EPIDERMAL GROWTH FACTOR STIMULATES PROSTAGLANDIN BIOSYNTHESIS BY CANINE KIDNEY (MDCK) CELLS

Lawrence Levine and Aviv Hassid

Department of Biochemistry, Brandeis University
Waltham, Massachusetts 02154

Received May 9,1977

SUMMARY

Serum and/or arachidonic acid stimulated prostaglandin production by dog kidney (MDCK) cells. Epidermal growth factor (EGF) at concentrations of 10^{-9} to 10^{-10} M stimulated the biosynthesis of prostaglandins by MDCK cells but not that by human fibroblasts (D-550), mouse fibroblasts (3T3), transformed mouse fibroblasts (MC5-5), and rabbit aorta endothelial cells (CLO). EGF also stimulated the release of radioactivity from MDCK cells radioactively labelled with [^{3}H]arachidonic acid.

INTRODUCTION

Many cell types are capable of synthesizing prostaglandins. When grown in culture these cells secrete the prostaglandins into the medium (1-4). Arachidonic acid, a substrate for prostaglandin cyclooxygenase, increases prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ production by mouse fibroblasts (3T3), transformed mouse fibroblasts (MC5-5), human fibroblasts (D550) (5), and rabbit aorta endothelial cells (CLO) (5,6). Among the substances that simulate MC5-5 cells to produce prostaglandins are the mitogens thrombin (4,7) and serum (4,8). This increased production results from stimulated deacylation of phospholipids (9,10).

We wish to report that: 1) a canine kidney cell line (MDCK) produces prostaglandins when grown in culture; 2) this cell produces three to ten times more $PGF_{2\alpha}$ than PGE_2; 3) in the presence of serum or arachidonic acid, two to three times more prostaglandins are produced by these cells; 4) purified epidermal growth factor (EGF), at 10^{-9} and 10^{-10} M levels, stim-

ISSN 0006-291X

ulates prostaglandin production by MDCK; and 5) purified EGF at 10^{-9} M stimulates the release of radioactively labelled prostaglandins and arachidonic acid from radioactively labelled MDCK cells.

MATERIALS AND METHODS

An established cell line from normal dog kidney with epithelial-like morphology (MDCK) was obtained from Dr. Charles D. Stiles, University of California, La Jolla. The rabbit aorta endothelial cells (CLO) were obtained from Dr. Vincent Buonassissi, University of California, La Jolla; and the human fibroblast cell line was purchased from The American Type Culture Collection, Rockville, Maryland. The cells were grown in Eagle's minimal essential medium containing 2 mM L-glutamate (MEM$^-$) and supplemented with 10% (v/v) fetal bovine serum. Exponentially growing cells were used in all experiments. Cells were seeded at 2 x 10^5 or 2 x 10^6 cells/60 mm Falcon tissue culture dishes in 4 ml of the serum-supplemented medium and grown for one day. The medium was removed, and the dishes were rinsed twice with 2 ml of the minimal essential medium without serum supplementation (MEM$^-$). Two ml of MEM$^-$ containing the increments of the experimental reagents (serum, arachidonic acid or EGF) were then added to the dishes, and the cells were incubated in a humidified incubator at 37° in an atmosphere of 95% air and 5% CO_2. After designated times, the medium was withdrawn from duplicate dishes, chilled, and stored at -20°. At the time of each medium collection, the cells, after dispersion with trypsin, were counted. The media were assayed for PGE_2 and $PGF_{2\alpha}$ with either anti-PGE_2 or anti-$PGF_{2\alpha}$. The anti-$PGF_{2\alpha}$ reacts with PGE_2 0.1%, and the anti-PGE_2 reacts with $PGF_{2\alpha}$ 0.01%. These antisera do not identify the prostaglandins as either monoenoic or dienoic prostaglandins. Growth and culture conditions and preparation of cells labelled with [^{3}H]arachidonic acid in the cellular lipids were identical to that described with MC5-5 cells (9). Analysis of the radioactive materials in the cell after extraction with 2:1 chloroform-methanol showed that 68% of the radioactivity was associated with phospholipids, 7% with triglycerides, 21% with unidentified materials, 3% with prostaglandins, and less than 1.0% with free arachidonic acid. Thin layer chromatographic analyses of the cellular radioactive materials and the radioactive materials released into the medium were carried out with silica gel and developed in the solvent system described by Marinetti (11). Purified EGF was given to us by Dr. Howard Green.

