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Control of the adrenocortical cell cycle: interaction between FGF2 and ACTH

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Abstract

FGF2 elicits a strong mitogenic response in the mouse Y-1 adrenocortical tumor cell line, that includes a rapid and transient activation of the ERK-MAPK cascade and induction of the c-Fos protein. ACTH, itself a very weak mitogen, blocks the mitogenic response effect of FGF2 in the early and middle G_1 phase, keeping both ERK-MAPK activation and c-Fos induction at maximal levels. Probing the mitogenic response of Y-1 cells to FGF2 with ACTH is likely to uncover reactions underlying the effects of this hormone on adrenocortical cell growth.

Key words
- Adrenocortical cell cycle
- ACTH
- FGF2
- ERK-MAPK
- c-fos proto-oncogene

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Introduction

The *in vivo* growth-promoting and the *in vitro* growth-inhibitory effects of ACTH on adrenocortical cells are well-documented phenomena, but the mechanisms by which ACTH exerts these apparently contradictory growth effects are essentially unknown.

The basic mechanisms of steroidogenesis regulation by ACTH are well known, mainly due to the enormous progress in this research field during the last 10 years (1-4). By comparison, during this same period, the studies on mechanisms of growth regulation by ACTH have experienced only modest progress. However, nowadays, state of the art research in the molecular biology of the cell cycle allows us to envision a rapid advance in the study of the effects of ACTH on growth.

About 10 years ago we observed (5,6) that ACTH induces the c-fos proto-oncogene in Y-1 mouse adrenocortical cells, a cell line widely used as a cell culture model for the adrenal cortex (7). This observation has made us aware of the fact that ACTH receptors initiate signals activating transcription of genes known to be turned on by the growth factor's tyrosine kinase receptors. Such resemblance between ACTH and growth factors was an interesting starting point to initiate a probe into the effects of ACTH on cell cycle regulation in Y-1 adrenal cells. Actually, years before (8) we had analyzed the interaction between purified pituitary FGF and ACTH in the growth response of Y-1 cells growth-arrested by serum starvation. At the time we showed that ACTH displays a growth-inhibitory effect by blocking the $G_1 \rightarrow S$ transition of Y-1 cells stimulated with FGF to initiate DNA synthesis (8). This growth inhibitory effect of ACTH was likely to be mediated by cAMP/PKA, but its molecular basis was then, and still is

now, unknown. Presently, we are reanalyzing this phenomenon using recombinant FGF2 and ACTH with Y-1 cells under well-defined experimental conditions (9-11). The present paper highlights some results of this research project.

The mitogenic response of Y-1 adrenocortical cells to FGF2

The cycle of Y-1 cells is arrested in a G_0-like state upon serum starvation: the BrdU labeling index is reduced to about 5% (10^{-4} M BrdU; 2-h incorporation) after 48-h incubation in serum-free medium (9,10).

In serum-free medium, G_0-arrested Y-1 cells growth stimulated with recombinant bovine FGF2 (1 to 20 ng/ml; short pulse or sustained treatment) display the following sequence of events, that culminates in mitogenesis: a) rapid and transient activation of p42 and p44 ERK-MAPK (ERK = extracellular regulated kinase; MAPK = mitogen-activated protein kinase), peaking between 2 and 5 min; b) induction of the c-Fos protein: 80% of the nuclei labeled with an anti-c-Fos monospecific polyclonal antibody by 1 h; c) the onset of DNA synthesis stimulation by 8 h, showing a 10-fold increase in BrdU-labeling index by 12 h; d) a sharp elevation in mitotic index by 18 h. This sequence delimitates the $G_0 \rightarrow G_1 \rightarrow S$ transition in G_0-arrested Y-1 cells treated with FGF2, allowing to estimate the minimal length for the G_1 and $S + G_2$ phases of the cell cycle as 8 and 10 h, respectively.

Ninety percent inhibition of c-Fos protein induction with antisense oligonucleotides is sufficient to block completely cell entry into the S phase stimulated with FGF2 (11). Therefore c-Fos induction seems to be an absolute requirement for the mitogenesis triggered by FGF2.

