Vol. 198, No. 1, 1994
January 14, 1994

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 298-303

PDGF-INDUCES THE GLUTATHIONE-DEPENDENT ENZYME PGH_2/PGE_2 ISOMERASE IN NIH3T3 AND pEJ TRANSFORMED FIBROBLASTS

Michael J. Kelner[1] and Slavomir F. Uglik[2]

[1] University of California, San Diego, Department of Pathology, 200 West Arbor Drive, San Diego, CA 92103-8320

[2] Polish Mother's Memorial Hospital, UL. Rzgowska 281/291, Lodz, Poland

Received November 29, 1993

Summary: Exposure of NIH3T3 and pEJ serum-starved cells to platelet derived growth factor results in a 16 fold increase in the glutathione-dependent enzyme prostaglandin H_2/prostaglandin E_2 isomerase activity (EC 5.3.99.3). The response is rapid as a detectable increase in NIH3T3 cells occurs after only 7 minutes of exposure to the growth factor. Only a mild increase in another microsomal glutathione-dependent enzyme, microsomal glutathione transferase (EC 2.5.1.18), was detected after a 2 hour exposure to the growth factor.

Stimulation of serum-starved NIH3T3 cells with platelet-derived growth factor (PDGF) results in cellular release of prostaglandin E_2 (PGE_2) that is detectable within minutes (1). This stimulation of PGE_2 release by PDGF is blocked in pEJ (ras-transformed) cells, although the pEJ cells have an equivalent basal rate of PGE_2 release (1). Addition of cycloheximide, a translational inhibitor, blocks PDGF-stimulated PGE_2 release in NIH3T3 cells suggesting that de novo synthesis of prostaglandin H synthase (cyclooxygenase) is required for growth factor stimulation of PGE_2 release (2). Subsequent studies, however, demonstrate that serum starved cells have ample capacity to support maximal PDGF-stimulated PGE_2 release without de novo synthesis of PGHS enzyme (3). Thus, alteration in prostaglandin H synthase activity is not the cause of PDGF-induced PGE_2 release. The overall increase in prostaglandin synthesis is due to of phospholipase C and A_2 activities by the growth factor (1), but the the exact mechanism by which PGE_2 production is preferentially increased (over other prostaglandins also

[1]Corresponding author. Fax: 619-543-3730.

0006-291X/94 $5.00

derived from PGH_2) has not been explained. We hypothesized that PDGF could stimulate the activity of the glutathione-dependent enzyme PGH_2/PGE_2 isomerase within minutes of exposure to the growth factor. We present such evidence here.

MATERIALS AND METHODS

Cell culture and chemicals- The NIH3T3 and pEJ-ras transformed fibroblasts were a gift from Dr. Martin Haas (Cancer Institute, UCSD) and were grown in DMEM with 10% calf serum and 2 mM glutamine as previously described (4). Fetal calf serum was from Hyclone (Logan, Utah) and contained 0.7 ng/ml of PDGF by bioassay. Nonradiolabeled receptor-grade PDGF was from R&D Systems (Minneapolis, MN). Prostaglandin H_2 was from Calbiochem (La Jolla, CA).

Plasma-derived serum (PDS) preparation - During the clotting process platelets release significant amounts of PDGF into the serum (5), and therefore commercially available serum contains significant amounts of PDGF. Therefore, PDGF-deficient serum was prepared (6). Only plasticware was used for the entire PDS preparation process. We obtained anticoagulated human blood by venipuncture into a sodium citrate solution such that the final concentration of the sodium citrate was 0.38% (weight/volume). The anticoagulated blood was transferred to prechilled, plastic tubes and centrifuged at 3,000 rpm for 20 minutes at 4°C in a Beckman Sorval 2C. The supernatant was then centrifuged at 13,500 rpm for 20 minutes at 4°C. Next 1.0 M calcium chloride was added to a final concentration of 20 mM. Ringer solution was then added at a ratio of 1 volume to 6 volumes of plasma. The plasma was incubated at 37° for 2 hours to allow clot formation. Tubes were centrifuged at 13,500 rpm for 30 minutes at 4°C. The supernatant was dialyzed against 0.1 molar TRIS, pH 7.4, at 4°C for 24 hours, then applied to a CM Sephadex column (Pharmacia Fine Chemicals). The concentrated PDS was dialyzed against 3 changes of Ringer solution over a 24 hour period. The dialyzed solution was heated to 56°C for 30 minutes to inactivate compliment. This solution was centrifuged at 13,500 rpm for 20 minutes and filtered. The final product was stored at -70°C.

