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COMMENTARY

Molecular Mechanisms Involved in the Regulation of Prostaglandin Biosynthesis by Glucocorticoids

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Abstract. The anti-inflammatory properties of glucocorticoids are attributed in part, to their interference with prostaglandin synthesis. Phospholipases A_2 and cyclooxygenases, the key enzymes of prostaglandin biosynthesis, are targets of glucocorticoid action; the molecular mechanisms, however, are not yet understood in detail. Obviously, glucocorticoids can act at different levels of gene regulation depending on cell type and inducing stimulus. The current knowledge of glucocorticoid interference with phospholipase A_2 and cyclooxygenase expression is summarized. In comparison with other nonsteroidal anti-inflammatory drugs, glucocorticoids are unique inasmuch as they also inhibit cytokine synthesis and expression of other inflammation-related enzymes. Based on a more detailed understanding of glucocorticoid action, it may be possible to therapeutically exploit the anti-inflammatory effects and at the same time avoid the unwanted metabolic actions of these steroids. BIOCHEM PHARMACOL 53;10:1389–1395, 1997. © 1997 Elsevier Science Inc.

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INTERFERENCE OF GLUCOCORTICOIDS WITH PROSTAGLANDIN SYNTHESIS

Glucocorticoids are potent anti-inflammatory and immunosuppressive drugs that act on almost all types of cells and show complex interactions with expression and function of multiple mediators. PGs† have long been recognized as targets of glucocorticoid action. PGs, especially PGE_2 and PGI_2, have proinflammatory properties, increasing vascular permeability and causing vasodilation and hyperalgesia [1]. In contrast to other inhibitors of PG synthesis, such as the nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids do not directly inhibit any of the enzymes of PG biosynthesis. Rather, the action of glucocorticoids is dependent on their interference with *de novo* protein biosynthesis, as will be outlined further. Consequently, basal PG synthesis is hardly affected by glucocorticoids, whereas induced synthesis is susceptible. This can be demonstrated *in vitro* and *in vivo*. An example is shown in Fig. 1. Differentiated and non-differentiated monocytic cells were incubated with different types of steroids [2]. PG synthesis was induced by the addition of exogenous arachidonic acid. Although both types of cells were capable of synthesizing and releasing PGs, only the activated synthesis in the differentiated cells was inhibited concentration dependently by dexamethasone and prednisolone. This inhibition was due to an interference of glucocorticoids with the expression of the inducible cyclooxygenase isoform Cox-2, as shown later [3]. At high concentrations, glucocorticoids act non-specifically, as demonstrated by the inhibition of PG synthesis by the gestagen progesterone. The steroids are related structurally to cholesterol, and the non-specific effect may relate to insertion of the steroids into cellular membranes. In *in vivo* studies, glucocorticoids were shown not to interfere with basal PG secretion in healthy human volunteers [4] or rabbits [5]. The basic enzyme machinery, necessary to synthesize prostanoids, was not changed by glucocorticoid treatment, whereas the inducible expression of PG-synthesizing enzymes was inhibited.

Glucocorticoids interfere with induction of PG synthesis caused by a wide variety of stimuli, notably the inflammatory cytokines interleukin-1β and tumor necrosis factor α. Not only do these cytokines induce cyclooxygenases, but they induce phospholipases as well. Thus, cyclooxygenases and phospholipases are potential targets of glucocorticoid-mediated inhibition of PG synthesis.

MOLECULAR BASIS OF GLUCOCORTICOID ACTION

It may appear somewhat surprising that the molecular mechanism of glucocorticoid inhibition of PG synthesis is still far from being understood in detail. To facilitate insight into the problems encountered by these investigations, a brief summary of the general mechanisms of glucocorticoid action is given. A more detailed discussion of glucocorticoid receptor action may be found in extended reviews (e.g. [6–9]). The basic features of glucocorticoid action can be summarized as follows: The free GR forms a heterocomplex with heat shock proteins hsp90, hsp56, and hsp70. This heterocomplex is located in the cytosol and, to a lesser

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† *Abbreviations*: Cox, cyclooxygenase; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; $sPLA_2$, secreted phospholipase A_2; $cPLA_2$, cytosolic phospholipase A_2; and PG, prostaglandin.

