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Pharmacology

Lipocortin 1 mediates dexamethasone-induced growth arrest of the A549 lung adenocarcinoma cell line

(glucocorticoids/RU38486/eicosanoids/annexins/neutralizing antibody/indomethacin)

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ABSTRACT The synthetic glucocorticoid dexamethasone (1 μM to 1 pM) strongly (maximum > 80%) inhibits proliferation of the A549 human lung adenocarcinoma line (EC_{50} > 1 nM) and leads to the appearance, or a further increase (≈3-fold) in the expression on the cell surface, of the calcium and phospholipid binding protein lipocortin (annexin) 1. Both these effects, which are shared by hydrocortisone (1 μM) but not by progesterone or aldosterone (1 μM), are inhibited by the antiglucocorticoids RU38486 and RU43044 (1 μM). The nonsteroidal antiinflammatory drugs indomethacin (1 μM) and naproxen (10 μM) and human recombinant lipocortin 1 (0.05–5.0 μg/ml) also produce growth arrest in this cell line. During proliferation A549 cells spontaneously release prostaglandin E_2 [10–20 ng (28–57 pmol) per ml per 5-day period] into the growth medium. In concentrations that cause growth-arrest, dexamethasone, indomethacin, and lipocortin 1 abolish the generation of this eicosanoid by A549 cells. Prostaglandin E_2 itself (0.01–1 pM) stimulates cell growth and partially reverses (≈50%) the inhibition of growth caused by dexamethasone and indomethacin. Addition of the neutralizing anti-lipocortin 1 monoclonal antibody 1A (5 μg/ml), but not the nonneutralizing anti-lipocortin monoclonal antibody 1B, substantially reversed (>80%) the inhibitory activity of dexamethasone on both growth and prostaglandin E_2 synthesis. The generation of prostaglandin E_2 by A549 cells seems to be an important regulator of cell proliferation *in vitro* and the dexamethasone-induced suppression of proliferation in this model is attributable to eicosanoid inhibition caused by lipocortin 1.

Glucocorticoids inhibit the proliferation of many cell types including thymocytes (1), lymphocytes (2, 3), fibroblasts (4), muscle (5), skin (6), leukemic cell lines (7, 8), and hepatoma cells (9) in culture. The molecular mechanisms involved in cell growth control have not been clarified although several studies implicate eicosanoids as modulators of this aspect of cell physiology (e.g., ref. 10). The biological actions of glucocorticoids in many cells are brought about by discrete changes in gene expression and protein synthesis (11, 12) after combination with specific intracellular receptors. Several, but apparently not all, cell types respond to glucocorticoids with increased intracellular or pericellular amounts of an eicosanoid-suppressive factor now identified as chiefly lipocortin 1 (also known as annexin 1; for review, see ref. 13). This protein, which is a member of a superfamily of proteins characterized by their ability to bind calcium and anionic phospholipids (14), has a variety of biological actions. For example, systemic administration or extracellular application of the protein inhibits eicosanoid generation (15), cytokine- and poly(I)·poly(C)-induced fever (16, 17), acute inflammatory oedema (18), neutrophil migration (19), and ischemic damage to the brain (20) and causes differentiation of squamous carcinoma cells (21). In addition, lipocortins may play a role in several other cellular processes including exocytosis (22), chemotaxis (19), anticoagulation (23), growth-factor signal transduction (24, 25), cellular differentiation (26), and cell growth (27).

We now report that the synthetic glucocorticoid dexamethasone potently inhibits the growth of A549 cells *in vitro* and that suppression of prostaglandin (PG) E_2 formation brought about by a steroid-induced increase in pericellular lipocortin 1 seems to be a major mechanism controlling this response.

MATERIALS AND METHODS

Cell Culture. A549 cells (Flow Laboratories) were maintained in continuous logarithmic-phase growth in Dulbecco's modified Eagle's medium/F-12 (DMEM/F-12; Sigma) containing phenol red (Sigma) and 10% (vol/vol) fetal calf serum (FCS; Flow Laboratories) in T-150 tissue culture flasks. The cells were not allowed to reach confluence at any time as this diminishes their response to steroids.

