Dexamethasone inhibits the induction of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase by phorbol ester in human promonocytic U937 cells

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · JULY 2000

Impact Factor: 4.66 · DOI: 10.1016/S0167-4889(00)00039-2 · Source: PubMed

2 AUTHORS, INCLUDING:

Hsin-Hsiung Tai
University of Kentucky
269 PUBLICATIONS **6,651** CITATIONS

ELSEVIER

Biochimica et Biophysica Acta 1497 (2000) 61–68

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Dexamethasone inhibits the induction of NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase by phorbol ester in human promonocytic U937 cells

Min Tong [1], Hsin-Hsiung Tai *

Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, USA

Received 29 December 1999; received in revised form 2 March 2000; accepted 9 March 2000

Abstract

Pro-inflammatory prostaglandins are known to be first catabolized by NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) to inactive metabolites. This enzyme is under regulatory control by various inflammation-related agents. Regulation of this enzyme was investigated in human promonocytic U937 cells. 15-PGDH activity was found to be optimally induced by phorbol 12-myristate 13-acetate (PMA) at 10 nM after 24 h of treatment. The induction was blocked by staurosporine or GF 109203X indicating that the induction was mediated by protein kinase C. The induction by PMA was inhibited by the concurrent addition of dexamethasone. Nearly complete inhibition was observed at 50 nM. Other glucocorticoids, such as hydrocortisone and corticosterone, but not sex hormones, were also inhibitory. Inhibition by dexamethasone could be reversed by the concurrent addition of antagonist mifepristone (RU-486) indicating that the inhibition was a receptor-mediated event. Either induction by PMA or inhibition by dexamethasone the 15-PGDH activity correlated well with the enzyme protein expression as shown by the Western blot analysis. These results provide the first evidence that prostaglandin catabolism is regulated by glucocorticoids at the therapeutic level.

Keywords: Dexamethasone; Prostaglandin; Dehydrogenase; Inflammation; Monocyte

1. Introduction

Prostaglandins are a family of pro-inflammatory fatty acids derived from arachidonic acid through the action of two isoforms of prostaglandin endoperoxide synthase or cyclooxygenase (COX) [1]. COX-1 appears to be responsible for the synthesis of prostaglandins necessary for housekeeping functions, whereas COX-2 is induced to synthesize prostaglandins in tissues in response to mitogens, growth factors, tumor promoters and pro-inflammatory cytokines. Anti-inflammatory steroids, such as dexamethasone, are able to inhibit the induction of COX-2 as a plausible mechanism of action [2].

Abbreviations: PG, prostaglandin; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; DTT, dithiothreitol; PMA, phorbol 12-myristate 13-acetate; PDD, 4α-phorbol 12,13-didecanoate; LXA_4, lipoxin A_4; AP, activator protein; CRE, cyclic AMP response element; CREB, cyclic AMP response element binding protein; PMSF, phenylmethylsulfonyl fluoride; DEX, dexamethasone

* Corresponding author. Fax: +1-606-257-7585; E-mail: htai1@pop.uky.edu

[1] On leave from the College of Pharmacy, West China University of Medical Sciences, Chengdu, China.

0167-4889/00/$ – see front matter
PII: S0167-4889(00)00039-2

Prostaglandins are rapidly metabolized and excreted in the urine. The first step in the metabolism of prostaglandins is catalyzed by NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) [3]. This enzyme is considered the key enzyme responsible for the biological inactivation of prostaglandins since the product 15-ketoprostaglandins possess greatly reduced biological activities [4]. The enzyme is ubiquitously present in mammalian tissues [5]. However, only a few cell types, including HL-60 cells [6], HEL cells [7], and human monocytes [8–10], have been identified to express the enzyme activity.

Regulation of the expression of this enzyme at the cellular level has been studied in some instances. Phorbol ester induces the synthesis of 15-PGDH in HL-60 cells [6] and in HEL cells [7] possibly mediated by protein kinase C activation. Dexamethasone and other glucocorticoids at the micromolar level stimulate the induction of 15-PGDH in HEL cells and the induced enzyme can be inactivated by PMA through a protein kinase C-mediated mechanism [7]. 1,25-Dihydroxyvitamin D_3 also induces 15-PGDH in human neonatal monocytes [10]. Molecular basis of the induction by these different agents is yet to be determined. However, the presence of a number of potential regulatory elements, such as Sp1, CRE, GRE, AP1 and AP2 in the promoter region of the 15-PGDH gene may provide potential binding sites for these agents to initiate gene transcription [11].

