Journal of Cellular Biochemistry 62:251–261 (1996)

Dexamethasone Alters Rapidly Actin Polymerization Dynamics in Human Endometrial Cells: Evidence for Nongenomic Actions Involving cAMP Turnover

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Abstract Glucocorticoids, in addition to their well characterized effects on the genome, may affect cell function in a manner not involving genomic pathways. The mechanisms by which the latter is achieved are not yet clear. A possible means for this action may involve the actin cytoskeleton, since the dynamic equilibrium of actin polymerization changes rapidly following exposure to several stimuli, including hormones. The aim of the present work was to find out if glucocorticoids exert rapid, nongenomic effects on actin polymerization in Ishikawa human endometrial cells, which represent a well characterized in vitro cell model expressing functional glucocorticoid receptors. Short term exposure of the cells to the synthetic glucocorticoid dexamethasone resulted in an overall decrease of the G/total-actin ratio in a time- and dose-dependent manner. Specifically, in untreated Ishikawa cells the G/total-actin ratio was 0.48 ± 0.01 ($n = 26$). It became 0.35 ± 0.01 ($n = 13, P < 0.01$) following exposure to 10^{-7} M dexamethasone for 15 min. This was induced by a significant decrease of the cellular G-actin level, without affecting the total actin content, indicating a rapid actin polymerization. This conclusion was fully confirmed by direct fluorimetry measurements, that showed a significant increase of the F-actin content by 44% ($n = 6, P < 0.001$) in cells treated with dexamethasone (10^{-7}M, 15 min). The rapid dexamethasone-induced alterations of the state of actin polymerization were further supported by fluorescence microscopy. The latter studies showed that the microfilaments of cells pretreated with 10^{-7}M dexamethasone for 15 min were more resistant to various concentrations of the antimicrofilament drug cytochalasin B, compared to untreated cells, implying microfilament stabilization. The action of dexamethasone on actin polymerization seems to be mediated via specific glucocorticoid binding sites, since the addition of the glucocorticoid antagonist RU486 completely abolished its effect. Moreover, it appears to act via non-transcriptional pathways, since actinomycin D did not block the dexamethasone-induced actin polymerization. In addition, cell treatment with 10^{-7}M dexamethasone for 15 min fully reversed the forskolin-, but not the 8-bromo-cAMP-induced actin depolymerization. In line with these findings, the cAMP content of Ishikawa cells was decreased by 29.2% after a 15 min treatment with 10^{-7}M dexamethasone ($n = 4, P < 0.01$). In conclusion, our results showed that dexamethasone induces rapid, time-, and dose-dependent changes in actin polymerization dynamics in Ishikawa cells. This action seems to be mediated via cAMP, involving probably nongenomic pathways. The above findings offer new perspectives for the understanding of the early cellular responses to glucocorticoids. © 1996 Wiley-Liss, Inc.

Key words: dexamethasone, actin, polymerization, Ishikawa cells, cAMP, actinomycin D

INTRODUCTION

Polymerized actin microfilaments are in a dynamic equilibrium with the monomeric actin forms. A major characteristic of this monomer-polymer actin equilibrium is its rapid response to extracellular effectors including a number of hormones [Rao et al., 1985; Theodoropoulos et al., 1992; Ding et al., 1991; Simon et al., 1993; Wu et al., 1992]. Rapid changes in actin polymerization may contribute to the way by which hormones induce rapid cellular events such as secretion, exocytosis, and membrane trafficking [Mils et al., 1994; Hesketh and Pryme, 1991; Annis and Bader, 1988; Burgess and Kelly, 1987; Castellino et al., 1992; Kiley et al., 1992]. Indeed, in response to nicotine, normal chromaffin cells undergo depolymerization of their F-actin and subsequent loss of their microfilament network [Sontag et al., 1988; Cheek and Burgoyne,

Received October 31, 1995; accepted January 18, 1996.

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1987; Trifaro et al., 1989], thus promoting the secretion of catecholamines. Similarly, vasopressin depolymerizes F-actin of the apical region of amphibian bladder granular cells and of mammalian inner medullary collecting duct cells, allowing the rapid fusion of water channels, which carry vesicles to the apical membrane [Ding et al., 1991; Simon et al., 1993]. Furthermore, TRH-mediated prolactin secretion by GH_4C_1 rat pituitary cells is associated with acute reorganization of actin cytoskeleton [Kiley et al., 1992]. On the other hand, actin polymerization is promoted by insulin, glutamine, or hypotonic exposure [Rao et al., 1985; Theodoropoulos et al., 1992], conditions that induce cell volume changes in rat hepatocytes [Theodoropoulos et al., 1992]. Glucocorticoids appear to affect actin microfilament stabilization albeit in a long-term manner [Castellino et al., 1992, 1995]. This mode of action has been proposed as the mechanism by which glucocorticoids inhibit ACTH secretion from the anterior pituitary corticotrophs [Castellino et al., 1992]. Moreover, glucocorticoids cause permanent changes in the organization of microfilaments in the trabecular meshwork cells [Clark et al., 1994]. However, evidence has also accumulated suggesting that glucocorticoids, in addition to their effects mediated via genomic pathways, may affect cell function by rapidly-responding nongenomic interactions [Edwardson and Bennett, 1974; Hua and Chen, 1989; Ffrench-Mullen et al., 1994]. Based on these reports, we inquired whether these rapid effects of the glucocorticoids may involve the actin cytoskeleton.

