyclase in liver broken-cell homogenates by PGE_1, the dependence of this activation on purine nucleotides, and the comparison of prostaglandin activation to that measured in the presence of glucagon.

MATERIALS AND METHODS

Pyruvate kinase (Type II), phospho-enol-pyruvate (trisodium salt), ATP (disodium salt), cyclic AMP (sodium salt), GTP (Type I), dithioerythritol (DTE), and glucagon were purchased from Sigma Chemical Co. Prostaglandins were provided by Dr. John Pike, Upjohn Co., Kalamazoo, Michigan. Radioactive materials were obtained from New England Nuclear Corp., Dowex 50W-X4 from Bio-Rad, Alumina (neutral, activity I) from Merck. Rats (250-350 g) were purchased from Holtzman Co. Mice, rabbits, and mongrel dogs were obtained from the University of Utah Vivarium.

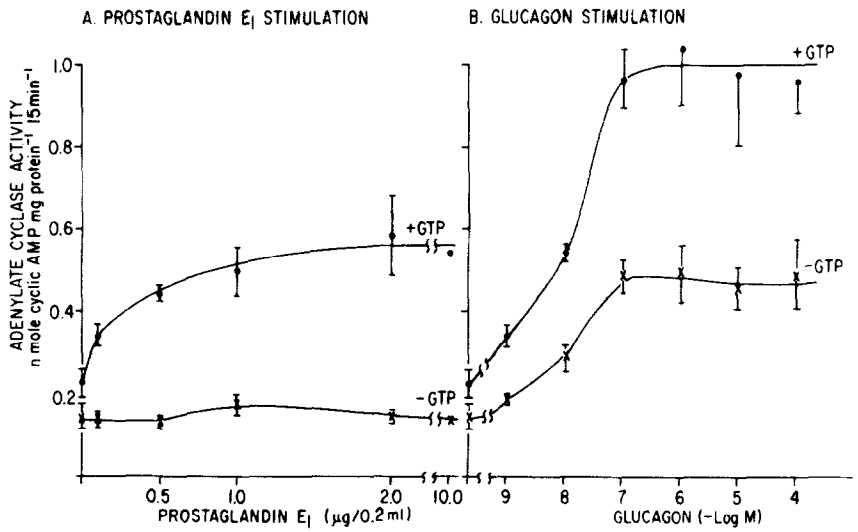

Fig. 1. Incubation and assay conditions are described in Methods. Protein concentrations varied from 150-300 µg per incubation sample. GTP, when present, equaled 10^{-4} M. Values given are the means of 4 measurements, glucagon; 8 measurements, PGE_1. Brackets indicate standard error of the mean (S.E.M.).

Rats and mice were anesthetized with diethyl ether, dogs and rabbits with phenobarbital. Livers were perfused with 0.9% saline to remove blood, excised, minced with scissors, and disrupted with a Potter-Elvehjem homogenizer in 10 volumes (per gm weight) of 0.01 M Tris-HCl pH 7.5 containing 1 mM DTE (buffer). Six strokes of a teflon pestle turning at 1,000 rpm were used to homogenize tissue. The homogenate was filtered through cheese cloth and then centrifuged at 1,200 x g for 15 min. Pellets were resuspended in 10 volumes of buffer (based on original liver weight), homogenized as above, and centrifuged at 1,200 x g for 10 min. A second suspension was made in 5 volumes of buffer which was centrifuged as above. The particulate preparation used in incubations was made by suspending these pellets in buffer.

Incubation. The incubation volume of 0.2 ml contained: 22 mM Tris-HCl, pH 7.5; 10 mM Mg^{++}; 0.6 mM theophylline; 0.03 mg bovine serum albumin; 9 mM phospho-enol-pyruvate; 7 units pyruvate kinase; 1 mM cyclic AMP; 5 mM KCl; 22 mM $(NH_4)_2SO_4$, added with pyruvate kinase; 0.25 mM DTE, added with enzyme; 0.5 mM ATP; and [α^{32}P]-ATP (2-4 x 10^6 CPM). Membrane protein in incubations was 0.75 to 1.5 mg/ml as determined by the procedure of Lowry et al. (4).

