PRODUCTION OF PROSTAGLANDINS BY CELLS IN VITRO: RADIOIMMUNOASSAY MEASUREMENT OF THE CONVERSION OF ARACHIDONIC ACID TO PGE_2 AND $PGF_{2\alpha}$

Fredrick Cohen and Bernard M. Jaffe
Departments of Biology and Surgery, Washington University,
St. Louis, Missouri 63110

Received September 25,1973

SUMMARY: A specific radioimmunoassay has been applied to the measurement of the conversion of arachidonic acid to PGE_2 and $PGF_{2\alpha}$. PGE_2 and $PGF_{2\alpha}$ biosynthesis was linearly related to the amount of arachidonic acid added and was significantly inhibited by indomethacin in concentrations as low as 10^{-10} M. Sonicated Hela, L, and HEp-2 cells synthesized 244.0, 42.3, and 22.6 ng PGE_2 per mg of protein, but made substantially less $PGF_{2\alpha}$.

Using a sensitive radioimmunoassay for prostaglandins developed in our laboratory[1,2], we have measured PGE in the media of cells in log phase growth in vitro[3,4,5]. In order to asses the biosynthetic ability of specific cell lines, it is necessary to measure their ability to convert known quantities of arachidonic acid to PGE_2[6,7]. Previously described techniques, including thin layer chromatography[8], bioassay[9], spectrophotometry[10], and mass spectroscopy[11] are not sensitive enough. In this report we describe the application of prostaglandin radioimmunoassay to the measurement of PGE_2 synthesis from precursor arachidonic acid, as well as preliminary data on the synthetic ability of Hela, L, and HEp-2 cells.

METHODS: The biosynthetic technique applied was a modification of that of Pace-Asciak and Wolfe[12] using two sources of cyclizing enzyme, homogenates of rat stomach and of cells in vitro.

Adult rats were deeply anesthetized by intraperitoneal injection of urethane, 1.0 gm/kg body weight. The stomachs were surgically removed and washed three times with ice cold buffer (50 mM KH_2PO_4-NaOH, pH 7.4). Duplicate 1.0 cm^2 pieces of stomach were mechanically homogenized by Vertis homogenizer directly into 1.0 ml of ice-cold buffer containing 56 µg glutathione, 0.57 µg hydroquinone, 20 mM EDTA, and 12.5 µg arachidonic acid (Sigma Chemical

Supported in part by NIH Training Grant 371. Address reprint requests to Bernard M. Jaffe, Department of Surgery, Washington U. 4960 Audubon, St. Louis

Company), transferred to conical bottom tubes, and incubated in 95% O_2, 5% CO_2 at 37° for one hour.

Cell lines were maintained in Eagle's MEM containing 10% fetal calf serum at 37 ± 0.5° in a 5% CO_2-95% O_2 atmosphere. The cells were harvested from 75 cm^2 flasks with 0.01% trypsin at 37° for 10 minutes. Washed cells (0.5×10^6) were centrifuged, resuspended in 1.0 ml of buffer, and disrupted by sonication before use in biosynthesis experiments.

After incubation, prostaglandins were extracted, chromatographed on silicic acid columns, and measured by radioimmunoassay as previously described [2,13]. For each experiment, data was corrected by using duplicate tissue, arachidonic acid, and buffer blanks which were identically processed.

RESULTS: Using rat stomach homogenates under standard conditions (1.0 cm^2 tissue, 1 hour incubation, 12.5 µg of added arachidonic acid) 21.04 ± 4.56 ng (n=12) of PGE_2 and 2.45 ± 1.33 ng (n=11) of $PGF_{2\alpha}$ were synthesized de novo, corresponding to mean conversion rates of 0.17% and 0.02%, respectively. In control incubations performed without added arachidonic acid precursor, significant concentrations of prostaglandins were noted in the gastric tissue blanks amounting to averages of 6.0 and 3.5 ng of PGE_2 and $PGF_{2\alpha}$, respectively. These concentrations represented prostaglandin already present in rat stomach and were subtracted for calculation of the amounts of prostaglandin synthesized. Arachidonic acid and buffer blanks were negligible.

