Dexamethasone Alters Arachidonate Release from Human Epithelial Cells by Induction of p11 Protein Synthesis and Inhibition of Phospholipase A₂ Activity*

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The effect of the glucocorticosteroid, dexamethasone, on arachidonic acid (AA) release and on protein levels of p11 and cytosolic phospholipase A₂ (cPLA₂) was studied in two epithelial cell lines, HeLa cells and BEAS-2B cells. Dexamethasone treatment of HeLa cells and BEAS-2B cells increased cellular p11 protein and mRNA levels in a time- and dose-dependent manner. It had little effect on levels of cPLA₂ protein. In order to determine if increased p11 protein expression resulted in increased interaction between p11 and cPLA₂, anti-cPLA₂ antibodies were used to immunoprecipitate p11-cPLA₂ complexes and Western blots of the immunoprecipitate were used to detect p11. In cells treated with dexamethasone, more p11 was detected in the anti-cPLA₂ immunoprecipitate compared with control cells. Dexamethasone treatment of HeLa cells prelabeled with [³H]AA decreased the release of [³H]AA under basal conditions and after stimulation with the calcium ionophore A23187 (10⁻⁶ m). In order to determine if altering the p11 protein levels in HeLa cells independent of glucocorticosteroid treatment could also produce an effect on [³H]AA release, cells were stably transfected with plasmids expressing either p11 antisenase mRNA or p11 mRNA. Cloned HeLa cells expressing p11 antisenase mRNA exhibited less cellular p11 protein compared with control cells and greater [³H]AA release compared with cells transfected with a control vector. Cloned HeLa cells stably transfected with a p11 expression vector exhibited increased p11 cellular protein and diminished [³H]AA release under basal conditions and in response to A23187. Therefore, dexamethasone alteration of epithelial cell AA release may be due in part to induction of p11 protein expression.

Phospholipase A₂s (PLA₂s)¹ are a group of enzymes that hydrolyze the ester bond of fatty acids from the sn-2 position of glycerophospholipids. The release of arachidonic acid (AA) from membranes by PLA₂ and its subsequent conversion into leukotrienes, prostaglandins, and other eicosanoids plays an important role in inflammation (1–4). The mammalian calcium-dependent PLA₂s can be grouped into major classes based on their molecular mass and cellular distribution, including the low molecular mass (10–14 kDa) secreted forms (sPLA₂) and the structurally unrelated high molecular mass (85 kDa) cytosolic PLA₂ (cPLA₂) (1, 3, 5).

To date, five different sPLA₂ isozymes have been described in mammalian cells. The 14-kDa sPLA₂ enzyme from synovial fluid and platelets (Group IIA) may be involved in the pathogenesis of inflammatory reactions (3, 6, 7). The 14-kDa PLA₂ lacks apparent selectivity for the sn-2 fatty acids of phospholipids and requires much higher Ca²⁺ concentrations (millimolar) than normal intracellular Ca²⁺ levels (nanomolar to micromolar) for activity. The 85-kDa high molecular mass cPLA₂ has higher selectivity to hydrolyze phospholipids containing AA esterified in the sn-2 position (1, 3, 5–11). Its activity is regulated by phosphorylation, G-protein activation, and physiologically relevant concentrations of calcium. Because cPLA₂ may play a central role in producing AA and lysophospholipid for subsequent metabolism to prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids, and platelet-activating factor, all potent lipid mediators of inflammation, the activation of cPLA₂ may play an important role in modulating the airway inflammatory response (1, 3, 5).

S-100 proteins are a family of proteins first described by Moore (12) who initially characterized a group of abundant low molecular weight (10–12 kDa) acidic proteins in nerve tissue. S-100 proteins are a group of Ca²⁺-binding proteins that are expressed in a cell type-dependent fashion. This family includes S-100α, S-100β, and p11/calpain light chain (13). p11 was described as a member of the S-100 family of EF hand type Ca²⁺-binding proteins but does not have the ability to bind Ca²⁺ ions due to crucial amino acid deletions and substitutions in the two EF hand loops of the protein (14, 15). p11 binds to and inhibits the phosphorylation of a 36-kDa protein known as p36, also known as annexin II as well as calpain heavy chain (16, 17).

