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Glucocorticoids Have Opposite Effects on Ornithine Decarboxylase and Cell Growth in Pancreatic Acinar AR42J Cells

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This paper reviews the relationships between the effects of glucocorticoids on rat pancreatic acinar AR42J cell polyamine levels and cellular growth and differentiation. Glucocorticoids inhibit the growth of AR42J cells. Glucocorticoids either stimulate or inhibit the formation of polyamines in a variety of cell types. Cells require polyamines for normal growth. Therefore, we tested the hypothesis that polyamines mediate the effects of glucocorticoids on AR42J cells. First, to confirm that AR42J cells required polyamines for growth we examined the effects of inhibiting ornithine decarboxylase (ODC). ODC is the most important and generally rate-limiting enzyme in the synthesis of the polyamines. As expected, the ODC inhibitor difluoromethylornithine (DFMO) inhibited AR42J cell DNA synthesis, and the addition of exogenous putrescine reversed this effect. The levels of growth inhibition by glucocorticoids and DFMO treatment were similar. Second, we examined the effects of glucocorticoids on ODC. Surprisingly, glucocorticoids increased levels of AR42J cell ODC mRNA, ODC activity, and putrescine. Glucocorticoids increased these parameters over a similar time-course as they decreased DNA synthesis. Analog specificity studies indicated that a glucocorticoid receptor mediated both the growth inhibitory and ODC stimulatory effects. Dose-response studies indicated, however, that growth inhibition was more sensitive to dexamethasone (DEX) than were ODC levels. Therefore, polyamines do not account for the effects of glucocorticoids on AR42J cell growth. In these cells, glucocorticoids have opposite and independent effects on ODC and growth.

INTRODUCTION

Polyamines are naturally occurring organic cations. Although they are extensively studied and known to be important in a large number of cellular functions, the actual physiologic roles of polyamines remain incompletely understood (for reviews, see [1,2]). That polyamines are required for normal cell growth is indisputable. The mechanisms involved in polyamine effects on cell growth remain, however, unclear. The most useful tool for investigating the roles of polyamines has been the ornithine decarboxylase inhibitor difluoromethylornithine (DFMO). Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the synthesis of the polyamines. A

Abbreviations: DEX: dexamethasone DFMO: difluoromethylornithine ODC: ornithine decarboxylase

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variety of growth factors and hormones stimulate ODC activity and ODC gene expression in a manner suggesting a role in growth regulation. Induction of ornithine decarboxylase activity is one of the earliest events that occurs during the transition of cells from dormancy to active proliferation [3]. Inhibition of ODC with DFMO leads to a depletion of polyamines and blocks proliferation in virtually all cells [1,2]. In a few tissues, directly increasing polyamines appears to stimulate cell division [4,5], although this process is not generally the case. Polyamines may be of particular importance in the regulation of proliferation in the gastrointestinal mucosa [6].

Polyamines are also thought to play an important role in pancreatic growth regulation [7]. Stimulation of pancreatic growth *in vivo* has been shown to stimulate ODC and lead to increased polyamines [8,9]. Furthermore, inhibition of ODC with DFMO inhibits normal pancreatic growth [10]. These studies conducted on pancreatic growth have, however, been carried out *in vivo* where the inherent difficulties of controlling polyamine levels and the complications of secondary effects make it difficult to establish whether increased ODC activity and polyamines are necessary or sufficient for stimulating acinar cell growth.

In order to investigate the regulation of pancreatic acinar cell growth *in vitro,* our laboratory has made use of rat pancreatic acinar AR42J cells. AR42J cells were developed from an azoserine-induced pancreatic tumor and are relatively well differentiated [11]. While AR42J cells are cancer cells and have some obvious differences from normal pancreatic acinar cells, they serve as a useful model for the investigation of cellular mechanisms. Our laboratory has investigated the time and concentration dependence and steroid specificity of dexamethasone (DEX)-induced growth inhibition [12] and ODC induction [13] in the rat pancreatic acinar AR42J cell line. We have also analyzed the role of polyamines in the effects of glucocorticoids on AR42J cell growth and differentiation [14]. We found that, in AR42J cells, glucocorticoids have opposite effects on ODC and cell growth. This paper reviews the data from these AR42J cell studies, presents comparisons, and discusses the implications of this discrepancy.

EFFECTS OF DEXAMETHASONE AND DFMO ON AR42J CELL GROWTH

An early observation concerning the effects of glucocorticoids on AR42J cells was a marked inhibition of cell growth [11]. In further studies, cells cultured in the presence or absence of dexamethasone showed no significant difference in DNA content over the course of the first 24 hours [unpublished observation]. After 48 hours, however, the control cultures continued to double, with a doubling time of ~34 hours, while the dexamethasone-treated cells were inhibited by over 95 percent [12]. Thus, after 48 hours, dexamethasone-inhibited cultures contained approximately 50 percent of the DNA found in the control cultures (Fig. 1).