In the present study, using quantitative biochemical and fluorimetric assays for the determinations of cellular actin, we examined whether glucocorticoids affect rapidly the dynamic equilibrium between monomeric and polymerized actin in Ishikawa human endometrial cells. This is a well characterized cell line expressing functional glucocorticoid receptors, in which the transcriptional activation mediated by glucocorticoids has been studied extensively [Makrigiannakis et al., 1992]. Having found that dexamethasone rapidly alters the state of actin polymerization we studied the molecular mechanism of this interaction. In particular, we examined whether this effect a) is dependent on gene transcription, b) is mediated by glucocorticoid binding sites, and c) involves cAMP regulation.

MATERIALS AND METHODS

Materials

Fetal bovine serum (FBS), Minimum essential medium (MEM), L-glutamine, penicillin, streptomycin, and Hank's balanced salt solution (HBSS) were from Gibco (Life Technologies Inc., Gaithersburg, MD). Dulbecos-MEM/Ham's F-12, DNA from herring testes, cytochalasin B, forskolin, 8-bromo-cAMP, and actinomycin D were from Sigma (St. Louis, MO). Rhodamine-phalloidin was from Molecular Probes (Eugene, OR). The cAMP determination kit, cyclicAMP[^{125}I] assay system (dual range) RPA 509, was from Amersham (Braunschweig, Germany). The Bio-Rad protein assay kit II (500-0002) was from Bio-Rad Laboratories (Palo Alto, CA). [9-(Tetrahydro-2-furyl)adenine] (THFA) was from Calbiochem (Lucerne, Switzerland). Actin from rabbit muscle was our own preparation [Faulstich et al., 1984]. All other chemicals were obtained from the usual commercial sources at the purest grade available.

Cell Culture and Treatments

Ishikawa cells were established as a permanent cell line from a well differentiated endometrial adenocarcinoma [Gravanis and Gurpide, 1986]. The cells were cultured in 25 cm^2-flasks in MEM containing 15% FBS in a 5% CO_2 95% air atmosphere at 37°C. Near confluence the cells were washed twice and cultured for 12 h in DMEM/Ham's F-12 in the absence of serum, but supplemented with 10mM L-glutamine, 15mM HEPES, and 1% antibiotic-antimycotic solution to final concentrations of 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were exposed to various concentrations of dexamethasone (10^{-6}–10^{-9} M) and/or RU486 (10^{-6}–10^{-9} M) for different periods of time (15–120 min). For the measurements of the cellular G- and total-actin content and total protein levels, cells were washed twice with cold HBSS containing 0.05% EDTA and removed from flasks using scrapers. For the determinations of the actin dynamic equilibrium in the presence of forskolin (50 μM), 8-bromo-cAMP (1mM) or THFA (0.1mM), cells were incubated for 15 min with each of these agents, followed by a 15-min treatment with 10^{-7}M dexamethasone. In a set of separate experiments, dexamethasone, and forskolin at the appropriate concentrations were added simultaneously to the cell preparations and the monomer-polymer actin equilibrium was determined after 15 min incubation. In the experiments with actinomycin D, cells were preincubated for 90 min with 1mg/l actinomycin D before adding 10^{-7}M dexamethasone for 15 min to the incubation medium.

DNase I Inhibition Assay for Actin Quantitations

The monomeric (G-) and total actin content were measured in Ishikawa cells by the G-actin-dependent DNase I inhibition assay [Blikstad et al., 1978], with minor modifications, as described previously [Theodoropoulos et al., 1992]. The intracellular actin content was quantified by reference to a standard curve for the inhibition of DNase I activity, prepared from rabbit muscle actin, isolated as previously described [Faulstich et al., 1984]. The G- and total-actin content in Ishikawa cells was related to the total protein content. Protein concentrations were measured by the Bio-Rad protein kit.

Fluorescence Measurements of F-Actin

F-actin content, in the absence or presence of dexamethasone, was determined in Ishikawa cells by fluorescence measurements of rhodamine-phalloidin labelled samples, according to the method described [Wu et al., 1992; Cable et al., 1995], with some minor modifications. Briefly, cells were fixed by addition of 0.3 ml of formaldehyde (3.7% in PBS), followed by 15 min incubation at room temperature. The cells were then permeabilized by adding 0.3 ml Triton X-100 (0.2% in PBS). 0.3 ml of rhodamine-phalloidin (1.5 μM in PBS) were added and samples were incubated for 30 min. The cells were then washed with PBS (3 × 1 ml) and dissolved in 0.5 ml of 0.1 M NaOH. Fluorescence of the samples was measured in a Perkin-Elmer LS 3B fluorimeter using excitation and emission wavelengths of 550 and 580 nm respectively.

Fluorescence Microscopy

For fluorescence microscopy, Ishikawa cells were grown for 24 h on 22 × 22 mm cover slips in MEM medium, containing 15% FBS, followed by 12 h incubation in DMEM/Ham's F-12 in the absence of serum. Then, cells were incubated for 10 min with cytochalasin B (10^{-6} M and 2×10^{-6} M), dissolved in culture medium. In the appropriate experiments, cells were preincubated for 15 min with 10^{-7} M dexamethasone before adding cytochalasin B. The procedure of cell fixation and the direct fluorescence staining of microfilaments by rhodamine-phalloidin included incubation of the cell preparations with 3.7% formaldehyde for 4 min, washing in PBS and then immersing in acetone at −20°C for 3–5 min. The cells were then washed twice in PBS, incubated for 40 min at room temperature with rhodamine-phalloidin at a dilution 1:20 in PBS and washed four times for 3 min in PBS [Katsantonis et al., 1994; Fostinis et al., 1992]. The cover slips were inverted in a 1:1 v/v mixture of glycerol and PBS and visualized in a Leitz Dialux 20EB microscope (Vetzlar, Germany) equipped with epifluorescent illumination. Micrographs were obtained with a 35 mm (C-35AD-4) camera on Kodak P3200 black and white films.

cAMP Assay

cAMP levels in Ishikawa cells were determined by a method based on that described by Newsome et al. [1994]. Immediately after collection, untreated and dexamethasone-treated cells (10^{-7}M, 15 min) were rapidly frozen in liquid nitrogen and divided into two equal parts. One part was applied to an Amprep SAX 500 mg minicolumn (Amersham), dried by lyophilization, and assayed directly for the determination of cAMP by means of a commercially available radioimmunoassay kit (Amersham). The other part of each sample was used for the determination of the protein content per μl of cell extract by the Bio-Rad kit. Values of cAMP levels in cell extracts, expressed as fmol cAMP/mg protein, were calculated in triplicates from four distinct experiments.