Cell Proliferation Experiments. Subconfluent A549 cells were removed by treatment with 0.05% trypsin (Sigma)/0.02% EDTA (Sigma) in phosphate-buffered saline (PBS) for ≈5 min. The detached cells were seeded into 12-place multiwell plates (Flow Laboratories) at 5 × 10^4 cells per ml per well in DMEM/F-12/10% FCS and incubated overnight. The medium was replaced with DMEM/F-12 (without phenol red), containing 10% dextran/charcoal-stripped FCS and dilutions of the steroid or other drug or the appropriate vehicle. During culture the medium was replenished every other day. On the days indicated, cells were removed with trypsin and counted in triplicate by using a Neubauer hemocytometer. Viability was assessed routinely using the trypan blue exclusion assay. Dexamethasone, hydrocortisone, aldosterone, progesterone, indomethacin, and naproxen were from Sigma. RU38486 and RU43044 were generous gifts from D. Philibert (Roussel-Uclaf, Romainville, France).

Lipocortin 1 Induction on the Cell Surface. To provide a sufficient number of cells for measurement of cell surface lipocortin 1 induction, subconfluent A549 cells were seeded into T-75 flasks (Flow Laboratories) at 0.5 × 10^6 cells per flask in 10 ml of DMEM/F-12/10% FCS and incubated overnight. The medium was changed to 20 ml of DMEM/F-12 without phenol red but with 10% dextran/charcoal-stripped FCS, and the cells were incubated for a further 5 days. The appropriate steroids, steroid antagonists, or ethanol vehicle were then added directly to each flask without a further change of medium as fresh FCS itself can result in an induction of lipocortin 1 (27). At various times after treatment the medium was aspirated and the cell monolayers were washed with 2 ml of PBS containing 1 mM EDTA. Total protein in the washing was precipitated with 10% (wt/vol) trichloroacetic acid, resuspended in 100 μl of electrophoresis

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Abbreviations: PG, prostaglandin; FCS, fetal calf serum.

sample buffer, and subjected to Western blot analysis for lipocortin 1 expression.

Western Blot Analysis and Determination of Lipocortin 1 Expression. Western blot analysis employed conventional procedures using the specific rabbit anti-lipocortin 1 antiserum 842 [a generous gift from J. Browning (Biogen Research)]. Quantification of immunoreactive bands was made using a video-scanning densitometer analysis system designed by Roger Jagoe (Royal Postgraduate Medical School, Hammersmith Hospital, London).

Lipocortin 1 and Neutralizing Anti-Lipocortin 1 Antibody Experiments. Subconfluent A549 cells were seeded into 12-place multiwell plates in DMEM/F-12/10% FCS at 5×10^4 cells per ml per well and incubated for 18–24 h. The medium was changed to DMEM/F-12 without phenol red and serum supplements. Dexamethasone was added at a final concentration of 2 nM and the neutralizing monoclonal antibody [McAB1A (28), a generous gift from J. Browning] was added at a final concentration of 5 μg/ml. Recombinant CHO-derived lipocortin 1 [a gift from J. Browning, prepared essentially as described (29)] was added in a serial dilution of 5, 0.5, and 0.05 μg/ml (140–1.4 nM). Fresh antibody or lipocortin 1 was added each day and the cells were counted after 4 days of treatment.

Measurement of PGE_2 Production. Medium from steroid- or lipocortin-treated cell cultures was stored frozen at −70°C and PGE_2 was subsequently estimated using a conventional commercially available RIA procedure (Amersham).

Statistical Analysis. Results are expressed as the mean ± 1 SD. Student's *t* test (unpaired) was used to determine statistical significance with $P < 0.05$ as the minimal value indicating a significant result.

