Vol. 82, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 14, 1978 Pages 879-886

PROSTAGLANDIN E_1 EFFECTS ON CYCLIC AMP AND GLYCOGEN METABOLISM IN RAT LIVER*

Floyd W. Sweat and Lindsey Yamashita

Department of Biochemistry, University of Utah
College of Medicine, Salt Lake City, Utah 84132

Received March 28,1978

SUMMARY: Prostaglandins E_1 or E_2 (PGE_1, PGE_2)[1] stimulated adenylate cyclase(s) from particulate fractions of whole liver homogenates 5- to 6-fold, but caused only slight (1.5- to 2-fold) stimulation of the enzyme from homogeneous hepatocytes. In contrast, glucagon stimulated enzyme from hepatocytes 12- to 15-fold and enzyme from whole liver 8- to 10-fold. Accordingly, most of the total prostaglandin-sensitive adenylate cyclase in cell suspensions was recovered in fractions containing non-parenchymal cells, and most of the total glucagon-sensitive activity was recovered with hepatocytes. PGE_1 did not change adenosine-3',5'-monophosphate (cyclic AMP) concentrations, or alter cyclic AMP increases caused by glucagon in hepatocytes. Glucagon consistently increased hepatocyte cyclic AMP concentrations and stimulated glycogenolysis by 35 to 40%. PGE_1 did not affect basal or glucagon-stimulated glycogenolysis in the intact cells.

We have shown that prostaglandins of the E series stimulate particulate adenylate cyclase(s) from livers of several species (1), and these results have been well substantiated (2-5). PGE_1 acts synergistically with guanosine-5'-triphosphate (GTP) to cause stimulation equal to that caused by glucagon, but less than that by glucagon + GTP (6). These findings furnish one of several criteria necessary to establish cyclic AMP as a second messenger for PGE_1 effects in liver. On the other hand, attempts to determine the action(s) of prostaglandins on hepatic carbohydrate metabolism have led to conflicting results: in some studies, PGE_1 increased glucose output from perfused livers (7,8), but contrary results have also been reported (9-11). PGE_1 decreased basal and

*This research was supported by National Institutes of Health Grant AM 18208.

[1]Abbreviations: Cyclic AMP, adenosine-3',5'-monophosphate; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2.

0006-291X/78/0823-0879$01.00/0

glucagon mediated glucose production in liver slices (12), but PGE_1 is also reported to increase cyclic AMP concentrations 5-fold in slices (13). PGE_1 has increased (3), had no effect on (9,10), and suppressed (14) increases in cyclic AMP caused by glucagon in perfused rat livers. PGE_1 has been reported to increase cyclic AMP concentrations and stimulate glycogenolysis in hepatocytes (15,16), with later conclusions by the same authors that PGE_1 has no significant effect on cyclic AMP concentrations in liver parenchymal cells (17).

Our earlier work indicated that a portion of prostaglandin-sensitive adenylate cyclase activity in whole liver homogenates does not originate from hepatocytes (18). Results presented here confirm and extend this conclusion by showing that most of the PGE_1-sensitive enzyme comes from non-parenchymal cells, and that exogenous PGE_1 has no significant effect on glycogenolysis or cyclic AMP metabolism in hepatocytes.

MATERIALS AND METHODS

Rats were mature (300-350 g) albino males from the Holtzman Co., Madison, WI. Prostaglandins were donated by the Upjohn Co., Kalamazoo, MI; prostaglandin homogeneity was routinely verified by thin-layer chromatography. Radioisotopes were purchased from New England Nuclear; other reagents and chemicals are from Sigma Chemical Co., St. Louis, MO.

