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Glucocorticoids inhibit prostaglandin synthesis not only at the level of phospholipase A_2 but also at the level of cyclo-oxygenase/PGE isomerase

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1 Prostanoid synthesis was induced in bone marrow-derived macrophages by addition of exogenous arachidonic acid to the cell cultures. When the cells were preincubated with dexamethasone (10^{-7} and 10^{-6} M) overnight, prostaglandin synthesis was inhibited by $66.5 \pm 2.8\%$ and $56.7 \pm 2.9\%$ (mean $\pm$ s.d.; $n = 3$) respectively.

2 Endogenous membrane bound phospholipase A_2 was measured with labelled phospholipids used as substrates. The enzyme activity with phosphatidylcholine and phosphatidylethanolamine as substrates was inhibited by $27.0 \pm 8.3\%$ and $23.3 \pm 11.1\%$ ($n = 4$) respectively, in dexamethasone-treated macrophages compared to control cells. Neither the distribution of radiolabelled arachidonic acid among the different phospholipid species nor the release of arachidonic acid from prelabelled cells were significantly impaired by pretreatment of the macrophages with dexamethasone (1 μM).

3 The enzyme activity of the cyclo-oxygenase/prostaglandin E (PGE) isomerase was measured in cell membranes from control cells and dexamethasone-treated cells. It was inhibited by $40.0 \pm 8.4\%$ ($n = 4$) in dexamethasone-treated cells as compared to control cells. Thus, glucocorticoids inhibit not only phospholipase A_2 in these cells, but predominantly inhibit arachidonic acid metabolism subsequent to its release from phospholipids.

Introduction

Glucocorticoids exert their multiple metabolic functions by binding to cytoplasmic receptors and subsequent induction of catalytic or regulatory proteins (Rousseau, 1984). Their anti-inflammatory properties have been attributed in part to the liberation and enhanced synthesis of proteins, collectively called lipocortins, which inhibit phospholipase A_2, leading to a decreased eicosanoid synthesis (Flower, 1984). Such proteins have been isolated from various cells (Sato *et al.*, 1988, and references therein) and cloned from the human histiocytic lymphoma line U937 (Wallner *et al.*, 1986). Lipocortins were shown to be identical with another group of proteins, membrane cytoskeletal proteins which bind calcium and phospholipids including calpactins I and II, as well as other proteins (Huang *et al.*, 1986; Brugge, 1986). These proteins are very abundant and may account for up to 2.5% of total cell protein as shown in endothelial cells (Hullin *et al.*, 1989). Their exact physiological function has not yet been defined but it has been supposed that they may play a role in membrane-to-cytoskeleton linkage (Glenney *et al.*, 1987) or regulation of cell functions such as secretion (Hutton, 1986).

The effect of glucocorticoids on phospholipase A_2 is most often measured by the decreased release of fatty acids from prelabelled cellular lipids (e.g. Hong & Levine, 1976; Flower & Blackwell, 1979; Hirata *et al.*, 1980). An attempt to measure reduced endogenous phospholipase activity with exogenous substrate was not successful (Russo-Marie & Duval, 1982). The phospholipase A_2 inhibitory capacity of lipocortins is usually measured with pancreatic phospholipase and various labelled phospholipids, most often labelled *E. coli*, as substrate. It was shown recently at least in the case of some substrates, that lipocortins do not interact directly with the phospholipase A_2 but inhibit the enzyme by interaction with the substrate phospholipids (Davidson *et al.*, 1987; Aarsman *et al.*, 1987).

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By comparing arachidonic acid release and PGE_2 synthesis in unstimulated mouse peritoneal macrophages, Wood *et al.* (1984) found an inhibition of PGE_2 synthesis but not arachidonic acid release in hydrocortisone-treated cells. The molecular mechanism underlying these effects of hydrocortisone, however, was not further investigated. Therefore, in the present study we investigated the molecular mechanism of the inhibition of prostaglandin synthesis in macrophages by dexamethasone. For the first time we were able to measure a decreased enzyme activity of membrane-bound endogenous phospholipase A_2 after treatment of cells with dexamethasone. In addition, a marked decrease of cyclo-oxygenase/PGE isomerase activity was demonstrated which was even more pronounced than the inhibition of phospholipase A_2 activity. These findings indicate a more complex interference of glucocorticoids with arachidonic acid metabolism than has often been suggested.

