Unmasking a Growth-promoting Effect of the Adrenocorticotropic Hormone in Y1 Mouse Adrenocortical Tumor Cells*

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The adrenocorticotropic hormone (ACTH) inhibits the growth of Y1 mouse adrenocortical tumor cells as well as normal adrenocortical cells in culture but stimulates adrenocortical cell growth in vivo. In this study, we investigated this paradoxical effect of ACTH on cell proliferation in Y1 adrenal cells and have unmasked a growth-promoting effect of the hormone. Y1 cells were arrested in the G1 phase of the cell cycle by serum starvation and monitored for progression through S phase by measuring [3H]thymidine incorporation into DNA and by measuring the number of nuclei labeled with bromodeoxyuridine. Y1 cells were stimulated to progress through S phase and to divide after a brief pulse of ACTH (up to 2 h). This effect of ACTH appeared to be cAMP independent, since ACTH also induced cell cycle progression in Kin-8, a Y1 mutant with defective cAMP-dependent protein kinase activity. The growth-promoting effect of ACTH in Y1 was preceded by the rapid activation of p44 and p42 mitogen-activated protein kinases and by the accumulation of c-FOS protein. In contrast, continuous treatment with ACTH (14 h) inhibited cell cycle progression in Y1 cells by a cAMP-dependent pathway. The inhibitory effect of ACTH mapped to the midpoint of G1. Together, the results demonstrate a dual pathway. The inhibitory effect of ACTH on cell cycle progression, a cAMP-independent growth-promoting effect early in G1 possibly mediated by mitogen-activated protein kinase and c-FOS, and a cAMP-dependent inhibitory effect at mid-G1. It is suggested that the growth-inhibitory effect of ACTH at mid-G1 represents an ACTH-regulated check point that limits cell cycle progression.

The growth-inhibitory effects of the adrenocorticotropic hormone (ACTH) on adrenal cells in vitro are well documented. ACTH-induced inhibition of cell proliferation has been observed in the Y1 mouse adrenocortical tumor cell line (1) as well as in normal adrenocortical cells isolated from a variety of species including rat, cow, and human (for review, see Ref. 2). ACTH arrests dividing adrenal cells by interfering with progression through the G1 phase of the cell cycle (3) and inhibits the initiation of DNA synthesis in G1-arrested cells following addition of serum or growth factors (4, 5). Several lines of evidence indicate that the growth-inhibitory effect of ACTH is mediated by cAMP with the most compelling data arising from studies of Y1 adrenal tumor cells harboring dominant inhibitory mutations in cAMP-dependent protein kinase (PKA) that specifically disrupt cAMP-dependent signaling pathways (6). These PKA mutants are resistant to the growth-inhibitory actions of ACTH and cAMP analogs (7, 8), indicating that cAMP and PKA are obligatory components of this effect of ACTH on cell proliferation. The inhibition of proliferation seen in isolated adrenocortical cells contrasts sharply with the growth-promoting effects of ACTH on the adrenal gland in vivo and has led to the widely held view that ACTH serves as an indirect mitogen for the adrenal cortex in intact animals (2). Paradoxically, however, ACTH induces expression of genes often associated with enhanced cell proliferation such as ornithine decarboxylase (9) and fos and jun protooncogenes (10–12) in isolated adrenocortical cells, raising the possibility of an underlying growth-promoting action of the hormone.

The MAP kinase cascade, an important regulator of cell cycle progression, has been used recently as a biochemical marker to evaluate the status of hormones and growth factors as mitogens. Activation of the MAP kinase pathway is involved in the mitogenic effects of growth factors such as epidermal growth factor, platelet-derived growth factor, and FGF (13, 14), acting via receptor tyrosine kinases and also appears to mediate the mitogenic effects of thyrotropin on thyrocytes (15), angiotensin II on smooth muscle cells (16), and thrombin on fibroblasts (17), each acting through a G protein-coupled receptor. Conversely, inhibition of the MAP kinase cascade accompanies the growth-inhibitory effects of cAMP observed in fibroblasts and other cell types (for review, see Ref. 18). In the present study, we examined the regulation of the MAP kinase pathway in Y1 mouse adrenocortical tumor cells to reconcile the growth-inhibiting effect of ACTH in vitro with the conflicting biochemical data that suggests an underlying mitogenic effect of the hormone. Although we expected that ACTH would inhibit MAP kinase activity in Y1 cells, consistent with the growth-inhibitory effects of the hormone, we find that ACTH activates the MAP kinase cascade via a signaling mechanism that is cAMP-independent. This effect of ACTH on MAP kinase prompted us to reexamine the effects of ACTH on Y1 cell proliferation. We find that ACTH promotes the transition of Y1 cells from G1 to S in a cAMP-independent manner and stimulates cell division when administered to Y1 cells as a short pulse early in the G1 phase of the cell cycle. In contrast, administration of ACTH later in the cell cycle inhibits the transition of cells from G1 to S. These
results suggest that we have unmasked a CAMP-independent, growth-promoting effect of ACTH that has new implications for the mitogenic effects of the hormone on the adrenal cortex.