Glucocorticoids are effective in the treatment of immune and inflammatory disorders affecting the lung and other organs. One mechanism of glucocorticoid modulation of the inflammatory response is inhibition of the release of AA from cellular lipids (18, 19) and inhibition of prostaglandin H synthase-2 synthase or cyclooxygenase-2 expression in a number of tissues (20–24). The rate of eicosanoid synthesis may be regulated by the availability of free AA that can be metabolized into prostaglandins and leukotrienes via the cyclooxygenase and lipooxygenase pathways. The decreased synthesis of bioactive eicosanoids may represent an important mechanism of the anti-inflammatory action of glucocorticoids. Glucocorticoids can induce annexins which might inhibit sPLA₂ activity in vitro (25–30). A recent study has demonstrated that p11 can directly

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* The abbreviations used are: PLA₂, phospholipase A₂; cPLA₂, cytosolic PLA₂; sPLA₂, secreted PLA₂; AA, arachidonic acid; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; HBSS, Hank’s balanced salt solution; RPA, ribonuclease protection assay; bp, base pair(s); GAPDH, glyceradehyde 3-phosphate dehydrogenase; BSA, bovine serum albumin; HPLC, high-pressure liquid chromatography.
interact with the COOH-terminal region of 85-kDa cPLA2 and inhibit cPLA2 enzyme activity (31). Therefore, it was of interest to study whether p11 plays a role in glucocorticoid induced changes in cellular arachidonic release.

EXPERIMENTAL PROCEDURES

Cell Culture—HeLa cells were obtained from the American Type Culture Collection (Rockville, MD) and grown in DMEM medium with 10% fetal bovine serum. BEAS-2B cells, a human bronchial epithelial cell line, were a gift from Curtis Harris and John Lechner, National Cancer Institute, Bethesda, MD. BEAS-B cells were grown in L15 medium (Biofluids, Rockville, MD) in 150-mm culture flasks by the single step guanidinium thiocyanate-phenol-chloroform extraction method (Tri-reagent, Molecular Research Inc., Cincinnati, OH). The RNA pellet was precipitated with isopropanol, washed with 70% ethanol, and redissolved in diethyl pyrocarbonate water. To construct the probe for cPLA2 mRNA, a 306-bp product of cPLA2 cDNA was amplified by polymerase chain reaction using the following set of sense and antisense primers: 5′-CAACACCGAGAAGTTAAGGAT-3′ (799–823); 3′ primer, 5′-AAATAAGTCCGGACGACATTAAT-3′ (1104–1084) (Biosynthesis Inc., Lewisville, TX). The product for cPLA2 gene was cloned into the TA cloning vector (Invitrogen, San Diego, CA). Orientation of the insert was determined by DNA sequencing. The cPLA2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA probes were prepared by in vitro transcription using T7 polymerase with [α-32P]CTP. The RNA probes were prepared by in vitro transcription using SP6 polymerase with [α-32P]CTP. An RPA assay kit (RPAI, Ambion, Austin Texas) was used. Hybridization was performed at 45 °C for 16 h with and with 10 μg for (GAPDH) or 20 μg for (cPLA2 and 40 μg for (p11) of total RNA. 106 dpm of RNA were added to the labeled probe. After hybridization, the unhybridized RNA was digested by addition of 1:100 diluted RNaseA/T1 mix at 37 °C for 60 min. Digestion was terminated by the addition of RNase inactivation and precipitation mixture. The protected RNA fragment was analyzed by autoradiography after separation on 6% polyacrylamide, 8% urea gels (Novex).

Effect of RU486 on Dexamethasone-induced p11 Expression—The HeLa cells grown on 175-cm2 flasks were treated with dexamethasone (10−9 M) with or without the glucocorticoid receptor antagonist, RU486 (10−5, 10−7, 10−9, 10−11, and 10−12 M) for 24 h. At the end of incubation time, crude cytosolic extracts of treated and control cells were prepared and Western blots were done as described in the experimental procedures section for immunoblot of p11 protein.