Methods

Bone marrow cultures

Bone marrow-derived macrophages were cultivated in a similar way to that described by Fischer *et al.* (1984). Cells were flushed from the femora of six to eight week-old C57/B16 mice and cultivated in DMEM (4.5 g ml^{-1} glucose, Gibco, Karlsruhe, F.R.G.), supplemented with 1 mM sodium pyruvate, 2 mM glutamine, 1% non essential amino acids, 5×10^{-5} M 2-mercaptoethanol, 10% new born calf serum (Gibco), 5% horse serum (Boehringer Mannheim, F.R.G.) and 30% L-cell conditioned medium. Bone marrow cells were seeded in petri dishes (Greiner Nuertingen, F.R.G.) (10 ml, 1×10^5 cells ml^{-1}) and cultured at 37°C in a humidified atmosphere containing 10% CO_2. At day 5, 5 ml of the medium, containing non adherent cells, were removed and supplemented by 10 ml fresh medium. Medium (10 ml) was also exchanged on day 7. At day 8, the medium was removed and the adherent cells were washed twice with warm phosphate buffered saline. To remove adherent cells, the monolayer was incubated for 10 min on ice with 10 ml HEPES (5 mM) buffered RPMI 1640 (Gibco). Then the cells were flushed with a pipette, centrifuged and washed once with HEPES buffered RPMI. The cells were subcultured with 5 to 6×10^6 cells per petri dish in culture medium as described above. At day 11 the cells were flushed from the dishes and used for the experiments. Cell viability always exceeded 90% as determined by trypan blue exclusion. Homogeneity of the macrophage preparation was assessed by staining according to Giemsa and non specific esterase staining (Ichikawa *et al.*, 1966; Gould *et al.*, 1975).

L-cell conditioned medium

L 929 S cells were kindly provided by E. Ferber, Freiburg, F.R.G. They were kept in DMEM containing 10% foetal calf serum (Flesch & Ferber, 1986). Conditioned medium was prepared in 160 cm^2 culture flasks (Costar, Technomara, Fernwald, F.R.G.) with 5×10^6 cells per 50 ml medium. At day 8, the medium was collected, centrifuged and kept batchwise at −20°C.

Release of prostaglandins

Macrophages were preincubated over night in DMEM supplemented with 5 mg ml^{-1} bovine serum albumin (essentially fatty acid-free) in flat bottom micro titer plates (Nunc, Wiesbaden, F.R.G.) at a cell density of 5×10^5 cells ml^{-1}. If not indicated otherwise, dexamethasone was added during this preincubation period. Then the medium was removed and the cells were further incubated with DMEM and the respective concentration of dexamethasone in the presence or absence of arachidonic acid to induce prostaglandin synthesis. After the times indicated, the supernatant was removed, centrifuged and an aliquot used for the determination of the prostaglandin concentration.

The concentration of PGE_2 and thromboxane B_2 (TXB_2) was determined by a double antibody radioimmunoassay as described recently (Kaever *et al.*, 1988). The antibodies were kindly provided by V. Kaever, Hannover, F.R.G.

Release of arachidonic acid

Release of arachidonic acid was determined according to Wood *et al.* (1984). Macrophages were incubated over night as described above with 0.04 μCi [^{14}C]-arachidonic acid (Amersham Buchler, Braunschweig, F.R.G., specific activity 50 mCi mmol^{-1}). The cells were washed once with medium containing 5 mg ml^{-1} albumin and further incubated in medium supplemented with albumin in the presence or absence of thimerosal, an inhibitor of fatty acid reacylation. At the end of the incubation time, an aliquot of the supernatant was removed and the radioactivity therein determined by liquid scintillation counting. In control experiments, the liberated activity was shown to be more than 95% arachid-

onic acid when analyzed by thin layer chromatography (t.l.c.). However, all of the activity was once arachidonic acid esterified to complex lipids. Thus the determination of the total release activity could be taken as a measure of liberated arachidonic acid.