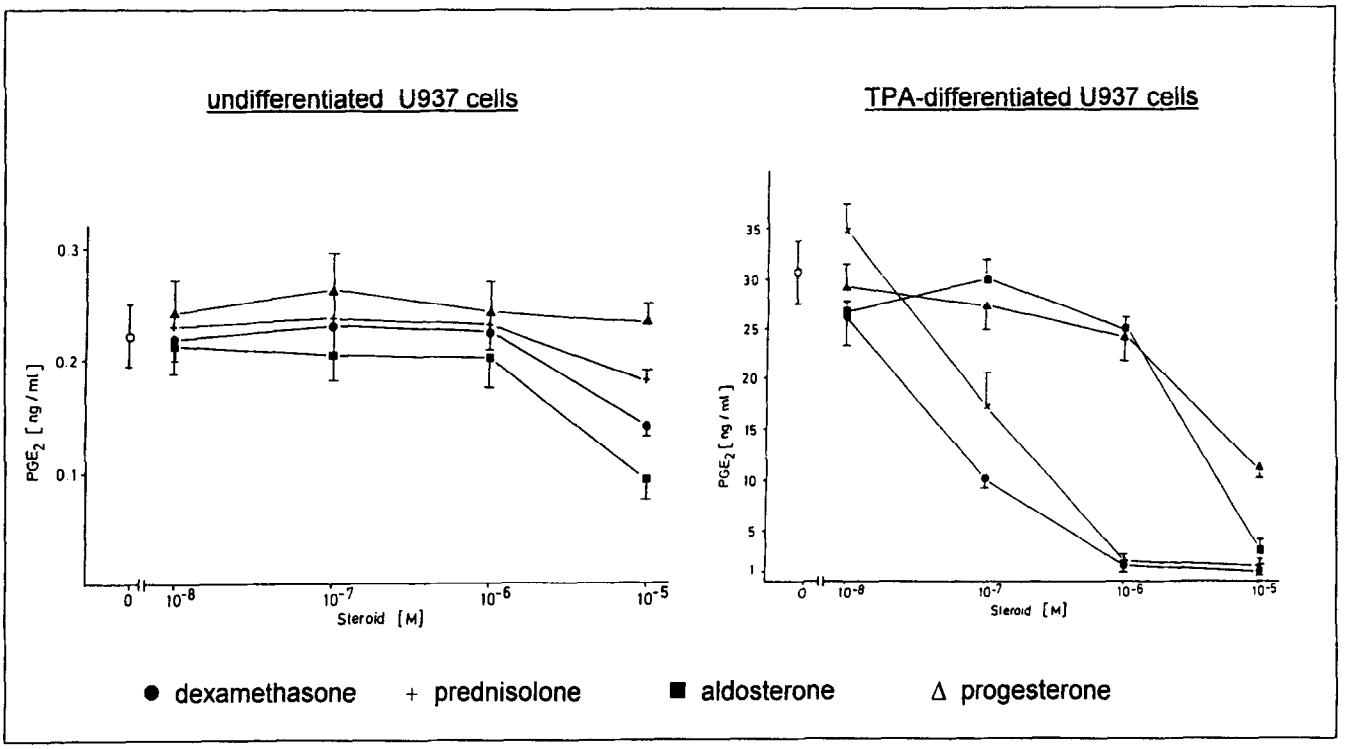

FIG. 1. Interference of glucocorticoids with PGE_2 synthesis. Undifferentiated U937 cells or cells differentiated with tetradecanoyl phorbol acetate (TPA, 5×10^{-9} M) for 72 hr were incubated for 24 hr with the steroids as indicated. Arachidonic acid (10^{-5} M) was added for the last 4 hr. PGE_2 synthesis was determined in the cell culture supernatants by radioimmunoassay [2]. Data are means ± SD of two separate wells assayed in duplicate. Reprinted by permission of the publisher from *Biochem Pharmacol* 40: 1307–1316, 1990. Copyright 1990 by Elsevier Science Inc. [Ref. 2].