Immunoprecipitation of Native p11 Protein from HeLa and BEAS-2B Cells—The HeLa or BEAS-2B cells grown on 175-cm2 culture flasks with and without dexamethasone and/or insulin were centrifuged. After four washings with PBS, 20 μl of protein loading buffer was added to the sample, and the cells were boiled at 100 °C for 10 min before electrophoresis on 10% polyacrylamide gels (Novex) using Tris-glycine SDS buffer. The crude cytosolic protein was isolated as described above for immunoblot for p11 protein. For immunoprecipitation, the isolated crude cytosolic fraction (200 μl, 400 μg of protein) was added to a microcentrifuge tube containing 1 ml HBSS (with calcium and magnesium) and 10 μl of rabbit anti-human cPLA2 antibody. The supernatant was incubated at 4 °C for 30 min, 25 μl of Protein G Plus/Protein A-agarose (Pierce) was then added to each sample, and the mixture was incubated at 4 °C for 4 h, followed by centrifugation in a microcentrifuge at 2500 rpm for 5 min at 4 °C. The supernatant was aspirated, and the pellet was washed four times with 1 ml cold PBS (phosphate-buffered saline, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) with repeated centrifugation. After four washings with PBS and 20 μl of protein loading buffer was added to the sample and the cells were boiled for 10 min before electrophoresis on 10% acrylamide gels (Novex) using Tris-glycine SDS buffer. The separated proteins were electrophoretically transferred onto a nitrocellulose membrane blocked with 5% non-fat milk and then probed with a 1:2000 dilution of mouse anti-human p11 monoclonal antibody. The antibody (IgG) was second antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). The blot was developed using the ECL Western blotting detection system (Amersham Pharmacia Biotech).

Immunoblot of p11 Protein—HeLa or BEAS-2B cells were grown on 175-cm2 flasks and treated with dexamethasone (Calbiochem) (10−7, 10−9, and 10−11 M) for 24, 36, or 48 h. For time course experiments, the culture medium was changed at the same time, and all cells were harvested at the same time. Dexamethasone (10−7 M) was added at the indicated times prior to harvesting. At the indicated times treated and control cells were rinsed three times with cold PBS. After washing, the cells were transferred to 0.5 ml of homogenization buffer; 50 mM Hepes, pH 8.0, 1 mM EDTA, 1 mM EGTA, 100 μM leupeptin, 1 mM dithiothreitol, 10 mM phenylmethylsulfonyl fluoride, 0.5 mM soybean trypsin inhibitor, and 0.5% Triton X-100. Cells in homogenization buffer were sonicated for 15 s times three using a microprobe. Total protein was assayed by BCA reagent (Pierce). Samples containing 20 μg of cell lysate protein were separated on 18% Tris-glycine gels (Novex, San Diego, CA) using Tris-glycine SDS running buffer. The separated proteins were electrophoretically transferred onto a nitrocellulose membrane (Novex), then blocked with 5% non-fat dry milk overnight. p11 protein expression was detected by using 1:2000 dilution of mouse anti-human p11 monoclonal antibody (Transduction Laboratories, Lexington, KY) and 1:5000 dilution horseradish-peroxidase-conjugated donkey-anti-mouse IgG as second antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). The blot was developed using the ECL Western blotting detection system (Amersham Pharmacia Biotech).

Ribonuclease Protection Assay (RPA) for cPLA2 and p11 mRNA Levels—The HeLa cells were treated with dexamethasone (10−7, 10−9, and 10−11 M) for 24 to 48 h. Total cellular RNA was extracted from 175 cm2 culture flasks by the single step guanidinium thiocyanate-phenol-chloroform extraction method (Tri-reagent, Molecular Research Inc., Cincinnati, OH). The RNA pellet was precipitated with isopropanol, washed with 70% ethanol, and redissolved in diethyl pyrocarbonate water. To construct the probe for cPLA2 mRNA, a 306-bp product of cPLA2 cDNA was amplified by polymerase chain reaction using the following set of sense and antisense primers: 5′-CCACACCGAGAAGTTAAGGAT-3′ (799–823); 3′ primer, 5′-AAATAAGTCCGGACGACATTAAT-3′ (1104–1084) (Biosynthesis Inc., Lewisville, TX). The product for cPLA2 gene was cloned into the TA cloning vector (Invitrogen, San Diego, CA). Orientation of the insert was determined by DNA sequencing. The cPLA2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA probes were prepared by in vitro transcription using T7 polymerase with [α-32P]CTP. The RNA probes were prepared by in vitro transcription using SP6 polymerase with [α-32P]CTP. An RPA assay kit (RPAI, Ambion, Austin Texas) was used. Hybridization was performed at 45 °C for 16 h with and with 10 μg for (GAPDH) or 20 μg for (cPLA2 and 40 μg for (p11) of total RNA. 106 dpm of RNA and 20 μl of cPLA2 and 20 μl of p11 RNA probes were used. After hybridization, the unhybridized RNA was digested by addition of 1:100 diluted RNaseA/T1 mix at 37 °C for 60 min. Digestion was terminated by the addition of RNase inactivation and precipitation mixture. The protected RNA fragment was analyzed by autoradiography after separation on 6% polyacrylamide, 8% urea gels (Novex).