To analyze the distribution of labelled arachidonic acid among the different phospholipid classes, macrophages were incubated in multi well plates (24 wells, Costar) with 0.05 μCi [^{14}C]-arachidonic acid in the presence or absence of various concentrations of dexamethasone for 20 h. Then the medium was removed, and the cells lysed with 500 μl cold H_2O. The suspension was briefly sonicated and the lipids were extracted by chloroform/methanol and separated by t.l.c. (Goppelt & Resch, 1984). The amount of radioactivity of the individual lipid fractions was determined by liquid scintillation counting.

Determination of enzyme activities

Enzyme activities were determined in a crude membrane fraction from macrophages. For this purpose, the macrophages were incubated over night with or without dexamethasone in petri dishes in medium supplemented with albumin. At the end of the incubation period, the supernatant was replaced by cold buffer (20 mM Tris/HCl, pH 8.0, 5% saccharose). The plates were either kept on ice for 15 min or frozen to −20°C before use. The cells were scraped off by a rubber policeman and briefly sonicated in the cold. To prepare crude membranes, the homogenate was centrifuged at 600 *g* and the pellet resuspended in buffer and again sonicated. The supernatants were combined, supplemented with 0.1 mM $CaCl_2$ and centrifuged at 100,000 *g* for 1 h. The pellet was resuspended in buffer (Tris/HCl, saccharose) and kept on ice or frozen to −80°C. Protein was determined by measuring the native fluorescence at 280/340 nm (Resch *et al.*, 1972) with bovine serum albumin used as standard.

Determination of phospholipase A_2 activity

Phospholipase A_2 activity was determined essentially as described by Flesch & Ferber (1986) using phosphatidylcholine L-a-1-palmitoyl-2-arachidonyl (arachidonyl-1-[^{14}C]) (NEN, Boston, MA, U.S.A.) as substrate. The phospholipid was suspended in twice distilled water containing bovine serum albumin (essentially fatty acid-free) by brief sonication in the cold (final concentration in the assay: 1 μM lipid corresponding to about 25,000 c.p.m., 5 mg ml^{-1} albumin). The assay mixture contained 50 mM Tris/HCl, pH 8.5, 10 mM $CaCl_2$, 10 to 20 μg protein and the labelled lipid as substrate in a total volume of 250 μl in glass tubes. Control assays were performed with 10 mM EDTA instead of $CaCl_2$. After incubation for 60 min at 37°C the reaction was terminated by the addition of 375 μl cold isopropanol/1 N HCl (1/0.086, v/v). The glass tubes were kept in an ice bath and 700 μl heptane was added. The solution was shaken vigorously in order to obtain a homogeneous one phase system and the tubes were warmed up to room temperature to develop a two-phase system. After 30 min, 500 μl of the heptane phase were transferred to an Eppendorf tube containing 200 μl heptane and about 200 mg of silica to adsorb extracted phospholipid. The well mixed tubes were centrifuged at 13,000 *g* for 5 min and the radioactivity of 500 μl of the supernatant determined by liquid scintillation counting. The enzyme activity was calculated from the percentage of liberated fatty acid. In control experiments the heptane phase was analyzed by t.l.c. and contained exclusively free arachidonic acid. No diacylglycerol was detectable, which might have been generated by a phospholipase C activity.

Determination of cyclo-oxygenase/PGE isomerase activity

The synthesis of PGE_2 from arachidonic acid was determined in a crude membrane fraction similar as described by Kosaka *et al.* (1987). The assay mixture contained 50 mM HEPES, pH 7.4, 4 mM glutathione, 1 μM haemoglobin, 1 mM tryptophan, 20 to 40 μg protein and 1 μM arachidonic acid (sodium salt) as substrate in a total volume of 200 μl. As control, 5×10^{-5} M diclophenac was added. After incubation for 10 min at 37°C the reaction was terminated by the addition of 20 μl acetylsalicylate (5×10^{-4} M) and quick freezing in liquid nitrogen. The PGE_2 concentration was determined by radioimmunoassay. There were no crossreactivities with any of the constituents of the assay.