MATERIALS AND METHODS

Cells and Cell Culture—The cells used in this study are the Y1 mouse adrenocortical tumor cell line (19) and a CAMP-resistant, PKA-defective mutant of the Y1 cell line designated Kin-8 (20). Cells routinely were grown either in Ham's nutrient mixture F10 supplemented with 15% heat-inactivated horse serum and 2.5% heat-inactivated fetal bovine serum or in DMEM with 10% fetal bovine serum as described in detail previously (21). Cells were arrested early in the G1 phase of the cell cycle by incubation in serum-deficient medium for the times specified. All cultures were maintained at 36.5 °C in a humidified atmosphere of 5% CO2, 95% air.

Phosphorylation of MAP Kinase—Cells were replicate plated at a density of 5 × 10^4 cells per 10-cm tissue culture dish, cultured for 4 days in F10 growth medium plus serum, and then transferred to serum-free alpha minimal essential medium for 72 h to induce growth arrest. Cells then were treated with various reagents to modulate the MAP kinase cascade, rinsed twice with phosphate-buffered saline, and solubilized in 0.5% digitonin at 4 °C in lysis buffer containing 1% Triton-X-100, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1% (w/v) Nonidet P-40, 0.5% (v/v) sodium deoxycholate, 0.1% SDS, protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml benzamidine, 5 μg/ml aprotinin), and phosphatase inhibitors (10 mM sodium fluoride, 10 mM disodium pyrophosphate, and 1 mM sodium orthovanadate) (22). Samples were clarified by centrifugation at 4 °C. Samples (10 μg of protein) were boiled in SDS sample buffer for 3–5 min, electrophoresed on 10% polyacrylamide gels in the presence of SDS (23), and electroblotted onto polyvinylidene difluoride membranes (NEN Life Science Products) using a Bio-Rad transblot apparatus. Phosphorylated forms of MAP kinase were detected by immunoblotting using a PhosphoPlus MAPK antibody kit (New England Biolabs, Missisauga, Ontario). The kit provides a primary rabbit antibody that specifically recognizes the tyrosine-phosphorylated forms of pp44 MAP kinase (Erk1) and pp42 MAP kinase (Erk2) and a secondary alkaline phosphatase-conjugated anti-rabbit antibody for chemiluminescent detection.

Incorporation of 3HThymidine into DNA—The incorporation of [methyl-3H]thymidine into DNA was determined essentially as described previously (5, 24). Cells were replicate plated at a density of 8 × 10^4 cells per 6-cm tissue culture dish and cultured for 2 days in growth medium plus serum to ensure logarithmic growth. Cells were transferred to alpha minimal essential medium without serum for 72 h to induce growth arrest. Cells then were treated with various reagents to modulate the MAP kinase cascade, rinsed twice with phosphate-buffered saline, and solubilized in 0.5% digitonin at 4 °C in lysis buffer containing 1% Triton-X-100, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1% (w/v) Nonidet P-40, 0.5% (v/v) sodium deoxycholate, 0.1% SDS, protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml benzamidine, 5 μg/ml aprotinin), and phosphatase inhibitors (10 mM sodium fluoride, 10 mM disodium pyrophosphate, and 1 mM sodium orthovanadate) (22). Samples were clarified by centrifugation at 4 °C. Samples (10 μg of protein) were boiled in SDS sample buffer for 3–5 min, electrophoresed on 10% polyacrylamide gels in the presence of SDS (23), and electroblotted onto polyvinylidene difluoride membranes (NEN Life Science Products) using a Bio-Rad transblot apparatus. Phosphorylated forms of MAP kinase were detected by immunoblotting using a PhosphoPlus MAPK antibody kit (New England Biolabs, Missisauga, Ontario). The kit provides a primary rabbit antibody that specifically recognizes the tyrosine-phosphorylated forms of pp44 MAP kinase (Erk1) and pp42 MAP kinase (Erk2) and a secondary alkaline phosphatase-conjugated anti-rabbit antibody for chemiluminescent detection.