RESULTS

When MDCK cells, incubating in MEM$^-$, were treated with increasing amounts of serum or arachidonic acid for one hour, prostaglandins were found in the medium with stimulations of 2.5 with serum for both $PGF_{2\alpha}$ and PGE_2 (Fig. 1A) and 2.8 with arachidonic acid for $PGF_{2\alpha}$ (PGE_2 was not determined) (Fig. 1B). These stimulations of MDCK cells were much less (10 to 50 times) than that found with other cell lines (5). MDCK cells

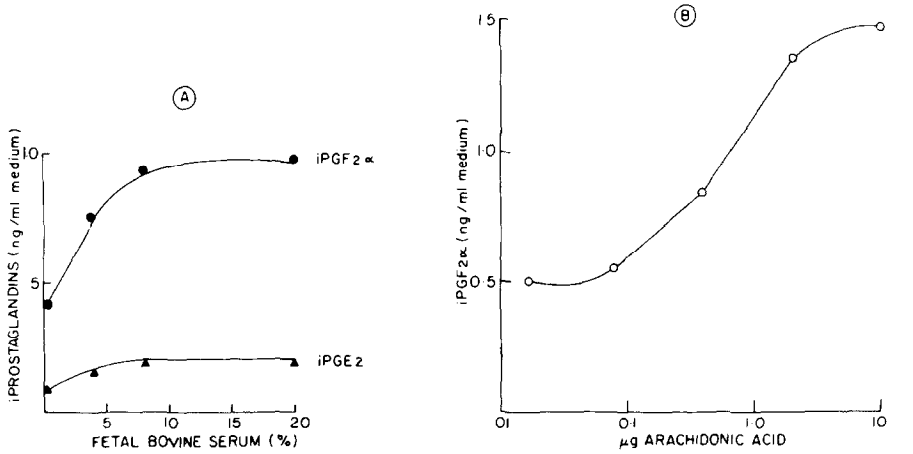

Fig. 1. Effect of fetal bovine serum and arachidonic acid on prostaglandin production by MDCK cells. MDCK cells at 2.2 x 10^6 cells/dish were incubated with MEM⁻ containing increments of fetal bovine serum (A) or arachidonic acid (B). After one hour, media were collected and assayed for (A) $PGF_{2\alpha}$ (●) and PGE_2 (▲); or (B) $PGF_{2\alpha}$ (o). Duplicate dishes were used and the values obtained agreed within 10% of the mean values. In (B), only $PGF_{2\alpha}$ was measured because the levels of arachidonic acid used in the media interferred with the radioimmunoassay for the PGE_2 produced by MDCK cells.

produced three to ten times more $PGF_{2\alpha}$ than PGE_2. Human fibroblasts (D550), mouse fibroblasts (3T3) and transformed mouse fibroblasts (MC5-5) produce more PGE_2 than $PGF_{2\alpha}$, whereas a rat aorta endothelial cell produces equal amounts of $PGF_{2\alpha}$ and PGE_2 (5).

Purified EGF stimulated MDCK cells to produce prostaglandins (Fig. 2A). This stimulation increased with time. During the incubation in MEM⁻ there was no appreciable growth of MDCK cells; the number of cells per dish varied over the nine hours between 5 and 5.5 x 10^5 cells/dish. As little as 0.5 ng/ml (8 x 10^{-11} M) of EGF stimulated production of both $PGF_{2\alpha}$ and PGE_2 after twelve hours of incubation (1.7 ng $PGF_{2\alpha}$ and 0.2 ng PGE_2/ml compared to 1.2 ng $PGF_{2\alpha}$ and 0.16 ng PGE_2/ml in MEM⁻). The human fibroblasts (D-550), the mouse fibroblasts (3T3), the transformed mouse fibroblasts (MC5-5) and the rabbit aorta endothelial cells (CLO) treated in an identical manner were not stimulated to produce prostaglandins by EGF (30