extent, in the nucleus. According to present knowledge, glucocorticoids enter the cells without the help of a carrier protein. Binding of the hormone leads to conformational changes of the receptor complex, dissociation of the heat shock proteins, and translocation of the receptor-hormone complex to the nucleus. The receptor-hormone complex acts as a transcription factor: It binds to certain DNA sequences (GRE) in the promoter regions of sensitive genes. Gene transcription can be enhanced or decreased by the GR. Recent data from several laboratories consistently indicate that the outcome of GR action does not depend solely on GR-DNA interaction. Interaction with other transcription factors is an essential feature of GR-mediated effects and seems to be a key to understanding cell- and stimulus-dependent effects of glucocorticoids.

Basically, three possible mechanisms of gene regulation by GRs have to be considered:

1. Direct enhancement of transcription of the target gene (examples in Table 1): This list contains proteins involved in protein and carbohydrate metabolism pointing to the physiological role of glucocorticoids. Surprisingly, Cox-2, the key enzyme of PG synthesis, can be a target of positive regulation by glucocorticoids in certain cell types. It was shown before that glucocorticoids enhance PG synthesis in amnion cells [18] and recently evidence was provided that in these cells Cox-2 is a direct target of positive glucocorticoid action [17].
2. Direct inhibition of transcription of the target gene via binding to negative GR elements or interaction with other transcription factors (examples in Table 2): The negative effects of glucocorticoids on gene transcription seem to be highly dependent on protein-protein interaction with binding of the GR to DNA being less important.
3. Indirect modulation of gene expression: In this case, the GR-sensitive gene product is an intermediary factor that modulates gene expression of the apparently glucocorticoid-sensitive target gene. The latter mechanism may be of particular relevance for the immunosuppressive effects of glucocorticoids and possibly also for their anti-

TABLE 1. Positive regulation of gene expression by glucocorticoids

Regulated gene	Interacting transcription factors	Ref(s).
Aspartate aminotransferase	GR tetramer	[10]
Phosphoenolpyruvate carboxykinase	GR/CREB	[11]
Tyrosine aminotransferase	HNF3	[12, 13]
Somatostatin	GR/CREB	[14]
Angiotensin II Type 1A receptor		[15, 16]
Cyclooxygenase-2		[17]

Examples of gene promoters are given that have been shown to be activated directly by the glucocorticoid receptor.

TABLE 2. Negative regulation of gene expression by glucocorticoids

Regulated gene	Interacting transcription factors	Ref(s).
Prooplomelanocortin		[19]
Proliferin	AP-1	[20]
Collagenease	AP-1	[21–23]
Interleukin-2	AP-1	[24, 25]
Interferon-γ	AP-1 CREB-ATF	[26]
MCP-1*	AP-1	[11]
c-*fos*	SRF	[27]
Interleukin 8	NF-κB	[28]
Interleukin-6	NF-κB	[29]
ICAM-1†	NF-κB	[30]

Examples of genes are given, the transcription of which is regulated negatively by the glucocorticoid receptor.

* MCP, monocyte chemoattractant protein.

† ICAM, intracellular adhesion molecule.

inflammatory actions, because most of the cytokines and enzymes involved in eicosanoid metabolism do not contain negative GREs in their promoters. Interference of glucocorticoids with one of the main transcription factor families, the NF-κB/-κB family, was shown recently by several groups [31, 32].

Thus far, none of the mechanisms discussed has been shown to mediate the effects of glucocorticoids on the enzymes involved in PG biosynthesis, although all of the essential enzymes are targets of glucocorticoid action.

INHIBITION OF PHOSPHOLIPASES A_2 BY GLUCOCORTICOIDS

Glucocorticoids inhibit both types of phospholipases involved in prostanoid synthesis under pathological conditions; the secreted "inflammatory" phospholipase A_2 ($sPLA_2$ type II) and the cytosolic enzyme ($cPLA_2$, type IV).