RESULTS

Regulation of the MAP Kinase Pathway

Regulation of the MAP kinase pathway in Y1 cells was monitored by measuring the phosphorylation and activation of two MAP kinase isoforms, Erk1 and Erk2. Y1 cells were maintained in serum-free medium for 72 h to arrest cell growth early in G1. Cells were then transferred to medium that was fully supplemented with serum, harvested at different time periods, and subjected to Western blot analysis using an antibody that specifically recognizes tyrosine-phosphorylated forms of Erk1 and Erk2. As shown in Fig. 1a, serum rapidly and transiently stimulated the appearance of phosphorylated forms of Erk1 and Erk2. These phosphorylated forms of MAP kinase were detectable within 1 min after serum addition, reached a maximum after 5 min, and declined toward control levels by 20 min. FGF, another mitogen for Y1 cells (25), also stimulated the phosphorylation of Erk1 and Erk2 but with somewhat delayed kinetics (Fig. 1b). The appearance of phosphorylated forms of MAP kinase in response to serum or FGF reflected activation of MAP kinase as determined by direct measures of enzymatic activity (data not shown).

To determine if the growth-inhibitory effects of ACTH and 8-Br-cAMP in Y1 cells were associated with inhibition of the MAP kinase cascade, Y1 cells were preincubated with porcine ACTH (Acthar; Rhône-Poulenc Rorer Canada Inc., Montreal, Quebec) or 8-Br-cAMP for 10 min and then treated with serum
for varying periods of time. Although this same experimental paradigm had been used previously to correlate the growth-inhibitory effects of cAMP with an inhibitory effect on the MAP kinase pathway in rat and hamster fibroblasts (26, 27), pretreatment of Y1 adrenal cells with ACTH or 8-Br-cAMP failed to inhibit serum-stimulated MAP kinase phosphorylation (Fig. 1, c and d). To the contrary, the addition of ACTH to Y1 cells arrested by serum starvation rapidly stimulated the phosphorylation of Erk1 and Erk2 (Fig. 2a) and increased MAP kinase activity (data not shown). The hormone was effective at concentrations as low as 0.1 microunit/ml. Synthetic human ACTH₁₋₃₉ also stimulated MAP kinase phosphorylation (Fig. 2a) over a similar concentration range (data not shown), excluding the possibility that the observed effects were due to growth factors contaminating the pituitary-derived Acthar preparation.

The concentrations of ACTH that effectively stimulated MAP kinase activity were 50-fold lower than the concentrations of hormone required to produce detectable changes in cAMP levels in these cells (28), raising the possibility that ACTH exerted its effects on MAP kinase via a cAMP-independent pathway. To test this hypothesis, we examined the ability of ACTH to stimulate MAP kinase phosphorylation in the cAMP-resistant, PKA-defective mutant Kin-8. As shown in Fig. 2b, ACTH effectively activated MAP kinase phosphorylation in the Kin-8 mutant over the same concentration range as seen in parent Y1 cells, supporting the hypothesis that activation of the MAP kinase cascade occurred through a cAMP-independent mechanism. In both parental Y1 cells and the PKA-defective mutant, Kin-8, the effect of ACTH was mimicked by the phorbol ester PMA (Sigma) but not by the inactive 4α-hydroxyl isomer of PMA, implicating a protein kinase C-dependent pathway of regulation (Fig. 2, c and d). Although MAP kinase phosphorylation also was stimulated by 8-Br-cAMP, a similar effect was achieved with 5′-AMP (Fig. 2, c and d), suggesting that the action was not specific for the cyclic nucleotide and possibly involved signaling via purinergic receptors.