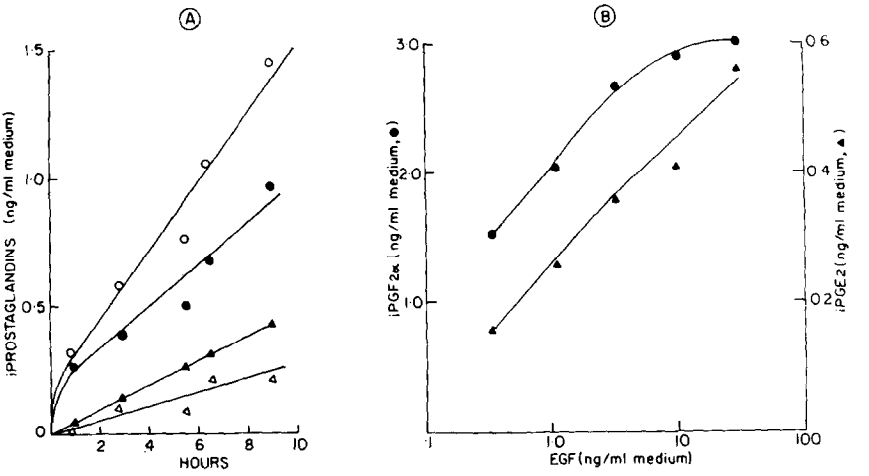

Fig. 2. Effect of EGF on prostaglandin production by MDCK cells. MDCK cells at 2 x 10^5 cells/dish were incubated with MEM⁻ or EGF (30 ng/nl) for various periods of time (A) or with increments of EGF for twelve hours (B). Duplicate dishes were used for each time point and each EGF concentration. The media were collected and assayed (A) for $PGF_{2\alpha}$ in the presence (o) and absence of EGF (●) and PGE_2 in the presence (▲) and absence of EGF (Δ). In the presence of increments of EGF (B), the media were assayed for $PGF_{2\alpha}$ (●) and PGE_2 (▲). In the absence of EGF, 1.2 ng/ml of $PGE_{2\alpha}$ and 0.16 ng/ml PGE_2 were produced. The values obtained for the media in the duplicate dishes agreed within 10% of the mean values.

ng/ml). An inhibitor of prostaglandin synthetase, indomethacin (0.01 μM), blocked the EGF-stimulated prostaglandin production by MDCK cells.

Earlier studies with MC5-5 cells showed that stimulators of prostaglandin production, such as serum, thrombin and bradykinin, also stimulated the release of [^{3}H]arachidonic acid from cellular phospholipids. Incubation of MDCK cells, in which [^{3}H]arachidonic acid had been incorporated into cellular lipids, with EGF also stimulated the release of radioactive materials from the cells into the medium. This stimulation was observed as early as two hours after addition of the EGF. The radioactive materials that were released into the medium after eight hours of incubation with EGF (and control MEM⁻) were extracted with ethylacetate and analysed by thin layer chromatography. Increased levels of radioactive prostaglandins

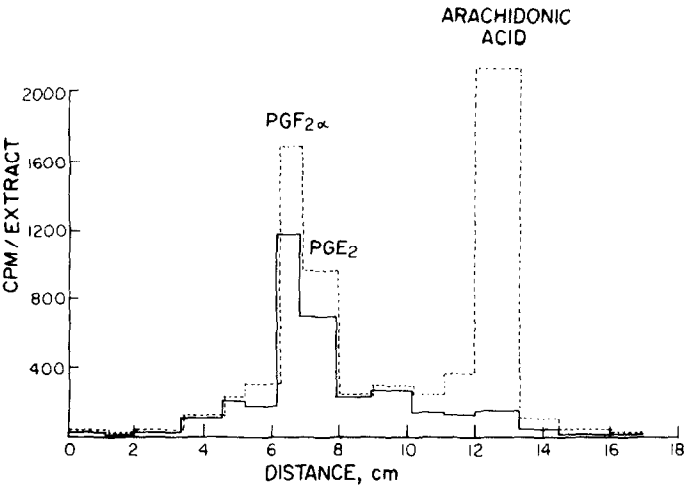

Fig. 3. Effect of EGF on release of radioactive materials from radioactively labelled MDCK cells. MEM⁻ (2.8 ml out of a total of 4 ml) from radioactively labelled MDCK cells (0.4 x 10^6 cells/dish) after incubation for eight hours in the presence or absence of EGF (20 ng/ml) was acidified with 1 ml of 0.1 N HCl and extracted twice with 3 ml ethylacetate. The extract was evaporated and applied to a silica gel 60 plate (E. Merck, Darmstadt, Germany) along with carrier lipids. The latter were located after development with iodine vapor. The zones corresponding to the carrier lipids as well as short segments from the rest of the plate were scraped off the plate and counted for radioactivity. (------) represents the distribution of radioactivity from medium incubated with EGF, and (———), the distribution of radioactivity from the MEM⁻ control. The values shown are the average of duplicate dishes from one experiment. These values agreed within 10% of the mean values. A second experiment gave similar results. The ratio of $PGF_{2\alpha}$ to PGE_2 appears to be lower than that calculated from radioimmunoassay data. This presumably is due to unidentified material comigrating with PGE_2.

and arachidonic acid as well as some unidentified compounds were found (Fig. 3).