Statistical Analysis

Results were expressed as mean ± SE from n = cell preparations. Statistical analysis of the monomeric (G-) and the total-actin levels, the G/total-actin ratio, the F-actin content, and cAMP levels in treated Ishikawa cells was performed by one way analysis of variance (ANOVA) and by unpaired Student's test. For comparing the effects of dexamethasone, RU486, forskolin, 8-bromo-cAMP, and THFA on G/total-actin ratio we used the Kruskal-Wallis nonparametric test, since results were normalized to control.

RESULTS

Dexamethasone Alters Rapidly the Polymerization State of Actin

In untreated Ishikawa cells the G- and total-actin content amounted to 24.98 ± 1.27 and

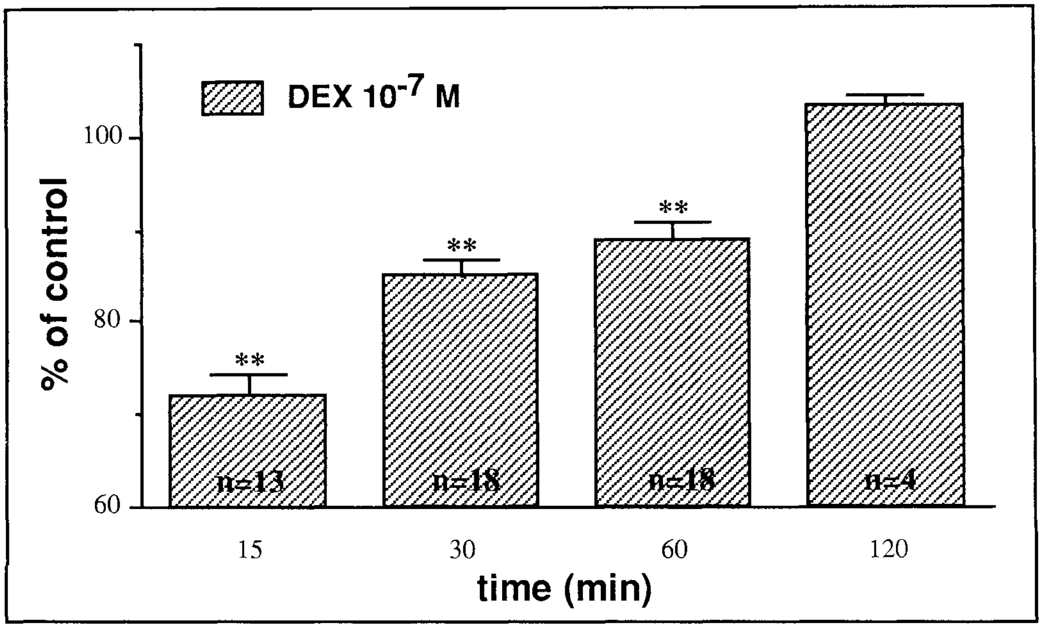

Fig. 1. Time-course of the effect of dexamethasone on G/total-actin ratio. G/total-actin ratios were determined in Ishikawa cells incubated for 15, 30, 60, and 120 min with 10^{-7}M dexamethasone. Results are expressed as percentage of control value (untreated cells), and represent mean ± SE from n = number of cell preparations. **Denotes significant difference ($P < 0.01$) compared to untreated cells (control).

TABLE I. Effect of 15 Min Treatment With Dexamethasone (10^{-6}–10^{-9}M) on Monomeric (G-) and Total-Actin Content (μg actin/mg Protein) and on G/Total-Actin Ratio in Ishikawa Cells

Treatment (15 min)	G-actin content μg/mg cell protein	total actin content μg/mg cell protein	G/total-actin ratio
Control (n = 26)	24.98 ± 1.27	52.43 ± 2.23	0.48 ± 0.01
DEX 10^{-6}M (n = 13)	17.50 ± 1.29*	54.53 ± 3.51	0.32 ± 0.02*
DEX 10^{-7}M (n = 13)	17.42 ± 1.21*	50.06 ± 2.67	0.35 ± 0.01*
DEX 10^{-8}M (n = 5)	23.82 ± 2.79	54.10 ± 4.32	0.44 ± 0.03
DEX 10^{-9}M (n = 5)	22.60 ± 1.54	49.00 ± 3.45	0.46 ± 0.03

*Denotes statistically significant differences ($P < 0.01$) in G-actin content and G/total-actin ratios, compared to control incubations. No statistically significant differences were observed for total actin levels.