Incubations were for 15 min at 37° and were terminated by immersing the samples in boiling water for 3 min. Tracer amounts of [^{3}H] cyclic AMP were added to monitor cyclic AMP recovery.

Cyclic AMP assay. An initial chromatography was a modification of that described by Krishna et al. (5). The samples (final volume of 0.25 ml) were applied to 0.5 x 4 cm columns of Dowex 50W-X4 previously rinsed with 0.01 M Tris-HCl pH 7.6 (elution buffer). After the sample entered the Dowex, columns were washed with 4 ml elution buffer, and the effluent containing most of the ATP and ADP was discarded. Alumina columns (0.5 x 6 cm) were placed under the Dowex so that further effluent would flow onto the alumina. Four ml of elution buffer was used to elute the cyclic AMP from the Dowex. Effluent obtained from the alumina column during this step was discarded. The Dowex columns were removed, and elution of the cyclic AMP from the alumina column continued with 8 ml of elution buffer. The total 8 ml of effluent was collected in a scintillation counting vial. This solution was warmed and taken to dryness under an air stream. Scintillation solution (Bio-solv - Beckman, PPO, and dimethyl POPOP in toluene, plus 0.2 ml H_2O) was added, and radioactivity measured with a Packard tri-carb spectrometer. Blank levels of radioactivity were less than 100 cpm using 2-6 x 10^6 cpm of substrate.

RESULTS

Considerable variation in the action of prostaglandins in different species has been noted; therefore prostaglandin E_1 (PGE_1) activation of rat, mouse, rabbit, and dog liver adenylate cyclase was verified. Data given in Table I show that the liver adenylate cyclase from these animals responds to PGE_1, although there is some variation in the degree of stimulation. In all cases, stimulation by PGE_1 is dependent upon the presence of guanosine-5'-triphosphate (GTP), and stimulation by PGE_1 + GTP is comparable to that caused by glucagon alone but surpassed by stimulation with glucagon + GTP (Table I). Activation by fluoride ion, which is considered to cause near optimal enzyme activity, is also given in Table I. Under the assay conditions described, cyclic nucleotide phosphodiesterase has a negligible effect on the accumulation of cyclic AMP. Thus, it is concluded that the increases in cyclic AMP indicated in Table I are due to direct stimulation of adenylate cyclase.

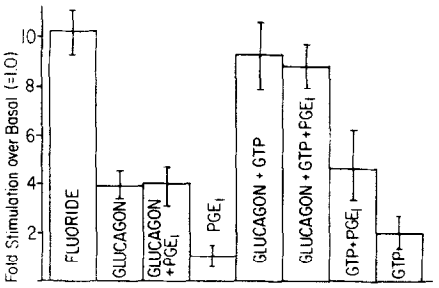

Fig. 2. Incubation and assay conditions are described in Methods. Components were present as indicated at the following concentrations: Fluoride = 10 mM, glucagon = 10^{-5} M, PGE_1 = 10 µg/ml, GTP = 10^{-4} M. Basal specific activity was 0.24 nmole cyclic AMP mg^{-1} protein 15 min^{-1}. Brackets indicate ± S.E.M. for six measurements.

Fig. 1A describes PGE_1 activation and its dependence on GTP in more detail, using the particulate fraction from rat liver. Threshold stimulation occurred with 0.5 µg/ml (1.4 µM) and optimal stimulation with 2.5 µg/ml (7.0 µM) of PGE_1. Concentrations of PGE_1 to 100 µg/ml gave the same stimulation as the 2.5 µg/ml concentration. In comparison, Krishna *et al.* have reported optimal stimulation of platelet membrane adenylate cyclase with 2 µM PGE_1 (6), and Wolff *et al.* have shown that the adenylate cyclase from thyroid is stimulated by 30 µM PGE_1 (7).

Fig. 1B shows glucagon stimulation measured using identical incubation conditions and the same particulate preparations with which the data for Fig. 1A was obtained. Comparison of Figures 1A and 1B shows that GTP + PGE_1 gives greater stimulation than glucagon but that glucagon + GTP stimulates more than PGE_1 + GTP. Glucagon is more potent with effects being observed with 0.01 µM concentrations as compared to 1.41 µM with PGE_1.

