Vol. 180, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1991 Pages 46-52

CYTOKINE- AND FORSKOLIN-INDUCED SYNTHESIS OF GROUP II PHOSPHOLIPASE A_2 AND PROSTAGLANDIN E_2 IN RAT MESANGIAL CELLS IS PREVENTED BY DEXAMETHASONE

Casper Schalkwijk*, Margriet Vervoordeldonk*, Josef Pfeilschifter#, Fritz Märki# and Henk van den Bosch*

*Centre for Biomembranes and Lipid Enzymology, Padualaan 8, 3584 CH Utrecht, The Netherlands

Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland

Received August 12, 1991

We have previously described that treatment of rat glomerular mesangial cells with interleukin-1β, tumor necrosis factor or forskolin stimulates the synthesis and secretion of prostaglandin E_2 and group II phospholipase A_2. We now report that pretreatment of the mesangial cells with dexamethasone dose-dependently suppresses the cytokines- and forskolin-induced synthesis of prostaglandin E_2 as well as the induced synthesis and secretion of group II phospholipase A_2. These observations implicate that the inhibition of the cellular or secreted phospholipase A_2 activity by dexamethasone in rat mesangial cells is not due to induced synthesis of phospholipase A_2 inhibitory proteins but caused by direct inhibition of phospholipase A_2 protein expression. © 1991 Academic Press, Inc.

PLA_2 catalyses the hydrolysis of ester bonds at the sn-2-position of membrane phospholipids and plays a key role in the liberation of arachidonic acid. Cellular free arachidonic acid serves as precursor for the biosynthesis of a variety of biologically active lipids including the prostaglandins and leukotrienes (1). These potent lipid mediators are involved in several physiological and pathological events such as inflammation (2).

PLA_2's described thusfar can be discriminated into a class of high molecular weight PLA_2's of 60-110 kDa and a class of low molecular weight PLA_2's of 14 kDa. The latter can be divided into group I and group II PLA_2 (3). Group II PLA_2 is found in many tissues and cell types and is believed to participate in inducing and maintaining inflammatory reactions. In accord with this hypothesis, high amounts of group II PLA_2 are found at inflammatory sites (4-8) and injections of this purified PLA_2 proved pro-inflammatory (9,10). Furthermore, the potent inflammatory mediators IL-1 and TNF have been shown to enhance PLA_2 activities in rabbit chondrocytes (11,12), rat smooth muscle cells (13) and HepG2 cells (14) accompanied with increased levels of mRNA coding for group II PLA_2.

Abbreviations: PLA_2, phospholipase A_2; IL-1β, interleukin 1β; TNF, tumor necrosis factor; PBS, phosphate-buffered saline; PGE_2, prostaglandin E_2.

0006-291X/91 $1.50
Copyright © 1991 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Mesangial cells play an important role in glomerular haemodynamics and inflammation in the kidney. Recently we described the formation of PGE_2 in parallel with synthesis and secretion of group II PLA_2 from rat renal mesangial cells in response to IL-1β, TNF and forskolin (15-17). The cytokine-stimulated formation of PGE_2 could be prevented by dexamethasone (18). The anti-inflammatory activity of glucocorticosteroids has been attributed to inhibition of prostaglandin synthesis. The mechanism by which glucocorticosteroids inhibit prostaglandin synthesis is still unclear. Initially, this inhibition has been ascribed to glucocorticosteroid-induced synthesis of PLA_2 inhibitory proteins termed lipocortins (19). However, recent reports challenged this model by showing that inhibition of PLA_2 by lipocortin is most likely caused by substrate sequestration (20,21).

In view of the fact that cytokines stimulate the synthesis of PGE_2 and PLA_2 in rat mesangial cells and that the induced formation of PGE_2 could be prevented by dexamethasone we investigated the effect of dexamethasone on synthesis and secretion of group II PLA_2 in cells stimulated with IL-1β, TNF and forskolin. The induced synthesis of group II PLA_2 was dose-dependently prevented by pretreatment of cells with dexamethasone.