52.43 ± 2.23 μg/mg total protein (n = 26), respectively. The ratio of monomeric to total cellular actin, i.e., the G/total-actin ratio was 0.48 ± 0.01 (n = 26) (Table I). Dexamethasone (10^{-7}M) increased actin polymerization in a time-dependent manner, as indicated by the decreased G/total-actin ratios shown in Figure 1. The onset of the decrease of the actin ratio was detectable within 15 min and persisted for up to 60 min. When the cells were incubated for 2 h or even longer (24 h, not shown) with 10^{-7}M dexamethasone, the actin ratio was reversed to control levels indicating a transient event. The effect of dexamethasone on the G/total-actin ratio in Ishikawa cells was dose-dependent. As shown in Table I and Figure 2, incubation of the cells (15 min) with dexamethasone (10^{-6}M and 10^{-7}M) lowered the G/total-actin ratios from 0.48 ± 0.01 (n = 26) to 0.32 ± 0.02 (n = 13, $P < 0.01$) and 0.35 ± 0.01 (n = 13, $P < 0.01$) respectively, indicating a maximal corresponding decrease of the ratios by 33% and 27%, compared to untreated cells. Since the cellular G-actin level was significantly lowered, whereas the total actin content did not change significantly (Table I), it was concluded that under these experimental conditions a significant and rapid stimulation of actin polymerization took place.

To confirm the above findings we performed direct fluorescence measurements of rhodamine-phalloidin-labelled F-actin in Ishikawa cells incubated with dexamethasone. Indeed, a significant

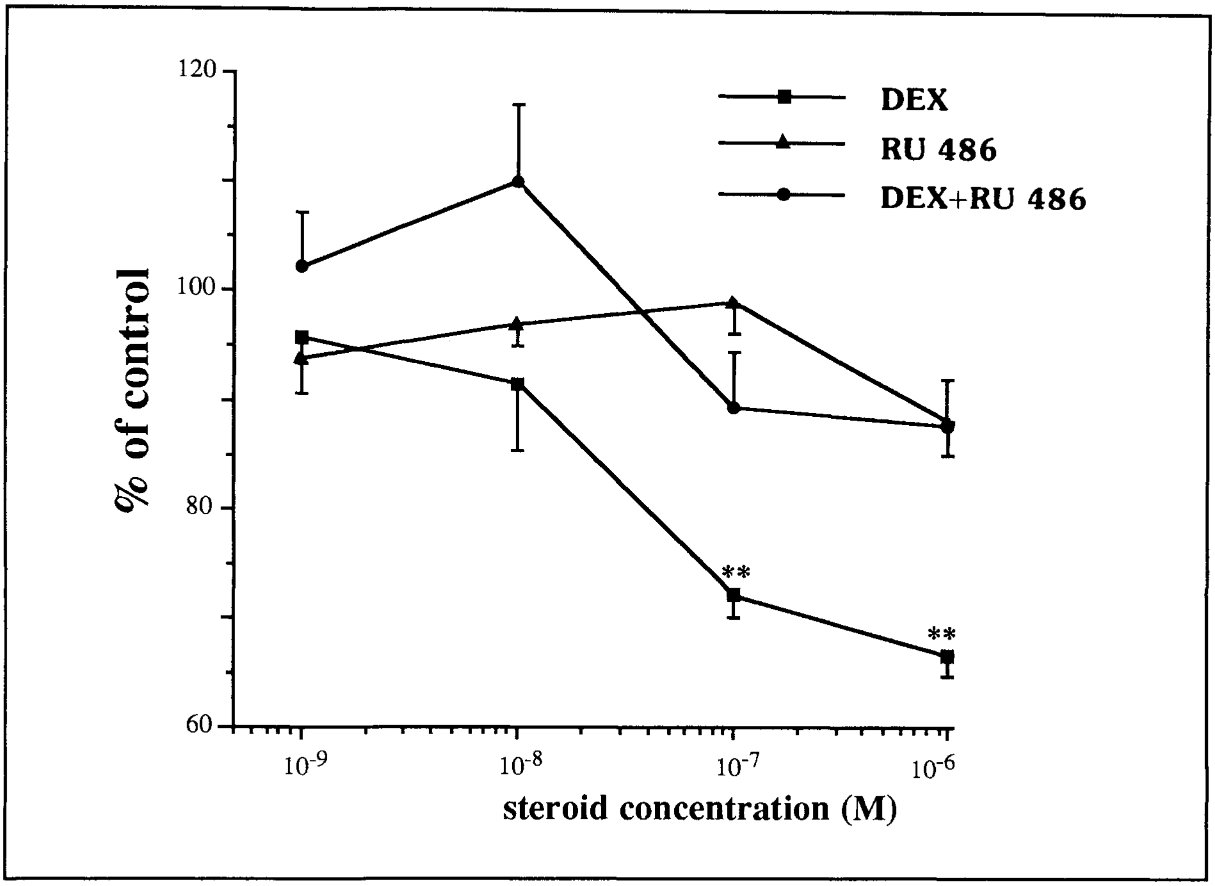

Fig. 2. Dose-response of the effect of dexamethasone (DEX), RU486 and DEX plus RU486 on G/total-actin ratio. G/total-actin ratios were determined in Ishikawa cells, treated for 15 min with dexamethasone (-■-, n = cell preparations indicated in Table I), RU486 (-▲-, n = 5), and dexamethasone plus 10^{-6}M RU486 (-●-, n = 5), at the indicated concentrations. Results are expressed as percentage of control (untreated cells) and represent mean ± SE. **Denotes significant difference ($P < 0.01$) compared to untreated cells (control).

increase of the fluorescence was measured when cells were treated for 15 min with 10^{-7}M dexamethasone, compared to untreated cells (from 16.7 ± 2.3 units/mg protein to 29.9 ± 2.7 units/mg protein; $n = 6, P < 0.001$), indicating a rapid increase of polymerized F-actin.

Involvement of Glucocorticoid Binding Sites

The effect of the glucocorticoid antagonist RU486 on the actin polymerization state in the absence or presence of dexamethasone (10^{-6}M to 10^{-9}M) was also examined. As shown in Figure 2, the rapid (15 min), dose-dependent effect of dexamethasone on actin polymerization was fully prevented when Ishikawa cells were incubated in the presence of RU486 (10^{-6}M). Moreover, the monomer-polymer actin equilibrium did not change significantly when cells were incubated with 10^{-6}M to 10^{-9}M of the glucocorticoid antagonist for 15 min (Fig. 2). These results indicate that the dexamethasone induced decrease of the G/total-actin ratio is probably mediated via specific glucocorticoid binding sites.