Materials

Dexamethasone phosphate (9α-fluoro-16α-methylprednisolone phosphate, Decadron) and acetylsalicylate were obtained from MSD Sharp and Dohme, München, F.R.G. In the dilutions used, the solvent of dexamethasone did not influence the parameters measured. Thimerosal (ethylmercurythiosalicylate), arachidonic acid (sodium salt) and actinomycin D were from Sigma, München, F.R.G. Arachidonic acid (free acid) was obtained from NuChek Prep, Elysian, U.S.A. The fatty acid was kept in ethanol/toluol (4/1, v/v). Before use, the organic solvent was evaporated and the arachidonic acid diluted in the

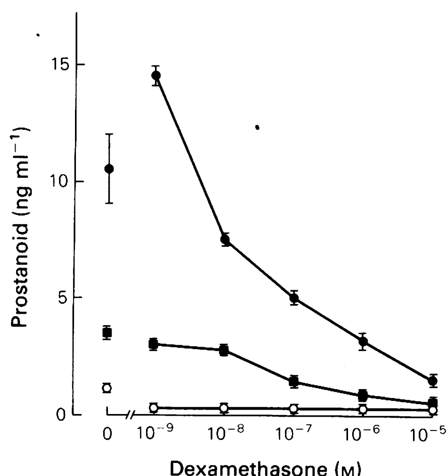

Figure 1 Concentration-dependent inhibition of prostanoid synthesis from exogenous arachidonic acid by dexamethasone. Macrophages were preincubated for 20 h in the presence or absence of dexamethasone in the concentrations indicated. Then the medium was exchanged and the cells were further incubated in the presence (● PGE_2; ■ thromboxane) or absence (○ PGE_2) of arachidonic acid (3×10^{-6} M) for 1 h. The concentration of thromboxane in the absence of arachidonic acid was below the detection limit. Data are results of a representative experiment out of a series of 3 similar ones. Vertical bars are the range of duplicate incubations.

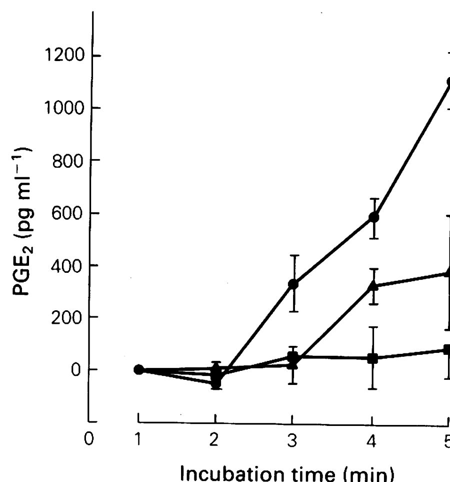

Figure 2 Short time kinetics of the induction of prostaglandin synthesis in bone marrow-derived macrophages. After preincubation for 20 h without (●) or with dexamethasone (▲ 10^{-7} M; ■ 10^{-6} M), the cells were kept in fresh medium under the same conditions for about 30 min. Then arachidonic acid was added (3×10^{-6} M) and the prostaglandin release determined after the times indicated. Vertical bars are the range about the means of two experiments each with duplicate incubations.

appropriate medium by brief sonication. Diclofenac (*o*-(2,3 dichloranilino)-phenylacetic acid) was kindly provided by Ciba-Geigy, Werth, F.R.G.

