Glucocorticoid Deficiency Increases Phospholipase A_2 Activity in Rats

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Abstract

An important mechanism for the antiinflammatory effect of pharmacological doses of glucocorticoids is the inhibition of arachidonic acid release from phospholipids by phospholipase A_2 (PLA_2). As a corollary, one might predict that low endogenous concentrations of glucocorticoids favor inflammatory disease states. Indeed, clinical and experimental observations revealed an association between glucocorticoid deficiency and disease states caused by immunological and/or inflammatory mechanisms. The purpose of the present investigation was to study the regulation of PLA_2 mRNA, protein, and enzyme activity in adrenalectomized (ADX) rats where glucocorticoid concentrations were below physiological levels. The mRNA of group I and II PLA_2 were measured by PCR. Group II PLA_2 mRNA was increased by 126±9% in lung tissue of ADX rats, whereas group I PLA_2 was increased only by 27±1.5%. The increase in group II mRNA in ADX rats was reflected by a corresponding increase of group II PLA_2 protein (70–100%) in lung, spleen, liver, and kidney. This increase was reversed by the administration of exogenous corticosterone. After ADX, the percentage increase in total PLA_2 activity was higher than that of mRNA or PLA_2 protein, suggesting that the activity of the enzyme was modulated by inhibitors or activators. The concentration of lipocortin-I, an inhibitor of PLA_2 enzyme was strongly correlated with the activity of PLA_2 in the tissues (lung, spleen, liver, and kidney). In all these tissues, the concentrations of lipocortin-I declined after ADX. Thus upregulation of PLA_2 enzyme and downregulation of lipocortin-I might account for the enhanced inflammatory response in hypoglucocorticoid states. (*J. Clin. Invest.* 1993. 92:1974–1980.) Key words: phospholipase A_2 • glucocorticoids • adrenalectomy • inflammation • lipocortin

Introduction

The biological and clinical relevance of high endogenous or exogenous glucocorticoid concentrations in mammals has been widely discussed (1–4). During stress, for example, increased concentrations of glucocorticoid hormones were hypothesized to turn off the defensive inflammatory reactions that may overshoot after noxious stimuli (4). This effect is used in clinical practice by prescribing pharmacological doses of glucocorticoids to patients with inflammatory diseases. The antiinflammatory effect of glucocorticoids is mediated through a number of mechanisms, among which the inhibition of eicosanoid production by phospholipase A_2 (PLA_2)[1] enzyme appears to play a key role (5). PLA_2 enzymes characterized so far are classified into group I and group II based on their primary and secondary structures (6). Group II (but not group I PLA_2) has been suggested to be involved in the pathogenesis of inflammatory reactions (7–11). Several lines of evidence reveal that high doses of glucocorticoids decrease PLA_2 activity by two different mechanisms: (*a*) by generating proteins such as lipocortin-I, which were shown to inhibit PLA_2 activity in vitro (for a review, see reference 5), through mechanisms that are not yet well defined (12–13), and (*b*) by reducing the expression of group II PLA_2 enzyme (14–15).

In contrast to the high glucocorticoid levels, the relevance of abnormally low endogenous glucocorticoid concentrations to the inflammatory response has rarely been addressed. Previous investigations have shown an increased duration and magnitude of inflammatory reactions and higher concentrations of various prostaglandins after adrenalectomy (ADX) or treatment with a glucocorticoid receptor antagonist in animals (16–20). It was hypothesized that the increased concentrations of prostaglandins were caused by an increased activity of PLA_2 (17, 20). In the present study, we report the effect of glucocorticoid deficiency induced by ADX in rats on mRNA, protein levels of group II PLA_2, and total enzyme activity of PLA_2 in different tissues. The results revealed an upregulation of group II PLA_2 after ADX, which was reversed after the administration of corticosterone.

Methods

Preparation of ADX rats. ADX with and without replacement of corticosterone pellets and sham-operated ADX (SHAM-ADX) were performed as described previously (21). Male Sprague Dawley rats, weighing 160–180 g (Holtzman Laboratory Animals, Madison, WI), were kept in a temperature-, humidity-, and light (12 h on)-controlled room and were allowed food and water ad libitum. The rats were anesthetized with ether and treated in the following ways: Group 1: SHAM-ADX. Adrenal glands were exposed but not touched. Group 2: SHAM-ADX + V. Adrenal glands were exposed but not touched (vehicle [V] = DMSO). Group 3: ADX + corticosterone. Rats were ADX and given a subcutaneous 20% corticosterone/80% cholesterol pellet that created plasma corticosterone values of ~ 25 μg/liter. Group 4: ADX. Adrenal glands were removed completely, resulting in plasma corticosterone concentration < 1 μg/liter.