MATERIALS AND METHODS

MATERIALS

Recombinant IL-1β was prepared by the Biotechnology Department of Ciba Geigy Ltd., Basel, Switzerland; recombinant TNF was from Boehringer Mannheim, F.R.G. and forskolin from Calbiochem, Luzern, Switzerland. [1-^{14}C] Linoleic acid was obtained from Amersham International. Nitrocellulose membranes were from Schleicher and Schull, Dassel, F.R.G. Alkaline-phosphatase conjugated goat anti-mouse immunoglobulines and prestained markers were products of Bio-Rad Laboratories. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate were obtained from Sigma, St. Louis, MO. Dexamethasone-21-acetate was from Sigma.

METHODS

Cell culture. Cultivation and characterization of rat mesangial cells was performed as described previously (22). Briefly, the cells were grown in RPMI 1640 supplemented with 20% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and bovine insulin at 0.66 units/ml (Sigma). For the experiments passages 26-29 of mesangial cells were used. Confluent mesangial cells cultured in 16 mm diameter wells were rinsed twice with PBS and incubated with 1 ml of DMEM containing 0.1 mg/ml of fatty acid-free bovine serum albumin (Sigma) with or without agents for the indicated time periods. Thereafter, the medium was withdrawn and centrifuged for 10 min at 1000 rpm in an IEC Centra-7R lab centrifuge. The supernatant was removed, frozen in liquid nitrogen and stored until assayed for PGE_2 by radioimmunoassay (15) and PLA_2 activity (17). The cells were washed once and dissolved in PBS and frozen in liquid nitrogen until further analysis of protein content and PLA_2 activity. Protein was determined by the method of Bradford (23) with bovine serum albumin as standard.

PLA_2-assays. PLA_2 activity was assayed using 0.2 mM sn-2-labeled [1-^{14}C] linoleoylphosphatidylethanolamine (specific activity 3000 dpm/nmol) in 0.5 ml of 0.1 M Tris/HCl pH 8.5 in the presence of 10 mM $CaCl_2$ and 0.05% Triton X-100 as previously described (24,25)

Western blotting. For protein blotting the samples were resolved by SDS-PAGE using a 15% polyacrylamide gel according to Laemmli (26). The proteins were transferred to nitrocellulose (2 mA/cm^2 for 45 min) and immunodetected with monoclonal antibodies against

the rat mitochondrial group II PLA_2 (27,28). After the primary antibody incubation the blot was incubated with alkaline phosphatase conjugated goat anti-mouse IgG. After washing the alkaline phosphatase activity was detected by developing the blots in p-nitro blue tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate (17).

RESULTS

Our previous experiments have shown that treatment of rat glomerular mesangial cells with IL-1β, TNF or forskolin stimulated the secretion of PGE_2 into the culture medium accompanied with induced synthesis and secretion of group II PLA_2 (15-17). In view of the hypothesis that the anti-inflammatory action of glucocorticosteroids is due to inhibition of prostaglandin synthesis we first examined the effect of dexamethasone on the PGE_2 release from stimulated mesangial cells. Addition of either IL-1β or IL-1β plus forskolin to mesangial cells caused a 100-fold stimulation of PGE_2 synthesis as detected in the culture supernatant (Fig. 1). In both cases, the synthesis of PGE_2 can be blocked by pretreatment of the mesangial cells with increasing concentrations of dexamethasone.This result is in agreement with previous findings (18). Next we determined the effect of dexamethasone on the induced synthesis of group II PLA_2. With Western blot analysis using anti-group II PLA_2 IgG no 14 kDa group II PLA_2 could be detected in the supernatant of control mesangial cells (Fig. 2). In accord with previous results (17), enzyme protein became detectable in the culture supernatants of IL-1β or IL-1β plus forskolin stimulated cells. This induced secretion of PLA_2 was completely prevented by pretreament of the cells with dexamethasone (Fig. 2). Dexamethasone-mediated inhibition of PGE_2 and PLA_2 secretion was also observed when cells were stimulated with TNF or forskolin alone (data not shown).