Statistical tests

When arachidonic acid or prostaglandin release was measured, means of duplicate incubations were determined under each experimental condition. When enzyme activities were measured, duplicate assays were performed for each cell preparation. To compare the values obtained from different primary cell cultures, the mean of each set of control cells was taken as 100% and the mean of the respective cells incubated with hexamethasone calculated as a percentage of the control. Percent data are given as means $\pm$ s.d. of n different cells cultures. The statistical significance of the inhibition values so obtained was calculated using the non-paired two-tailed Student's t test. A P value <0.05 was considered to indicate a statistically significant difference between two sets of data.

Results

Inhibition of prostanoid synthesis from exogenous arachidonic acid in dexamethasone-treated macrophages

Bone marrow-derived macrophages were obtained in high yield (about 20×10^6 cells from 1×10^6 stem cells) as a homogeneous preparation of mature macrophages. After cultivation in the presence of serum and L-cell conditioned medium as a source of colony stimulating factor, the cells were further incubated in medium supplemented with bovine serum albumin (5 mg ml^{-1}). The cells were induced to increase prostaglandin synthesis by exogenously added arachidonic acid. Prostanoid synthesis was inhibited by dexamethasone-pretreatment in a concentration-dependent manner. A representative experiment of the inhibition of PGE_2 and thromboxane synthesis after 1 h incubation with arachidonic acid (3×10^{-6} M) is shown in Figure 1. The synthesis of PGE_2 was inhibited by $66.5 \pm 2.8\%$ and $56.7 \pm 2.9\%$ by 10^{-6} M and 10^{-7} M dexamethasone respectively ($n = 3$, $P < 0.0001$ versus control). Thromboxane synthesis, which was about one third

of PGE_2 synthesis, was inhibited to the same extent. The amount of prostanoids released was not significantly different after 1, 3 and 5 h of incubation with arachidonic acid, indicating a fast synthesis during the first hour of incubation. Thus prostaglandin synthesis was measured during the first 5 min of arachidonic acid incubation (Figure 2). The value determined after 1 min was considered as background and thus set to zero. In control cells, an increase in PGE_2 synthesis was evident already after 3 min of exposure to arachidonic acid. The synthesis was almost completely inhibited by pretreatment with 10^{-6} M dexamethasone and to a lesser extent by pretreatment with 10^{-7} M dexamethasone.

When macrophages were incubated with dexamethasone and arachidonic acid simultaneously, no inhibition of prostanoid synthesis was observed (Figure 3). They had to be preincubated with dexamethasone for at least 3–5 h before an inhibitory effect became apparent. To show the necessity of RNA synthesis for the dexamethasone-induced inhibition of prostaglandin synthesis, macrophages were treated with actinomycin D prior to dexamethasone incubation (Table 1). At the end of the incubation time, cell viability was 60 ± 6% compared to control cells under the different conditions used. RNA synthesis was inhibited completely (>97%) as determined by [^{3}H]-uridine incorporation. The inhibitory effect of dexamethasone on PGE_2 synthesis was reversed by pretreatment of the cells with actinomycin D.

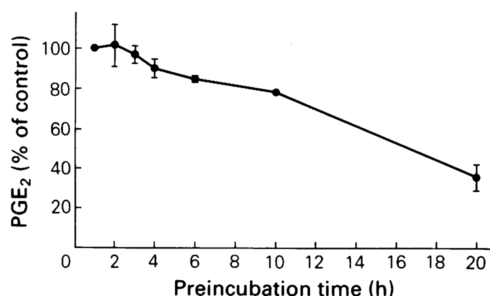

Figure 3 Importance of the preincubation for dexamethasone-induced inhibition of prostaglandin synthesis. Macrophages were preincubated with or without dexamethasone (10^{-6} M) for the times indicated. Then the medium was removed and medium containing arachidonic acid (3×10^{-6} M) with or without dexamethasone was added to the cultures for 1 h. PGE_2 was determined in the supernatants by radioimmunoassay. Data are percentage PGE_2 compared to non-treated control cells (means ± s.d. of 3 experiments).