Actinomycin D Does Not Block the Rapid Effect of Dexamethasone on Actin Polymerization

To examine whether new gene transcription is necessary for the dexamethasone-induced actin polymerization, Ishikawa cells were incubated with 1 mg/l actinomycin D for 90 min, followed by treatment with 10^{-7}M dexamethasone for 15 min. As shown in Figure 3, in the presence of actinomycin D, dexamethasone (10^{-7}M) still influences the actin polymerization state, since the G/total-actin ratio is decreased to 0.37 ± 0.01 ($n = 5, P < 0.01$). These findings strongly suggest that glucocorticoids may act via rapidly-responding, non-transcriptional pathways.

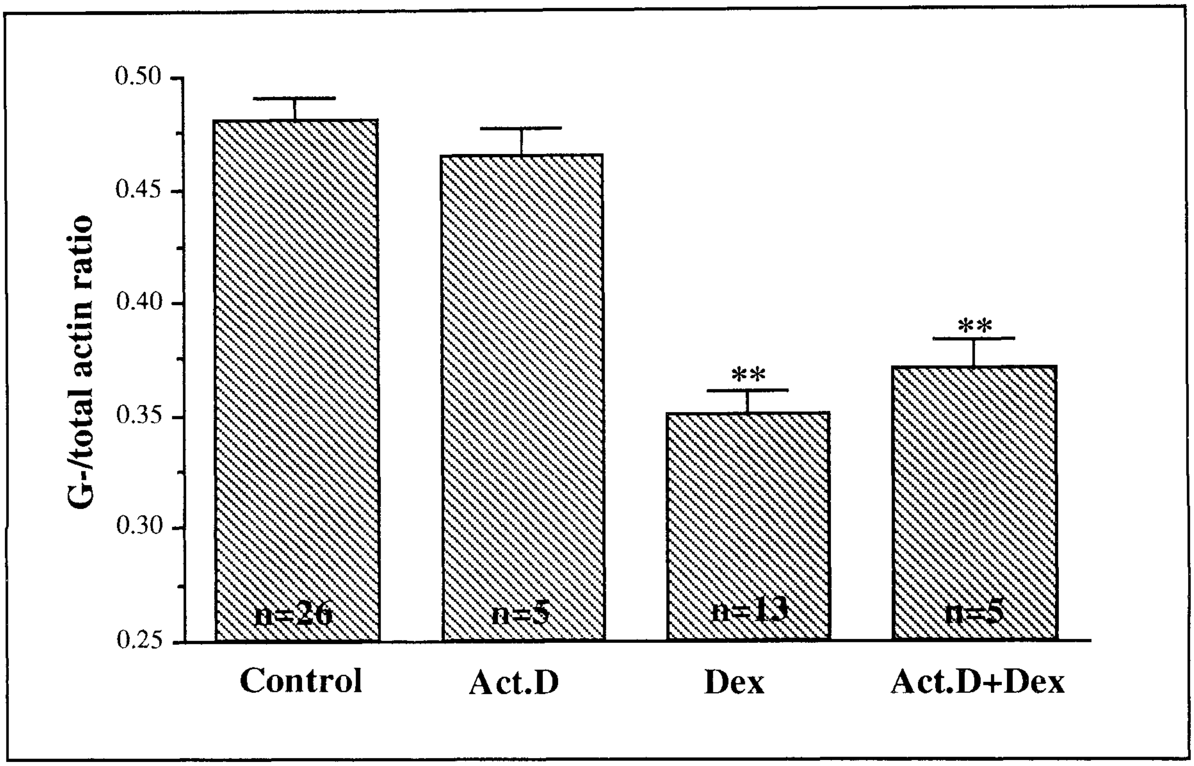

Fig. 3. Effect of actinomycin D on dexamethasone-induced changes of the G/total-actin ratio. G/total-actin ratios were determined in untreated Ishikawa cells (control), in cells treated with 10^{-7}M dexamethasone for 15 min (Dex) and in cells pretreated for 90 min with 1 mg/l actinomycin D (Act.D), followed by treatment with 10^{-7}M dexamethasone for 15 min (Act.D + Dex). The G/total-actin ratios are expressed as mean values ± SE from n = cell preparations. **Denotes significant difference ($P < 0.01$) compared to untreated cells (control).

Dexamethasone Increases Microfilament Resistance to Cytochalasin B

Rapid alterations of actin microfilament organization were examined by direct fluorescence microscopy of Ishikawa cells, preincubated for 15 min with dexamethasone (10^{-7}M), followed by 10 min incubations with graded concentrations of cytochalasin B (a fungal toxin inducing actin filament shortening). This approach represents an established procedure for the semiquantitative study of actin filament stabilization [Castellino et al., 1992; Katsantonis et al., 1994, Papakonstanti et al., 1996]. As shown in Figure 4a and a', the microfilamentous network of cytochalasin B-untreated Ishikawa cells in the absence or presence of dexamethasone (10^{-7}M) respectively, had very similar morphological characteristics. Incubation of Ishikawa cells with cytochalasin B (10^{-6}M) induced some disruption of actin filaments in untreated cells (Fig. 4b), since small and larger spots of phalloidin binding material became apparent in the cell interior. However, the microfilament network was still basically intact. On the other hand, in dexamethasone-treated cells, the microfilamentous structures appeared to be unaffected (Fig. 4b'). The differential microfilament shortening became even clearer after exposure to cytochalasin B (2×10^{-6}M). Under these conditions, in dexamethasone-treated Ishikawa cells only few of the microfilaments underwent depolymerization, whereas their overall network appeared largely intact (Fig. 4c'). In contrast, the incubation of untreated cells with cytochalasin B (2×10^{-6}M) induced a marked actin microfilament shortening, as indicated by the almost complete disappearance of the characteristic microfilamentous network (Fig. 4c). The above observations indicate that the rapid increase of polymerized actin after short term exposure of Ishikawa cells to dexamethasone renders actin filaments more resistant to the action of cytochalasin B, suggesting microfilament stabilization.