In this study, we report that 15-PGDH is induced by PMA in human promonocytic U937 cells and the induction is inhibited by dexamethasone and other glucocorticoids through a receptor-mediated mechanism. This is the first report documenting that dexamethasone is able to inhibit prostaglandin catabolism through inhibition of catabolic enzyme induction at subnanomolar concentrations.

2. Materials and methods

2.1. Materials

Phorbol 12-myristate 13-acetate (PMA), 4α-phorbol 12,13-didecanoate (PDD), NAD^+, α-ketoglutarate, dithiothreitol (DTT), staurosporine, bovine liver glutamate dehydrogenase (40 U/mg), RPMI 1640, penicillin, streptomycin, dexamethasone (DEX), mifepristone (RU-486), hydrocortisone, corticosterone, estradiol, progesterone, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma. GF 109203X was purchased from Alexis. PGE_2 was supplied by Cayman. ECL^+ plus Western Blotting Detection System RPN 2132 was from Amersham. Rabbit antiserum against human 15-PGDH was generated as described previously [12]. 15(*S*)-[15-^{3}H]PGE_2 was prepared according to a previously published procedure [13]. Human promonocytic U937 cells were obtained from the American Type Culture Collection. Other reagents were obtained from the best commercial sources.

2.2. Cell culture

U937 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 U penicillin/ml and 100 μg streptomycin/ml at 37°C in a humidified atmosphere of 5% CO_2. The cells were plated in 6-well plates (2 ml/well) at about 5×10^5 cells/ml in duplicate and grown overnight before treatment.

2.3. Treatment of U937 cells

After U937 cells were grown overnight, cells in each well received treatment with phorbol esters, dexamethasone, protein kinase C inhibitors or other steroids either alone or in different combinations as described in the figure legends.

2.4. Preparation of U937 cell homogenate

U937 cells from the above culture were spun down at maximal speed in a microfuge for 2 min and washed once with saline. About 1×10^6 cells were suspended in 1 ml of 0.05 M Tris–HCl buffer, pH 7.5 containing 1 mM DTT and sonicated in an ice bath for 3×10 s by an ultrasonic sonicator set at 4. The crude homogenate was used as an enzyme preparation.

2.5. Enzyme assay

15-PGDH was routinely assayed by measuring the transfer of ^{3}H from 15(*S*)-[15-^{3}H]PGE_2 to glutamate by coupling 15-PGDH with glutamate dehydrogen-

ase as described previously [13]. Briefly, the reaction mixture contained NH_4Cl (5 μmol), α-oxo-glutarate (1 μmol), NAD^+ (1 μmol), 15(*S*)-[15-^{3}H]PGE_2 (1 nmol, 30 000 cpm), glutamate dehydrogenase (100 μg), DTT (1 μmol) and 15-PGDH enzyme preparation in a final volume of 1 ml of 0.05-M Tris–HCl, pH 7.5. The reaction was allowed to continue for 10 min at 37°C, and was terminated by the addition of 0.3 ml of 10% aqueous charcoal suspension. After incubation for 5 min, the mixture was centrifuged at 2000×*g* for 5 min. The radioactivity in the supernatant was determined by liquid scintillation counting. Calculation of the amount of PGE_2 oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(*S*)-hydroxyl group of 15(*S*)-[15-^{3}H]PGE_2 as substrate. Enzyme activity of U937 sample was always assayed in duplicate.

2.6. SDS–PAGE and immunoblot analysis

U937 cells either treated or incubated with different stimuli were homogenated in phosphate-buffered saline containing 1 mM PMSF by sonication in an ice bath for 3×10 s. About 150 μg of cellular extract was resolved by SDS–PAGE (12% gel) according to the method of Laemmli [14]. Electrophoretic transfer of proteins from the gel to PVDF membrane was performed according to the method of Towbin et al. [15]. The membrane was blocked with 5% non-fat dry milk in 0.03 M Tris–HCl, pH 7.4 containing 120 mM NaCl and 0.05% Tween-20 (TBST) followed by incubation with a rabbit antiserum against the human placental 15-PGDH (1:2000 dilution in TBST with 5% non-fat milk) at room temperature for 2 h. After washing with TBST three times, the membrane was incubated with Protein A-HRP (1:5000 dilution in TBST with 5% non-fat milk) for 1 h at room temperature. The immunoreactive bands were detected with ECL$^+$ Plus Western Blotting Detection System. The intensity of the immunoreactive bands was determined by NIH 1.61 image.