In the 1980s the hypothesis was put forward and readily accepted that glucocorticoids induce proteins called lipocortins (later termed annexins) that were characterized by their ability to bind calcium and phospholipids and thus inhibit phospholipase A_2 activity [33]. In our own investigations, we could not show any correlation between lipocortin expression and glucocorticoid effects on PG synthesis or phospholipase activity. In differentiated U937 cells, expression of lipocortin I and II was, if at all, reduced by glucocorticoids [2]. Similar results were obtained by several other groups (e.g. Refs. 34–36). Lipocortins (or annexins) are abundant proteins found in all cells. Depending on the cellular system, expression of some of the isoforms was reported to be regulated by glucocorticoids (e.g. Ref. 37). In general, however, lipocortins are no longer considered to be essential mediators of glucocorticoid-induced inhibition of phospholipase A_2, although they inhibit prostanoid release when applied exogenously [38]. *In vivo*, an increase in phospholipase activity and a decrease in lipocortin expression were detected in adrenalectomized rats; however, this does not necessarily demonstrate a causal relation [39].

Depending on cell type and stimulus, inhibition of $sPLA_2$ by glucocorticoids is seen at the mRNA or at the protein level, suggesting transcriptional as well as posttranscriptional mechanisms that have not been defined yet at the molecular level [36, 40, 41]. The secreted phospholipase A_2 is also involved in defense mechanisms and secreted as acute phase protein (e.g. Refs. 42–45). Expression of acute phase proteins, in general, is either enhanced or not influenced by glucocorticoids. Recent data from our group show that glucocorticoids also interfere with induction of $sPLA_2$ expression in hepatocytes.[1]

Activity of $cPLA_2$ is rapidly regulated by changes in intracellular Ca^{2+} levels, phosphorylation, and membrane association. Depending on the stimulus, glucocorticoids may interfere with the signaling cascade leading to activation of $cPLA_2$, as shown recently in macrophages [46]. When incubation is prolonged, $cPLA_2$ mRNA expression is enhanced by cytokines and is sensitive to glucocorticoid inhibition. We first demonstrated regulation by tumor necrosis factor α and dexamethasone in epithelial cells [47]. Our findings have since been confirmed and investigated in more detail in other cellular systems [48]. The molecular mechanisms of glucocorticoid-mediated inhibition of $cPLA_2$ expression have not been worked out. The promoter of $cPLA_2$ gene contains two positive GREs, but functionality, i.e. induction of $cPLA_2$ by glucocorticoids, has not been shown thus far [49, 50].

Inhibition of phospholipases alone, however, could not account for the effects observed on PG synthesis. In experiments with bone marrow-derived macrophages, we found the enzyme activity of the cyclooxygenase more strongly inhibited by dexamethasone than phospholipase A_2 activity, suggesting cyclooxygenase as an additional target for glucocorticoid action [51].

INTERFERENCE OF GLUCOCORTICOIDS WITH CYCLOOXYGENASE ISOZYME ACTIVITY

Intensive investigation of the two cyclooxygenase isoforms over the last 5 years has provided a lot of information regarding the structure of the genes, including the promoter region and the regulation of gene expression by various bioactive mediators (summarized in Refs. 52 and 53). It became evident that both enzymes, in particular Cox-2, are regulated in a cell- and stimulus-dependent way. Therefore, generalizations regarding enzyme regulation should be made rather cautiously.

Cox-1 is often referred to as a "constitutive" enzyme, alluding to the observation that Cox-1 expression remains unaffected by most stimuli in cell culture systems. The promoter region of the gene, however, contains binding