Table 1 Effect of actinomycin D on the dexamethasone-induced inhibition of prostanoid synthesis

Macrophage incubation mixture	*Pretreatment conditions* Control	*Actinomycin D*
Medium	1033 ± 113	1436 ± 123
Dexamethasone	559 ± 75	1549 ± 175
Medium + arachidonic acid	4716 ± 760	5668 ± 761
Dexamethasone + arachidonic acid	1251 ± 27	6181 ± 347

Bone marrow-derived macrophages were preincubated for 3 h with actinomycin D (10 μg ml^{-1}). They were further incubated with dexamethasone (10^{-6} M) for 14 h. Part of the cells were stimulated during the last hour of the incubation period with exogenous arachidonic acid (10^{-5} M). PGE_2 (pg ml^{-1}) was determined in the supernatants by radioimmunoassay. Data are means ± s.d. of triplicate incubations.

Effect of conditioned media on PGE_2 synthesis

Macrophages were preincubated overnight without dexamethasone. Then the medium was removed and conditioned medium added from cells that had been preincubated in the presence or absence of dexamethasone (10^{-6} M). To induce prostaglandin synthesis, arachidonic acid or thimerosal were added as stimuli. Thimerosal is a mercury compound, which inhibits the reacylation of liberated endogenous arachidonic acid and thus increases the pool of free arachidonic acid available for prostanoid synthesis (Goppelt-Struebe *et al.*, 1986). The calculation of PGE_2 synthesis was corrected for the different amount of PGE_2 introduced by the conditioned medium. Compared to medium from control cells, the conditioned medium from dexamethasone-treated cells was able to inhibit prostaglandin release: after 60 min stimulation with thimerosal (10^{-4} M) or arachidonic acid (10^{-5} M) PGE_2 synthesis was inhibited by 18 ± 5% and 23 ± 9% ($n = 3$, $P < 0.05$ versus control), respectively. Inhibition of arachidonic acid release from prelabelled cells was not detectable under the same experimental conditions.

Effect of dexamethasone on arachidonic acid distribution among phospholipids

When bone marrow-derived macrophages were incubated with [^{14}C]-arachidonic acid, the fatty acid was rapidly incorporated into phospholipids, primarily

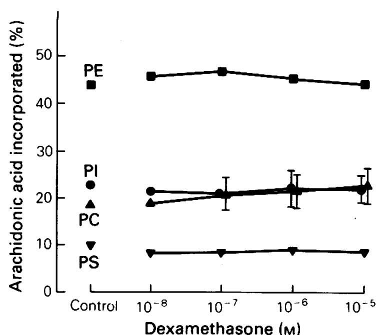

Figure 4 Distribution of arachidonic acid among different phospholipid species. Macrophages were incubated for 20 h with [^{14}C]-arachidonic acid in the presence of dexamethasone in the concentrations indicated. Lipids were extracted and separated as described in Methods. Vertical bars are the range of 2 experiments. If not indicated otherwise, the range was smaller than the symbols used. PE, phosphatidylethanolamine, PI phosphatidylinositol, PS, phosphatidylserine, PC phosphatidylcholine.

phosphatidylcholine, and neutral lipids. With prolonged incubation, arachidonic acid was rearranged among the different lipid species; after 20 h of incubation the fatty acid was esterified almost exclusively to phospholipids with most of the activity associated with phosphatidylethanolamine. After incubation of the macrophages for 20 h the distribution of arachidonic acid was not significantly altered, when the cells were incubated in the presence of various concentrations of dexamethasone (Figure 4).

Release of arachidonic acid from prelabelled cells

Macrophages were prelabelled for 20 h with arachidonic acid in the presence or absence of dexamethasone (10^{-6} M). Then the washed cells were further incubated in medium supplemented with albumin to trap liberated fatty acid. To enhance the arachidonic acid release the reincorporation was inhibited by thimerosal, which inhibits the lysophosphatide acyltransferase (Goppelt-Struebe *et al.*, 1986). The ratio of fatty acid released from dexamethasone-treated cells (10^{-6} M) to that released from control cells, was 0.94 ± 0.05 ($n = 6$) in the absence and 0.91 ± 0.25 ($n = 4$) in the presence of thimerosal (10^{-4} M), measured after 1 h. As can be seen from the large standard deviation, in some experiments there was even an increase in arachidonic acid release from dexamethasone-treated macrophages, but no significant decrease as expected from the prostanoid synthesis, which was inhibited by over 60% under the same conditions.