2.7. Statistical analysis

Each enzyme sample was performed in duplicate. The data were expressed as the mean ± S.E. Statistical significance was assessed by Student's *t*-test using a *P* value of < 0.05. Each figure is a representative of 2–4 replications.

3. Results

Human promonocytic U937 cells were found to express 15-PGDH activity following treatment with PMA. The time course of 15-PGDH induction by PMA is shown in Fig. 1. Maximum induction was observed at 24–36 h following the addition of PMA as shown by the activity assay (Fig. 1a) and by immunoblot analysis (Fig. 1b). The effect of increasing concentrations of PMA on 15-PGDH induction is shown in Fig. 2. Maximal stimulation of 15-PGDH activity by PMA was observed at 10 nM as shown in Fig. 2. Higher concentrations of PMA were not as effective. PDD, an inactive phorbol ester, was shown

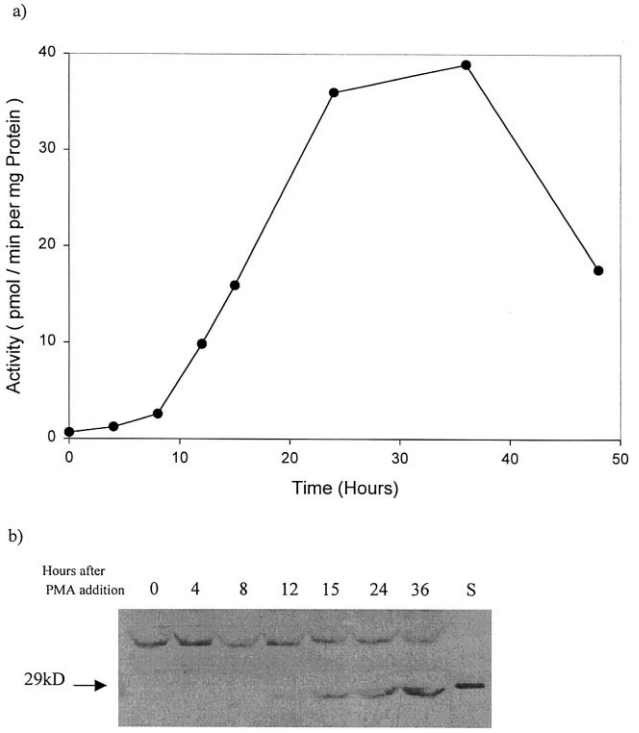

Fig. 1. The time course of 15-PGDH induction by PMA. U937 cells were treated with 10 nM of PMA for the indicated length of time. Cells were then washed and sonicated for the determination of 15-PGDH activity (a) and 15-PGDH immunoreactivity (b) as described in Section 2. Purified recombinant 15-PGDH (*S*) detected as a 29-kDa protein was included in the immunoblot. The higher molecular weight band was considered a non-specific protein detected by the antibodies.

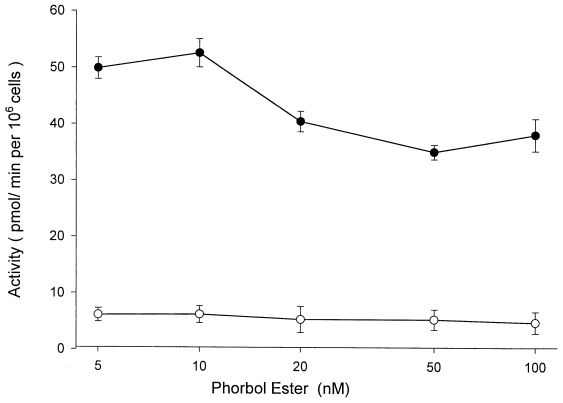

Fig. 2. Effects of PMA and PDD on 15-PGDH activity. U937 cells were treated with the indicated concentrations of PMA (●) or PDD (○) for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in Section 2.

to be unable to stimulate 15-PGDH activity at the concentrations tested. Stimulation of 15-PGDH activity by PMA was found to be inhibited by the addition of the protein kinase C inhibitors, staurosporine and GF 109203X [16,17]. Fig. 3 shows the effects of increasing concentrations of staurosporine or GF 109203X on PMA-induced stimulation of 15-PGDH activity. Almost total inhibition by staurosporine was observed at 100 nM, whereas about 75% inhibition by GF 109203X was seen at 1 μM.