Homogenates of whole liver, or of cell suspensions, were prepared as previously described in 0.01 M Tris-HCl buffer, pH 7.6, containing 1.0 mM dithioerythritol (18). Plasma membrane-enriched fractions were obtained by centrifuging homogenates 1200 x g for 15 min; pellets were homogenized in an equivalent volume of the original buffer and the mixture centrifuged again. This procedure was repeated twice; suspensions of the final precipitate were used in adenylate cyclase incubations (18). Adenylate cyclase was assayed by sequential Dowex and alumina chromatography using α-^{32}P-adenosine-5'-triphosphate as substrate (19).

Endogeneous concentrations of cyclic AMP were measured by competitive binding with protein kinase isolated from beef diaphragm (20). Standard curves were determined with solutions of recrystallized cyclic AMP which were carried through the same procedures as were the cell extracts. Protein concentrations were determined by the method of Lowry et al. (21).

Adenyl nucleotide pools were labeled by incubating hepatocytes (15 to 20 x 10^6 cells/ml) with 10 μM ^{3}H-adenine (ca 15 μCi/10^6 cells) in isotonic Krebs Ringer phosphate (KRP) buffer for 5 to 10 min. Cells were collected by centrifugation (50 x g), and then were suspended in fresh KRP buffer. The effects of stimulatory agents on the formation of ^{3}H-cyclic AMP were determined on aliquots of this suspension. Incubations

Table I. Specific Activities of Adenylate Cyclases from Different Liver Cell Types

Source of adenylate cyclase[1]	Adenylate cyclase activity (relative to activity measured with fluoride = 100%)[2]			
	Basal	GTP	PGE_1 + GTP	Glucagon + GTP
Whole liver	8	15	49	79
Hepatocytes	10	14	22	117
Kupffer cells	18	22	29	23
Non-parenchymal fraction	10	21	47	47

[1] Cells were isolated as previously described (18), except that the non-parenchymal fraction ("hepatocyte wash") was suspended in isotonic KRP buffer and then centrifuged 50 x g for 5 min to precipitate hepatocytes (repeated twice). Non-parenchymal cells were collected by centrifuging the resulting supernatant 1200 x g for 15 min. The preparation of homogenates and the assays used are described in Methods. Agents included in assays were present at the following final concentrations: GTP, 10 μM; PGE_1, 56 μM; glucagon, 10 μM.

[2] Activities with adenylate cyclase from each cell type are expressed relative to that measured with 5 or 10 mM sodium fluoride (fluoride = 100%). Specific activities measured with fluoride were 0.82, 0.53, 1.58, and 2.19 nmole cyclic AMP/mg/15 min incubation, with enzyme from whole liver, hepatocytes, Kupffer cells, and the non-parenchymal cell fraction, respectively.

(total volume = 0.1 ml) were terminated by the addition of 0.2 ml of cold methanol: 1 N HCl (1:1), and the samples then centrifuged to precipitate proteins (2500 x g, 5 min). Aliquots of each supernatant were chromatographed in Whatman No. 1 paper strips (isobutyric acid:2 N ammonium hydroxide, 66:34) for total product analysis. ^{3}H-Cyclic AMP was isolated from the remainder of the sample by chromatography (19). Ten percent of the original ^{3}H-adenine was recovered as adenyl nucleotides, and 0.02 to 0.1% of this radioactivity was recovered as ^{3}H-cyclic AMP.

RESULTS

Relative activities of adenylate cyclases from different liver cell types are presented in Table I. Fluoride ion causes expression of near maximum activity regardless of hormone specificity; so the ratio of hormone- to fluoride-stimulated activity should reflect the fraction of total adenylate cyclase which is hormone sensitive. Glucagon stimulated enzyme from whole liver to 79% of that caused by fluoride, suggesting that a portion of the total enzyme was not glucagon sensitive. Glucagon stimulation was increased relative to fluoride stimulation when homogeneous hepatocytes were used as the enzyme source, indicating that all adenylate cyclase from hepatocytes is glucagon

Table II. Contribution to Total Adenylate Cyclase Activity by Different Cell Types

Enzyme source[1]	Units of adenylate cyclase in the presence of:[2]		
	Basal	PGE_1 + GTP	Glucagon + GTP
Hepatocytes	0.117	0.624	2.577
Kupffer cells	0.079	0.104	0.128
Non-parenchymal fraction	0.349	1.842	2.204

[1] Different cell fractions were prepared as noted in Table I. Preparation of homogenates and assays are described in Methods. The final concentration of GTP, PGE_1, and glucagon were the same as noted in Table I. Data are from a single representative experiment.