PDGF stimulation studies - For PDGF stimulation studies (1) the cells were grown in culture dishes to about 50% confluency. The media was removed and replaced with DMEM plus 1.25% PDS. The cells were then incubated overnight at 37°C. The cultures were washed twice with 5 ml of DMEM, and DMEM plus 1.25% PDS was added to each culture. At this point PDGF in 4 mM HCL was added so the final concentration of PDGF was 2 units/ml of media. The equivalent volume of 4 mM HCl (without PDGF) was added as a control.

PGH_2/PGE_2 isomerase activity (EC 5.3.99.3) - 2 x 10^6 cells collected by scraping and resuspending in 100 ul of buffer (50 mM TRIS, pH 7.5, 0.1 M sodium chloride, 0.1 mM EDTA). Cells were disrupted by rapid freeze thawing twice in a dry ice acetone bath then centrifuged at maximum speed in a microcentrifuge (Beckman). The supernatant was transferred to tubes and either placed in a 37°C water bath for 1 minute, or for microsomal preparations was centrifuged at 110,000 x g for one hour, then placed in a 37°C water bath for 1 minute. Next, 10 ul of GSH solution (6 mg reduced glutathione/1 ml of TRIS/sodium chloride/EDTA buffer) (7,8) was added and followed by a 1 minute incubation. Then 100 ng of PGH_2 (stock solution is 0.01 mg PGH_2 in 0.2 ml acetone) was added to the tube and placed just above the surface of the solution. Cells were vortexed and placed in a water bath for exactly 1 minute. The reaction was terminated by adding 10 ul of iron solution (25 mM $FeCl_2$) to the tube and vortexing. Tubes were incubated at room temperature for 15 minutes and then returned to the ice bath. The concentration of PGE_2 in the tubes at 0 and 1 minute was determined by the PGE_2 radioimmunoassay described later. Cross reactivity of the PGE_2 antibody to PGH_2 was less than 0.01%.

Prostaglandin E_2 radioimmunoassay - After sonication the reconstituted sample was mixed with 100 µl of methyl oxamination reagent (9), then incubated overnight at room temperature. The prostaglandin E_2 content was then assayed using the Amersham (Amerlex-M) magnetic separation kit. The assay was performed entirely according to the manufacturers' instructions. The cross-reactivity of the antibody was 0.3% for prostaglandin E_1, less than 0.01% for PGH_2, and less than 0.001% for prostaglandin D_2, PGF_2(alpha), 6-keto-PGE_2, thromboxane B_2, 6-keto-PGF_1(alpha), and arachadonic acid.

Glutathione transferase activity (EC 2.5.1.18) - Glutathione transferase activity was determined in microsomal (pellet) and cytosolic (supernatant) fractions using 1 mM 1-chloro-2,4,-dinitrobenzene and 1 mM glutathione as substrates (10).

Data analysis - Statistical analysis of data included the Student's T-test (unpaired two tailed) and probability (p) values which were derived using GraphPad Instat Software (version 2.02)(La Jolla, California). Probability values less than 0.05 were considered statistically significant.