Concurrent addition of dexamethasone with 10 nM PMA inhibited PMA-induced stimulation of

Table 1
The effect of steroids on the induction of 15-PGDH activity by PMA in U937 cells

	Activity (pmol/min/ 10^6 cells)	Inhibition (%)
Vehicle	5.9 ± 1.3	
PMA	52.5 ± 2.8	0
PMA+dexamethasone	10.1 ± 0.9	90.9
PMA+hydrocortisone	25.1 ± 3.0	58.7
PMA+corticosterone	23.6 ± 4.1	62.1
PMA+estradiol	47.6 ± 3.5	10.5
PMA+testosterone	46.6 ± 3.1	12.1
PMA+progesterone	46.2 ± 2.6	13.5
PMA+mifepristone	47.3 ± 3.9	11.1

U937 cells were treated either with vehicle, PMA (10 nM) alone or PMA plus various steroids (1 μM each) for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in Section 2. % inhibition was calculated after substraction of basal activity.

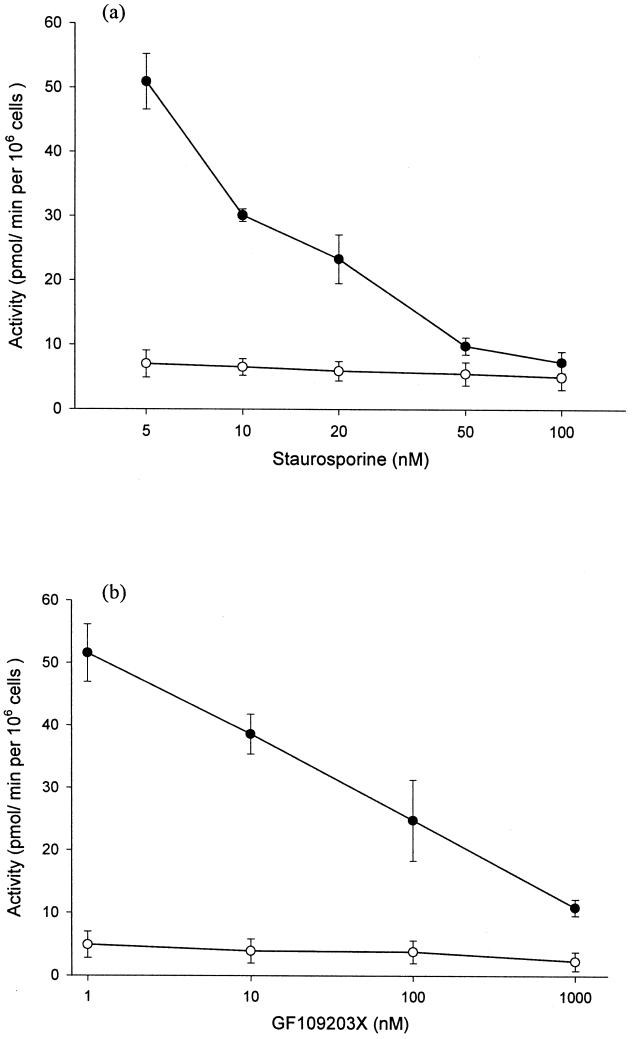

Fig. 3. Effect of staurosporine and GF 109203X on the induction of 15-PGDH activity by PMA. (a) U937 cells were treated either with staurosporine (○) or with 10 nM PMA plus staurosporine (●) at the indicated concentrations for 24 h. (b) U937 cells were treated either with GF 109203X (○) or with 10 nM PMA plus GF 109203X (●) at the indicated concentrations for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in Section 2.

15-PGDH activity in a concentration-dependent manner as shown in Fig. 4. Approximately 75% inhibition was seen at 10 nM. More than 90% inhibition was observed at 100 nM. The induction of 15-PGDH activity by PMA and the inhibition of dexamethasone were accompanied by the expression of enzyme protein as shown in Fig. 5. Western blot indicated that expression of enzyme protein was stimulated by PMA as revealed by the appearance