After surgery, all rats were provided with 0.5% saline for their drinking solution. Starting on the evening after the surgery, all rats, except those in group 4, were injected with DMSO three times a day to act as controls for a separate experiment. When the plasma adrenocorticotropic hormone and corticosterone concentrations were analyzed in the SHAM-ADX rats that received either subcutaneous injections of

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1. *Abbreviations used in this paper:* ADX, adrenalectomy; CORT, corticosterone; PLA_2, phospholipase A_2; SHAM, sham-operated; TBS, Tris-buffered saline; V, vehicle.

DMSO or no injections, there were no differences, indicating that the stress involved with the injections did not affect corticosteroid concentrations. On the morning and evening of the 5th d after the surgery, the rats were decapitated, and lung, spleen, kidney, and liver tissues were collected, immediately frozen in liquid nitrogen, and stored at −80°C until further use. This protocol was approved by the University of California, San Francisco Committee on Animal Research.

RNA extraction and modification of group II PLA_2 transcript. Total RNA from the tissues of different experimental rats was extracted by the lithium chloride–urea procedure (22). The RNA concentration was determined by absorbance at 260 nm. The quality of RNA was controlled by running an aliquot on a 1% agarose formaldehyde gel. Full-length group II PLA_2 cDNA (23) (750 bp) inserted in pGEM-3Z plasmid was obtained as gift from J. Ishizaki (Shinogi and Co. Ltd., Osaka, Japan). For the quantitative measurements of group II PLA_2 mRNA, this plasmid was modified as follows to construct an internal standard. An XbaI restriction site was introduced at the 5′ end of the PLA_2 cDNA by PCR to remove the BamHI site in the polylinker of pGEM-3Z plasmid. A BamHI fragment of 446 bp from positions 170–616 was removed from the PLA_2 cDNA resulting in a smaller group II PLA_2 cDNA of 254 bp. A transcript of this modified PLA_2 cDNA was prepared using SP6 RNA polymerase. This transcript was used as an internal standard for the quantitative measurement of group II PLA_2 mRNA. The transcript was constructed such that for reverse transcription and PCR the same primers could be used for the mRNA of both group II PLA_2 and the internal standard (24).

Reverse transcription of mRNA. The reverse transcription reaction mixture of 20 μl contained 50 mM Tris-HCl pH 8.2, 6 mM $MgCl_2$, 10 mM DTT, 100 mM NaCl, 200 μM dNTPs, 11 U RNAse inhibitor, 10 pmol 25-mer primer (3′ primer of the corresponding cDNA, position 695–719 of group II PLA_2 cDNA from Microsynth (Windisch, Switzerland), 100–1600 ng total RNA and 18 fg modified group II PLA_2 transcript, 1 U avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Biochemicals, Indianapolis, IN). Initially, the 3′ primer and total RNA, with and without modified group II PLA_2 transcript, were incubated together for 5 min at 65°C, then cooled at room temperature for 15 min. The remaining reaction components, including avian myeloblastosis virus reverse transcriptase, were then added and incubated at 42°C for 60 min.

PCR. PCR was performed in a total volume of 30 μl with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 50 pmol of the appropriate 3′ and 5′ cDNA primers (5′ primer: position 58–82 of PLA_2 cDNA) 6.0 μg BSA, 1 μCi [α-^{32}P]dCTP; 4 μl reverse transcribed group II PLA_2 cDNA, 1 U *Thermus aquaticus* DNA polymerase (Perkin Elmer Cetus Corp. Norwalk, CT). Before adding the enzyme and cDNA the samples were irradiated under ultraviolet light for 20 min to eliminate any contaminating DNA (25). The mixture was overlaid with mineral oil, and cDNA was then amplified with a DNA thermal cycler (Perkin Elmer Cetus Instruments) for 35 cycles. The amplification profile involved denaturation at 94°C for 1 min and 15 s, primer annealing at 72°C for 2 min and elongation of annealed primers at 72°C for 2 min. For group I PLA_2 (26) 100–1,600 ng total RNA was reverse transcribed (3′ primer: position 340–359) without internal standard. 6 μl of reverse transcribed material was used as a template for cDNA amplication by PCR, using the same conditions as for group II PLA_2 cDNA (5′ primer: position 43–62). 10 μl of each PCR reaction mixture were mixed with 2 μl of sixfold concentrated loading buffer and applied on 1.5% agarose gel containing ethidium bromide. Electrophoresis was carried out in Tris-borate-EDTA buffer, pH 8.0 with a constant voltage of 50 V for 90 min. Bands were visualized under ultraviolet light and excised from the gel. The radioactivity of the bands was measured in a scintillation counter using a Cerenkov program.