RESULTS

Effect of Glucocorticoids and Other Steroids on A549 Cell Proliferation. Dexamethasone (1 μM to 1 pM) inhibited A549 cell proliferation in a dose-dependent fashion with a half-maximal inhibition at ≈1 nM (Fig. 1). Cells treated for as little as 2 h with 1 μM dexamethasone and then transferred to fresh medium without steroid were still growth-arrested compared to control cells after 3 days of culture (Fig. 1 *Inset*).

At 10 nM, dexamethasone significantly ($P < 0.001$) inhibited A549 cell proliferation after 8 and 12 days of treatment (Fig. 2). This effect was prevented by 1 μM RU38486 at days 8 and 12 and by 1 μM RU43044 at day 12. The antiglucocorticoids themselves (1 μM) have no significant effect on A549 cell proliferation. These data are congruent with the hypothesis that the effect of dexamethasone is mediated through the glucocorticoid receptor and additional evidence for this is the fact that progesterone (1 μM) or aldosterone (1 μM, data not shown) produces no significant inhibition of cell growth whereas hydrocortisone (1 μM), another glucocorticoid, produces a qualitatively similar effect to dexamethasone (35 ± 5.4% inhibition; $P < 0.001$; $n = 2$ experiments). Cells displayed no morphological evidence of injury even after exposure to the highest concentration of dexamethasone used (1 μM) for 4 days and were >99% viable at this time according to the trypan blue exclusion test.

In addition to the steroids, indomethacin, in a concentration that completely inhibits the cyclooxygenase enzyme (1 μM) caused 98 ± 8.3% inhibition of cell growth and naproxen (10 μM) reduced cell replication to 53.7 ± 6.2% of the control values.

Increases in Cell Surface Lipocortin 1 Induced by Glucocorticoids. In addition to decreasing cellular proliferation, dexamethasone treatment concomitantly increased, in a time-dependent manner, the amount of lipocortin 1 that could be removed from the extracellular cell surface by washing with EDTA. Fig. 3*A* shows that the amount of lipocortin 1 recovered from the cell surface was maximal 2–6 h after dexamethasone treatment and returned to basal levels (within 24 h). Progesterone and aldosterone (1 μM) had no effect on lipocortin 1 expression at any time point (data not shown).

Fig. 3*B* also shows a Western blot analysis of an experiment in which dexamethasone, in a dose-dependent fashion, increased lipocortin 1 expression on the cell surface with a maximum effect observed at 10 μM (densitometric analysis; 3.4-fold increase) after 4 h of treatment. The appearance of lipocortin 1 at the cell surface could be caused by fresh

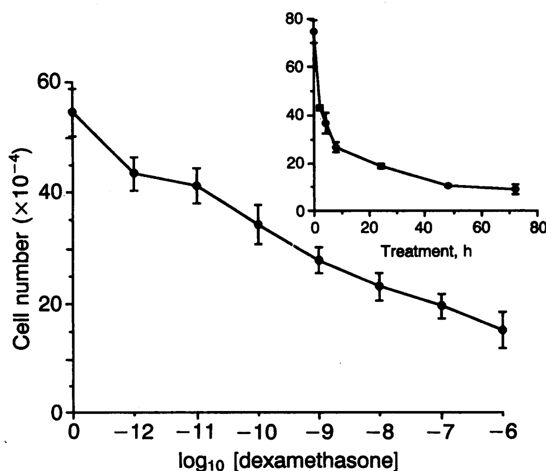

FIG. 1. Dexamethasone arrests A549 cell proliferation in a dose-dependent manner. Dexamethasone at 1 pM and 0.1 nM causes significant inhibition relative to control ($P < 0.01$). All higher concentrations are likewise inhibitory ($P < 0.001$). Each point represents the cell count from three wells (mean ± 1 SD). The data shown are from one of three experiments that yielded similar results, although the onset of the maximum inhibitory effect of the steroid varied between 4 and 8 days of treatment. (*Inset*) A549 cells incubated with dexamethasone (1 μM) for 2, 4, 8, 24, 48, and 72 h are growth-arrested compared to control ($P < 0.001$, all points). Each point is the cell count from three wells (mean ± 1 SD). This graph shows one of two experiments yielding near identical results.