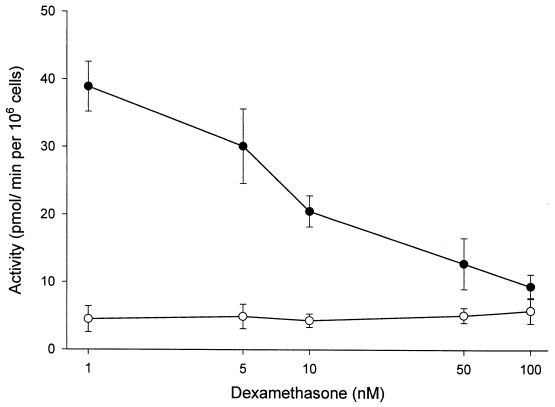

Fig. 4. Effect of dexamethasone on the induction of 15-PGDH activity by PMA. U937 cells were treated either with dexamethasone (○) or with 10 nM PMA plus dexamethasone (●) at the indicated concentrations for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in Section 2.

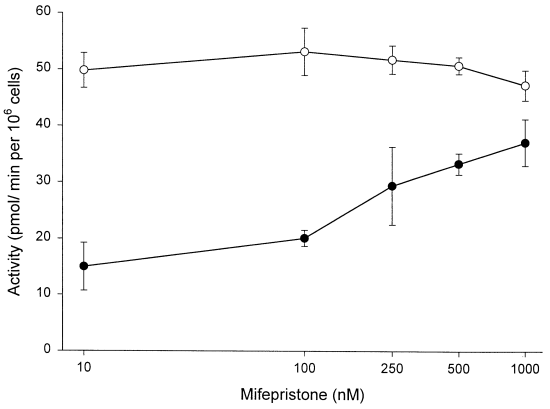

Fig. 6. Inhibition of PMA-induced 15-PGDH activity by dexamethasone is reversed by mifepristone. U937 cells were treated with 10 nM PMA plus the indicated concentrations of mifepristone (○) or 10 nM PMA plus 100 nM dexamethasone and the indicated concentrations of mifepristone (●) for 24 h. Cells were washed and sonicated for determination of 15-PGDH activity as described in Section 2.

of a 29-kDa protein which is the subunit size of 15-PGDH. The expression of 15-PGDH was nearly abolished by the addition of 10 nM dexamethasone and was completely blocked by the addition of 100 nM dexamethasone. Inhibition of PMA-mediated induction of 15-PGDH by dexamethasone was also observed with other glucocorticoids as shown in Table 1. More than 50% of the 15-PGDH activity induced by PMA was found to be inhibited by hydrocortisone and corticosterone at 1 μM. Other sex hormones and steroid receptor antagonist, mifepristone [18] showed much less inhibitory activity.

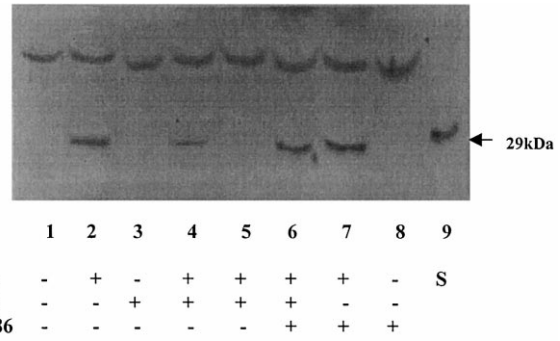

Fig. 5. Western blot analysis of 15-PGDH from U937 cells treated with PMA, dexamethasone, mifepristone (RU-486) or any combinations of the three different compounds. U937 cells were treated with vehicle (lane 1), PMA alone (10 nM, lane 2), dexamethasone alone (100 nM, lane 3), PMA (10 nM) plus dexamethasone (10 nM, lane 4), PMA (10 nM) plus dexamethasone (100 nM, lane 5), PMA (10 nM) plus dexamethasone (100 nM) and RU-486 (1 μM, lane 6), PMA (10 nM) plus RU-486 (1 μM, lane 7), or RU-486 alone (1 μM, lane 8) for 24 h. Cells were lysed and 150 μg protein was applied to each lane for SDS–PAGE and immunoblot analysis as described in Section 2. Purified Recombinant human 15-PGDH (S, lane 9) detected as a 29-kDa protein was run at the same time. The higher molecular band was considered a non-specific protein detected by the antibodies.