Further studies to determine any effect(s) of PGE_1 on the stimulation caused by glucagon are summarized in Fig. 2. Prostaglandin E_1 (10 µg/ml) does not alter the stimulation caused by glucagon or glucagon + GTP. However, inclusion of glucagon with optimal concentrations of PGE_1 increases the stimulation to that measured with glucagon (± GTP). Thus, the individual hormone stimulations are not additive, but glucagon will increase the activity measured in the presence of PGE_1.

DISCUSSION

The *in vitro* stimulation of liver adenylate cyclase by PGE_1 reported here

TABLE I

ADENYLATE CYCLASE ACTIVITY

Adenylate cyclase activity is expressed as nmole cyclic AMP biosynthesized mg protein^{-1} 15 min^{-1}. Fold increases over basal activity are given in parentheses. All determinations are in duplicate except for rat, which are the means of 20 measurements on nine animals (± S.E.M.). Preparation of particulate fractions used is described in Methods. Activity was measured within 60 min of enzyme preparations. The incubation volumes (0.2 ml) contained 150-300 µg (rat), 150 µg (mouse), 86 µg (dog), and 80 µg (rabbit) of protein. Components were present as indicated at the following concentrations: fluoride = 10 m$\underline{M}$, glucagon = $10^{-5}\underline{M}$, PGE_1 = 10 µg/ml, GTP = $10^{-4}\underline{M}$.

Animal	Basal	Fluoride	PGE_1	PGE_1 + GTP	GTP	Glucagon + GTP	Glucagon
Rat	0.168 (1.0) ± 0.036	1.535 (9.1) ± 0.349	0.179 (1.06) ± 0.026	0.688 (4.1) ± 0.088	0.354 (2.1) ± 0.108	1.283 (7.6) ± 0.324	0.745 (4.4) ± 0.159
Mouse	0.086 (1.0)	0.516 (6.0)	0.098 (1.1)	0.148 (1.7)	0.108 (1.3)	0.453 (5.3)	0.222 (2.6)
Dog	0.051 (1.0)	0.601 (11.8)	0.068 (1.3)	0.251 (4.9)	0.099 (1.9)	0.447 (8.8)	0.281 (5.5)
Rabbit	0.722 (1.0)	3.321 (4.6)	0.671 (0.93)	1.558 (2.2)	0.911 (1.3)	2.616 (3.6)	1.225 (1.7)

establishes the possibility that this hormone has some role in controlling glycogenolysis. Previous results have not suggested this possibility.

Examining rat liver, Curnow et al. reported that intravenous injection of PGE_1 caused decreases in glycogen synthetase (I) and increases in phosphophosphorylase activity (8). Both of these changes are compatible with increased cyclic AMP in liver. Although the above observations were interpreted in terms of PGE_1 mediated release of catecholamines from the adrenal (8), the possibility that PGE_1 has a more direct effect on hepatic glycogenolysis is supported by our data.

Exton et al. concluded that perfusion of PGE_1 in rat liver does not change cyclic AMP levels or alter glucose production, but detailed results were not presented (9). Others have reported increased glucose production caused by PGE_1 perfusion in rat liver (10). Similarities between glucagon and PGE_1 effects on cultured fetal liver cells are apparent in that both stimulate arylhydrocarbon hydroxylase (11), but PGE_1 suppressed induction of tyrosine aminotransferase by glucagon in adrenalectomized rats (12).

Binding of glucagon to liver plasma membranes has been related to adenylate cyclase activation (13), and specific binding of prostaglandins by purified liver plasma membranes has also been noted (14), supporting the possibility of an adenylate cyclase-prostaglandin interaction. In light of the above discrepancies, more data will be required before any direct in situ role of PGE_1 on liver glycogenolysis can be established.