[2] Activities are expressed as total units (from 1 gram of rat liver) during 15 min incubation with the specified agent(s). One unit of activity = 1 nmol cyclic AMP. Data were calculated by multiplying the mg of recovered membrane protein from each cell fraction (per gram of liver) by the respective specific activities.

sensitive. Glucagon stimulation of adenylate cyclase from homogeneous hepatocytes actually exceeded fluoride stimulation by 10 to 20%.

Most stimulation by PGE_1, relative to activity measured with fluoride or glucagon, was observed with the "non-parenchymal fraction" which contrained hepatocyte debris, erythrocytes, Kupffer and endothelial cells, and possibly other cells derived from the liver vasculature (Table I). Glucagon stimulation of enzyme from the "non-parenchymal fraction" is attributed to contamination by hepatocyte debris generated during liver disperson (see below) (18), and this stimulation is less than that measured with enzyme from whole liver or from hepatocytes (Table I). These data show, in terms of specific activity, that glucagon-sensitive adenylate cyclase comes from hepatocytes, and also indicate that prostaglandin-sensitive activity is associated with non-parenchymal liver cells.

The distribution of total prostaglandin and glucagon-sensitive adenylate cyclase is estimated in Table II. About 92% of total liver protein comes from hepatocytes, but a maximum of 30% of the total PGE_1-sensitive adenylate cyclase activity originates from these cells. The contribution of PGE_1-sensitive activity from hepatocytes reflects only

Table III. PGE_1 and Glucagon Effects on Cyclic AMP Metabolism in Hepatocytes[1]

Conditions	Cyclic AMP	
	pmol/10^6 cells[2]	^{3}H-Cyclic AMP[3] (cpm/10^6 cells)
Control (zero time)	6.45 ± 0.98	-
Incubated control	6.01 ± 1.29	265
Incubated + 56 μM PGE_1	6.82 ± 0.15	273
Incubated + 20 μM glucagon	13.63 ± 0.57	923
Incubated + 1 μM glucagon	11.22 ± 0.65	-
1 μM glucagon + 0.7 μM PGE_1	10.26 ± 0.93	-
1 μM glucagon + 7.0 μM PGE_1	9.81 ± 0.57	-
1 μM glucagon + 28 μM PGE_1	9.57 ± 0.91	1007

[1] Freshly prepared hepatocytes were incubated for 10 min at 37° in an atmosphere of 100% oxygen in KRP buffer, pH = 7.50, containing 1.3 mM calcium. Hepatocyte concentrations were from 19 to 20 x 10^6 cells/ml.

[2] The cyclic AMP content of cells plus medium was measured by protein binding. Values given are averages of four determinations (± SD). Two different cell preparations were used in obtaining data shown in the top and bottom halves of the table.

[3] Values give cpm of ^{3}H-cyclic AMP formed from nucleotide pools labeled with ^{3}H-adenine (see Methods). Data from one representative experiment are given; values are averages of duplicate measurements which differed by no more than 150 cpm. Data in the top and bottom halves of the table were obtained with the same hepatocyte preparation.

a 1.57-fold stimulation of basal activity (calculated from Table I), but this represents a significant portion of the total because of the large contribution of protein from hepatocytes. In comparison, PGE_1 stimulated activity from the non-parenchymal fraction 4.7 fold. About half of the total glucagon-sensitive activity was recovered with the "non-parenchymal fraction" (Table II) reflecting hepatocyte contamination (18). Purified hepatocyte nuclei and Golgi did not have PGE_1-sensitive adenylate cyclase (22). Hepatocyte survival after perfusion of liver with collagenase depends upon the efficiency of digestion, which is variable, and subsequent care taken in tissue dispersion. As a result, the distribution of glucagon-sensitive activity between the "hepatocyte" and "non-parenchymal" fractions varied among different preparations. Despite this, most of the prostaglandin-sensitive activity was always recovered in the "non-parenchymal fraction".