DISCUSSION

EGF increased the release of arachidonic acid from MDCK cells about ten times. However this accounts only for 1 to 2% of the total radioactivity of the cells and is not significantly greater than the 0.7% free arachidonic acid found in the labelled cells. Thus, this stimulation of arach-

idonic acid released by EGF could have come from increased secretion of cellular arachidonic acid. The increased radioactive $PGF_{2\alpha}$ and PGE_2 in the medium also could have come from stimulated secretion of free prostaglandins. Based on our earlier experiences with radioactively labelled MC5-5 cells (4,5), we believe that this increase of labelled arachidonic acid and prostaglandins resulted from stimulated deacylation of esterified arachidonate.

EGF is a polypeptide of molecular weight 6045 found in extracts of male mouse submaxillary glands (12). Several biological properties have been described for EGF: 1) it enhances the growth and maturation of epidermis in newborn mice and in organ cultures of epithelial tissues (12-17); 2) it is cocarcinogenic for epidermal carcinogenesis (18,19); 3) it is mitogenic for fibroblasts as well as other cell types (20-25) and 4) it increases the lifetimes of cultures of epidermal cells of newborn humans (26). While the evidence is strong that EGF plays a role in growth and development, little is known about its biochemical mechanisms of action. Deacylation of phospholipids and or triglycerides and conversion of some of this arachidonic acid into prostaglandins E_2 and $F_{2\alpha}$ appears to be an early reaction of MDCK cells to EGF.

ACKNOWLEDGMENTS

We thank Dr. Howard Green, Massachusetts Institute of Technology for the generous gift of the purified EGF, and the National Institute of Health (HD-07966 and CA-17309) for support. L. Levine is a Professor of Biochemistry of the American Cancer Society (Award PRP-21).

REFERENCES

1. Levine, L., Hinkle, P.M., Voelkel, E.F. and Tashjian, A.H., Jr. (1972) Biochem. Biophys. Res. Commun., 47, 888-896.

2. Jaffe, B.M. (1974) Prostaglandins, 6, 453-461.

3. Ritzi, E.M., and Stylos, W.A. (1976) J. Natl. Cancer Inst., 56, 529-533.

4. Hong, S.L., Polsky-Cynkin, R., and Levine, L. (1976) J. Biol. Chem., 251, 776-790.

5. Levine, L., Pong, S.S., Hong, S.L. and Tam, S. in New Biochemical Aspects of Prostaglandins and Thromboxanes (eds. N. Kharasch and J. Fried) in press (Academic Press New York).

6. Buonassissi, V. and Venter, J.C. (1976) Proc. Natl. Acad. Sci. USA, 73, 1612-1616.

7. Chen, L.B. and Buchanan, J. (1975) Proc. Natl. Acad. Sci. USA, 72, 131-135.

8. Todaro, G.J., Lazar, G.K. and Green, H. (1965) J. Cell Physiol., 66, 325-334.

9. Hong, S.L. and Levine, L. (1976) Proc. Natl. Acad. Sci. USA, 73, 1730-1734.

10. Hong, S.L. and Levine, L. (1976) J. Biol. Chem., 251, 5814-5816.

11. Marinetti, G.V. (1962) J. Lipid Res., 3, 1-20.

12. Cohen, S. (1962) J. Biol. Chem., 237, 1555-1562.

13. Cohen, S. (1963) J. Invest. Dermatol., 40, 1-5.

14. Cohen, S. (1965) Devl. Biol., 12, 394-407.

15. Turkington, R.W. (1969) Expl. Cell Res., 57, 79-85.

16. Bertsch, S. and Marks, F. (1974) Nature, 251, 517-519.

17. Starkey, R.H., Cohen, S. and Orth, D.N. (1975) Science, 189, 800-802.

18. Reynolds, V.H., Boehm, F.H. and Cohen, S. (1965) Surgical Forum, 16, 108-109.

19. Rose, S.P., Stahn, R., Passovoy, D.S. and Herschman, H. (1976) Experientia, 32, 913-914.

20. Armelin, H.A. (1973) Proc. Natl. Acad. Sci, USA, 70, 2702-2706.

21. Hollenberg, M.D. and Cuatrecasas, P. (1973) Proc. Natl. Acad. Sci. USA, 70, 2964-2968.

22. Cohen, S., Carpenter, G. and Lembach, K.J. (1975) Adv. Metabolic Disorders, (eds. Lust, R. and Hall, K.) 8, 265-284 (Academic Press New York).

23. Westermark, B. (1976) Biochem. Biophys. Res. Commun., 69, 304-310.

24. Hollenberg, M.D. (1975) Archs. Biochem. Biophys., 171, 371-377.

25. Rose, S.P., Pruss, R.M. and Herschman, H.R. (1975) J. Cell Physiol., 86, 593-598.

26. Rheinwald, J.G. and Green, H. (1976) Nature, 265, 421-424.