Stable Transfection of a p11 Antisense Plasmid in HeLa Cells—Individual Sep-Pak C18 cartridges were prepared with 15 ml of methanol followed by 5 ml of 5 mM EDTA and 10 ml of water. Samples were loaded onto the cartridges washed with 10 ml of water and eluted with 4 ml of methanol. The methanol fraction was collected and evaporated to dryness in a stream of nitrogen gas and resuspended in 200 μl of methanol for analysis by HPLC. An ultrahydro C18 column (4.7 × 250 mm) (Beckman Instruments) with 5-μm particle size was used. A gradient program was used with mobile phase A, water/acetonitrile/phosphoric acid (75:25:0.025), and mobile phase B, methanol/acetonitrile/trifluoroacetic acid (60:40:0.0016), at a flow rate of 1.5 ml/min. The AA fraction of HPLC elution was collected and measured for radioactivity. The AA-agarose (Pierce) was added to the sample and the mixture was incubated at 4 °C for 4 h, followed by centrifugation in a microcentrifuge at 2500 rpm for 5 min at 4 °C. The supernatant was aspirated, and the pellet was washed four times with 1 ml cold PBSDTS (phosphate-buffered saline, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) with repeated centrifugation. After four washings with PBS, 20 μl of protein loading buffer was added to the pellet and the sample was boiled for 10 min before electrophoresis on 10% acrylamide gels (Novex) using Tris-glycine SDS buffer. The separated proteins were electrophoretically transferred onto a nitrocellulose membrane blocked with 5% non-fat milk and then probed with a 1:2000 dilution of mouse anti-human p11 monoclonal antibody. The antibody (IgG) was second antibody (Jackson ImmunoResearch Laboratories, Inc.). The blot was developed using the ECL Western blotting detection system.
flask. Subsequent cultures of selected HeLa cells were routinely grown in the presence of selective pressure. Transfected HeLa cells were cloned by limiting dilution and clones used for Western blot and AA release.

For [H]AA release studies, equal numbers of cells transfected with pcDNA3.1(+) vector alone as control, and the cells transfected with the p11 antisense plasmid A5p11-pcDNA3.1(+) were grown in T-75-cm² culture flasks. Cells were labeled for 18 h with 1 μCi/ml [3H]AA in DMEM medium with 10% fetal calf serum and 1000 μg/ml Geneticin. Following repeated washing with media, 12 ml of fresh medium with 10% serum and 1000 μg/ml Geneticin were added to each flask. For studies of AA release after calcium ionophore stimulation, following repeated washing with HBSS(+) with 0.5% BSA for three times, 12 ml of calcium ionophore A23187 (10⁻⁶ M) in HBSS(+) with 0.5% BSA or HBSS with 0.5% BSA without A23187 were added to each flask, and the cells were incubated at 37 °C for 30 min. The supernatants were extracted by Sep-Pak C₁₈ cartridges and chromatographed by reverse phase HPLC as described above. The AA fraction of HPLC elution was collected and measured for radioactivity.

Stable Transfection of a p11 Expression Plasmid in HeLa Cells—A cDNA containing the coding region of the p11 gene was cloned into the mammalian expression vector pcDNA3.1(+) (Invitrogen) to create p11-pcDNA3.1(+) (+). The identity and orientation of construct was confirmed by DNA sequencing. The pcNDA3.1(+) vector carries the human cytomegalovirus immediate early enhancer-promoter sequences to promote constitutive expression of the cloned p11 insert in mammalian cells. The HeLa cells grown in 175-cm² flasks were exposed to 120 μl of LipofectAMINE Reagent (Life Technologies, Inc.) with 20 μg of p11-pcDNA3.1(+) expression plasmid alone. Cells were exposed to the mixture of LipofectAMINE and plasmid for 4 h. Following removal of the transfection reagent, fresh DMEM with 10% serum and 1000 μg/ml Geneticin (G418 sulfate) (Calbiochem) was added to HeLa cells. Subsequent cultures of selected HeLa cells were routinely grown in the presence of selective pressure. Transfected HeLa cells were cloned by limiting dilution and clones were used for Western blot and AA release after four passages.