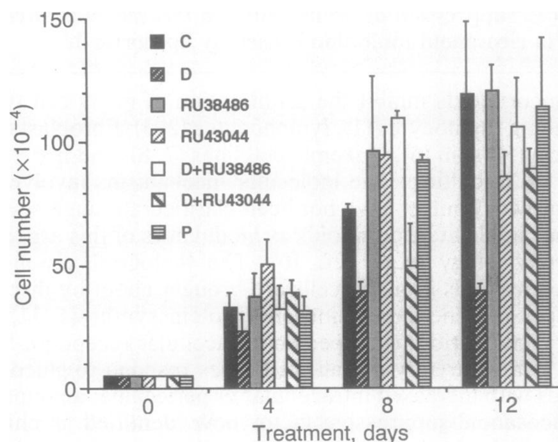

FIG. 2. Time-dependent growth arrest of A549 cells by dexamethasone (D; 10 nM). The growth of dexamethasone-treated cells is significantly inhibited relative to controls (C) at day 8 ($P < 0.001$) and day 12 ($P < 0.001$). Growth arrest is blocked by RU38486 (1 μM); dexamethasone plus RU38486 samples are significantly different from dexamethasone alone at day 8 ($P < 0.001$) but are not significantly different from untreated controls at day 12. Growth arrest is also blocked by RU43044 (1 μM) at day 12, but dexamethasone plus RU43044 is not significantly different from control. RU38486 or RU43044 (1 μM) alone does not significantly affect growth. Progesterone (P; 1 μM) does not significantly affect growth. Each point is the cell count of three wells (mean ± 1 SD). The figure is a typical example of three experiments.

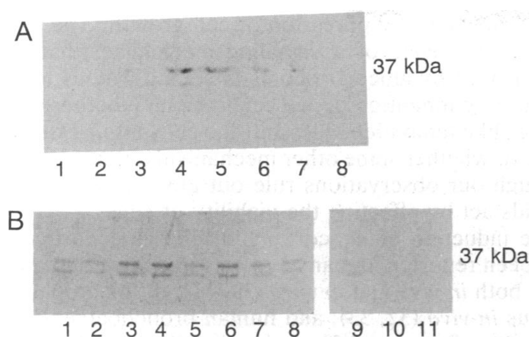

FIG. 3. Western blot analysis of lipocortin 1 expression on the surface of A549 cells. (*A*) Time course of cell surface lipocortin 1 expression induced by 1 μM dexamethasone. The effect is maximal at 2 h and returns to basal by 24 h. Densitometer readings are as follows. Lanes: 1, 0 (0 h); 2, 0 (0.5 h); 3, 2.06 (1 h); 4, 16.37 (2 h); 5, 12.59 (4 h); 6, 3.06 (6 h); 7, 2.9 (8 h); 8, 0 (24 h). (*B*) Dose-dependent induction of cell surface lipocortin 1 by dexamethasone. In this experiment some lipocortin 1 was already present on the control cells. Drugs were added as follows. Lanes: 1, none; 2, 0.1 μM dexamethasone; 3, 1 μM dexamethasone; 4, 10 μM dexamethasone; 5, 1 μM dexamethasone plus 1 μM RU38486; 6, 1 μM dexamethasone plus 1 μM RU30433; 7, 1 μM RU38486; 8, 1 μM RU30433; 9, 1 nM dexamethasone; 10, 1 nM dexamethasone plus 1 μM RU38486; 11, 1 nM dexamethasone plus 1 μM RU30433. Dexamethasone at 10 μM caused a further 3.4-fold increase. The action of 1 μM dexamethasone was reduced by equimolar RU38486 but not by RU43044 (lanes 1–8) but expression caused by much lower concentrations (1 nM) was strongly inhibited by both antiglucocorticoids at 1 μM (lanes 9–11). Densitometer readings are as follows. Lanes: 1, 3.76; 2, 4.35; 3, 10.4; 4, 12.7; 5, 4.93; 6, 9.54; 7, 4.89; 8, 3.76; 9, 4.01; 10, 0.49; 11, 0.9.

synthesis of the protein or by translocation of preexisting intracellular protein to the pericellular domain. Extracellular lipocortin 1 always appeared as a doublet with molecular masses of 37 and 33 kDa. The former is undoubtedly the native species whereas the latter is a commonly encountered N-terminal cleavage product.