The dependency of PGE_1 stimulation on GTP (Fig. 1A) is another uncertainty in relating these results to the action of PGE_1 in liver. Enhancement of in vitro hormone responses by GTP was first reported by Rodbell et al. (15), and similar effects of this nucleotide on hormone stimulation in other tissues has been observed. As reported by others (6,15), the observation of hormone-stimulation dependency on GTP requires low ATP concentrations in the incubation medium. In our studies (results not shown), the dependency of PGE_1 stimulation on GTP is observed with ATP concentrations from 0.1 to 2.0 mM. Furthermore, the requirement for GTP can be replaced by 10^{-4} M ITP or XTP. A similar lack of nucleotide specificity has been reported with prostaglandin stimulation of thyroid adenylate cyclase (7).

Prostaglandins also antagonize cyclic AMP increases caused by epinephrine in fat

cells (16) and inhibit hormonally induced responses in several other tissues (17). It was therefore of interest to examine effects of PGE_1 on glucagon-stimulated adenylate cyclase in liver. These results (Fig. 2) show that glucagon stimulation is unaffected by addition of PGE_1.

Prostaglandins cause increases or decreases in cyclic AMP levels in many tissues. However, studies using intact cells have not determined whether prostaglandins act by directly stimulating adenylate cyclase or by inhibiting cyclic AMP degrading phosphodiesterase. On the other hand, in vitro experiments have shown the direct stimulation of adenylate cyclase from thyroid (7), rat erythrocytes (18), platelets (6,19), cell culture preparations (20), corpus luteum (21), and in this paper, liver. Therefore, there is increasing evidence that prostaglandins may be general stimulators of adenylate cyclase in a number of tissues.

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REFERENCES

1. Robison, G.A., Butcher, R.W., and Sutherland, E.W. Cyclic AMP, Academic Press, New York, 1971.
2. Ramwell, P.W., and Shaw, J.E., Rec. Prog. Horm. Res., 26, 139 (1970).
3. Kuehl, F.A., Humes, J.L., Cirillo, V.J., and Ham, E.A., Advan. Cyclic Nucleotide Res., 1, 493 (1972).
4. Lowry, O.H., Rosebrogh, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
5. Krishna, G., Weiss, B., and Brodie, B.B., J. Pharmacol. Exp. Ther., 163, 379 (1968).
6. Krishna, G., Harwood, J.P., Barber, A.J., and Jamieson, G.A., J. Biol. Chem., 247, 2253 (1972).
7. Wolff, J., and Cook, G.H., J. Biol. Chem., 248, 350 (1973).
8. Curnow, R.T., and Nuttall, F.Q., J. Biol. Chem., 247, 1892 (1972).
9. Exton, J.H., Robison, G.A., Sutherland, E.W., and Park, C.R., J. Biol. Chem., 246, 6166 (1971).
10. Lemberg, A., Wilcinski, R., Izurieta, E.M., Halperin, H., Paglione, A.M., De Neuman, P., and Jauregui, H., Biochim. Biophys. Acta, 248, 198 (1971).
11. Gielan, J.E., and Nebert, D.W., J. Biol. Chem., 247, 7591 (1972).
12. Kajita, Y., and Hayaishi, Q., Biochim. Biophys. Acta, 261, 281 (1972).
13. Birnbaumer, L., and Pohl, S.L., J. Biol. Chem., 248, 2056 (1973).
14. Smigel, M., and Fleischer, S., Fed. Proc. Abstracts, 32, No. 1298 (1973).
15. Rodbell, M., Birnbaumer, L., Pohl, S.L., and Krans, H.M., J. Biol. Chem., 246, 1877 (1971).
16. Butcher, R.W., and Baird, C.E., J. Biol. Chem., 243, 1713 (1968).
17. Ramwell, P.W., and Rabinowitz, I., in Effects of Drugs on Cellular Control Mechanisms (eds. B.R. Rabin and R.B. Freedman), University Park Press, London, 1972.

18. Sheppard, H., and Burghardt, C.R., Molecular Pharmacol., 6, 425 (1970).
19. Robison, G.A., Arnold, A., and Hartmann, R.C., Pharmac. Res. Commun., 1, 325 (1969).
20. Makman, M.H., Proc. Nat. Acad. Sci. U.S.A., 68, 2127 (1971).
21. Marsh, J.M., Fed. Proc. Abstracts, 29, No. 803 (1970).