cAMP Turnover

Agents raising cellular cAMP levels have been described to induce actin depolymerization [Ding et al., 1991; Valitutti et al., 1993]. Thus, we

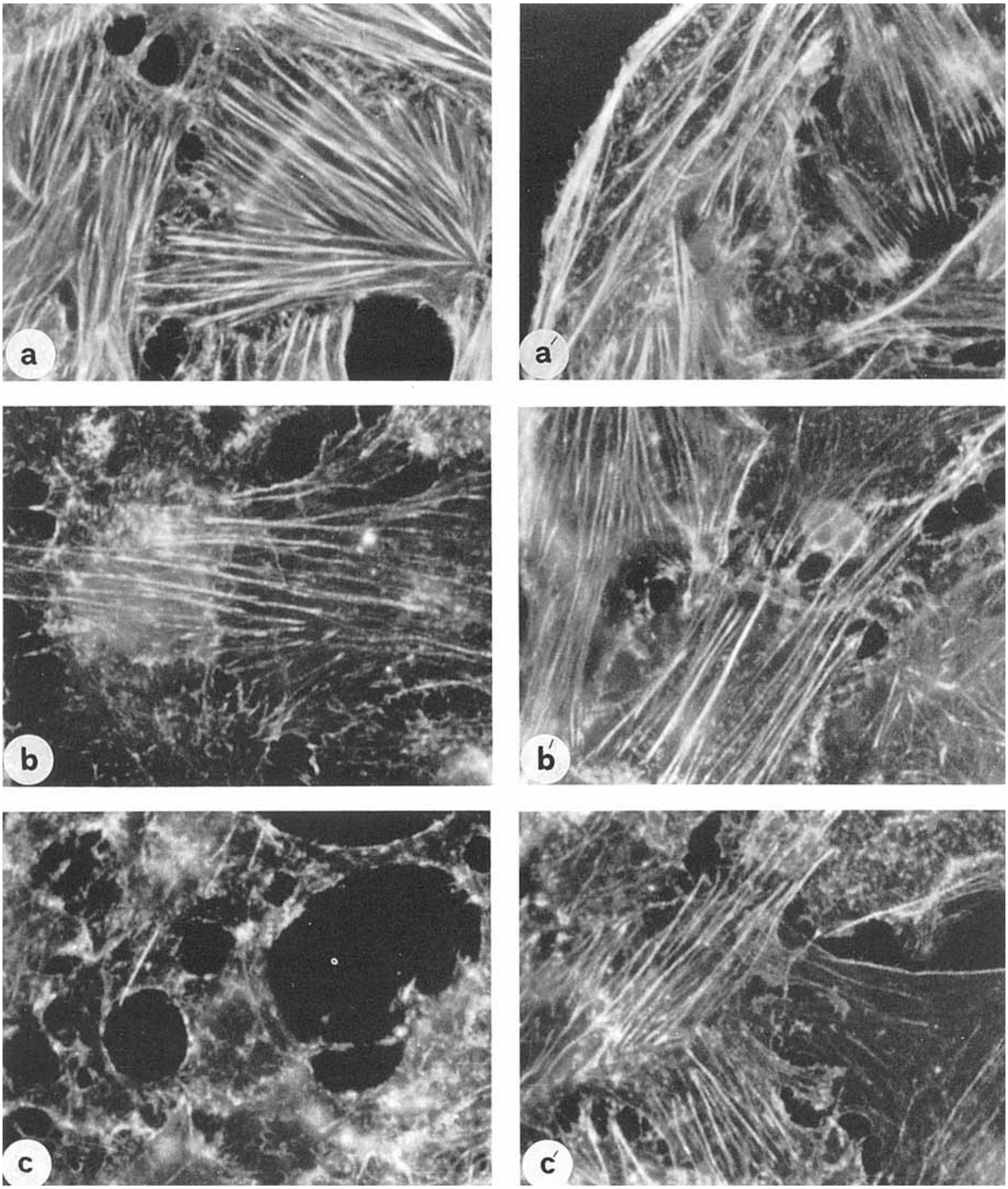

Fig. 4. Dexamethasone effect on the shortening action of cytochalasin B on actin filaments. Rhodamine-phalloidin staining of microfilaments in untreated Ishikawa cells (**a, b, c**) and cells treated with 10^{-7}M dexamethasone for 15 min (**a', b', c'**). Resistance of actin filaments to cytochalasin B was evaluated after cell incubation for 10 min with 10^{-6}M and 2×10^{-6}M of this drug. Magnification × 1000. (a,a'): Cytochalasin B-untreated cells; (b,b'): effect of 10^{-6}M cytochalasin B; (c,c'): effect of 2×10^{-6}M cytochalasin B.