RESULTS

Basal cellular content of PGE_2 was similar in the NIH3T3 and the pEJ-transformed fibroblasts at 12 ± 3 picograms/million cells for NIH3T3 cells and 12 ± 2 for pEJ transformed cells. PDGF rapidly induced PGE_2 release in NIH3T3 cells and slowly induced PGE_2 release in pEJ transformed cells in agreement with previous reports (figure 1). Basal PGH_2/PGE_2 isomerase activity was nearly

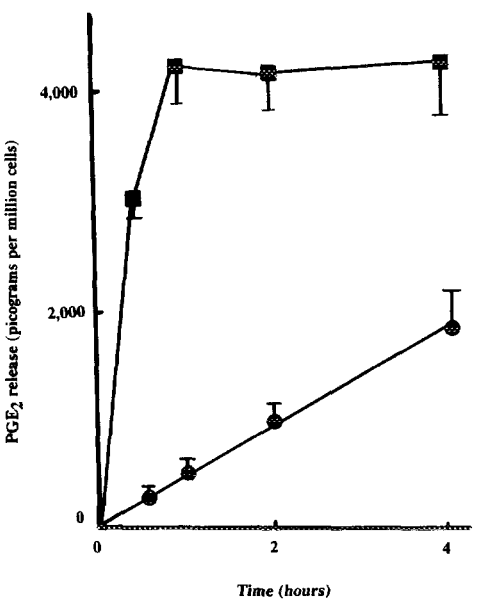

Figure 1. Prostaglandin E_2 release from NIH3T3 (■ – ■) and pEJ transformed (● – ●) cells in response to a 4 hour stimulation of minutes of PDGF (2 units/ml). Results expressed as total picograms of PGE_2 released into media per million cells (N = 3; X± SD).

undetectable in the absence of PDGF (table 1) in NIH3T3 and was moderately increased in pEJ-ras transformed cells. Stimulation of both NIH3T3 and pEJ-ras transformed fibroblasts with 2 units/ml PDGF for 2 hours resulted in a marked stimulation of PGH_2/PGE_2 isomerase activity (Table 1). A time course analysis with NIH3T3 cells revealed that the PDGF-induced increase in PGH_2/PGE_2 isomerase activity occurred within minutes after exposure to the growth factor (Figure 2).

PGH_2/PGE_2 isomerase (EC 5.3.99.3) is a glutathione-dependent microsomal enzyme that has been only partially purified (7,8). Therefore, we determined the effect of PDGF stimulation of microsomal glutathione transferase activity by assaying enzyme activity in microsomal fractions. A 2 hour exposure to 2 units/ml of PDGF resulted in only a mild increase in microsomal glutathione transferase activity from 3.3 $\pm$ 0.4 nanomoles/minute/10 million cells to 5.9 $\pm$ 0.9 nanomoles/minute/10 million cells. This increase is far less than the 18 fold increase induced by PDGF in PGH_2/PGE_2 isomerase activity. Addition of PDGF had no effect on cytosolic glutathione transferase activity (data not shown).

DISCUSSION

PGHS activity (the putative regulatory point in prostaglandin synthesis), based on mRNA and immunoassay (protein) is reportedly not induced by PDGF (1). When assaying PGHS in microsomal fractions by enzyme activity, however, we detect a mild increase (< 30%) after PDGF stimulation for 2 hours (data not shown), which is in agreement with another reports (2). In NIH3T3 cells the activity of

Table 1: Effect of a 2 hour PDGF stimulation on PGH_2/PGE_2 isomerase activity

	PGH_2/PGE_2 isomerase activity	
Cells	Basal	After PDGF
NIH3T3	6 $\pm$ 1	102 $\pm$ 8 **
pEJ transformed	20 $\pm$ 3	130 $\pm$ 22 **

PDGF (2 units/ml) stimulation of serum and analysis of PGH_2/PGE_2 isomerase activity was performed as described in methods. Analysis of a control mixture of PGH_2 and glutathione revealed no detectable nonenzymatic conversion of PGH_2 to PGE_2. Units are in picograms/minute/million cells (N=3, X$\pm$ SD). "**" refers to a $p < 0.01$ when compared to basal value.