Protein extraction and quantitative analysis of group II PLA_2. A small amount of tissue frozen at −80°C was powdered, and acid was extracted according to the method of Märki and Franson (27). The protein concentration was determined using the bicinchonic acid protein assay reagent (Pierce Chemical Co., Rockford, IL).

Western blotting. For Western blots, 2–10 μg of total protein (these protein samples were not boiled, and the treatment buffer did not contain any reducing agents) were loaded and resolved on a 12.5% SDS-polyacrylamide gel (28). The proteins were transferred to nitrocellulose membrane by electroblot using Tris-glycine buffer containing 20% methanol. The transfer was performed at a constant voltage of 60 V for 60 min. Protein transfer was monitored by staining the nitrocellulose membrane with Ponceau S (Sigma Chemical Co., St. Louis, MO) and the gel with Coomassie blue. The blots were agitated with Tris-buffered saline (TBS), pH 7.5, containing 5% BSA for 60 min at room temperature, washed three times with TBS and incubated with primary antibody (2E7 and 2B9 hybridoma supernatants specific for group II PLA_2 enzyme, a gift from Henk van den Bosch, State University of Utrecht, Utrecht, The Netherlands) diluted 1:5,000 in TBS containing 0.5% Tween 20 and 0.01% BSA. The incubation with monoclonal antibodies was performed for 4 hours at 37°C. Membranes were washed three times with TBS containing 0.05% Tween 20 and incubated with peroxidase conjugated rabbit anti-mouse IgG (H + L) (Sigma Chemical Co.) for 1 h at room temperature diluted 1:6,000 in TBS containing 0.05% Tween 20 and 0.01% BSA. Membranes were washed three times with TBS containing 0.05% Tween 20 and three times with TBS only to remove Tween 20. After washing, the peroxidase activity was detected using the enhanced chemiluminescence Western blotting detection system (Amersham International, Aylesbury, Buckinghamshire, United Kingdom) according to the manufacturer's instructions. The bands on the x-ray films were scanned with a transmittance scanning densitometer (Camag TLC Scanner II; Camag, Muttenz, Switzerland) to get an absorbance profile across each band. The peak areas were weighed for measurements.

Assay of PLA_2 activity. PLA_2 activity was assayed using [^{3}H]-oleate–labeled, autoclaved *Escherichia coli* as the substrate (29). The reaction mixture of 350 μl contained 100 mM Tris-HCl pH 8.0; 5 mM Ca^{2+}, 11.4×10^9 cells of autoclaved *E. coli* cells (corresponding to 10,000 cpm and 4.6 nmol phospholipid), and 0.3–70 μg tissue acid extract. The amount of tissue protein was chosen such that 6–15% hydrolysis of substrate was obtained when incubated at 37°C for 30 min. The reaction was terminated by adding 100 μl of 2 N hydrochloric acid. 100 μl of fatty acid–free BSA (100 mg/ml) was added, and the tubes were vortexed and centrifuged at 13,000 *g* for 5 min. An aliquot (140 μl) of the supernatant containing released [^{3}H]oleic acid was mixed with scintillation cocktail (Dynagel, J. T. Baker, B. V. Deventer, Holland) and counted in a liquid scintillation counter.

Results

The mRNA of group II PLA_2 was quantified by PCR using a constant amount of a modified group II cDNA transcript as an internal standard. Both specific target mRNA and internal standard were coamplified in one reaction using the same primers (24). Fig. 1 shows the PCR products of group II PLA_2 cDNA and the internal standard of rat lung tissue separated on an agarose gel; three concentrations of total RNA from control (SHAM-ADX) rats, SHAM-ADX + V, ADX + CORT, and ADX rats were used. Visual observation of the bands revealed that group II PLA_2 mRNA was increased in ADX rat lungs compared with the values in the other three groups. Similar results were obtained in two additional experiments. The inclusion of an internal standard excluded the possibility that increased concentrations of group II PLA_2 in ADX rats were caused by an artifact during the PCR amplification procedure (24).