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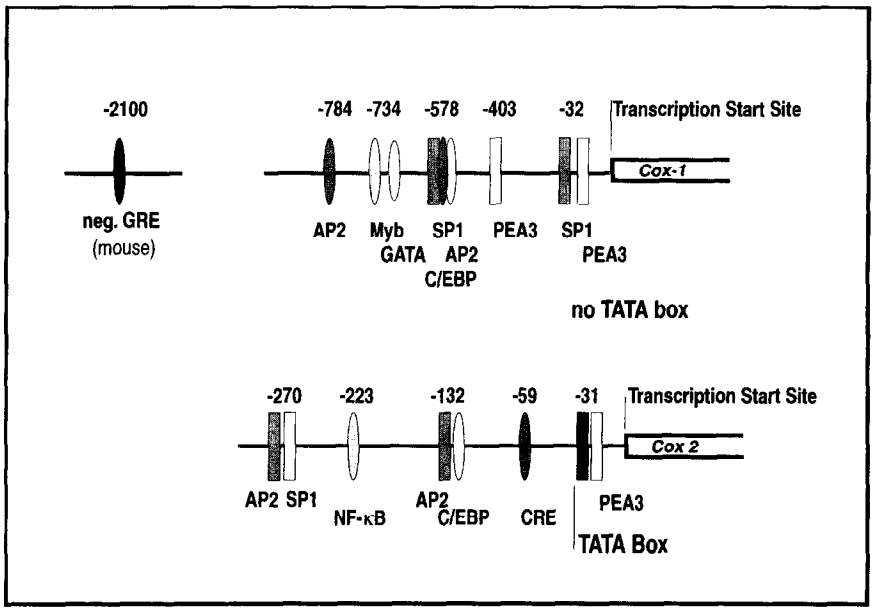

FIG. 2. Regulatory elements of Cox-1 and Cox-2 promoters. Location of the regulatory elements is according to Inoue *et al.* [54]. Additional regulatory elements are located further upstream in the Cox-2 promoter [55].

sequences for several transcription factors (Fig. 2 modified according to Ref. 54; additional binding sites for transcription factors were identified further upstream in the promoter [55]). Regulation of Cox-1 expression was observed during monocytic differentiation and was sensitive to inhibition by glucocorticoids [3]. Whether the inhibition is related to the putative negative GR element detected in the promoter region of the Cox-1 gene was not investigated further. Similarly, Cox-1 expression was increased modestly by serum [56] or long-term incubation with platelet-derived growth factor [57]. Inflammatory stimuli, however, usually affect Cox-2 expression without any changes in Cox-1 mRNA or protein expression. Nevertheless, recent data obtained with Cox-1 knock-out mice provided evidence that Cox-1 plays a role in certain states of inflammatory reactions [58]. It may be predicted that, under those circumstances, phospholipases may be the up-regulated enzymes.

In general, modulation of Cox-2 expression—positive or negative—can be envisaged at three different levels: mRNA translation, mRNA stability, or gene transcription.

Regulation at the translational level has hardly been investigated. Glucocorticoids were assumed to interfere at this level, because in some systems protein expression of Cox-2 was more sensitive to inhibition by glucocorticoids than mRNA levels [56, 59]. Thus far, no mechanistic model has been proposed to explain the glucocorticoid-mediated inhibition at the translational level.

In its 3′-untranslated region, Cox-2 mRNA contains a high number of so-called Shaw-Kamen sequences AUUUA, which are found in many immediate-early genes and have been shown to relate to enhanced mRNA degradation [55]. Consistent with this finding, the half-life of Cox-2 mRNA seems to be short, the shortest time reported being 30 min [60]. An increase in Cox-2 mRNA stability was described in several cellular systems, most notably in cells stimulated by interleukin-1 [60, 61]. Conclusions regarding mRNA stability are usually drawn from experiments where transcriptional activity is blocked by actinomycin D. These experiments should be viewed with some caution: half-lives obtained by this method may far extend the half-life observed in cells in the absence of actinomycin D, suggesting interference of actinomycin D with short-lived regulatory proteins [62]. Kinetic experiments also argued in favor of an interference of glucocorticoids with mRNA stability: even when dexamethasone was added to cells after the stimulus, at a time when the maximal transcription rate had been achieved already, it was still able to suppress steady-state mRNA levels [56, 63]. Several years ago, a proposal was put forward by Bailey *et al.* [64] that glucocorticoids might induce protein(s) that was assumed to interfere with mRNA accessibility and further processing by binding to the 3′-untranslated region. This hypothesis has neither been confirmed nor rejected.

