Glucocorticoids Suppress Group II Phospholipase A2 Production by Blocking mRNA Synthesis and Post-transcriptional Expression*

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We investigated the effects of glucocorticoids on group II phospholipase A2 (PLA2) expression in rat cultured smooth muscle cells. Both forskolin-induced and tumor necrosis factor (TNF)-induced PLA2 release responses were almost completely blocked by 10 and 100 nm dexamethasone, respectively, as assayed by protein blotting and PLA2 activity assays. Dexamethasone-mediated inhibition of PLA2 release appeared to be mediated by the glucocorticoid receptor. Dexamethasone at concentrations >10 nm inhibited forskolin-induced elevation of the group II PLA2 mRNA level but not TNF-induced elevation. These data suggest that the mechanism mediating forskolin-induced mRNA accumulation is sensitive to glucocorticoids, but the mechanism mediating the TNF-induced accumulation is not. Inhibition of TNF-induced PLA2 release by glucocorticoids may be explained by the blocking of post-transcriptional synthesis of the group II PLA2.

Glucocorticoids are among the most potent and widely used anti-inflammatory agents. However, the mechanism of their action is not fully understood. At least part of their effects are thought to be due to a decrease in the formation of pro-inflammatory prostaglandins and lipoxigenase products. It has been widely accepted that glucocorticoids induce the formation of phospholipase A2 (PLA2) inhibitory proteins known as lipocortins, thereby decreasing the release of arachidonic acid from lipids (1, 2). However, proteins of the lipocortin family, which are also known as calcapsins (3), are present in rat kidney in high concentrations in many cell types, and it is not clear whether they are glucocorticoid-inducible or are PLA2 inhibitors rather than phospholipid-binding proteins (4). Moreover, some researchers have recently reported that the effects of glucocorticoids for inhibiting PLA2 activity and prostanoid synthesis are independent of lipocortin (5, 6). The present study was performed to elucidate the mechanism mediating forskolin-induced mRNA accumulation and to characterize further the anti-inflammatory action of glucocorticoids, and we found that dexamethasone inhibited the production of group II PLA2 by blocking the accumulation of group II PLA2-encoding mRNA and the post-transcriptional expression of the protein.

**EXPERIMENTAL PROCEDURES**

**Isolation and Cultivation of SMCs**—Rat thoracic aortic SMCs were isolated by enzymatic digestion of media of thoracic aorta from male Sprague-Dawley rats as described by Charnley-Campbell et al. (20). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and subcultured every 3-4 days.

**Activation of SMCs**—Confluent SMCs in 90-mm diameter dishes were washed twice with Dulbecco's modified Eagle's medium and incubated with 8 ml of medium containing 0.1 mg/ml bovine serum albumin with or without the agents as described in the figure legends. At the end of incubation, the medium was removed for use in the PLA2 assay and protein blotting, and the cells for use in the RNA blotting and protein blotting.

**PLA2 Activity Assay**—Phospholipase activity was measured by the hydrolysis of [3H]oleic acid (Amersham Corp.)-labeled Escherichia coli phospholipid (21). The assay mixture contained 100 mM Tris, pH 7.4, and 1 mM CaCl2. The medium of the culture was diluted to produce hydrolysis of up to 10% of the substrate. Reaction mixtures were incubated for 1 h at 57 °C, and the released [3H]oleic acid was extracted and measured as described elsewhere (21).

**Protein Blotting**—Rabbit anti-rat group II PLA2 IgG was produced against PLA2 released from thrombin-stimulated rat platelets which was purified as described previously (22). One percent of the medium or the cells collected from a 90-mm diameter dish was subjected to sodium dodecyl sulfate-gel electrophoresis. Proteins were transferred onto a nitrocellulose membrane, and immunoblotting with the anti-rat group II PLA2 IgG or anti-lipocortin I antiserum was done using a blotting detection kit (Amersham Corp.).

**RNA Blotting**—Total cellular RNA was prepared by the method of Chomczynski and Sacchi (23). The RNA (15 μg) was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred onto a nylon membrane by a standard method (24). After immobilization of the RNA by baking and UV cross-linking, hybridization was performed as described by Church and Gilbert (25). Rat group II PLA2 cDNA (26) was labeled with [α-32P]dCTP (Du Pont-New England Nuclear, 5000 Ci/mmol) using a random primer labeling system (Amersham Corp.) and was used as a probe. After hybridization, the membrane was washed several times with 1.5 M sodium citrate, 15 mM NaCl, 0.1% sodium dodecyl sulfate at 65 °C. The signal was detected by autoradiography.

**Materials**—Human recombinant TNF-α was obtained from Gen-
zyme. The amount of TNF was expressed in units as defined by the supplier. Forskolin and steroids were purchased from Sigma. Other chemicals were of reagent grade.