studied the involvement of cAMP turnover in the rapid glucocorticoid mediated effects on actin polymerization. For this, Ishikawa cells were incubated for 15 min with forskolin (50 μM) or 8-bromo-cAMP (1mM), two agents that raise cAMP levels, and with [9-(tetrahydro-2-furyl)adenine, THFA] (0.1mM), an adenylyl cyclase inhibitor [Goldsmith and Abrams, 1991]. Subsequently, changes of the G/total-actin ratio were measured, following a 15-min cell treatment with dexamethasone (10^{-7}M). In all experiments the total-actin content did not change significantly. Figure 5 summarizes the obtained results. In line with earlier observations in another cell model [Ding et al., 1991], both forskolin and 8-bromo-cAMP induced depolymerization of F-actin in Ishikawa cells, as shown by the significant increase of the corresponding G/total-actin ratios (Fig. 5A, B). Dexamethasone, when added simultaneously or after forskolin, reversed fully the actin depolymerization induced by this agent, as indicated by the decrease of the G/total-actin ratio to levels very similar to those determined in Ishikawa cells treated solely with dexamethasone (Fig. 5A). Interestingly, the F-actin depolymerization induced by the non-hydrolysable 8-bromo-cAMP could not be restored by dexamethasone (Fig. 5B). Finally, in the presence of THFA, the G/total-actin ratio decreased significantly and remained equally suppressed in the presence of dexamethasone (Fig. 5C). These results indicate that the glucocorticoid-induced actin polymerization may be associated with alterations in cellular cAMP levels. To further evaluate this hypothesis, we determined directly the cAMP content of Ishikawa cells treated with dexamethasone. In cells incubated for 15 min with dexamethasone (10^{-7}M), cAMP amounted to 1637 ± 46 fmol/mg protein (n = 4) compared to 2312 ± 96 fmol cAMP/mg protein (n = 4) in untreated cells, indicating a significant decrease of the cellular cAMP content by 29.2% ($P < 0.01$). The above findings suggest that dexamethasone-induced actin polymerization may be directly associated with very rapid changes of cellular cAMP levels.

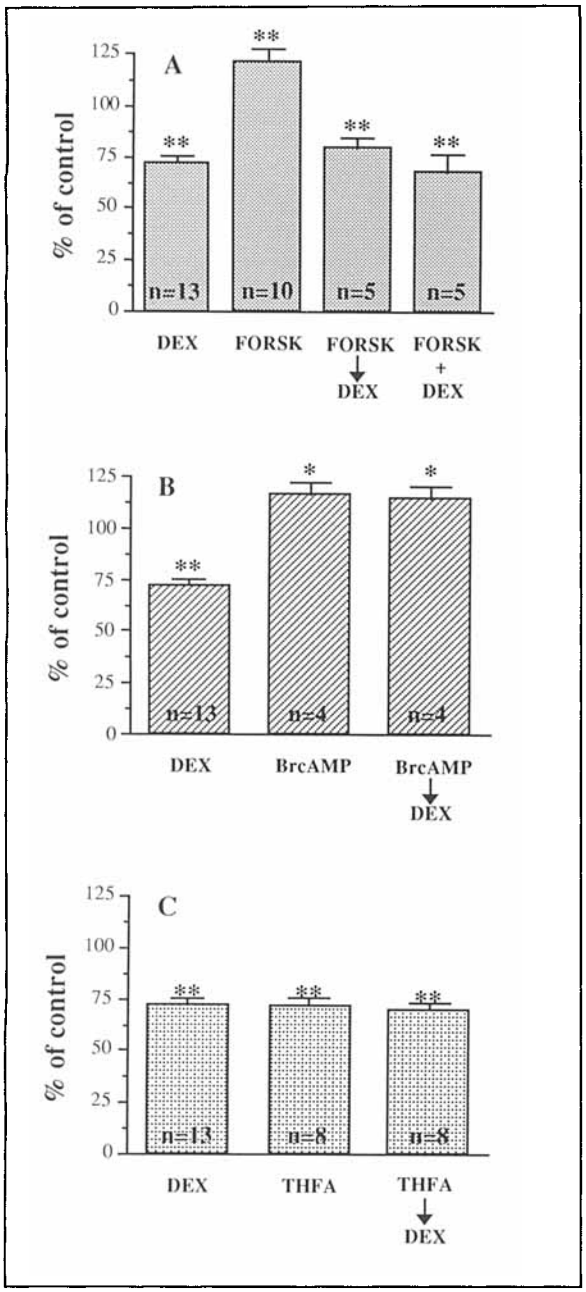

Fig. 5. Effects of dexamethasone on G/total-actin ratios in the presence of agents modifying cellular cAMP. **A:** G/total-actin ratios were determined in Ishikawa cells preincubated for 15 min with forskolin (FORSK, 50 μM), followed by a 15-min treatment with dexamethasone (FORSK → DEX, 10^{-7}M), or in cells incubated simultaneously with forskolin and dexamethasone for 15 min (FORSK + DEX). **B:** Ishikawa cells were preincubated for 15 min with 8-bromo-cAMP (BrcAMP, 1mM), followed by a 15-min treatment with dexamethasone (BrcAMP → DEX, 10^{-7}M). **C:** Ishikawa cells were preincubated for 15 min with 9-(tetrahydro-2-furyl)adenine (THFA, 0.1 mM), followed by a 15-min treatment with dexamethasone (THFA → DEX, 10^{-7}M). In A, B, and C, (DEX) represents the effect of 15 min cell treatment with dexamethasone (10^{-7}M, see Fig. 1). All results are expressed as percentage of control (untreated cells) and represent mean ± SE from n = cell preparations. ** and * denotes significant differences ($P < 0.01$ and $P < 0.05$ respectively) of the G/total-actin ratios, compared to untreated cells.