To assess quantitatively the effect of ADX on group II PLA_2 mRNA, [α-^{32}P]dCTP was included during the PCR. The incorporation of radioactivity into group II PLA_2 cDNA was determined at 20, 80, and 320 ng of RNA, with and without the internal standard (Fig. 2). Three independent experiments re-

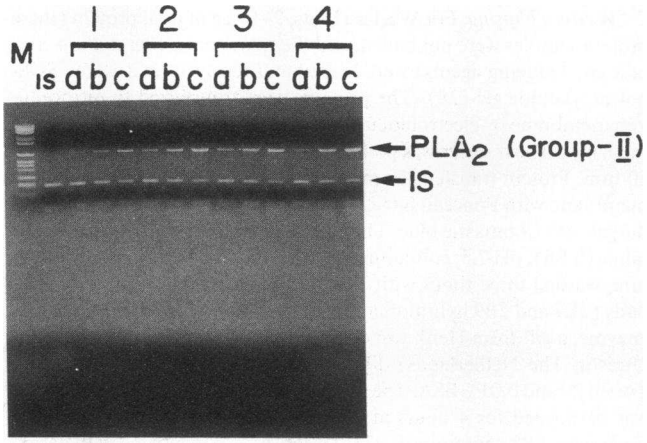
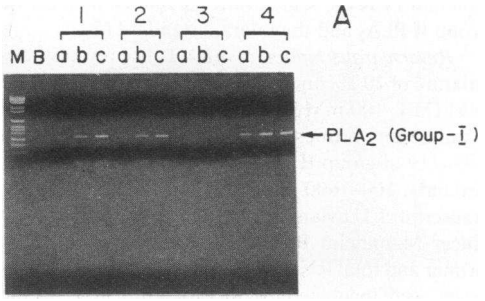


Figure 1. Agarose gel (1.5%) electrophoresis of PCR products of group II PLA_2 from rat lung tissue. Group II PLA_2 mRNA was reverse transcribed in the presence of 18 fg internal standard (*IS*) and 20 ng (lane *a*), 80 ng (lane *b*), and 320 ng (lane *c*) of reverse transcribed material was used as template for PCR reactions. *M*, DNA molecular weight marker VI (Boehringer Mannheim Biochemicals); *1*, SHAM-ADX; *2*, SHAM-ADX + V; *3*, ADX + CORT; *4*, ADX.

vealed that the internal standard did not affect the generation of group II PLA_2 cDNA. In all experiments, no significant difference in the group II PLA_2 mRNA was observed between SHAM-ADX and SHAM-ADX + V groups (Fig. 2, *A* and *B*). In ADX rats compared to SHAM-ADX + V rats, an increase of 126 ± 9% of group II PLA_2 mRNA was observed at 320 ng total RNA. In ADX rats substituted with glucocorticoids (ADX-CORT), group II PLA_2 mRNA was decreased by 33±15% when compared with ADX rats. These results were furthermore supported by the analysis of the ratios of cpm of group II PLA_2/cpm of internal standard. The results of one of three identical experiments are shown in Fig. 2 *B*.

The impact of glucocorticoids on group I PLA_2 has not been investigated previously. Therefore, we extended our study to group I PLA_2 also. Quantitative studies with [α-^{32}P]dCTP-labeled PCR products revealed that in ADX compared to SHAM-ADX + V rats, there was an increase of 27±1.5% of group I PLA_2 mRNA (Fig. 3 *A*). In all ADX rats treated with glucocorticoids (ADX + CORT) the levels of group I PLA_2 mRNA decreased by 71±4% below that of control groups; no signal was detected on agarose gel after PCR (Fig. 3 *A*).