The dose-response effect of dexamethasone on the inhibition of induced secretion of PLA_2 was next investigated. PLA_2 activity and protein in the culture supernatant increased sharply

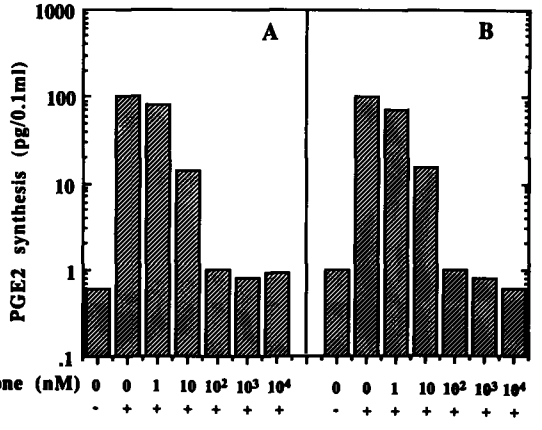


Figure 1. Dose-dependent inhibition by dexamethasone of the PGE_2 release of stimulated mesangial cells. Rat mesangial cells were stimulated with A; Il-1β (1 nM) or B; Il1β plus forskolin (10 μM) for 24 h with or without a 6 h pretreament with the indicated concentrations of dexamethasone.

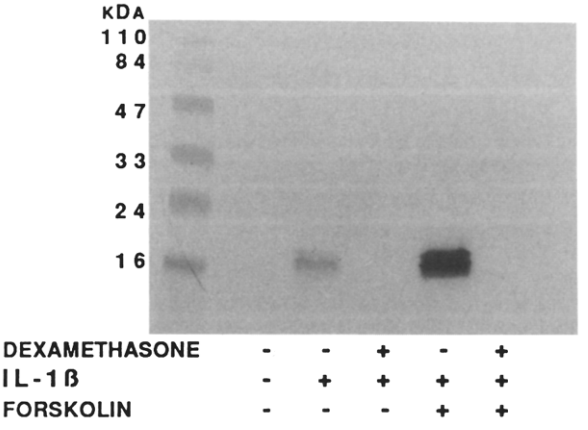
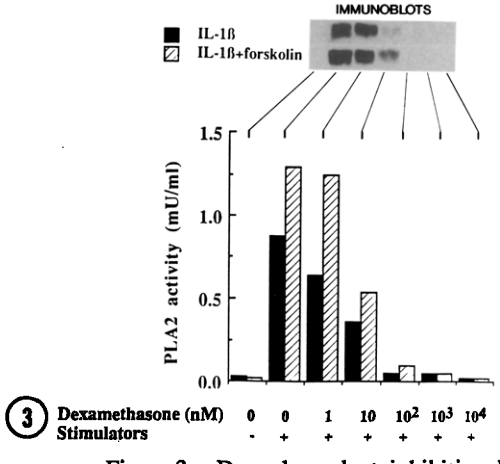


Figure 2. Effect of dexamethasone on the release of PLA_2 protein. Rat mesangial cells were stimulated with IL-1β (1 nM) or IL-1β plus forskolin (10 μM) for 24 h with or without a pretreament with dexamethasone (1 μM) for 6 h. Thereafter, 200 μl of the culture supernatants were used for Western blotting and PLA_2 immunostaining.

upon stimulation with either IL-1β or IL-1β plus forskolin (Fig. 3). As with PGE_2 secretion (Fig. 1) pretreatment of mesangial cells with increasing concentrations of dexamethasone gradually decreased the secretion of PLA_2 activity and protein in the culture medium as shown by activity measurements and immunoblotting, respectively (Fig. 3). The relation between the inhibition of PLA_2 protein levels and PLA_2 activities implicate that the inhibitory effect of