Table I

| Treatment          | Thymidine incorporation | Statistical significance |
|--------------------|-------------------------|--------------------------|
| None               | 1.0                     |                          |
| Serum              | 15.0 ± 3.25             | p = 0.008*               |
| ACTH               | 0.2 ± 0.04              | p = 0.001*               |
| ACTH + serum       | 2.6 ± 0.42              | p = 0.001*               |
| 8-Br-cAMP + serum  | 0.20 ± 0.01             | p = 0.0001*              |
| PMA + serum        | 6.1 ± 1.9               | p = 0.001*               |

*p values for significance relative to serum-starved controls. b p values for significance relative to serum-treated samples.

Effects of ACTH on Progression of Cells from G₁ to S Transition—The ability of serum-starved Y1 cells to progress from the G₁ phase of the cell cycle to S phase was monitored by measuring [³H]thymidine incorporation into DNA or by measuring the percentage of nuclei labeled with BrdUrd. Incubation of Y1 cells with serum for 14 h led to a 15-fold increase in [³H]thymidine incorporation into DNA (Table I). As determined from BrdUrd-labeling experiments, the changes in [³H]thymidine incorporation into Y1 cell DNA reflected the progression of cells from the G₁ phase of the cell cycle to S phase. As shown in Fig. 3, fetal bovine serum and FGF both stimulated the entry of Y1 cells into S phase with a lag of approximately 8 h and resulted in the labeling of approximately 80% of nuclei after 24 h. ACTH, 8-Br-cAMP, or PMA, when added together with serum during the entire incubation period, dramatically and significantly reduced the amount of [³H]thymidine incorporated (Table I). Even when added alone for 14 h, ACTH caused a significant reduction in [³H]thymidine incorporation compared with growth-arrested Y1 cells.

A Short Pulse of ACTH Early in G₁ Stimulates G₁ to S Transition—The observation that ACTH rapidly activated the MAP kinase cascade when added to growth-arrested Y1 cells (Fig. 2) prompted us to reexamine the effects of ACTH on the cell cycle particularly when administered over a short period to coincide with the activation of MAP kinase. As shown in Fig. 4, treatment of serum-starved Y1 cells with fetal bovine serum or FGF for varying periods of time caused a progressive increase in the percentage of nuclei labeled with BrdUrd, reaching a maximum stimulation of 3.5–5.5-fold (p < 0.05) after 24 h. At the end of this period, 80–90% of nuclei were labeled with BrdUrd. Using this experimental paradigm, we found that treatment with ACTH for short periods up to 2 h increased the labeling index of Y1 cells almost 2-fold (p < 0.05) and approached the effects of serum and FGF over the same periods (Fig. 4). In contrast, treatments with ACTH for longer periods of time were inhibitory. A short pulse of ACTH also stimulated proliferation of Y1 cells from G₁ to S as measured by incorporation of [³H]thymidine into DNA (Table II). Treatment of serum-starved Y1 cells with ACTH for 5 min produced a 4-fold increase in thymidine incorporation over controls, which approached the effects obtained with FGF over the same interval. Similar results were obtained after a brief exposure of Y1 cells to synthetic ACTH₁₋₃₉ in equimolar amounts (data not
shown), ensuring that the transition of cells from $G_1$ to $S$ resulted specifically from the hormone and not from another contaminating growth factor in the pituitary-derived hormone preparation.

To determine if the cells that were stimulated by ACTH to undergo progression from $G_1$ to $S$ also underwent mitosis, serum-starved Y1 cells were pulsed with ACTH for 5 min and incubated in serum-free medium for an additional 32 h to allow for cell division. Cells were then washed and transferred to DMEM without serum for the remainder of the incubation. All the experimental groups were incubated in medium containing 100 $\mu$M BrdUrd during the 12–24 h of incubation and were then processed to estimate the percentage of BrdUrd-labeled nuclei. Results are presented as $\times$-fold increases in labeling index over a control set that received serum-free medium throughout the entire experiment.