Several variables in the experimental protocol were studied, including the time of incubation and the amount of rat stomach homogenate employed. In duplicate experiments PGE_2 biosynthesis did not vary using incubation periods of 30, 60, and 120 minutes, (yielding 31.6, 29.7, and 38.8 ng PGE_2, respectively). When homogenates of 0.25 and 0.56 cm_2 of rat stomach served as source of cyclizing enzyme, 1.5 and 1.7 ng of PGE_2 (mean of duplicate experiments), respectively were synthesized; simultaneously, duplicate experiments using homogenates of 1.0 cm^2 of rat stomach yielded 17.4 ng PGE_2 under identical incubation conditions.

The amount of PGE_2 and $PGF_{2\alpha}$ synthesized was linearly related to the amount of arachidonic acid added to the reaction mixture (Figure 1).

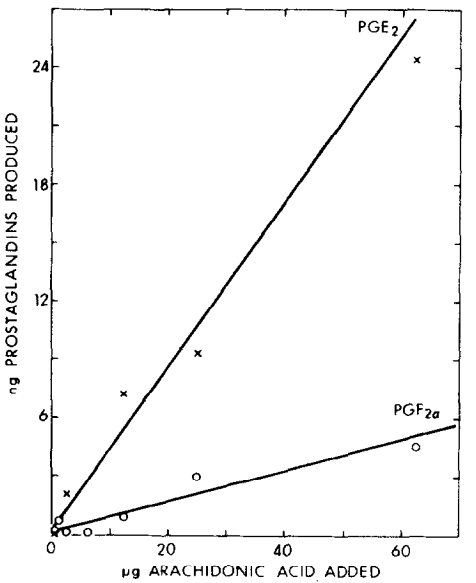

FIGURE 1: The relationship between PGE_2 and $PGF_{2\alpha}$ biosynthesis and the amount of arachidonic acid added in simultaneous, duplicate experiments (homogenates of 1.0 cm^2 rat stomach, 37°C, 60 minutes, 95% O_2 - 5% CO_2).

Although relatively small amounts of prostaglandins were synthesized in these experiments, PGE_2 was preferentially synthesized, maintaining a 5:1 E:F relationship of synthesized product. Indomethacin effectively inhibited synthesis of PGE_2. At 10^{-6} and 10^{-8}M, indomethacin resulted in approximately 45% inhibition of PGE_2 biosynthesis; at 10^{-9} and 10^{-10}M, indomethacin caused inhibition of synthesis to the extent of 49 and 68%, respectively.

For comparison with rat stomach, the biosynthetic ability of rat colon, liver, renal medulla, and lung was studied (Table 1). Protein concentrations were determined by the method of Lowry and co-workers.[14]

Prostaglandin synthesis by cultured mammalian cells was also studied (Table 1). Endogenous, intracellular concentrations of PGE_2 and $PGF_{2\alpha}$ were less than 150 pg per 500,000 cells. Only Hela cells synthesized significant amounts of $PGF_{2\alpha}$. However, synthesis of large amounts of PGE_2 was demonstrated

TABLE 1

PGE_2 and $PGF_{2\alpha}$ Biosynthesis

		mg protein in homogenate	prostaglandin synthesized ng/mg protein	
			PGE_2	$PGF_{2\alpha}$
Rat Tissue	Stomach	2.70	17.23	0.25
	Liver	1.75	2.55	<0.05
	Colon	0.45	17.71	1.08
	Renal medulla	1.85	1.57	0.63
	Lung	3.23	1.41	0.16
Cell Lines	Hela	0.19	244.0	4.90
	L	0.15	42.3	<0.30
	HEp-2	0.19	22.6	<0.30

by all three cell lines, with Hela cells being responsible for the greatest degree of conversion of arachidonic acid, and HEp-2 cells being responsible for the least.