The role of polyamines in AR42J cell growth was examined by observing the effects of blocking polyamine production with the ODC inhibitor difluoromethylornithine (DFMO) [14]. Total cellular DNA was measured for cells grown in dialyzed fetal bovine serum and in the presence or absence of DFMO after 48 hours in culture. Dialysis of the fetal bovine serum was necessary in order to reduce the normal levels of putrescine. DFMO (5 mM) inhibited AR42J cell growth to a level similar to that achieved with dexamethasone [14] (Fig. 1) or the combination of DFMO and dexamethasone [unpublished observation]. Addition to the medium of

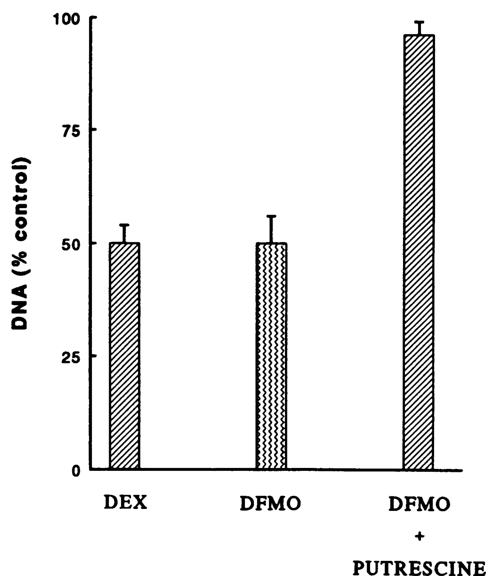

FIG. 1. Effects of dexamethasone and DFMO on AR42J cell growth. Cells were cultured for 48 hours with or without addition of dexamethasone (10 nM), DFMO (5 mM), or DFMO (5 mM) with added putrescine (100 μM). Cells were then scraped and analyzed for DNA content. Values are expressed as percentages of control DNA per culture well and are the means ± SEM of three experiments. Adapted from [12] and [14].

exogenous putrescine completely blocked the inhibitory effects of DFMO [14] (Fig. 1). Direct addition of putrescine had no effect itself on AR42J cell growth, nor did it reverse the growth-inhibitory effects of dexamethasone [unpublished observation].

Thus, the effects of glucocorticoids and DFMO on AR42J cell growth were similar except that the effects of glucocorticoids were not reversed by the addition of putrescine. This finding was the first evidence that the glucocorticoid-induced inhibition was unlikely to be accounted for by a decrease in polyamines.

TIME-COURSE OF DEXAMETHASONE EFFECTS ON AR42J CELL GROWTH AND ODC

The time-course of dexamethasone-induced decreases in AR42J cell DNA synthesis was analyzed by measurement of (^{3}H)-thymidine incorporation [12]. Dexamethasone treatment significantly reduced DNA synthesis after six hours. Half-maximal inhibition occurred after 12 hours of treatment with dexamethasone. Maximal inhibition occurred after 18 hours of DEX treatment (9.0 ± 1.0 percent of control).

To determine the effects of glucocorticoids on AR42J cell ODC, changes in the level of ODC mRNA and activity were measured [13]. Dexamethasone (100 nM) increased ODC mRNA levels after six hours. Maximal, threefold increases in the level of ODC mRNA occurred after 12 hours, and ODC mRNA levels returned to control values after 48 hours [13]. Dexamethasone also increased ODC activity. A maximal stimulation of ODC activity of approximately twofold was observed after 12 hours of dexamethasone treatment [13]. These increases in ODC resulted in a 27-fold increase in AR42J cell content of putrescine four hours after addition to culture media [14].

A comparison of the time-courses of glucocorticoid effects on DNA synthesis, ODC activity, and ODC mRNA levels is shown in Fig. 2.

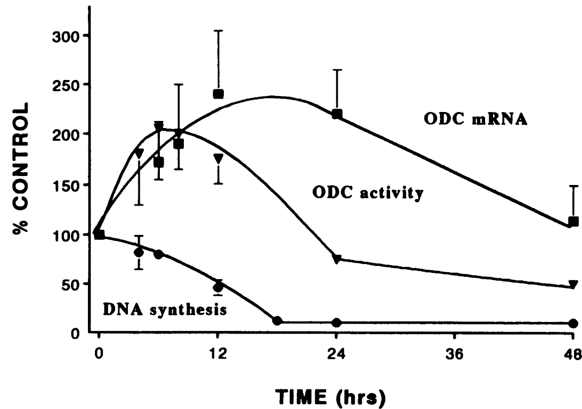

FIG. 2. Time-course of DEX action on DNA synthesis and ODC mRNA and activity. (^{3}H)-thymidine incorporation was assessed over one hour, after prior 10 nM DEX treatment for the indicated durations. (^{3}H)-thymidine incorporation is expressed as percentage of control (cpm/μg DNA). For ODC measurements, cells were incubated with dexamethasone for the indicated periods of time, and ODC activity was then determined in the cell homogenates. Ornithine decarboxylase mRNA concentrations were quantitated, using slot-blot analysis. All data are expressed as percentages of average control and are expressed as mean ± SEM of three to seven experiments. Adapted from [12,13,14].