DISCUSSION

The biological action of steroid hormones is mainly mediated via genomic pathways. This classical mechanism of action involves receptor activation, binding to DNA and gene activation, regulation of mRNA transcription, followed by de novo protein synthesis [for reviews see Grody et al., 1982; Savouret et al., 1989; Murdoch and Gorski, 1991; O'Malley et al., 1991]. Thus, the biological effects attributed to steroid hormone action are generally characterized as relatively long-term events. Recent evidence suggests, however, that steroid hormones may also exert short-term, nongenomic effects [for reviews see Wehling, 1994; Brann et al., 1995]. Indeed, rapid effects have been reported for almost every steroid [Wehling, 1994], including cortisol [Hua and Chen, 1989; Ffrench-Mullen et al., 1994; Steiner et al., 1988; Borski et al., 1991], and other glucocorticoids [Edwardson and Bennett, 1974; Hall, 1982]. The finding that dexamethasone binds to plasma membrane-enriched liver fractions [Qelle et al., 1988] further supports the notion that glucocorticoids may affect cellular pathways independently of their cytoplasmic receptors. Accordingly, in the present work we considered the possibility that glucocorticoids induce rapid changes in actin cytoskeleton, which may be involved in rapid cellular events. This hypothesis would imply rapid polymerization or depolymerization of the filamentous actin network, which underlies the plasma membrane.

Our results showed a rapid and dose-dependent decrease of the G/total-actin ratio in Ishikawa endometrial cells treated with dexamethasone. This effect was detectable within 15 min and persisted for up to 1 h. It was due to a significant decrease of the G-actin content, while the total actin level was not seriously affected by dexamethasone. In line with these findings, direct fluorimetric measurements showed increased F-actin content in rhodamine-phalloidin-labelled cell preparations treated with dexamethasone. From these results we concluded that short-term incubation with dexamethasone induces rapid actin polymerization in Ishikawa cells. The changes in the polymerization state of actin cytoskeleton, quantitated by using biochemical and fluorimetric assays were corroborated by morphological observations with fluorescence microscopy. In accordance with the increased actin polymerization during short-term incubation with dexamethasone, microfilaments demonstrated increased resistance to the desintegrating effect of graded cytochalasin B concentrations, suggesting stabilization of the actin filaments.

The rapid effect of dexamethasone on actin polymerization did not appear to involve nuclear events since transcriptional inhibitors, such as actinomycin D, could not block them. This finding indicates a post-translational glucocorticoid action, independent of new gene transcription. Moreover, the event appears to be specific, mediated via interaction with glucocorticoid binding sites, suggested by the complete restoration of the G/total-actin ratio by the glucocorticoid antagonist RU486. It remains unclear, however, whether dexamethasone binding to Ishikawa cells involves also binding to the nuclear or cytoplasmic receptors, or to plasma membrane receptors, as indicated recently for liver plasma membrane preparations [Quelle et al., 1988].

The results of several studies suggest that cellular signal transduction can enhance or impair, in a variety of ways, glucocorticoid-mediated transcriptional effects [Nordeen et al., 1994]. The rapid alterations in actin polymerization dynamics by dexamethasone seem to be associated with rapid changes of cellular cAMP levels. Supporting experimental evidence was provided by the observation of differential actions of dexamethasone on actin depolymerization, induced by agents such as forskolin and the non-hydrolysable cAMP analogue 8-bromo-cAMP (Fig. 5A, B) that increase cAMP levels. These results indicate a possible involvement of cAMP hydrolysis in actin polymerization mediated by dexamethasone. In line with these findings, direct measurements of cellular cAMP levels in Ishikawa cells treated with dexamethasone, showed a significant decrease of cAMP, thus indicating that changes of intracellular cAMP content may be correlated to dexamethasone treatment and the subsequent actin polymerization. This notion is further supported by other experiments, that showed the same decrease in G/total-actin ratios in cells treated with THFA, an inhibitor of cAMP-generating adenylate cyclase, in the presence or absence of dexamethasone (Fig. 5C).

The dexamethasone mediated rapid actin polymerization in Ishikawa cells is compatible with the hypothesis that actin cytoskeleton may be involved in the regulation of glucocorticoid-induced rapid cellular events. The physiological mechanisms that may control the rapid glucocorticoid effects on microfilaments, shown in our study, require very fast responses. These can not be easily attributed to induction of specific proteins regulating F-actin stabilization, as it has been recently proposed for the observed long-term inhibition of ACTH release from pituitary cells by dexamethasone [Castellino et al., 1992]. Thus, the observed rapid (15 min) modifications in actin polymerization, implying microfilament stabilization, may represent an alternative physiological way of regulating rapid, nongenomic glucocorticoid effects. Such a hypothesis would imply a dual mode of glucocorticoid action, addressing both the rapid, probably nongenomic actions and the genomic mediated slower effects. According to this model, glucocorticoids could trigger cellular responses through the rapid changes in actin polymerization dynamics, reported in this study. On the other hand, induction and de novo synthesis of actin binding proteins, which could regulate microfilament organization and stabilization as well [Castellino et al., 1992, 1995], may delineate the molecular mechanism for the long-term, genomic dexamethasone action on actin cytoskeleton. A dual model for steroid action has been recently proposed for mineralcorticoids, which were reported to influence electrolyte transport systems through both nongenomic and genomic pathways [Wehling, 1994].

In conclusion, our results showed that dexamethasone induces rapid modifications in actin polymerization dynamics in Ishikawa cells mediated probably via non-transcriptional pathways. These findings are compatible with the assumption that actin cytoskeleton may be involved in the mechanisms regulating rapid cellular responses to glucocorticoids.

ACKNOWLEDGMENTS

The authors thank Dr. K. Thermos and Dr. D.S. Emmanouel for fruitful discussions. This work was supported by grants from the Biomedical Research Committee of the Greek Ministry of Health (ΚΕΣΥ) and the Greek Secretariat for Research and Technology (ΠΕΝΕΔ 91) to C.S.

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