The induction by dexamethasone of lipocortin 1 on the cell surface was inhibited (51%) by an equimolar concentration (1 μM) of RU38486 but an equimolar amount of RU43044, which has a lower affinity for the glucocorticoid receptor, was ineffective. However, when A549 cells were maintained in medium containing lower concentrations of dexamethasone (1 nM), RU38486 or RU43044 (1 μM) substantially reduced (90% and 80%, respectively) the appearance of lipocortin 1 at the cell surface (Fig. 3*B*).

Effect of Recombinant Human Lipocortin 1 on A549 Cell Proliferation. In three experiments, exogenous human recombinant lipocortin 1 was added to A549 cell cultures and proliferation was assessed after 3 days. Fig. 4 shows one such experiment in which a dose-related inhibition of cell proliferation by lipocortin 1 was observed with significant effects being detectable at 0.05 μg/ml (1.4 nM). In this particular experiment the original cell number was 5×10^4, which tripled during the 4-day culture. Maximum inhibition of replication was observed with lipocortin 1 at 0.5 μg/ml (14 nM), which gave ≈77% inhibition, approximately equivalent to the inhibition produced by 0.5 μM dexamethasone.

Effect of the Anti-Lipocortin 1 Neutralizing Monoclonal Antibody on Dexamethasone-Induced Cell Growth Arrest. The dexamethasone-induced inhibition of A549 cell growth is substantially reversed by the lipocortin 1 neutralizing monoclonal antibody 1A. Fig. 4 *Inset* shows the result of an experiment (one of four) in which addition of the antibody to the culture medium at 5 μg/ml almost completely reversed the inhibition of cell proliferation induced by 2 nM dexamethasone while having no effect on its own. To be fully effective, it was necessary for the antibody to be replenished

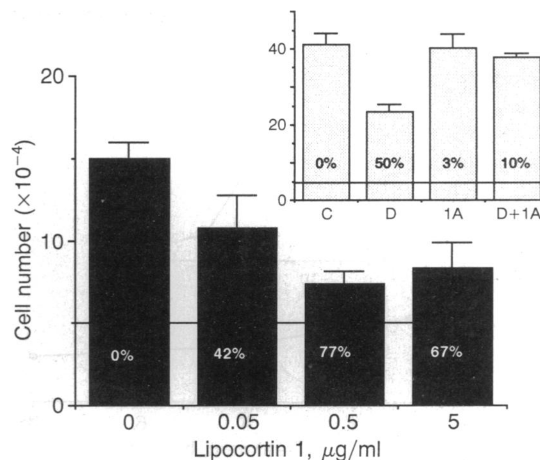

FIG. 4. Proliferation of A549 cells is inhibited by addition of exogenous lipocortin 1 in a dose-dependent manner. Lipocortin 1 (0.05 μg/ml) was significantly inhibited relative to control ($P < 0.04$) as was lipocortin (0.5 μg/ml) vs. control ($P < 0.001$) and lipocortin (5 μg/ml) vs. control ($P < 0.01$). Each point represents the cell count of three wells (mean ± 1 SD). The histogram is one of two similar experiments. The horizontal bar indicates the cell numbers at the start of the study. The figures in the columns represent inhibition of cell proliferation. (*Inset*) Growth arrest induced by dexamethasone (20 nM) is reversed by the neutralizing monoclonal antibody 1A at 5 μg/ml. Dexamethasone (D)-treated samples showed significant inhibition of cell growth relative to control ($P < 0.001$). Monoclonal antibody 1A and dexamethasone plus monoclonal antibody 1A (D + 1A) samples were not significantly different from control. Each bar is the cell count from three wells (mean ± 1 SD). The histogram is typical of four experiments. The horizontal bar indicates the cell numbers at the start of the study. The figures in the columns represent inhibition of cell proliferation.

every day. The addition of the nonneutralizing anti-lipocortin 1 monoclonal antibody 1B had no effect at all at this concentration (data not shown).