**TABLE II**

| Treatment of Y1 or Kin-8 cells with short pulses of ACTH or PMA stimulates incorporation of $[^3H]thymidine$ into DNA |
|---|---|---|---|
| Cell line | Treatment | Thymidine incorporation | Statistical significance |
| Y1 | None | 1.0 | |
| | Serum | 6.2 ± 0.45 | $p = 0.0004$ |
| | FGF | 4.1 ± 0.30 | $p = 0.007$ |
| | ACTH | 4.0 ± 0.95 | $p = 0.001$ |
| | PMA | 2.1 ± 0.14 | $p = 0.01$ |
| Kin-8 | None | 1.0 | |
| | Serum | 5.0 ± 0.5 | $p < 0.001$ |
| | FGF | 4.4 ± 0.35 | $p < 0.001$ |
| | ACTH | 3.4 ± 0.5 | $p < 0.005$ |
| | PMA | 4.3 ± 0.52 | $p < 0.0006$ |

The Role of PKA in the Growth-promoting Effects of ACTH—To evaluate the contribution of the cAMP-signaling pathway to the stimulatory effect of ACTH on cell cycle progression, PKA-defective Kin-8 cells were growth-arrested and monitored for incorporation of $[^3H]thymidine$ into DNA in the presence of different stimuli. Kin-8 cells responded to a brief pulse of serum as did parental Y1 cells and exhibited a 5- to 0.5-fold increase in thymidine incorporation (Table II) and a 1.6-fold increase in cell number ($p < 0.001$). ACTH also stimulated thymidine incorporation into Kin-8 DNA with a 3.4-fold increase that compared favorably with the 4-fold increase seen in parental Y1 cells (Table II) and with a 1.4-fold increase in cell number ($p < 0.001$) that was not significantly different.
ACTH Stimulates Cell Cycle Progression in Y1 Adrenal Cells

Y1 cells were plated on coverslips, serum-starved for 48 h, and then treated with 100 milliunits/ml ACTH (porcine, Sigma), 20 ng/ml recombinant bovine FGF, or a combination of ACTH + FGF for 2 h as indicated. Cells then were washed and transferred to DMEM without serum and incubated for an additional 22 h. One set of coverslips was treated with FGF for 2 h, transferred to serum-free medium, and challenged with ACTH from the 4th to 6th h of incubation. During the 12–24 h of incubation, all the experimental groups were incubated in serum-free medium containing 100 μM BrdUrd and were then processed to estimate the percentage of nuclei labeled with BrdUrd. Results from three independent experiments were pooled such that 5330–6400 cells were counted per condition. As determined by χ² analysis with 1 degree of freedom, all differences between means were significant (at < 0.001%) except for the difference between means for the pair marked with asterisks (*).

### Table III

| First treatment | Second treatment | Labeled nuclei | Increment over control |
|-----------------|-----------------|---------------|----------------------|
| 0–2 h           | 4–6 h           | %             | %                    |
| None            | FGF             | 9.4           | 0.0                  |
| FGF             | ACTH            | 17.4*         | 8.0*                 |
| FGF + ACTH      | ACTH            | 24.6          | 15.2                 |
| FGF + ACTH      | ACTH            | 18.3*         | 8.9*                 |

from the effect of serum. Together, these results indicate that ACTH stimulated adrenal cell proliferation through a cAMP- and PKA-independent mechanism. In both parent Y1 cells and mutant Kin-8 cells, PMA stimulated the transition from G1 to S (Table II), suggesting a possible role for protein kinase C as a mediator of ACTH action.

Temporal Localization of the Growth-inhibitory Effect of ACTH—To determine if the stimulatory and inhibitory effects of ACTH on cell cycle progression could be temporally dissociated, labeling indices were determined after incubating serum-starved Y1 cells with FGF together with ACTH added at two different intervals in G1. As shown in Table III, the addition of FGF or ACTH to serum-starved Y1 cells during the first 2 h of G1 increased labeling indices 3.6- and 1.8-fold, respectively, over serum-starved controls. The addition of FGF together with ACTH for the first 2 h of G1 only modestly inhibited the increase in cell cycle progression (38%), whereas the addition of ACTH 2 h after the pulse with FGF inhibited cell cycle progression more dramatically (64%). These observations suggest that ACTH acts at a point early in the G1 phase of the cell cycle to stimulate progression to S phase and acts later in G1 to arrest cell cycle progression.

Effects of Growth Factors and ACTH on Expression of c-fos
c-fos is one of the earliest genes induced by mitogens in G0-arrested fibroblasts and is required for progression of cells through the G1 phase of the cell cycle (29–32). c-FOS protein was not detected in serum-starved Y1 cells but accumulated with different kinetics after treatment of serum-starved Y1 cells with ACTH, FGF, or fetal bovine serum; after 2 h, c-FOS was detected in at least 80% of nuclei (Fig. 5). ACTH and fetal bovine serum caused c-FOS to accumulate more rapidly than did FGF; however, the effects of ACTH and FGF on c-FOS accumulation were sustained over 6 h, whereas the effects of fetal bovine serum were transient and declined after 4 h.