DOSE-RESPONSE FOR DEXAMETHASONE EFFECTS ON DNA SYNTHESIS AND ODC

To establish the dose-dependency of the dexamethasone effects on DNA synthesis and ODC mRNA, AR42J cells were cultured in the presence of various concentrations of dexamethasone and DNA synthesis, and ODC mRNA levels were then measured [12,13]. Figure 3 compares these dose-response relationships. Inhibition of DNA synthesis was half-maximal at 0.5 nM and maximal at 10 nM dexamethasone [12]. ODC mRNA induction by dexamethasone also occurred in a dose-dependent manner [13]. AR42J cells were incubated for 12 hours with various concentrations of dexamethasone; then ODC mRNA concentrations were determined quantitatively by slot-blot hybridization. Half-maximal effects were seen at 10 nM and maximal

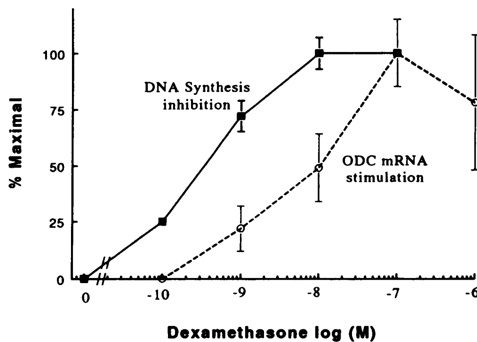

FIG. 3. Dependence of DNA synthesis and ODC mRNA content on dexamethasone concentration. (^{3}H)-thymidine incorporation over one hour and ODC mRNA were measured after treatment with dexamethasone at the specified concentrations. Data are presented as percentages of the maximal response, either inhibition of DNA synthesis or stimulation of ODC mRNA levels. Each point represents the mean ± SEM of three to seven experiments. Adapted from [12] and [13].

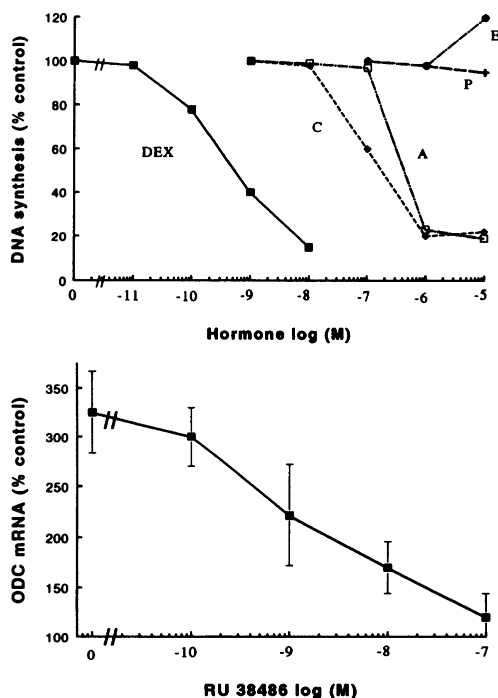

FIG. 4. Specificity of steroid effects on AR42J cell DNA synthesis and ODC mRNA levels. **A.** The effect of various steroid hormones on DNA synthesis. Cells were maintained for 20 hours in the specified concentration of **DEX**, corticosterone (**C**), aldosterone (**A**), progesterone (**P**), or estradiol (**E_2**) prior to one hour of (^{3}H)-thymidine incorporation. Results are expressed as percentage of control (cpm/μg DNA), means ± SEM for four to seven experiments. Adapted from [12]. **B.** Effects of the glucocorticoid antagonist RU 38486 on dexamethasone-induced ODC gene expression. AR42J cells were incubated for 12 hours with dexamethasone (100 nM) and the indicated concentrations of RU 38486. Ornithine decarboxylase mRNA concentrations were then determined and expressed as percentages of untreated control. These data represent the mean ± SEM of three experiments. Adapted from [13].

effects occurred at 100 nM dexamethasone [13]. Higher doses of dexamethasone had no additional effect on ODC mRNA concentrations [unpublished observation].