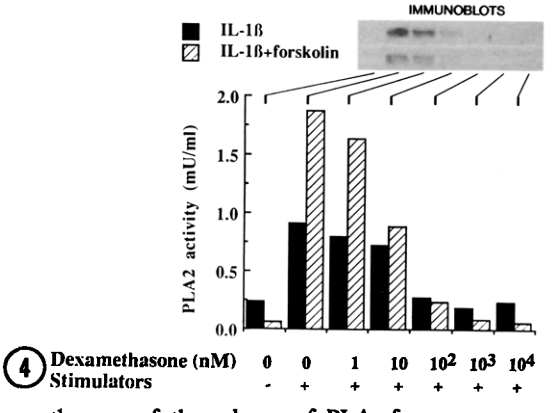


Figure 3. Dose-dependent inhibition by dexamethasone of the release of PLA_2 from stimulated mesangial cells. Rat mesangial cells were treated with dexamethasone at various concentrations for 6 h and thereafter stimulated with IL-1β (1 nM) or IL-1β plus forskolin (10 μM) for 24 h. The culture supernatants were analyzed for PLA_2 activities and for the presence of group II PLA_2 with immunoblotting.

Figure 4. Dose-dependent inhibition by dexamethasone of the synthesis of PLA_2 upon stimulation of mesangial cells. Rat mesangial cells were treated with dexamethasone at various concentrations for 6 h and thereafter stimulated with IL-1β (1 nM) or IL-1β plus forskolin (10 μM) for 24 h. The PLA_2 activities were determined in cell homogenates (protein concentration: 1 mg/ml) and 50 μg protein was used for Western blotting and PLA_2 immunostaining.

dexamethasone on PLA_2 activities is most likely not caused by dexamethasone-induced synthesis of inhibitory proteins of PLA_2. As shown by immunoblotting, the inhibition of PLA_2 activity is fully explainable by the fact that less enzyme is secreted. In order to determine whether only the secretion of induced PLA_2 from dexamethasone pretreated cells was inhibited or whether the *de novo* synthesis of PLA_2 was prevented we performed immunoblotting and PLA_2 activity measurements of the mesangial cells. A completely similar pattern compared with that of the culture media was observed when the corresponding cells were investigated (Fig. 4). Both PLA_2 activities and cellular levels of the enzyme as detected by immunoblotting decreased upon pretreatment of the mesangial cells with increasing doses of dexamethasone. Thus, not only the secretion but also the synthesis of IL-1β and IL-1β plus forskolin inducible PLA_2 in mesangial cells is dose-dependently prevented by pretreatment of the cells with dexamethasone. Similar responses of dexamethasone were observed in either TNF or forskolin stimulated mesangial cells (data not shown).

DISCUSSION

The IL-1β, TNF or forskolin induced synthesis and secretion of group II PLA_2 accompanying the induced formation of PGE_2 suggests a key role of this PLA_2 in the release of arachidonate and the regulation of prostaglandin formation in rat mesangial cells. Although it has been demonstrated in fibroblasts (29) and mesangial cells (30,31) that IL-1 not only augmented arachidonate release but also stimulates prostaglandin endoperoxide synthase activity, it is generally believed that the release of arachidonate, rather than prostaglandin endoperoxide synthase activity, is the rate limiting-step in prostaglandin formation.

It is well accepted that the anti-inflammatory effect of glucocorticosteroids is due to inhibition of prostaglandin synthesis. The glucocorticosteroid inhibition of prostaglandin production was initially attributed to decreased release of arachidonate mediated by the synthesis of the PLA_2 inhibitor protein lipocortin (19). Recent reports have questioned the involvement of lipocortin. Inhibition of PLA_2 by lipocortin *in vitro* was only demonstrated at very low substrate concentrations (20,21). Furthermore, several reports described lipocortin-independent inhibition of PLA_2 activities and prostanoid synthesis (32–35).