To establish whether the increase of group II PLA_2 mRNA is associated with increased group II PLA_2 protein, lung, spleen, liver, and kidney tissues were analyzed by Western blot using specific group II PLA_2 monoclonal antibodies (Fig. 4, *A* and *B*). In all these tissue samples ADX resulted in an increased concentration of group II PLA_2 protein ranging from 70 to 100% depending on the type of tissue analyzed (Fig. 4 *B*). In these tissues of ADX rats substituted with glucocorticoids, group II PLA_2 protein was decreased by 64–85%, when com-



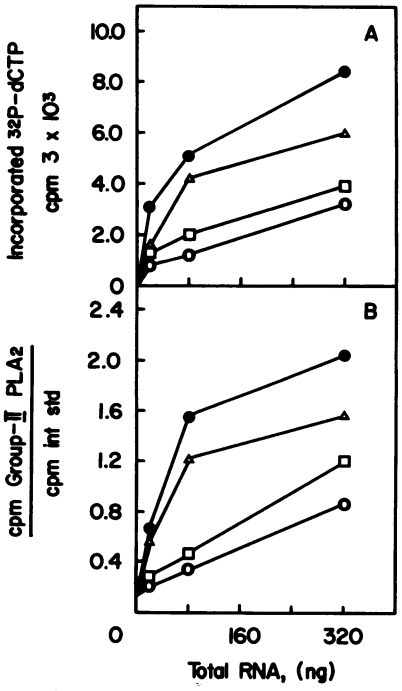


Figure 2. (*A*) Incorporation of [α-^{32}P]dCTP (cpm) into cDNA of group II PLA_2 as a function of total RNA used for reverse transcription. To exclude potential competition of primers for group II PLA_2 cDNA and internal standard during PCR, no internal standard was added to this reverse transcription. ○, SHAM-ADX; □, SHAM-ADX + V; △, ADX + CORT; ●, ADX. Each symbol represents one animal. (*B*) Incorporation of [α-^{32}P]dCTP (cpm) into cDNA of group II PLA_2 in the presence of 18 fg internal standard. The ratio of group II PLA_2 cpm/internal standard cpm is plotted against the total RNA concentration used during reverse transcription reaction. The symbols are the same as those used in *A*.

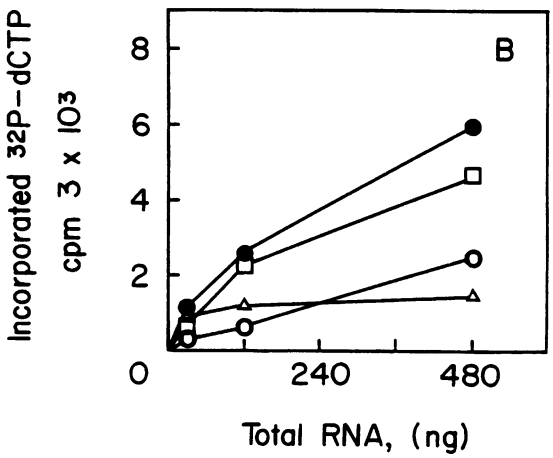


Figure 3. (*A*) Agarose gel (1.5%) electrophoresis of PCR products of group I PLA_2 from rat lung tissue. Group I PLA_2 mRNA was reverse transcribed and 30 ng (lane *a*), 120 ng (lane *b*), and 480 ng (lane *c*) of reverse transcribed material was used as template for PCR reactions. *M*, DNA molecular weight marker IV (Boehringer Mannheim Biochemicals); *1*, SHAM-ADX; *2*, SHAM-ADX + V; *3*, ADX + CORT; *4*, ADX. (*B*) Incorporation of [α-^{32}P]dCTP (cpm) into cDNA of group I PLA_2 cDNA as a function of total RNA used for reverse transcription. ○, SHAM-ADX; □, SHAM-ADX + V; △, ADX + CORT; ●, ADX.