RESULTS

Inhibition of PLA₂ Secretion from SMCs by Dexamethasone—Our previous results (19) have shown that group II PLA₂ gene expression and the release of the PLA₂ enzyme from SMCs are induced by at least two distinct mechanisms. One mechanism is mediated by cAMP-elevating agents such as forskolin, isobutylmethylxanthine, and dibutyryl cAMP and the other by inflammatory factors such as IL-1, TNF, and lipopolysaccharide. In this study, we first examined the effect of dexamethasone on the PLA₂ release caused by the two mechanisms. Fig. 1 shows that the release of PLA₂ activity from SMCs induced by forskolin and the release induced by TNF were both inhibited by dexamethasone. However, the dose-response curves were not identical. Inhibiting the TNF-induced response required higher concentrations of dexamethasone than inhibiting the forskolin-induced response.

To rule out the possibility of the decrease of the extracellular PLA₂ activity being caused by enhanced secretion of PLA₂ inhibitory proteins such as lipocortins, we performed protein blotting analyses using anti-rat group II PLA₂ IgG. As shown in Fig. 2, extracellular release of group II PLA₂ was actually inhibited by dexamethasone. Forskolin-induced PLA₂ release was almost completely inhibited by 10 nM dexamethasone and the TNF-induced release by 100 nM dexamethasone, which agrees with the results of Fig. 1 from the PLA₂ activity assay. Furthermore, a protein blotting study using anti-lipocortin I antiserum revealed that dexamethasone did not increase the release of lipocortin I from SMCs and the accumulation of the protein in the cells (data not shown).

Effects of Other Steroids on PLA₂ Release—In order to know whether dexamethasone-mediated inhibition of group II PLA₂ release from SMCs was specific for glucocorticoid activity, we examined other steroids for their effect on forskolin- or TNF-induced PLA₂ release. Interaction of SMCs with 100 nM hydrocortisone or 1000 nM aldosterone almost completely inhibited forskolin-induced PLA₂ release (Fig. 3A). The effect of progesterone, a sex steroid, was weak; it inhibited the PLA₂ release by only about 30%, even at 1000 nM. Similar results were obtained for TNF-induced PLA₂ release (Fig. 3B), but higher concentrations of steroids were necessary to inhibit the TNF-induced response. Although progesterone was shown to have much less glucocorticoid activity (Fig. 3), it has been known to compete with glucocorticoids for receptor occupancy (27). Next, we tested the effect of progesterone on dexamethasone-mediated suppression of PLA₂ release. As can be seen from Table I, progesterone abrogated the dexamethasone-mediated suppression of PLA₂ release.

Effect of Dexamethasone on the Induction of Group II PLA₂ mRNA—The above results indicate that one or more steps preceding the PLA₂ release are inhibited by the action of dexamethasone. Protein blotting analysis revealed that neither TNF nor forskolin enhanced the accumulation of PLA₂ protein in 10 μM dexamethasone-pretreated cells (data not shown), indicating that the steps inhibited by dexamethasone preceded the synthesis of the PLA₂ protein. Next, in order to examine whether the suppression of the group II PLA₂ release by dexamethasone was associated with suppression at the mRNA level, we carried out RNA blotting analyses. Dexamethasone, at concentrations >10 nM, remarkably suppressed forskolin-induced elevation of the group II PLA₂ mRNA level (Fig. 4A), which agrees with the results of the effect of dexamethasone on forskolin-induced PLA₂ release. On the other hand, as shown in Fig. 4B, 10–1000 nM dexamethasone had little effect on TNF-induced accumulation of the mRNA.

![Fig. 1. Inhibition of PLA₂ release by dexamethasone. SMCs were exposed to hydrocortisone (O), aldosterone (O), or progesterone (A) at various concentrations for 1 h and then stimulated with 10 μM forskolin (○) or 200 units/ml TNF (●), or vehicle (□) for an additional 24 h. The supernatants were collected and analyzed for PLA₂ activity. Data are the mean values from a representative experiment performed in duplicate.](image)

![Fig. 2. Inhibitory effect of dexamethasone on the release of PLA₂ protein. SMCs were incubated with dexamethasone at various concentrations for 1 h and then stimulated with 10 μM forskolin (A) or 200 units/ml TNF (B), or vehicle (Control) for 24 h. The supernatants were analyzed by protein blotting with anti-group II PLA₂ IgG.](image)

![Fig. 3. Effect of some steroids on PLA₂ release. SMCs were exposed to hydrocortisone (O), aldosterone (●), or progesterone (△) at various concentrations for 1 h and then stimulated with 10 μM forskolin (A) or 200 units/ml TNF (B). Stimulation-induced increase of PLA₂ activity in the medium is expressed with the value without steroids as 100%. Data are the mean values from a representative experiment carried out in duplicate.](image)

![Table I](image)
although the PLA₂ assay and protein blotting study (Figs. 1 and 2) demonstrated that dexamethasone at concentrations >100 nM severely suppressed TNF-induced PLA₂ release.