Table IV. Effects of PGE_1 and Glucagon on Glucose Production by Intact Hepatocytes

Conditions[1]	Glucose production[2] ($\mu g/10^6$ hepatocytes)
Control (0 min)	9.42
Incubated 30 min	85.76 ± 1.13
Incubated 30 min + PGE_1	85.64 ± 0.85
Incubated 30 min + glucagon	112.58 ± 2.89
Incubated 30 min + PGE_1 + glucagon	114.26 ± 1.81

[1] Fresh hepatocytes from fed rats were incubated under 100% oxygen as noted in Table III, in the presence of the indicated agent(s): PGE_1 = 28 μM, glucagon = 10 μM. The hepatocyte concentration was 20 x 10^6 cells/ml. Values are averaged from duplicate measurements on two different cell preparations (± SD).

[2] Glycogen and glucose were measured on the same incubation samples using glucose-6-phosphate dehydrogenase in conjunction with amyloglucosidase. Free glucose was measured first on a sample aliquot, and then total glucose + glycogen was measured after incubation of another aliquot with amyloglucosidase (23). Glycogen was also measured by ethanolic precipitation, hydrolysis in sulfuric acid, and colorimetric assay with phenol (24). Values obtained by the two methods were in agreement. The glycogen content of the fresh hepatocytes was 286.29 ± 34.7 $\mu g/10^6$ cells.

Table III compares the effects of PGE_1 and glucagon on cyclic AMP metabolism in intact hepatocytes. Cyclic AMP was measured by protein binding (20) and by recovery of ^{3}H-cyclic AMP in cells incubated with ^{3}H-adenine. Glucagon caused 2- to 3-fold increases in cyclic AMP, but PGE_1 had no effect. PGE_1 also did not significantly alter cyclic AMP increases caused by glucagon when both agents were included together. Basal and stimulated concentrations of cyclic AMP were slightly higher when theophylline was included, but PGE_1 still had no effect (data not shown).

The effects of glucagon and PGE_1 on hepatocyte glycogenolysis were also compared (Table IV). Glucagon caused predictable decreases in hepatocyte glycogen which were accounted for by stoichiometric increases in glucose. PGE_1 had no effect on basal- or glucagon-stimulated glycogenolysis.

DISCUSSION

Results presented here are in disagreement with those published by Tomasi and Barnabei et al., showing that PGE_1 causes dose- and time-dependent cyclic AMP

increases in hepatocytes, which are comparable in degree and more sustained than increases caused by glucagon (15,16). Likewise, glycogenolysis was reported to be enhanced by PGE_1 (15,16), but gluconeogenesis was unaffected (16). Later reports by Tomasi et al. are more consistent with our data, showing that PGE_1 has no significant effect on cyclic AMP metabolism in liver hepatocytes; however, the additional statement by these investigators that "PGE_1 dramatically increases cyclic AMP levels in a discrete hepatocyte population not involving parenchymal cells" leaves doubt of their intended conclusion(s) (17).