Production of PGE_2 by A549 Cells. To assess the production of PGE_2 by A549 cells, cells were cultured in T-150 flasks for 4 days to allow accumulation of easily measurable (10–15 ng/ml; 28–42 pmol) amounts of PGE_2 in the culture medium during the growth period. This release is suppressed by treatment of the cells with dexamethasone in a dose-dependent fashion with half-maximal inhibition at ≈1 nM (Fig. 5). The dexamethasone-induced suppression of PGE_2 release is very rapid with a significant ($P < 0.001$) effect occurring within 30 min of addition of the steroid and persisting throughout the ensuing days of culture even when the steroid is subsequently removed. In the presence of the neutralizing monoclonal antibody 1A (5 μg/ml), the inhibition of PGE_2 by 1 nM dexamethasone was reduced from 77.0 ± 0.1% to 46.2 ± 9.6% ($P < 0.001$; $n = 1$ experiment).

To test PGE_2, the eicosanoid was added to wells containing cells in fresh (i.e., containing no eicosanoids) medium. PGE_2 had a biphasic action on cell growth with a mild (≈25%, $P < 0.01$) stimulation observed at 0.01–1 pM but with inhibition of cell proliferation observed at higher concentrations (Fig. 6).

To determine whether PGE_2 could reverse the actions of dexamethasone or indomethacin, the eicosanoid was added to cultures in which growth had been inhibited by the addition of 1 nM dexamethasone or 1 μM indomethacin. Fig. 6 *Inset* shows that PGE_2 on its own caused an ≈38% stimulation of cell growth when added to cells in this experiment and that the inhibition by dexamethasone was substantially reduced in the presence of 1 pM PGE_2 (from ≈72% inhibition to 24% inhibition). A similar effect was seen with indomethacin, which itself caused 98.3 ± 8.3% inhibition of cell growth, 1 pM PGE_2 caused 24.7 ± 11% increase in cell proliferation,

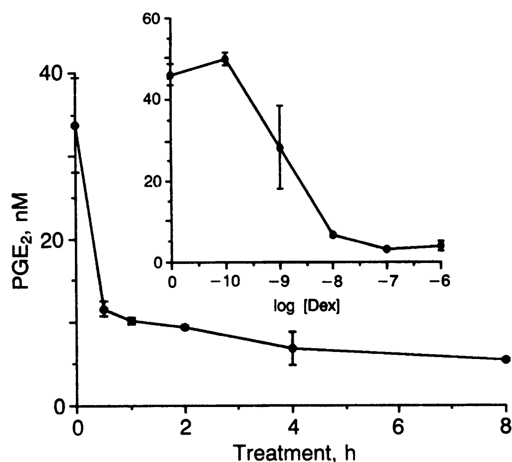

FIG. 5. Dexamethasone (Dex; 1 μM) inhibits PGE_2 production by A549 cells. Each point represents the cell count of two samples (mean ± 1 SD). At all times from 0.5 h to 8 h, PGE_2 production was inhibited relative to controls at time zero ($P < 0.001$). The graph is typical of four experiments. (*Inset*) Dexamethasone inhibits PGE_2 production by A549 cells in a dose-dependent manner. Each point is the cell count of two samples (mean ± 1 SD). $P < 0.001$, dexamethasone at 10 nM, 0.1 μM, and 1 μM vs. control; $P < 0.05$, dexamethasone at 1 nM vs. control. This experiment is one of two similar experiments.

and the addition of PGE_2 to cells treated with indomethacin reduced the drug-induced inhibition to 56 ± 9.8%.

DISCUSSION

In this paper we report that dexamethasone causes a substantial inhibition of cell proliferation in the A549 adenocarcinoma human cell line. The magnitude of the inhibition is typical of that observed in other glucocorticoid-sensitive cell lines (1–9).