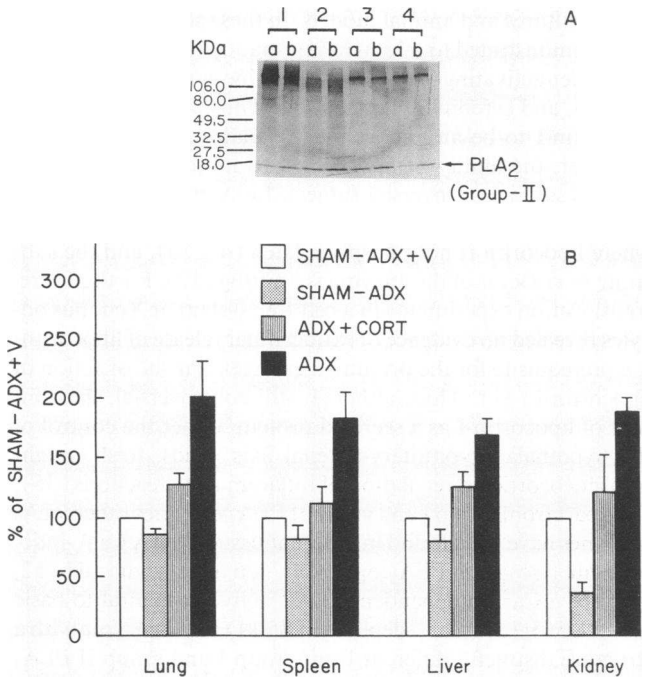


Figure 4. PLA_2 protein measurements in rat tissues. (*A*) Western blot analysis of group II PLA_2 in acid extracts of lung (*1*), spleen (*2*), liver (*3*), and kidney (*4*) tissues. A representative control group SHAM-ADX + V (lane *a*) and experimental group ADX (b) for each tissue is presented. To get a good signal different amounts of protein were used: 2 μg for lung, 5 μg for spleen and liver, and 10 μg for kidney. (*B*) Western blot analysis of group II PLA_2 protein of different tissues. SHAM-ADX + V–treated rats were used as a reference (100%). The values are expressed as the mean±SD (n = 3).

pared with ADX rats. In lung tissue, the percentage decline in protein was more pronounced (73%) than the corresponding decline of mRNA (33%). The reason for the very low PLA_2 protein content in kidneys of SHAM-ADX is not clear.

Activity of PLA_2 was assessed in tissues of lung, liver, spleen and kidney. For that purpose, the PLA_2 enzyme was solubilized after acid extraction. The PLA_2 enzyme extracted showed a pH optimum of 7.5–9.0 and required 5 mM Ca^{2+} for optimal activity. The results from PLA_2 activity measurements are shown in Fig. 5. Interestingly different tissues showed different amounts of PLA_2 specific activity; lung tissue had the highest and kidney tissue the lowest PLA_2 activity. In correlation with increased mRNA and PLA_2 protein, the total PLA_2 activity was also increased in the above studied tissues of ADX rats (Fig. 5). In all the tissues analyzed, there was a threefold increase of PLA_2 activity in ADX rats when compared with SHAM-ADX + V rats. The increased PLA_2 activity was restored to normal level when ADX rats were substituted with glucocorticoids.

The relative increase in total PLA_2 activity in ADX rats was twofold higher compared to the relative increase of mRNA and protein suggesting that PLA_2 activity is possibly modulated by changes in activators or inhibitors of PLA_2. Lipocortin-I has shown to inhibit PLA_2 activity (12, 13, 29). Previously, we have reported decreased levels of lipocortin-I mRNA and protein in ADX rats and restoration of lipocortin-I to control levels in ADX rats treated with glucocorticoids (21). When the lipocortin-I concentrations were plotted next to the corresponding PLA_2 activities (Fig. 5), a correlation was observed: at higher concentrations of lipocortin-I, a decreased PLA_2 activity was observed, and at lower concentrations of lipocortin-I an increased PLA_2 was observed when the same organ was analyzed in different groups of rats.

Discussion

The quantitative RNA-PCR study revealed that both group I and group II PLA_2 mRNA levels were elevated after ADX in lung tissue. The upregulation of PLA_2 in ADX rats was not a nonspecific phenomenon, since the mRNA levels of other proteins (metallothionein-II and c-*fos*, c-*myc*, c-*erbAβ* oncogenes) have been shown to remain unchanged (21). After ADX, the increase of mRNA of group II PLA_2 was more pronounced than that of group I PLA_2, and when the ADX rats were substituted with glucocorticoids, the mRNA of group II PLA_2 was decreased less than that of group I PLA_2, suggesting that group I PLA_2 and group II PLA_2 are regulated differently by glucocorticoids. Previous studies with rat vascular smooth vessel cells indicated that group II PLA_2 mRNA was regulated by two different mechanisms: First, by cAMP as a second messenger, and second, by a cAMP-independent pathway (11). It has been shown that glucocorticoids inhibited cAMP-dependent, but not cAMP-independent expression of group II PLA_2 mRNA (15). Provided these in vitro studies reflect the in vivo