In this report we demonstrate that dexamethasone pretreatment of rat glomerular mesangial cells prior to stimulation with either IL-1β or IL-1β plus forskolin negatively regulates the secretion of PGE_2 into the culture medium. This observation is in full agreement with previous results (18) and is in line with the general notion that glucocorticosteroids inhibit prostaglandin synthesis. Stimulation of mesangial cells with IL-1β or IL-1β plus forskolin induced secretion of PLA_2 activity and this secretion can be prevented by pretreatment of the cells with increasing concentrations of dexamethasone (Fig.3). By comparing PLA_2 activity with PLA_2 protein levels in immunoblotting experiments we were able to discriminate between steroid-induced inhibition of PLA_2 activity and steroid-induced inhibition of PLA_2 protein expression. It was shown that the inhibition of PLA_2 activities in both culture media and cells was paralleled by decreases in PLA_2 mass (Fig.3 and 4). This indicates that dexamethasone inhibits not only

secretion of induced PLA_2, with accumulation of PLA_2 protein in the cells, but inhibits PLA_2 protein synthesis. These data strongly suggest that the inhibitory effect of dexamethasone on the induced PLA_2 in mesangial cells is not mediated by induced synthesis of PLA_2 inhibitory proteins but can be explained by preventing the synthesis of an inducible PLA_2.

These data obtained in rat mesangial cells confirm a recent report of Nakano et al. (36) who described the effect of dexamethasone on the expression of group II PLA_2 in rat smooth muscle cells. It was demonstrated that dexamethasone suppressed forskolin and TNF induced synthesis of group II PLA_2 by blocking the forskolin induced accumulation of group II PLA_2 coding mRNA. In the case of TNF induced enzyme synthesis, mRNA levels were less affected and a blocking of, presumably, post-transcriptional expression of the protein was inferred. Furthermore, it was demonstrated *in vivo* that glucocorticoid suppressed the expression of the PLA_2-II gene in tissues of endotoxin-treated rats (37).

In summary, the observations described in this report showed that the dexamethasone-induced inhibition of PLA_2 and PGE_2 release from cytokines- and forskolin-stimulated mesangial cells is due to dexamethasone-induced inhibition of PLA_2 synthesis. Additional experiments are underway to establish whether dexamethasone exerts its effect at the transcriptional or translational phase of the cytokines- and forskolin-induced PLA_2 synthesis.