Table 2 Inhibition of phospholipase A_2 and cyclo-oxygenase/PGE isomerase activity in membranes of dexamethasone-treated macrophages

	% inhibition
Phospholipase A_2	
substrate: phosphatidylcholine	27.0 ± 8.3*
substrate: phosphatidylethanolamine	23.3 ± 11.1*
Cyclo-oxygenase/PGE isomerase	40.0 ± 8.4**

Membranes were prepared from macrophages which had been incubated for 20 h with or without dexamethasone (10^{-6} M). Enzyme activities were determined as described in Methods. Data are percentage inhibition compared to non-treated control cells ($n = 4$, *$P < 0.02$, **$P < 0.01$)

Inhibition of phospholipase A_2 by dexamethasone

The activity of phospholipase A_2 was measured in membranes obtained from macrophages that had been incubated with dexamethasone for 20 h. This incubation did not influence the adherence or the optical appearance of the cells. The amount of protein recovered in the cell homogenates and membranes from dexamethasone-treated cells and control cells was not significantly different. Phospholipase A_2 activity was determined with radiolabelled phospholipids as substrates. Cleavage of arachidonic acid from phosphatidylcholine and phosphatidylethanolamine, labelled with arachidonic acid in position 2, was inhibited in the membranes from dexamethasone-treated cells (Table 2).

Inhibition of cyclo-oxygenase/PGE isomerase activity

The conversion of exogenously added arachidonic acid to PGE_2 was measured in a crude membrane fraction. Membranes, prepared from dexamethasone-treated cells, showed a significantly reduced cyclo-oxygenase/PGE_2 isomerase activity (Table 2).

Discussion

The inhibitory effect of glucocorticoids on prostaglandin synthesis has been demonstrated in various cell types and tissues, especially in inflammatory tissues (Flower, 1988). It was attributed to the induction of lipocortins, which are characterized by their ability to inhibit phospholipase A_2. This mechanism, however, was not consistent with all the experimental evidence obtained in different types of inflamma-

tion models (Calignano *et al.*, 1985; Foster & McCormick, 1985). When healthy men or rabbits were treated with glucorticoids, the whole body production of prostanoids measured as urinary excretion was not diminished (Naray-Fejes-Toth *et al.*, 1984; Rosenkrantz *et al.*, 1985). The increased PGE_2 synthesis observed in some cells such as fibroblasts (Chandrabose *et al.*, 1978), mast cells (Robin *et al.*, 1985) or renal medullary interstitial cells (Erman *et al.*, 1986 and references therein), upon glucocorticoid administration may add to this effect. Thus, the effect of glucocorticoids on prostanoid synthesis varies depending on the cell system, adding to the complexity of glucocorticoid action. At variance with many other groups, Moore & Hoult (1980) could show a decrease in the activity of prostaglandin synthase and an increase in the activities of prostaglandin degrading enzymes in different tissues of rats by glucocorticoids and an action at a later stage than phospholipase A_2 was also suggested by Wood *et al.* (1984), investigating hydrocortisone effects in macrophages. Thus there was evidence that the action of glucocorticoids on arachidonic acid metabolism is more complex than a generalized phospholipase A_2 inhibition.

One of the arguments against a regulation distal to phospholipase A_2 was the reversibility of steroid-induced inhibition of prostanoid synthesis by exogenously added arachidonic acid (Russo-Marie & Duval, 1982; Parente *et al.*, 1984; Fan & Lewis; 1985).