SPECIFICITY OF GLUCOCORTICOID RESPONSE ON AR42J CELL GROWTH AND ODC

To determine the steroid specificity of the receptor responsible for the actions of dexamethasone on AR42J cell DNA synthesis, we examined the effectiveness of various steroids in inhibiting DNA synthesis [12] (Fig. 4). Dexamethasone was the most potent (half-maximal effect at 0.5 nM), followed by corticosterone (half-maximal effect at 200 nM) and aldosterone (half-maximal effect at 600 nM). Neither progesterone nor estradiol significantly inhibited DNA synthesis. These results were consistent with specific action on the glucocorticoid receptor.

The specific glucocorticoid antagonist RU 38486 was utilized to determine whether the observed effects on ODC mRNA levels were caused by specific interactions between dexamethasone and the glucocorticoid receptor [13] (Fig. 4B). The antagonist by itself had no effects on ODC mRNA concentrations at a final concentration of up to 100 nM (data not shown). When the antagonist was administered simultaneously with dexamethasone, however, it blocked the glucocorticoid induction of ODC gene expression in a dose-dependent manner. Again the data supported the hypothesis that a glucocorticoid receptor mediates the effects on ODC gene expression.

DISCUSSION

ODC and polyamines are required for AR42J cell growth, as proven by the ability of DFMO to block cell growth and the reversibility of this effect by exogenous

putrescine. The observations reviewed here argue, however, that glucocorticoids regulate ODC and DNA synthesis independently in AR42J cells. This hypothesis was indicated not only by the opposing effects of glucocorticoids on the two processes, but also by the differences in dose-dependency. Dexamethasone inhibition of DNA synthesis occurred at ten- to twentyfold lower concentration than its effects on ODC. Our data show that glucocorticoids simultaneously inhibit AR42J cell growth and stimulate ODC mRNA levels, activity, and cellular putrescine levels. This striking separation of growth from ODC activation and putrescine supports the proposition that ODC and polyamines are not strictly growth-related.

A dissociation of polyamine metabolism from proliferation has been previously reported in Swiss 3T3 cells [15]. Pronase treatment stimulated 3T3 cells' polyamine synthesis four- to sixfold but did not increase either DNA synthesis or cell number. In the same study, glucocorticoids led to substantial increases in DNA synthesis and cell number that were not accompanied by significant increases in the polyamine parameters.

The liver also shows a separation of the effects of glucocorticoids on ODC and cell growth. Glucocorticoids lead to a large increase in ODC mRNA [16] and activity [16,17] in the liver. Glucocorticoids also induce ODC in hepatocytes in primary culture [18,19]. Glucocorticoids are, however, potent inhibitors of hepatocyte growth both *in vivo* [20,21] and *in vitro* [22,23]. Furthermore, as was the case in the 3T3 cells, stimuli not inducing liver growth were able to increase ODC, and the extent of ODC stimulation was not necessarily proportional to the rate of growth [24].

Perhaps the best explanation for these discrepancies is that polyamines are also involved in a number of cellular functions other than growth. For example, polyamines are known to be important for protein and RNA synthesis [1,2]. In some cases, ODC induction appears to correlate more closely with RNA synthesis than with DNA synthesis [15]. Glucocorticoids increase the abundance of rough endoplasmic reticulum in AR42J cells [11]. It is possible that this effect involves de novo synthesis of ribosomes and that polyamines are involved. Polyamines were required for glucocorticoid induction of amylase activity, but not amylase mRNA in AR42J cells [14]. This finding indicates that protein synthesis may have a greater sensitivity to polyamine depletion than RNA synthesis. Therefore, it is likely that the induction of ODC by glucocorticoids in AR42J cells is related to changes in protein synthesis. These data suggest that interpretation of the effects of treatments on ODC and polyamines must take into account the fact that those parameters may be more closely linked to protein synthesis than to cell growth.

The mechanisms by which glucocorticoids affect cell growth remain largely unknown. In some cell types, glucocorticoids are growth-stimulatory [25–28], while in others glucocorticoids are growth-inhibitory [20–23]. The general mechanisms for steroid hormone action involve the induction of gene expression by an interaction between the hormone-receptor complex and DNA enhancer elements, leading to increased transcription [29]. In some cases, the actions of glucocorticoids are explained by alterations in the secretion of autocrine growth factors [30,31]. In other cases, effects may be due to changes in growth factor receptors. In most cases, however, the specific genes responsible for growth effects have not been elucidated. The AR42J cell may prove a valuable model in the search for the genes by which glucocorticoids inhibit cell growth.

CONCLUSIONS

Taken together, the data reviewed here suggest that polyamines should not be viewed as being strictly growth-related. Thus, increases in ODC activity should not automatically be interpreted as indications of growth stimulation. The AR42J cell may be a useful model for delineation of some of the non-growth-related roles of polyamines and for the investigation of the growth-inhibitory effects of glucocorticoids.

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