REFERENCES

1. Van den Bosch, H. (1980) Biochim. Biophys. Acta 604, 191-246.
2. Dewitt, D.L. (1991) Biochim. Biophys. Acta 1083, 121-134.
3. Heinrikson, R.L., Krueger, E.T. and Keim, P.S. (1977) J. Biol. Chem. 252, 4913-4921.
4. Kramer, R.M., Hession, C., Johansen, B., Hayes, G., McGray, P., Pinchang Chow, E., Tizard, R. and Pepinsky, R.B. (1989) J. Biol. Chem. 264, 5768-5775.
5. Forst, S., Weiss, J., Elsbach, P., Maraganore, J.M., Reardon, L. and Heinrikson, R.L. (1986) Biochemistry 25, 8381-8385.
6. Chang, H.W., Kudo, I., Tomita, M. and Inoue, K. (1987) J. Biochem. 102, 147-154.
7. Hara, S., Kudo, I., Chang, H.W., Matsuta, K., Miyamoto, Y. and Inoue, K. (1989) J. Biochem. 105, 395-399.
8. Seilhamer, J.J., Pruzanski, W., Vadas, P., Plant, S., Miller, J.A., Kloss, J. and Johnson, L.K. (1989) J. Biol. Chem. 264, 5335-5338.
9. Vadas, P. and Pruzanski, W. (1986) Lab. Invest. 4, 391-404.
10. Murakami, M., Kudo, I., Nakamura, H., Yokoyama, Y., Mori, H. and Inoue, K. (1990) FEBS Lett. 268, 113-116.
11. Lyons-Giordano, B., Davis, G.L., Galbraith, W., Pratta, M. and Arner, E. (1989) Biochem. Biophys. Res. Commun. 164, 488-495.
12. Kerr, J., Stevens, T., Davis, G., McLaughlin, J. and Harris, R. (1989) Biochem. Biophys. Res. Commun. 165, 1079-1084.
13. Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) FEBS Lett. 261, 171-174.
14. Crowl, R.M., Stoller, T.J., Conroy, R.R. and Stouer, C.R. (1991) J. Biol. Chem. 266, 2647-2651.
15. Pfeilschifter, J., Pignat, W., Vosbeck, K. and Märki, F. (1989) Biochem. Biophys. Res. Commun. 159, 385-394.
16. Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F. and Vosbeck, K. (1991) Biochem. J. 273, 199-204.
17. Schalkwijk, C.G., Pfeilschifter, J., Märki, F. and Van den Bosch, H. (1991) Biochem. Biophys. Res. Commun. 174, 268-275.
18. Pfeilschifter, J., Pignat, W., Vosbeck, K., Märki, F. and Wiesenberg, I. (1989) Biochem. Soc. Transact. 916-917.
19. Hirata, F., Schiffmann, E., Venkatasubramanian, K., Solomon, D. and Axelrod, J. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 2533-2536.

20. Davidson, F.F., Dennis, E.A., Powell, M. and Glenney, J.R. (1987) J. Biol. Chem. 262, 1698-1705.
21. Aarsman, A.J., Mijnbeek, G., Van den Bosch, H., Rothhut, G., Prieu, B., Comera, C., Jordan, L. and Russo-Marie, F. (1987) FEBS Lett. 219, 176-180.
22. Pfeilschifter, J., Kurtz, A. and Bauer, C. (1984) Biochem. J. 223, 855-859.
23. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
24. Van den Bosch, H. and Aarsman, A.J. (1979) Agents Action 9, 382-389.
25. Van den Bosch, H., Aarsman, A.J. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 348, 197-207.
26. Laemmli, U.K. (1970) Nature 227, 680-685.
27. Aarsman, A.J., De Jong, J.G.N., Arnoldussen, E., Neys, F.W., Van Wassenaar, P.D. and Van den Bosch, H. (1989) J. Biol. Chem. 264, 10008-10014.
28. De Jong, J.G.N., Amesz, H., Aarsman, A.J., Lenting, H.B.M. and Van den Bosch, H. (1987) Eur. J. Biochem. 164, 129-135.
29. Raz, A., Wyche, A. Siegel, N. and Needleman, P. (1988) J. Biol. Chem. 263, 3022-3028.
30. Coyne, D.W. and Morrison, A.R. (1990) Biochem. Biophys. Res. Commun. 173, 718-724.
31. Topley, N., Floege, J., Wessel, K., Hass, R., Radeke, H.H., Kaever, V. and Resch, K. (1989) J. Immunol. 143, 1989-1995.
32. Hullin, F., Raynal, P., Ragab-Thomas, J.M.F., Fauval, J., and Chap, H. (1989) J. Biol. Chem. 264, 3506-3513.
33. Bienkowski, M.J., Petro, M.A. and Robinson, L.J. (1989) J. Biol. Chem. 264, 6536-6544.
34. Beyaert, R., Suffys, P., Van Roy, F. and Fiers, W (1990) FEBS Lett. 262, 93-96.
35. Piltch, A., Sun, L., Fava, R.A. and Hayashi, J. (1989) Biochem. J. 261, 395-400.
36. Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) J. Biol. Chem. 264, 12745-12748.
37. Nakano, T. and Arita, H. (1990) FEBS Lett. 273, 23-26.