To examine if inhibition by dexamethasone is a receptor-mediated event, steroid receptor antagonist, mifepristone (RU-486), in increasing concentrations was added along with 10 nM dexamethasone during induction of 15-PGDH activity by PMA. Fig. 6 shows that mifepristone itself had little effect on PMA-induced 15-PGDH activity. However, dexamethasone-mediated inhibition of PMA-induced enzyme activity was reversed by mifepristone in a concentration-dependent manner. Mifepristone at 1 μM recovered most of the enzyme activity. Again, mifepristone did not affect the induction of the 29-kDa protein by PMA, but was able to antagonize the effect of dexamethasone since the expression of the 29-kDa protein still persisted when both dexamethasone and mifepristone were added as shown in Fig. 5. It appears that the induction of 15-PGDH activity coincided with the expression of the 29-kDa protein.

4. Discussion

Human promonocytic U937 cells have been shown

to exhibit prostaglandin biosynthetic activity following exposure to PMA [19]. The increase in biosynthetic activity was attributed to the induction of cyclooxygenase expression. Similar induction of prostaglandin biosynthetic activity by PMA was also observed with other leukemia cell lines, such as THP-1 [20] and HL-60 [21] cells. In addition to the induction of cyclooxygenase activity, PMA also stimulates the expression of 15-PGDH in HL-60 cells [6]. We examined, in this study, if PMA also induced the expression of 15-PGDH in addition to the expression of cyclooxygenase in U937 cells. The results indicate that the catabolic enzyme, 15-PGDH, was also induced by PMA. The expression of 15-PGDH activity in human monocytes was first suggested by Serhan's group who found that lipoxin A4 (LXA_4) was rapidly metabolized to 15-oxo-LXA_4, 13,14-dihydro-15-oxo-LXA4 and 13,14-dihydro-LXA_4 in adherent human monocytes [8]. The first step in the metabolism of LXA_4 is catalyzed by 15-PGDH which metabolizes hydroxy fatty acids as well as prostaglandins [22,23]. Recently, Dichaud et al. [10] reported that 15-PGDH-related mRNA was constitutively expressed in adult human monocytes and 15-PGDH activity could be induced by 1,25-dihydroxyvitamin D_3 in human neonatal monocytes. Whether 15-PGDH can be induced by PMA in these monocytes was not explored. Our finding in U937 cells suggests that 15-PGDH may be also induced by PMA in human monocytes.

The concentrations of PMA needed to induce 15-PGDH activity in U937 cells were comparable to those used in the induction of 15-PGDH in HL-60 cells [6]. PMA at 10 nM appeared to be optimal. Higher concentrations were found to be less effective. Studies in HL-60 cells [6] and HEL cells [7] indicate that higher concentrations of PMA may cause inactivation of 15-PGDH through PMA-mediated protein kinase C catalyzed phosphorylation of the enzyme. Whether this is true in U937 cells remains to be determined. The induction of 15-PGDH expression by PMA appears to be mediated by the activation of protein kinase C. This is supported by several lines of evidence. Firstly, PMA, a potent activator of protein kinase C, induced a concentration-dependent induction of 15-PGDH activity and enzyme protein, whereas inactive phorbol ester, PDD, did not show enzyme induction at any concentration tested. Secondly, two different types of protein kinase C inhibitors, staurosporine and GF 109203X, were able to inhibit PMA-induced expression of 15-PGDH activity. Thirdly, the induction of 15-PGDH by PMA is consistent with the fact that AP-1, AP-2 and CRE regulatory sequences, which are known to be involved in PMA-mediated protein kinase C-induced responses in other genes, are found in the 5′-flanking region of 15-PGDH gene [11].

The induction of 15-PGDH in U937 cells by PMA was found to be inhibited by dexamethasone at nanomolar concentrations. Inhibition by dexamethasone is shared by other glucocorticoids, but not significantly by sex hormones, indicating that inhibition of PMA-induced expression of 15-PGDH is a general property of anti-inflammatory glucocorticoids. Lack of effect by sex hormones may be also due to the absence of expression of the cognate receptors for these hormones in U937 cells. Glucocorticoids have also been demonstrated to block the induction of prostaglandin synthetic enzyme, cyclooxygenase-2 at nanomolar concentrations [2]. It was thought to be a mode of action of anti-inflammatory glucocorticoids. The inhibitory effect of dexamethasone on PMA-induced expression of 15-PGDH appeared to be a receptor-mediated event since the inhibition could be reversed by the presence of steroid receptor antagonist, mifepristone. However, the reversal was found to be incomplete. This could be due to the partial agonist activity of mifepristone since mifepristone itself showed some inhibition of PMA-induced 15-PGDH activity (Table 1). Partial agonist activity of mifepristone has been observed in other systems [24,25]. Repression of 15-PGDH expression by dexamethasone is in direct contrast to a previous report in which glucocorticoids were found to induce 15-PGDH in HEL cells [7]. However, the concentrations needed to induce 15-PGDH in HEL cells were at the submicromolar range of dexamethasone. Furthermore, progesterone was also found to be a potent inducer of 15-PGDH in HEL cells. This study shows that progesterone did not significantly inhibit PMA-induced expression of 15-PGDH activity in U937 cells. Micromolar concentrations of dexamethasone also did not induce expression of 15-PGDH activity in U937 cells. Therefore, it appears that steroid effect on 15-PGDH gene expression is cell type specific.