DISCUSSION

The results presented here demonstrate that ACTH, administered to Y1 cells as a short pulse early in the G1 phase of the cell cycle, activates MAP kinase (Fig. 2), promotes the progression of cells from the G1 phase of the cell cycle to S phase (Table II and Fig. 4), and ultimately stimulates cell division. These effects of ACTH appear to be independent of the cAMP-signaling cascade since ACTH also exerts these effects in the PKA-defective mutant, Kin-8, and may involve a PKC-dependent pathway since these actions of ACTH are mimicked by a pulse of PMA (Fig. 2 and Table II). ACTH is known to activate PKC in Y1 cells (33) lending further support to this latter hypothesis. There are, however, other potential pathways through which ACTH may activate the MAP kinase cascade and stimulate cell proliferation. For example, other hormones acting through G protein-coupled receptors have been shown to activate MAP kinase via a pathway involving activation of G protein β/γ subunits, phosphoinositide 3-kinase, and a src-like tyrosine kinase (34, 35). Therefore, the relative contribution of the protein kinase C pathway to the proliferative effects of ACTH has yet to be established.

ACTH also stimulates the rapid accumulation of c-FOS protein (Fig. 5). Although not rigorously established in our experiments, results from other systems suggest that this effect of ACTH is likely to be mediated by activation of the MAP kinase signaling cascade (36). Indeed, the ACTH-stimulated increase in c-FOS accumulation (Fig. 5) occurs subsequent to the activation of MAP kinase by ACTH (Fig. 2) and also appears to be mediated by a protein kinase C-dependent pathway rather than by a cAMP-dependent mechanism (10, 11). The observations that ACTH and serum stimulate c-FOS accumulation more rapidly than does FGF (Fig. 5) is also consistent with this hypothesis since ACTH and serum activate the MAP kinase cascade more rapidly than does FGF (Fig. 1).3 The transient effect of serum on c-FOS accumulation (Fig. 5) suggests the presence of specific serum factors that control either the stability of the c-fos transcript or c-FOS protein but which appear not to have an impact on the generation of the mitogenic signal.

Although Y1 cells are of tumor origin, the cell line behaves in many other respects like normal adrenocortical cells and has long been used as a model adrenocortical cell system (37, 38). Thus, the finding that ACTH under appropriate circumstances can stimulate the proliferation of Y1 adrenal cells may be physiologically relevant. The results presented here suggest that the trophic effects of ACTH on the adrenal cortex may result from a direct mitogenic effect of the hormone rather than from an indirect effect as postulated previously (2). In addition,
our results provide a rationale for the inductive effects of ACTH on genes associated with cell proliferation such as ornithine decarboxylase (9) and fos and jun protooncogenes (Fig. 5 and Refs. 10–12) that were previously considered to be paradoxical effects of the hormone. Our results also may bear on the controversy regarding the origins of the proliferative cells of the adrenal cortex. According to one hypothesis, proliferative adrenal cells arise from a population of undifferentiated stem cells that expand and differentiate as they move through the distinct zones of the cortex, whereas an alternate hypothesis suggests that proliferative cells arise from differentiated zones of the gland (39, 40). Our results that demonstrate proliferative effects of ACTH on a differentiated cell line would seem to be more consistent with the hypothesis that proliferating adrenal cells arise from differentiated zones of the gland rather than from an undifferentiated stem cell population.

With prolonged treatment, ACTH inhibits G$_1$ to S progression in Y1 cells (Table I). Our results indicate that the growth-inhibitory effect of ACTH does not result from inhibition of the MAP kinase isoforms Erk1 and Erk2 (Fig. 2). Instead, preliminary results suggest that growth inhibition may result from a post-transcriptional effect of ACTH leading to destabilization of c-myc transcripts (for discussion, see Ref. 8). Although cAMP analogs result in Y1 cells (Table I). Our results indicate that the growth-inhibitory effect of ACTH leading to stabilization of c-myc transcripts (for discussion, see Ref. 8).

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