Transcriptional regulation of Cox-2 has been investigated in more detail. The Cox-2 promoter was analyzed in several species (e.g. Refs. 55 and 65–67) and contains potential binding sites for various transcription factors (Fig. 2). Most of these elements are conserved between species, but located at different positions within the promoter. The AP-1 site, the CRE element, the NF-κB and the C/EBP (NF-IL6) site, which are located within the first 400 bp, were shown to be functional (Table 3). Again, these results cannot be generalized: overexpression of the oncogene v-*src* in fibroblasts led to an enhanced expression of Cox-2, whereas overexpression of v-*src* in rat mesangial cells did not enhance Cox-2 expression, but enhanced Cox-1 expression and rather prevented induction of Cox-2 by stimuli such as platelet-derived growth factor AB or BB [72].

Interference of glucocorticoids with transcription was observed in functional studies: reduced transcriptional ac-

TABLE 3. Transcriptional regulation of Cox-2

Response element	Cell type	Stimulus	Ref.
C/EBP	Rat granulosa cells	hCG	[65]
AP-1, CRE	Chicken fibroblasts	Serum	[68]
CRE	Mouse fibroblasts	v-src	[69]
NF-κB, C/EBP	Mouse osteoblastic cells	TNFα	[70]
CRE	Human monocytic cells	Differentiation	[71]
CRE, C/EBP	Human endothelial cells	LPS/TPA	[54]

Transcriptional regulation of Cox-2 has been analyzed in different cellular systems. Abbreviations: C/EBP is also called NF-IL6 or IL-6REBP; hCG, human chorionic gonadotropin; TNF, tumor necrosis factor; LPS, lipopolysaccharide; and TPA, tetradecaonyl phorbol acetate.

tivity by dexamethasone was reported in serum-stimulated fibroblasts [56]. In mesangial cells stimulated with platelet-derived growth factor, we could show that the reduced Cox-2 mRNA levels were not due to message destabilization, also suggesting that glucocorticoids interfere with transcription (unpublished results). The mechanism of the transcriptional inhibition remains unclear. The negative GRE shows some variability in sequence, but no putative glucocorticoid receptor binding site was detected in the Cox-2 genes of different species sequenced thus far. Attempts to prove glucocorticoid effects in promoter transfection experiments have not been successful [68]. These data suggest that either the GR itself may act outside the DNA regions investigated so far, or, more likely, a glucocorticoid-sensitive transcription factor interferes with transcriptional activation of the Cox-2 gene. Although there is ample evidence that glucocorticoids interfere with PG synthesis by diminishing Cox-2 mRNA, as determined by Northern blot analyses, the precise molecular mechanisms are still not known.

Cox-2 as a target of glucocorticoid regulation was confirmed by *ex vivo* studies [73]: Cox-2 levels were undetectable in macrophages from normal rats, but were elevated following adrenalectomy, and were not detectable in adrenalectomized animals receiving dexamethasone replacement therapy. These data show that also under normal physiological conditions, Cox-2 levels are regulated by endogenous glucocorticoids.

CONCLUSION

Investigations conducted over the last couple of years have provided us with concepts of how glucocorticoids work, as well as insights into the regulation of phospholipase and cyclooxygenase expression. The task ahead will now be to bring the different pieces of this complicated puzzle together to understand more clearly the molecular mechanisms of glucocorticoid-mediated inhibition of PG synthesis.

It is doubtful whether a unifying mechanism will emerge, which will explain phospholipase or cyclooxygenase inhibition in all cells and by all stimuli. An important aspect will be the analysis of the interaction between the glucocorticoid receptor and other transcription factors. This might then be the basis of pharmacological concepts of how to mimic interactions of the glucocorticoid receptor not with all but with certain transcription factors that are involved in the regulation of a limited number of genes. Following this hypothesis, it might be possible not only to affect enzymes of PG biosynthesis but also other proinflammatory proteins, such as cytokines, the inducible NO synthase, or chemokines, which are known targets of glucocorticoids. Ideally, transcription factors should be targeted which are not, or are to a lesser extent, involved in the regulation of enzymes related to basic cellular metabolism.

Cell specificity and stimulus dependency of glucocorticoid action may lead the way to novel drugs that may more specifically interfere with a certain subset of inflammation-related proteins than do the glucocorticoids of today.

Note added in proof: In a recent paper, Ristimäki *et al.* [74] showed evidence for post-transcriptional destabilization of cytokine-induced Cox-2 mRNA by dexamethasone.

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