Our earlier studies on adenylate cyclases from different liver cell types indicated that a significant portion of enzyme stimulated by PGE_1 did not originate from liver parenchymal cells (i.e., hepatocytes) (18). This is established here by a quantitative comparison of the total glucagon and PGE_1-sensitive enzyme obtained from different cell fractions (Table II), and by the relative changes in specific activities accompanying the resolution of liver into its cellular components (Table I). PGE_1 also does not change cyclic AMP concentrations or glycogenolysis rates in isolated hepatocytes (Tables III, IV), suggesting PGE_1 effects which are mediated by cyclic AMP involve non-parenchymal cells. Endothelial (25) and Kupffer cells (26) are distinct cell types of the liver reticuloendothelial system (RES) and together make up about 30% of the total liver cell population. This does not exclude possible actions of prostaglandins on hepatocytes which are unrelated to those of cyclic AMP metabolism; for example, changes in ion permeability (15).

REFERENCES

1. Sweat, F. W. and Wincek, T. J. (1973) Biochem. Biophys. Res. Commun. 55, 522-529.
2. Tomasi, V. and Ferretti, E. (1975) Mol. Cell. Endo. 2, 221-232.
3. Zenser, T. V., DeRubertis, F. R., and Curnow, R. T. (1974) Endocrinology 94, 1404-1410.
4. Pradhan, T. K. and Criss, W. E. (1976) Cancer Biochem. Biophys. 1, 239-243.
5. Hickie, R. A., Jan, S.-H., and Datta, A. (1975) Cancer Res. 35, 596-600.
6. Yamashita, L. and Sweat, F. W. (1976) Biochem. Biophys. Res. Commun. 70, 438-444.

7. Lemberg, A., Wikinski, R., Izurieta, E. M., Halperin, H., Paglione, A. M., De Neuman, P., and Jauregui, H. (1971) Biochim. Biophys. Acta 248, 198-204.
8. Sacca, L., Perez, G., Rengo, F., and Condorelli, M. (1974) Diabetes 23, 532-535.
9. Levine, R. A. (1973) Prostaglandins, Cyclic AMP, Biological Actions, Clinical Applications (Kahm, R. H., ed.) p. 75-117, Academic Press, New York.
10. Exton, J. H., Robison, G. A., Sutherland, E. W., and Park, C. R. (1971) J. Biol. Chem. 246, 6166-6177.
11. Levine, R. A. (1974) Prostaglandins 6, 509-521.
12. Wheeler, G. E. and Epand, R. M. (1975) Mol. Pharmacol. 11, 335-339.
13. Chayoth, R., Epstein, S. M., and Field, J. B. (1973) Cancer Res. 33, 1970-1974.
14. DeRubertis, F. R., Zenser, T. V., and Curnow, R. T. (1974) Endocrinology 95, 93-101.
15. Tomasi, V., Poli, A., Ferretti, E., and Barnabei, O. (1975) Advan. Enzyme Regul. 13, 189-200.
16. Barnabei, O., Poli, A., Ferretti, E., Migani, P., and Tomasi, V. (1976) Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies (Tager, J. M., Söling, H. D., and Williamson, J. R., eds.) p. 422-429, North-Holland Publishing Company, Amsterdam.
17. Ferretti, E., Biondi, C., and Tomasi, V. (1976) FEBS Letters 69, 70-74.
18. Wincek, T. J., Hupka, A. L., and Sweat, F. W. (1975) J. Biol. Chem. 250, 8863-8873.
19. Wincek, T. J. and Sweat, F. W. (1975) Anal. Biochem. 64, 631-635.
20. Gilman, A. G. (1970) Proc. Nat. Acad. Sci. U.S. 67, 305-312.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
22. Sweat, F. W. and Yamashita, L. Manuscript in preparation.
23. Keppler, D. and Decker, K. (1974) Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) p. 1127-1131, Academic Press, New York.
24. Lo, S., Russell, J. C., and Taylor, A. W. (1970) J. Appl. Phys. 28, 234-236.
25. Blouin, A., Bolender, R. P., and Weibel, E. R. (1977) J. Cell Biol. 72, 441-455.
26. Widmann, J.-J. and Fahimi, H. C. (1976) Lab. Invest. 34, 141-149.