In our experiments prostanoid synthesis was inhibited by dexamethasone when arachidonic acid was added to the cells as inducing agent. The discrepancy with the reports cited may be due to different experimental conditions: in those experiments arachidonic acid and dexamethasone were added simultaneously and PGE_2 secretion measured after a few hours. In our kinetic experiments we could show that arachidonic acid is very rapidly metabolized to prostaglandins: an increased PGE_2 synthesis was measurable after 3 min of incubation with arachidonic acid and came to an end within 1 h. As the action of glucocorticoids is dependent on protein synthesis, preincubation with dexamethasone was an absolute prerequisite to detect any effects. Inhibition of PGE_2 synthesis by dexamethasone was abolished by pretreatment of the cells with actinomycin D, excluding any direct glucocorticoid effects. When exogenous arachidonic acid is added to macrophages, the arachidonic acid is rapidly incorporated into complex lipids, phospholipids and neutral lipids. Dexamethasone did not significantly influence the distribution of [^{14}C]-arachidonic acid among the various phospholipid species, as was also reported for cultured rat kidney cells (Russo-Marie & Duval, 1982). Release of arachidonic acid from prelabelled macrophages (peritoneal macrophages from mouse and RAW_{264} cells) had been shown not to be influenced by hydrocortisone (Wood *et al.*, 1984). These results were confirmed by our experiments with bone marrow-derived macrophages and dexamethasone. Also the conditioned medium did not interfere with the arachidonic acid release, but inhibited prostaglandin synthesis. These findings correlate with the rather modest inhibition of phospholipase A_2 activity by about 25%.

Inhibition of the membrane-bound phospholipase A_2 was measured with two types of substrates, phosphatidylcholine and phosphatidylethanolamine. Lipocortin was shown recently to inhibit pancreatic phospholipase A_2 with phosphatidylethanolamine as substrate but not with phosphatidylcholine (Rothut *et al.*, 1987). At the moment, however, we do not have any evidence for the mechanism of the glucocorticoid-induced intracellular inhibition of phospholipase A_2, bound to the particular fraction of macrophages. As we could show recently, phospholipase A_2 activity is also inhibited in dexamethasone-treated phorbolester-differentiated U937 cells (Koehler *et al.*, 1989). However, the expression of lipocortins is not inducible by glucocorticoids in these cells (Isacke *et al.*, 1989). Thus other mechanisms of inhibition of phospholipase A_2 may also be operative in the glucocorticoid-treated cells.

The modest inhibition of phospholipase A_2 activity and the failure to detect effects of dexamethasone on phospholipid metabolism suggested additional regulatory mechanisms. Endogenous arachidonic acid is metabolized to cyclo-oxygenase or lipoxygenase products. It is not quite clear whether exogenous arachidonic acid is a direct substrate for the cyclo-oxygenase or whether it is first esterified to phospholipids and becomes a substrate after cleavage by a phospholipase A_2. Esterification seems to be necessary for leukotriene formation but not for prostanoid synthesis in resident macrophages (Scott *et al.*, 1982). The very fast PGE_2 synthesis observed in bone marrow-derived macrophages suggested a direct conversion, which was inhibited by dexamethasone. Thus the data indicated an effect of dexamethasone on the cyclo-oxygenase/PGE isomerase system. This could be confirmed by the direct measurement of the cyclo-oxygenase/PGE isomerase system. The conversion of arachidonic acid was inhibited in membranes from dexamethasone-treated macrophages compared to membranes from control cells. A similar inhibition of prostaglandin synthase in rats was also shown by Moore & Hoult (1980). After this manuscript was submitted, Bailey *et al.* (1988) showed decreased mRNA levels of cyclo-oxygenase in vascular smooth muscle cells and Raz *et al.* (1989) showed decreased levels of cyclo-

oxygenase in fibroblasts. This indicates that the effects of glucocorticoids on cyclo-oxygenase are not restricted to a certain cell type but seem to be of more general nature.

Thus we could show a dexamethasone-induced inhibition of an endogenous membrane-bound phospholipase A_2 activity. However, our results also indicate that there are additional regulatory mechanisms that do not lead to a general inhibition of arachidonic acid metabolism but to a more selective inhibition of prostanoid synthesis.

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