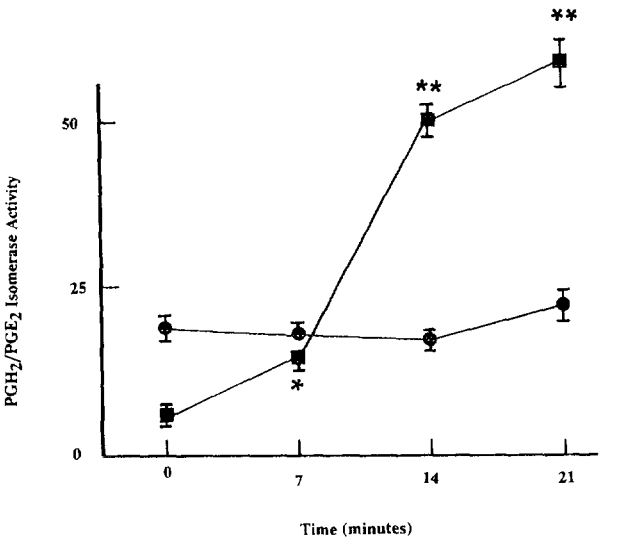

Figure 2. Induction of PGH_2/PGE_2 isomerase activity upon exposure to PDGF (2 units/ml) in NIH3T3 cells (■ — ■) and pEJ transformed cells (● — ●). Results expressed as picograms/minute/million cells (N=3; X± SD). "*" and "**" refer to respectively $p < 0.05$ and $p < 0.01$ when compared to initial value (t=0).

PGH_2/PGE_2 isomerase, however, was markedly stimulated (~ 18 fold) after PDGF stimulation for 2 hours. This PDGF-induced increase in PGH_2/PGE_2 isomerase activity occurs within minutes of exposure to the growth factor. PGHS synthase activity is sufficient to handle the initial release of arachadonic acid upon exposure to PDGF and a marked increase in PGH_2 production occurs (1). PGH_2 represents a branchpoint in the biosynthesis of a variety of eicosanoids and a common pool of PGH_2 serves as a substrate for metabolic production of these eicosanoids (reviewed in 11). Thus, the marked increase in PGH_2/PGE_2 isomerase allows conversion of the increased PGH_2 into PGE_2 before it can be converted to other prostaglandin moieties by enzymatic or nonenzymatic means. Although PGH_2/PGE_2 isomerase is glutathione-dependent and microsomal in location, our studies suggest it is not the classical microsomal glutathione transferase enzyme. We are currently attempting to purify PGH_2/PGE_2 isomerase and determine its cDNA sequence to allow further studies.

ACKNOWLEDGMENTS

This work was supported by funds from the National Cancer Institute (RO1CA52310), National Institute of Environmental Health Sciences (RO1ES04989), and National Institute of Aging (RO1AG10746).

REFERENCES

1) Benjamin, C.W., Tarpley, W.G., and Gorman, R.R. (1987) Proc. Natl. Acad. Sci USA 84,546-550.

2) Habenicht, A.J.R., Goerig, M., Grulich, J., Rothe, D., Gronwald, R., Loth, U., Schletter, G., Kommerell, B., and Ross, R. (1985) J. Clin. Invest. 75, 1381-1387.

3) Lin, A.H., Bienkowski, M.J., and Gorman, R.R. (1989) J. Biol. Chem. 264, 17379-17383.

4) Kelner, M.J., and Bagnell, R. (1990) J. Biol. Chem. 265, 10872-10875.

5) Busch, C., Wasteson, Å., and Westermark, B. (1976) Thromb. Res. 8, 493-500.

6) Vogel, A., Raines, E. Kariya, B., Rivest, M-J, and Ross, R. (1978) Proc. Natl. Acad. Sci USA 75, 2810-2814.

7) Moonen, P., Buytenhek, M., and Nugteren, D.H. (1982) Methods Enzymol. 86, 84-91.

8) Ogino, N., Miyamaoto, T., Yamamoto, S., and Hayaishi, O. (1976) J. Biol. Chem. 252, 890-895.

9) Kelly, R.W., Graham, B.J.M., and O'Sullivan, M.J. (1989) Prost. Leuk. Essent. Fatty Acids 37,187-196.

10) Habig, W.H., Pabst, M.J., and Jakoby, W.B. (1974) J. Biol. Chem. 249,7130-9.

11) Marnett, L.J., and Maddipati, K.R. (1991) Prostaglandin H synthase. In Peroxidases in Chemistry and Biology. (J. Everse, K. Everse, and M.B. Grisham, eds.) CRC Press, Boca Raton, FL., Vol. I, 294-334.