The mechanism(s) through which dexamethasone represses PMA-induced expression of 15-PGDH is not known. It is generally believed that dexamethasone acts by binding to the cytosolic glucocorticoid receptor which then translocates into the nucleus and engages in regulation of gene expression [26,27]. Most of the functions carried out by glucocorticoid receptor are at the level of transcriptional control, although some of which are post-transcriptional [28,29]. Transcriptional repression by the glucocorticoid receptor may be based on protein–protein interaction between the receptor and other transcriptional factors without the recruitment for DNA binding by the receptor [30]. Dexamethasone has been shown to repress the expression of cyclooxygenase-2 induced by PMA in part through CRE [31]. Although the mechanism by which dexamethasone elicits events that antagonize the activation of CRE is not clear, one plausible mechanism is that ligand–receptor complex interferes with CREB binding to its *cis*-acting element. A report indicating an interaction between CREB and the glucocorticoid receptor in human peripheral blood mononuclear cells has appeared [32]. The presence of CRE, AP-1 and AP-2, which are known to be involved in PMA-induced responses in other genes, in the 5′-flanking region of 15-PGDH gene indicates that dexamethasone may suppress the expression of 15-PGDH through inhibitory interaction with these potential regulatory elements.

Our finding that inhibition of PMA-induced expression of 15-PGDH by dexamethasone at the therapeutic level is a novel one. If prostaglandins are pro-inflammatory and dexamethasone is anti-inflammatory, one would expect that dexamethasone should enhance prostaglandin catabolism by stimulating the activity of 15-PGDH to facilitate removal of prostaglandins. In contrast, the inhibition of 15-PGDH expression, a previously unreported effect of dexamethasone, would appear to be a paradoxical effect of this anti-inflammatory drug. Similar paradoxical situation was found in the induction of cyclooxygenase-1 by dexamethasone and stem cell factor in mast cells [33]. However, recent evidence indicates that 15-PGDH may also regulate the action of endogenous anti-inflammatory eicosanoids, such as lipoxins and aspirin-triggered lipoxins (ATL) by oxidizing and inactivating these eicosanoids [8,34]. Several studies using stable lipoxin analogs and ATL lipoxin analogs, which resist rapid inactivation by 15-PGDH, showed that their anti-inflammatory actions were prolonged in vivo [34–36]. In view of these recent results and our findings, it appears that the anti-inflammatory action of dexamethasone may do so by prolonging the action(s) and in vivo biohalf-life of anti-inflammatory eicosanoids, such as lipoxins and ATL.

Acknowledgements

This work was supported in part by a grant from NIH (HL-46296).

References

[1] H.R. Herschman, Biochim. Biophys. Acta 1299 (1996) 125–140.
[2] J.L. Masferer, B.S. Zweifel, P.T. Manning, S.D. Hauser, K.M. Leahy, W.G. Smith, P.C. Isakson, K. Seibert, Proc. Natl. Acad. Sci. USA 91 (1994) 3228–3232.
[3] E. Anggard, B. Samuelsson, J. Biol. Chem. 239 (1964) 4097–4102.
[4] E. Anggard, Acta Physiol. Scand. 66 (1966) 509–510.
[5] E. Anggard, C. Larsson, B. Samuelsson, Acta Physiol. Scand. 81 (1971) 396–403.
[6] C.Q. Xun, Z.G. Tian, H.H. Tai, Biochem. J. 279 (1991) 553–558.
[7] C.Q. Xun, C.M. Ensor, H.H. Tai, Biochem Biophys. Res. Commun. 177 (1991) 1258–1265.
[8] C.N. Serhan, S. Fiore, D.A. Brezinski, S. Lynn, Biochemistry 32 (1993) 6313–6319.
[9] J.F. Maddox, C.N. Serhan, J. Exp. Med. 183 (1996) 137–146.
[10] F. Dichaud, S. Roux, J.L. Frendo, R. Delage-Mourroux, J. Maclouf, M.C. deVernejoul, M.S. Moukhtar, A. Jullienne, Blood 89 (1997) 2105–2112.
[11] M. Matsuo, C.M. Ensor, H.H. Tai, Biochem. Biophys. Res. Commun. 235 (1997) 582–586.
[12] C.L. Tai, O.T. Mak, T. Arai, H.H. Tai, Biochem. J. 267 (1990) 75–78.
[13] H.H. Tai, Biochemisty 15 (1996) 4586–4592.
[14] U.K. Laemmli, Nature 227 (1970) 680–685.
[15] H. Towbin, T. Staehelin, J. Gordon, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[16] T. Tamaoki, H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto, Biochem. Biophys. Res. Commun. 135 (1986) 397–402.
[17] D. Toullc, P. Dianetti, H. Coste, P. Bellevergue, T. Grand-Perret, M. Ajkane, V. Baudet, P. Boissin, E. Boursier, F.