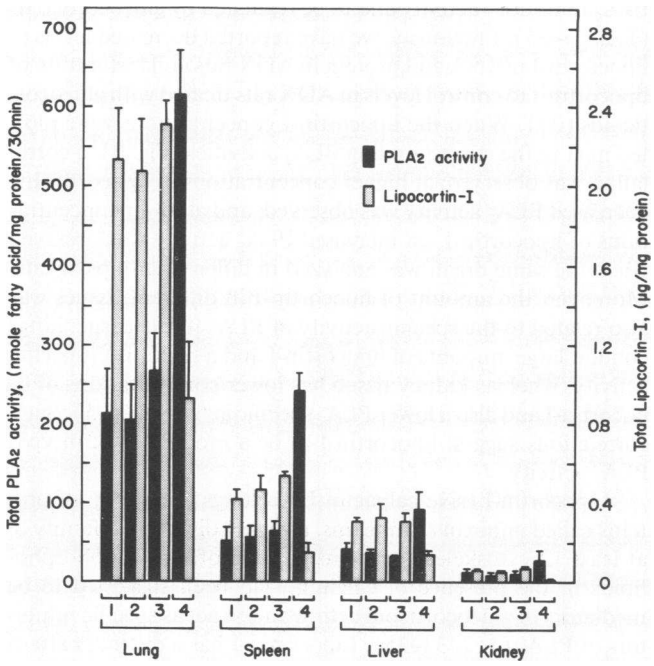


Figure 5. Total PLA_2 activity (nmol fatty acid/mg protein per 30 min) and lipocortin concentrations (μg/mg protein) in rat tissues. Lipocortin-I data previously published were used to correlate PLA_2 activity measurements with lipocortin-I protein content (21). In this approach total lipocortin-I concentration of monomer in lung, monomer and dimer in spleen, and dimer in liver and kidney were estimated using the standard curve of recombinant lipocortin-I. Each value represents the mean (±SD) of three different experiments. Lane *1*, SHAM-ADX; lane *2*, SHAM-ADX + V; lane *3*, ADX + CORT; lane *4*, ADX.

situation, then one might assume that the inhibition of mRNA of group II PLA_2 by glucocorticoids in ADX rats was mediated by the cAMP pathway. Since the cAMP-independent pathway seems to be regulated by factors other than glucocorticoids (15), the absence of a complete normalization of group II PLA2 mRNA in ADX rats with glucocorticoids is not surprising.

In lung tissue of ADX rats substituted with glucocorticoids, group II PLA_2 protein was decreased by 73% when compared with ADX rats; this percentage decline in protein was more pronounced than the corresponding decline of 33% in mRNA, suggesting that glucocorticoids decrease group II PLA_2 protein also by modulating posttranscriptional mechanisms. Separate transcriptional and posttranscriptional effects of pharmacological doses of glucocorticoids have been reported in inflammatory conditions on the synthesis of group II PLA_2, tumor necrosis factor, and interleukin-1 (14, 30, 31).

The increases of group II PLA_2 protein and mRNA in ADX rats were also reflected by increases of PLA_2 enzyme activity. The PLA_2 enzyme activity measured in these tissues exhibited biochemical properties similar to group II PLA_2 enzyme activity characterized from synovial fluid from rheumatoid patients and human platelets with regard to its optimum pH, Ca^{2+} dependence, substrate preference, and also stability towards acidic pH (32). The relative increase in total PLA_2 activity was two-fold higher compared with the relative increase of mRNA and protein following ADX, suggesting that PLA_2 activity was possibly modulated by an increase in activators or by removal of inhibitors. Lipocortins have been demonstrated to exhibit PLA_2 inhibitory activity and to be regulated by glucocorticoids (1, 2, 33–35). Previously, we have reported decreased levels of lipocortin-I mRNA and protein in ADX rats and restoration of lipocortin-I to control levels in ADX rats treated with glucocorticoids (21). When the lipocortin-I concentrations were plotted next to the corresponding PLA_2 activities (Fig. 5), a correlation was observed: at higher concentrations of lipocortin-I, a decreased PLA_2 activity was observed, and at lower concentrations of lipocortin-I, an increased PLA_2 activity was observed when the same organ was analyzed in different groups of rats. Moreover, the amount of lipocortin-I in different tissues was also related to the specific activity of PLA_2 in the tissue; lungs contain large amounts of lipocortin-I and a high PLA_2-specific activity, whereas kidney tissue has lower concentrations of lipocortin-I and also a lower PLA_2 specific activity (Fig. 5). Such correlations suggest lipocortin-I to be a modulator of in vivo PLA_2 activity.