Interestingly, it is not necessary for the glucocorticoid to be present throughout the entire experiment as incubating the cells for as little as 2 h with dexamethasone, followed by subculture into dexamethasone-free medium, is sufficient to cause substantial suppression of cell growth. The data suggest the presence of a signaling mechanism that may be switched off by glucocorticoids in such a way as to effect a long-lasting inhibition of cell replication. Whether glucocorticoids, like tamoxifen (30), shift the cells into a "quiescent" phase or whether some other mechanism obtains is not clear, although our observations rule out any suggestion that the steroids act by affecting the viability of cells.

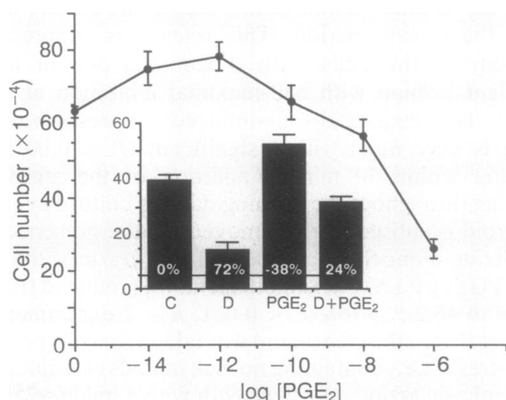

FIG. 6. PGE_2 alters growth of A549 cells in a dose-dependent manner. Growth with PGE_2 (1 pM) is significantly greater than in control wells ($P < 0.01$) and 0.01 pM PGE_2 also has a significant effect ($P < 0.02$). PGE_2 at 0.1 nM has no effect but PGE_2 at 10 nM or at 1 μM is significantly inhibitory relative to control ($P < 0.001$). Each point represents the cell count from three wells (mean ± 1 SD). The graph is typical of two experiments. (*Inset*) PGE_2 (1 pM) can partially reverse the dexamethasone (D; 1 nM)-induced growth arrest. Dexamethasone vs. controls (C) and dexamethasone plus PGE_2 vs. dexamethasone alone were both significant ($P < 0.001$). Each bar represents data from three wells (mean ± 1 SD). This graph is typical of two experiments. The horizontal bar indicates the cell number at the start of the experiment and the figures in the columns represent percentage inhibition of cell growth relative to the control.

The induction of lipocortin 1 by the glucocorticoids has now been reported in many systems including human monocytes both *in vivo* and *in vitro* (29, 31), rat macrophages and thymus *in vivo* (32, 33), and human bronchoalveolar lavage fluid (34). Some undifferentiated cells in culture do not respond to glucocorticoids with an induction of lipocortin 1 (35), but induction can occur once the cells are differentiated (36). Other work points to the importance of culture conditions in studying synthesis of lipocortin 1 by cultured cells. Schlaepfer and Haigler (27) observed that there was a peak of lipocortin 1 synthesis by cells immediately after plating or after replenishing the medium with fresh serum. This latter effect could be duplicated by the addition of growth factors such as epidermal growth factor. This clearly implies that factors other than glucocorticoids can regulate the synthesis of lipocortin 1 in cells and also suggests that if the cells are already maximally expressing the protein, it is very difficult to observe any further effect of the glucocorticoids, a fact that may account for much contradictory data in the literature.

In our experiments, we have sought to reduce the interference from other factors by taking the following precautions. (*i*) The cells were maintained in logarithmic-phase growth and were not allowed to reach confluence as this diminishes their responsiveness to corticosteroids (unpublished observations). (*ii*) We have used charcoal-stripped FCS in the culture medium so that endogenous steroids and possibly other low molecular weight cofactors in the serum that might influence lipocortin 1 synthesis are removed. (*iii*) We have incubated our cells for several days in steroid-free medium prior to the addition of glucocorticoids to allow the surge in lipocortin 1 synthesis that occurs after the plating procedure to subside, thus allowing a more facile determination of the freshly synthesized protein.