For [H]AA release, equal numbers of cells transfected with pcDNA3.1(+) vector alone as control and the cells transfected with the p11 expression plasmid p11-pcDNA3.1(+) were grown in T-75-cm² culture flasks. Cells were labeled for 18 h with 1 μCi/ml [3H]AA in DMEM medium with 10% fetal calf serum with 1000 μg/ml Geneticin. For studies of AA release after calcium ionophore stimulation, following three washes with HBSS(+) with 0.5% BSA, 12 ml of calcium ionophore A23187 (10⁻⁶ M) in HBSS(+) with 0.5% BSA or HBSS with 0.5% BSA without A23187 were added to each flask, and the cells were incubated at 37 °C for 30 min. The supernatants were collected and measured for radioactivity.

RESULTS

Dexamethasone Increases p11 Protein Levels in Human HeLa Cells and BEAS-2B Cells—The effect of dexamethasone treatment on human epithelial cell expression of p11 was studied by Western blot of two different epithelial cell lines, HeLa cells and BEAS-2B cells. Fig. 1A demonstrates the effect of dexamethasone treatment of HeLa cells on cellular p11 accumulation. Treatment of cells with dexamethasone (10⁻⁷ M) for 24–48 h resulted in a significant increase in p11 protein expression in cell lysates. In addition, treatment of cells with 10⁻⁷, 10⁻⁸, and 10⁻¹¹ M dexamethasone for 24 h resulted in a significant increase in p11 protein expression in cell lysates. Treatment of BEAS-2B cells with dexamethasone (10⁻⁷ M) for 24–48 h also resulted in a significant increase in p11 protein expression in cell lysates.

Effect of Dexamethasone on Steady State Levels of p11 mRNA—Steady state levels of mRNA for p11 were measured by RPA of total cellular RNA extracted from HeLa cells that were incubated without or with dexamethasone (10⁻⁷ M) for 24–48 h. As shown in Fig. 2A, these cells produce p11 mRNA and the steady state level of p11 mRNA was increased by dexamethasone treatment over 24–48 h. In addition, dexamethasone in concentrations of 10⁻⁷ to 10⁻¹¹ M induced a dose-related change in p11 mRNA levels.

Effect of Dexamethasone on cPLA₂ Protein and mRNA Levels in HeLa Cells—The effect of dexamethasone treatment on human epithelial cell expression of cPLA₂ was studied by Western blot of cell lysates. Treatment of cells with dexamethasone...
GAPDH and cPLA2-specific radiolabeled cRNA probes, respectively, by gel electrophoresis and immunoblotting.

B: The dose effect of dexamethasone on cPLA2 protein levels (Fig. 3). The results from RPA and Western blot studies indicated that the effect of dexamethasone on cPLA2 mRNA levels. Fig. 4 shows the effect of RU486 (10^-7 to 10^-12 M) on dexamethasone-induced increases in p11 protein levels.

Dexamethasone Inhibits AA Release from the HeLa Cells—The results from RPA and Western blot studies indicated that dexamethasone treatment had an effect on p11 mRNA levels and protein production but little or no effect on the mRNA expression or protein level of cPLA2. In these cells, dexamethasone treatment does alter the release of [^3H]labeled AA both at base line and after exposure to the calcium ionophore A23187. Fig. 6 demonstrates that labeled AA release from dexamethasone-treated HeLa cells (HD) is lower than that from untreated HeLa cells (HC). After treatment with A23187, the release of labeled AA from dexamethasone-treated HeLa cells (HD + A) is significantly decreased compared with untreated control cells (HC + A).