Lipocortin-I (sive calpactin-II) belongs to a group of proteins called annexins. Annexins, a widely distributed family of at least 12 cytoskeletal proteins capable of binding phospholipids in the presence of calcium, had been suggested to be mediators of glucocorticoid action and to act as specific inhibitors of PLA_2 (1, 2, 33–35). Lipocortin-I has a distinct pattern of organ distribution (Fig. 5) and a discrete pattern of cellular distribution (36). For example, lipocortin-I in skin is extremely abundant in the epidermis, especially in the basal keratinocytes, but sparse in the dermis (36). There exists evidence that the synthesis of lipocortin-I can be induced in monocytes and differentiated macrophage-like cell lines by glucocorticoids (37–41); however, in certain other cell lines the synthesis of lipocortin-I does not appear to be regulated by glucocorticoids (42, 43). Several pharmacological studies have used recombinant human lipocortin-I and derived peptide fragments in cell cultures and animal models. In these studies, lipocortin-I was demonstrated to inhibit inflammation (44–45), synthesis of platelet-activating factor (46), cytokine-mediated pyrogenesis (47), and cerebral ischemia (48). Thus potential exists for lipocortin-I to be an endogenous regulator of inflammation. However, the ability of lipocortin-I to inhibit the activity of PLA_2 is still a controversial subject. Lipocortin-I is indeed an inhibitor of PLA_2 in vitro (29), but only under conditions where lipocortin is not phosphorylated (49, 50), and the substrate or cofactor of the enzyme is limiting (12). Furthermore, translocation experiments in a cell-free system or Xenopus oocytes revealed no evidence of extracellular release of lipocortin-I, a prerequisite for the presumed extracellular site of action of lipocortin-I (51). Thus, although still controversial, the concept of lipocortin-I as a secreted protein, under the control of the hypothalamic-pituitary-adrenal axis yields, fresh insight into glucocorticoid regulation of inflammatory reactions (35).

Apart from group I and group II PLA_2 enzymes other PLA_2 isozymes have been found in different tissues: (*a*) A Ca^{2+}-independent lysosomal PLA_2 optimally active at acidic pH (52, 53); (*b*) a Ca^{2+}-independent PLA_2, active at neutral to basic pH (54, 55); (*c*) a Ca^{2+}-dependent 85-kD PLA_2 enzyme with a structure distinctly different from group I and group II PLA_2 enzymes (56, 57). The structure of both Ca^{2+}-independent PLA_2 enzymes is not known. Whether the 85-kD PLA_2 enzyme is involved in inflammatory reactions has not been determined. Most likely, different isoforms of PLA_2 enzymes may coexist in a single system, and might exert separately or cumulatively their effect in generating arachidonic acid. The other enzymes that generate arachidonic acid, depending on cell types are (*a*) sequential action of phospholipase C followed by diacylglycerol lipase or diacylglycerol kinase followed by phosphatidic acid specific PLA_2; and (*b*) phospholipase D followed by phosphatidic acid specific PLA_2 (58). It is evident that glucocorticoids inhibit the release of arachidonic acid and subsequent formation of eicosanoids, no matter which class or type of phospholipase enzyme releases the arachidonic acid. The present investigation demonstrates that for restoration of PLA_2 activity by glucocorticoids in ADX rats, apart from regulating PLA_2 enzyme at transcriptional and posttranscriptional level, an increase of an endogenous inhibitor is necessary. Lipocortin-I is a good candidate for such an inhibitory function, since lipocortin has been shown to inhibit Ca^{2+}-independent PLA_2 (52), phospholipase C (59), and phospholipase D (60).

The majority of chronic inflammatory diseases are of unknown etiology, and the factors modulating their severity are largely unknown. Physiological endogenous glucocorticoid levels may protect from these disease states and low levels promote these disease states. The latter hypothesis was proposed in 1922 by Képinov, who reported an increased sensitivity to bronchial anaphylactic reactions in ADX guinea pigs (16). That observation was in line with the later findings of an association between glucocorticoid deficiency and disease states explained by allergic and/or immunological mechanisms in experimental animals and humans (17, 61–64). The upregulation of group II PLA_2 enzyme and the downregulation of lipocortin-I by low levels of glucocorticoids indicate that clinically unrecognized partial glucocorticoid deficiency, either caused by suppression of endogenous glucocorticoid production after withdrawal of exogenous glucocorticoids or caused low cortisol synthesis for other reasons, may be relevant for the genesis of inflammatory diseases.

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