Loriolle, L. Duhamel, D. Charon, J. Kirilovsky, J. Biol. Chem. 266 (1991) 15771–15781.
[18] K.B. Horwitz, Endocr. Rev. 13 (1992) 146–163.
[19] T. Nanayama, S. Hara, H. Inoue, C. Yokoyama, T. Tanabe, Prostaglandins 49 (1995) 371–382.
[20] C.J. Smith, J.D. Morrow, L.J. Roberts, L.J. Marnett, Biochem. Biophys. Res. Commun. 192 (1993) 787–793.
[21] M. Goering, A.J.R. Habenicht, R. Heitz, W. Zeh, H. Katus, B. Kommerell, R. Ziegler, J.A. Glomset, J. Clin. Invest. 79 (1987) 903–911.
[22] Y. Liu, K. Yoden, R.F. Shen, H.H. Tai, Biochem. Biophys. Res Commun. 129 (1985) 268–274.
[23] J.M. Bergholte, R.J. Soberman, R. Hayes, R.C. Murphy, R.T. Okita, Arch. Biochem. Biophys. 257 (1987) 444–450.
[24] B.M. Vayssiere, S. Dupont, A. Choquart, F. Petit, T. Garcia, C. Marchandeau, H. Gronemeyer, M. Resche-Region, Mol. Endocrinol. 11 (1997) 1245–1255.
[25] J. Liden, F. Delaunay, I. Rafter, J.A. Gustabsson, S. Okrat, J. Biol. Chem. 272 (1997) 21467–21472.
[26] S.M. Hollenberg, V. Giguere, P. Segui, R.M. Evans, Cell 49 (1987) 39–46.
[27] M. Danielsen, J.P. Northrop, J. Jonklaas, G.M. Ringold, Mol. Endocrinol. 1 (1987) 816–822.
[28] P.J. Knudsen, C.A. Dinarello, T.B. Strom, J. Immunol. 139 (1987) 4129–4134.
[29] A. Tobler, R. Meier, M. Seitz, B. Dewald, M. Baggiiolini, M.F. Freg, Blood 79 (1992) 45–51.
[30] S. Heck, M. Kullmann, A. Gast, H. Ponta, H.J. Rahmsdorf, P. Herrlich, A.C. Cato, EMBO J. 13 (1994) 4087–4095.
[31] Z. Wang, H.H. Tai, Prostaglandins Leukotrienes Essent. Fatty Acids 60 (1999) 243–248.
[32] I.M. Adcock, C.R. Brown, C.M. Gelder, H. Shiraski, M.J. Peters, P.J. Barnes, Am. J. Physiol. 268 (1995) C331–C338.
[33] J.M. Samet, M.B. Fasano, A.N. Fonteh, F.H. Chilton, J. Biol. Chem. 270 (1995) 8044–8049.
[34] C.N. Serhan, Prostaglandins 53 (1997) 107–137.
[35] T. Takano, S. Fiore, J.F. Maddox, H.R. Brady, N.A. Petasis, C.N. Serhan, J. Exp. Med. 185 (1997) 1693–1704.
[36] C.N. Serhan, J.F. Maddox, N.A. Petasis, I. Akritopoulou-Zanze, A. Papayianni, H.R. Brady, S.P. Colgan, J.L. Madara, Biochemistry 34 (1995) 14609–14615.