DISCUSSION: The specificity of the radioimmunoassay for prostaglandins has been described in detail.[2,15] Long-chain fatty acids, steroids, and fat-soluble vitamins do not cross-react to a significant degree. Arachidonic acid cross-reacts with anti-prostaglandin antibodies (anti-PGE and anti-PGF) to only 10^{-5} to 10^{-6}. In addition, using the extraction-separation procedure described, recovery of each of the prostaglandins averages 70%, whereas only 2-4% of labeled arachidonic acid is recovered in any silicic acid fraction. By the combination of antibody specificity and the technique for processing samples for assay, arachidonic acid in microgram quantities do not interfere significantly with the ability of the radioimmunoassay to measure picograms of PGE_2 and $PGF_{2\alpha}$.

In each experiment, conversion of arachidonic acid to prostaglandins has been demonstrated. As shown by other investigators[12], approximately 5 times as much PGE_2 is synthesized as $PGF_{2\alpha}$. Rates of conversion described are

rather low compared to the data of Pace-Asciak and Wolfe[12] who described up 11-12% conversion. However, in their experiments, these investigators utilized almost 300 times more stomach homogenate and 17 times more arachidonic acid than we did. The purpose of this study was not to demonstrate high rates of conversion, rather to evaluate the methodology for examining prostaglandin synthesis as a test system for studies on sources of cyclizing enzyme in cells in vitro. Initial studies presented in this report using rat stomach homogenates demonstrate a linear relationship between precursor concentrations and prostaglandin production and document that prostaglandin synthesis is significantly inhibited by incubation in the presence of low doses of indomethacin.

Established cell lines in vitro had biochemical systems capable of synthesizing prostaglandins from precursors, preferentially synthesizing predominantly PGE. In previous studies we have measured PGE concentrations in media of the same cell lines during the active growth phase[4]. In these studies, PGE concentrations were inversely related to rates of cell proliferation. Associated with its slow growth during log phase growth, Hela released considerably more PGE into the media than did HEp-2 or L cells (5.74 vs 0.69 and 0.98 ng/10^6 cells/day, respectively). The correspondence in data between these studies with sonicated cells and known amounts of precursor and prior experiments studying PGE release into media during log phase cell proliferation suggests that cell lines have differing inherent abilities to synthesize prostaglandins E and F, and that perhaps, this synthetic ability may be related to rates of cell proliferation.

REFERENCES

1. Jaffe, B.M., Smith, J., Newton, W.T., and Parker, C.W., Science 171:494 (1971).
2. Jaffe, B.M., Behrman, H.R., and Parker, C.W., J. Clin. Invest. 52:398 (1973).
3. Jaffe, B.M., Parker, C.W., and Philpott, G.W., Surg. Forum 22:90(1971).
4. Jaffe, B.M., Philpott, G.W., Hamprecht, B., and Parker, C.B., Adv. in the Biosciences 9:179 (1973).
5. Hamprecht, B., Jaffe, B.M., and Philpott, G.W., FEBS Letters (in press).
6. Anggard, E. and Samuelsson, B., J. Biol. Chem. 240:3518 (1961).
7. Samuelsson, B., Fed. Proc. 31:1442 (1972).

8. Bygdeman, M. and Samuelsson, B., Clin. Chim. Acta 13:465 (1966).
9. Ferreira, S.H. and Vane, J.R., Nature 216:868 (1967).
10. Shaw, J.E. and Ramwell, P.W., Methods Biochem. Anal. 17:325 (1969).
11. Axen, U., Green, K., Horlin, D., and Samuelsson, B., Biochem. Biophys. Res. Comm. 45:519 (1971).
12. Pace-Asciak, C. and Wolfe, L.S., Biochem. Biophys. Acta 218:539 (1971).
13. Jaffe, B.M. and Parker, C.W., in "Third Conference on Prostaglandins in Fertility Control", S. Bergstrom, K. Green, and B. Samuelsson, B., eds., Karolinska Institutet, Stockholm (1972), p. 69.
14. Lowry, O.H., Roseborough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193:265 (1951)..
15. Jaffe, B.M., in "Radioassays in Clinical Medicine", R. Donati and W.T. Newton, eds., Charles C. Thomas, Springfield, Ill., (in press).