RU486 Inhibits Dexamethasone-induced p11 Protein Increases—We have shown that dexamethasone increases p11 expression and inhibits PLA2 activity in vitro. It has been reported that p11 can bind to cPLA2 and inhibit cPLA2 activity in vitro. In order to study whether dexamethasone might alter cPLA2 activity in part by increasing p11 expression in human cells, we performed two studies. First, we constructed a p11 antisense plasmid and then stably transfected HeLa cells to examine the AA release in these cells. Western blot studies of cloned transfected cells showed that p11 protein production was decreased in HeLa cells which were transfected with ASp11-pcDNA3.1(+) plasmid compared with HeLa cells, which were transfected with pcDNA3.1(+) plasmid alone (Fig. 7A). There was no change in cPLA2 expression in these cells (Fig. 7B). [^3H]AA release from the HeLa cells that were permanently transfected with p11 antisense plasmid increases both at base line and


**FIG. 6.** [3H]AA release from dexamethasone treated HeLa cells. The cells grown in T-75-cm² flasks were labeled for 18 h with 1 μCi/ml [3H]AA in 12 ml of DMEM medium and then treated with 10⁻² M dexamethasone for 24 h. After repeated washing, the cells were then incubated with 10⁻² M ionophore A23187 in 12 ml of HBSS (containing 1.3 mM Ca²⁺) for 30 min, and the supernatants were extracted by Sep-Pak C₁₈ cartridges and chromatographed by HPLC as described under “Experimental Procedures.” Data were expressed as AA release measured separately from 11–12 individual flasks from two separate sets of experiments. HC = HeLa control cells; HD = HeLa cells treated with dexamethasone; A = treatment with A23187. p < 0.001 for HC versus HD; p < 0.001 for HC+A versus HD+A.

**FIG. 7.** The p11 and cPLA₂ protein levels in HeLa cells transfected with a p11 antisense plasmid. A, cell lysates from cells transfected with the p11 antisense plasmid or cells transfected with a control vector were processed as described under “Experimental Procedures,” and 20 μg of total protein was subjected to gel electrophoresis and immunoblotting.

**FIG. 8.** [3H]AA release from HeLa cells transfected with a p11 antisense plasmid. The cells grown in T-75-cm² flasks were labeled for 18 h with 1 μCi/ml [3H]AA in 12 ml of DMEM with Geneticin. After repeated washing, some cells were then incubated with 10⁻⁶ M ionophore A23187 in 12 ml of HBSS (containing 1.3 mM Ca²⁺) or with HBSS without A23187 for 30 min, and the supernatants were extracted by Sep-Pak C₁₈ cartridges and chromatographed by HPLC as described under “Experimental Procedures.” Data were expressed as AA release measured separately from 10 to 12 individual flasks in each group. VC = vector control cells; ASp₁₁ = HeLa cells transfected with a plasmid expressing p11 antisense mRNA; A = HeLa cells treated with A23187. p < 0.001 for VC versus ASp₁₁; p < 0.05 for VC + A versus ASp₁₁ + A.

**DISCUSSION**

p11, or calpactin light chain, is a member of the S-100 family small calcium binding proteins; however, it has several unique features. S-100 proteins contain two EF hands that function as calcium binding domains (13). p11 does not have the ability to bind Ca²⁺ ions due to amino acid deletions and substitutions in the two EF hand motifs (14, 15). Instead, p11 is present in a variety of cells separately or as a heterotetramer binding to annexin II. The heterotetramer is composed of two copies of the 36-kDa heavy chain, annexin II subunits and two copies of the 11-kDa light chain, p11 subunits (p₃₆)(p₁₁), (32, 33).

Glucocorticosteroids are potent anti-inflammatory agents. This anti-inflammatory effect may be produced via a variety of mechanisms. A group of structurally related, calcium-dependent phospholipid-binding proteins, annexins, which were formerly known as lipocortins or calpactins, had been shown to be...
inducible by glucocorticoids. Annexin I has been reported to inhibit sPLA₂ activity in vitro (25–30). These observations led to the hypothesis that the inhibition of sPLA₂ by annexins is the mechanism of the anti-inflammatory action of glucocorticoids. Subsequent studies failed to show a direct interaction between the 14-kDa PLA₂ and annexins. Instead, this inhibition may be dependent on the concentration of substrate (34, 35), the extent of inhibition being more closely related to the inhibitor:substrate rather than the inhibitor:enzyme ratio. In addition, glucocorticoid treatment suppresses the induction of Group II sPLA₂ expression in a variety of cells (36–40).

**REFERENCES**

Dexamethasone Alteration of Cellular p11