Surprisingly, the MEK inhibitor PD98059 (12) at 50 µM does not inhibit ERK-MAPK activation by FGF2 in Y-1 cells, whereas it completely inhibits ERK-MAPK activation by ACTH (see below). This observation suggests that FGF2 receptors in Y-1 cells activate a pathway that does not utilize MEK1 to phosphorylate ERK-MAPK. PD98059 at 50 µM also fails to inhibit c-Fos protein induction and DNA synthesis stimulation by FGF2 in Y-1 cells.

The growth-response of Y-1 adrenocortical cells to ACTH

Short pulses (up to 2 h) of commercially available purified porcine ACTH (0.1 mU/ml) elicit in G_0-arrested Y-1 cells a growth-response that closely resembles the early steps triggered by FGF2 treatment, namely: rapid and transient activation of the ERK-MAPK and induction of the c-Fos protein. However, synthetic forms of ACTH (complete with 39 or just the first 24 amino acids) at saturating concentrations (10 to 100 mM), that fully stimulate steroidogenesis, do not activate ERK-MAPK and only induce c-Fos protein in 30% of the cells. On the other hand, both purified porcine ACTH and synthetic ACTH, in short pulses, have equally minor mitogenic activity causing a maximum 2-fold increase in basal BrdU labeling index. This low mitogenic activity of ACTH is also blocked by c-fos mRNA antisense oligonucleotides. In addition, the MEK inhibitor PD98059 (12) at 50 µM inhibits ERK-MAPK activation, c-Fos protein induction and DNA synthesis stimulation by ACTH. In conclusion, in Y-1 cells whose cell cycle has been arrested by serum deprivation, ACTH is a weak mitogen when compared with FGF2.

Interaction between FGF2 and ACTH

ACTH is a strong antagonist of the mitogenic activity of FGF2. Addition of ACTH combined with FGF2 for up to 2 h inhibits the mitogenic effect of FGF2 by 50%, keeping activation of ERK-MAPK and induction of c-Fos protein at maximal levels. The inhibitory effect of ACTH is more effective in

the middle of the G_1 phase, as shown by the following protocol: G_0-arrested Y-1 cells are stimulated for 2 h with FGF2 and then treated with ACTH for 4 to 6 h. This late ACTH treatment is sufficient to abolish the mitogenic effect of FGF2.

These results illustrate the complexity of the interaction between FGF2 and ACTH in the regulation of the $G_0 \rightarrow G_1 \rightarrow S$ transition of Y-1 adrenocortical cells. In early G_1, it is likely that ACTH somehow inhibits reactions initiated by FGF2-receptors, that are required to trigger mitogenicity, without antagonizing either MAPK cascade activation or c-fos gene induction. During G_1, temporally distant from the immediate activation process triggered by the FGF2 receptors, ACTH should inhibit reactions that promote G_1 phase traversing.

Concluding remarks

In the mitogenesis-triggering mechanisms initiated by FGF2 receptors in Y-1 cells whose cell cycle has been arrested by serum starvation, ERK-MAPK activation and c-fos gene induction are necessary steps. This observation is not surprising. First, growth factor tyrosine kinase receptors and some hormonal G protein-coupled receptors, capable of triggering mitogenesis, all activate the ERK-MAPK cascade (13). Second, induction of the c-fos gene seems to be an obligatory immediate early step in the $G_0 \rightarrow G_1 \rightarrow S$ transition of normal cells (14,15). All these considerations suggest that Y-1 adrenocortical tumor cells retain key reactions that regulate the initiation of the $G_0 \rightarrow G_1 \rightarrow S$ transition in normal cells. Thus, probing the growth responses of Y-1 cells with ACTH, particularly the growth response triggered by FGF2, is likely to uncover reactions underlying the growth effects of this hormone. Results obtained with Y-1 cells can be validated in primary cultures of adrenocortical cells.

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