**DISCUSSION**

In the present study, we clarified for the first time that glucocorticoids negatively regulate the synthesis and release of group II PLA₂ from SMCs, which is most likely mediated by activation of the glucocorticoid receptor. The inhibition of PLA₂ activity by glucocorticoids has long been attributed to the production of lipocortins (1, 2). However, some recent reports have described results negative to the theory (5–8), and the mechanism of PLA₂ suppression by glucocorticoids has become obscure again. Our findings demonstrating that glucocorticoids directly control the synthesis and the extracellular release of PLA₂ provide one possible explanation for the glucocorticoid-mediated PLA₂ suppression.

The RNA blotting study revealed that dexamethasone inhibited forskolin-induced elevation of the group II PLA₂ mRNA level but not TNF-induced elevation. We have reported that forskolin and TNF stimulate elevation of the mRNA level through distinct mechanisms (19), which was also supported by the different effects of cycloheximide on the two mechanisms. The different susceptibilities of the two pathways to dexamethasone found in this study further prove that forskolin and TNF stimulate the PLA₂ gene via different cellular machinery such as different trans-acting factors and different cis-acting elements, whose potencies to interact with the glucocorticoid receptor may be different.

Although the mechanism of gene regulation by TNF has not been well characterized, many of the cAMP-inducible genes have been shown to possess similar cAMP-responsive elements (28). The group II PLA₂ gene could be classified into this cAMP-responsive gene family based on the characteristic that forskolin-induced PLA₂ gene activation does not require prior protein synthesis, which is the case with the genes in the family. The glucocorticoid receptor is known to activate gene expression by binding to a specific glucocorticoid-responsive element (29); however, this cannot explain the mechanism by which glucocorticoid inhibits the transcription of specific genes. Recently, Akerblom et al. (30) have revealed that the glucocorticoid receptor negatively regulates gene expression by interfering with the cAMP-responsive elements. Although it is not known whether the group II PLA₂ gene has the cAMP-responsive elements, negative regulation of the PLA₂ gene by the glucocorticoid receptor may be conferred by interference with some elements regulating the PLA₂ gene. More precise analysis of the interaction of the glucocorticoid receptor with the PLA₂ gene is necessary to clarify the molecular mechanism of the regulation.

Inhibition of TNF-induced PLA₂ release by glucocorticoids without the inhibition of mRNA synthesis suggests that glucocorticoids can also block the PLA₂ expression at a post-transcriptional level. Since TNF did not induce the accumulation of the PLA₂ in dexamethasone-pretreated cells, glucocorticoids probably inhibit the synthesis of the PLA₂. The differences in the dose-response curves in Fig. 1 indicate that inhibition of the PLA₂ expression at the post-transcriptional level requires 10 times higher concentrations of glucocorticoids than inhibition of cAMP-mediated mRNA synthesis. Separate transcriptional and post-transcriptional effects of dexamethasone have been reported from the studies of Beutler et al. (31) on the synthesis of TNF and those of Knudsen et al. (32) on the synthesis of IL-1. Knudsen et al. (32) postulated that suppression by dexamethasone at the post-transcriptional level was mediated by dexamethasone-induced elevation of the AMP level. However, cAMP-mediated suppression cannot occur in our system because TNF and forskolin synergistically stimulate PLA₂ release from SMCs (19).

Therefore, the mechanism of glucocorticoid inhibition of PLA₂ expression at the post-transcriptional level remains obscure.

Although glucocorticoids often have been reported to up-regulate mRNA expression of eukaryotic genes, they generally act as negative regulators for mRNA expression of cytokines such as IL-1 (32), IL-2 (33), IL-3 (34), TNF (31), and interferon-γ (33). Similar regulation of the expression of group II PLA₂ and the cytokines by glucocorticoids offers further evidence for the important role of PLA₂ in inflammatory and immune responses.

This study using cultured rat SMCs suggests that group II PLA₂ plays an important role in the pathogenesis of vascular inflammatory processes such as vasculitis and atherosclerosis. Our recent study also showed that TNF and forskolin stimulated rat cultured chondrocytes to release group II PLA₂, suggesting that PLA₂ is also involved in the pathogenesis of articular joint inflammation and rheumatoid arthritis and that PLA₂ is similarly regulated in many cell types. However, in the vascular system, production of PLA₂ may be specific to SMCs because cultured rat endothelial cells did not respond to TNF or forskolin to release PLA₂. This finding also indicates that some tissue-specific factors may be involved in the expression of the gene. With these considerations in mind, it would be very important to clarify the regulation mechanism of the group II PLA₂ gene in detail on a molecular basis and to clarify the physiological role of PLA₂ in the progression of inflammatory diseases, which includes the problem of whether PLA₂ is involved in the formation of pro-inflammatory prostaglandins and lipoxygenase products.

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**REFERENCES**

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