Steroids can produce a dual effect promoting (*i*) the translocation of lipocortin 1 to the extracellular domain and then (*ii*) an increased synthesis taking place over a longer time span (37). Although lipocortin 1 has no signal sequence, there are several reports suggesting that the protein can be recovered from the pericellular domain of cells and in some cases is present in quite high concentrations in secretory fluids such as prostatic secretion (38). Browning *et al.* (29), in their study on human monocyte synthesis of lipocortin that used radioactive methionine-labeling techniques, clearly observed the presence of a labeled protein on the external cell surface. This has also been seen in other studies using an ELISA and other detection techniques (31). Because lipocortin 1 can produce biological actions when added directly to cells, or given systemically to animals, and because of the existence of a lipocortin binding protein on the surface of several cell types (39), we have speculated that this pericellular pool is biologically important. Therefore, in analyzing the effect of glucocorticoids on lipocortin 1 synthesis, we have paid special attention to this pool and indeed find that it increases after glucocorticoid treatment. These subtle changes in lipocortin 1 disposition are certain to be missed if the total cellular lipocortin is simply assayed after lysis of the cells in detergent-containing buffers and this may explain some contradictory data in the literature.

A further indication that cell surface lipocortin 1 is the biologically important pool in A549 cells comes from our experiments with the neutralizing monoclonal antibody 1A. This is a well-characterized monoclonal antibody and has

been reported (21) to reverse some actions of glucocorticoids. In our hands monoclonal antibody 1A, but not the nonneutralizing monoclonal antibody 1B, at 5 μg/ml was able virtually to reverse the inhibitory activity produced by an EC_{50} concentration of dexamethasone. There are now several reports (16, 20, 21) that antibodies to lipocortin 1 can interfere with the effects of antiinflammatory steroids and it is difficult to explain these data other than on the basis that there is an extracellular or pericellular pool of lipocortin that is biologically active and that this pool is altered by glucocorticoid steroids.

The mechanisms whereby dexamethasone and lipocortin 1 produce an arrest of cell growth are not entirely clear. Our results suggest that these agents interfere with the production of an eicosanoid, which is important in regulating cell proliferation. Many workers have suggested that eicosanoids can modulate cell growth although the actual nature of these metabolites and the direction of the regulation seem to vary from model to model. For example, some authors suggest that lipoxygenase products are important mediators of cell proliferation (e.g., ref. 40) whereas eicosanoids such as PGE_2 have generally been shown to be growth inhibitory (e.g., ref. 41).

Our data clearly show that A549 cells constitutively release large amounts of PGE_2 into the culture medium. Glucocorticoids are well-known to suppress PGE_2 release and an early experiment with indomethacin showing that this too had growth inhibitory effects led us to believe that it was the PGs rather than the lipoxygenase products that were active. Our dose–response studies with PGE_2 indicate that the effects of PGE_2 on cell growth are biphasic with a moderate stimulation occurring at low doses and growth suppression occurring at concentrations >0.1 nM. Although we were able to reverse drug-induced inhibition of proliferation with PGE_2, we were never able totally to restore proliferation to its control level. This observation could indicate that there are other factors (possibly other eicosanoids) that are also important in controlling proliferation in this system.

It is interesting to note that the time course for inhibition of PGE_2 production by dexamethasone corresponded very closely to that of the induction of lipocortin 1, that the addition of the recombinant lipocortin 1 protein also leads to a total inhibition in PGE_2 in this system, and that the neutralizing monoclonal antibody provided some protection from the dexamethasone effect. This provides strong evidence for a causal link between the two events.

In summary these experiments show that glucocorticoids have a dramatic effect on the growth of the A549 human adenocarcinoma cell line in culture and point to one mechanism whereby this may occur; namely, the induction of cell surface lipocortin 1 followed by a fall in eicosanoid production, possibly PGE_2, leads to changes in cell growth. No doubt there are many mechanisms whereby such complex agents as steroids can regulate cell replication (42) and it will be interesting to see whether this mechanism is common to many other cell types or is unique to the A549 adenocarcinoma cell line.

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