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Dexamethasone Induces Rapid Actin Assembly in Human Endometrial Cells Without Affecting Its Synthesis

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Abstract Dexamethasone exerts a stimulatory effect of rapid-onset on the polymerization of actin. This has been documented in human endometrial adenocarcinoma Ishikawa cells, resulting in an acute, dose-dependent decrease in the G/total-actin ratio. In the present study we completely characterized this fast and apparently nongenomic effect of dexamethasone on actin assembly. We followed the morphological alterations of actin cytoskeleton and measured the time-dependent dynamics of actin polymerization both by ruling out any changes of total actin in the cells and by measuring its transcript. Rapid changes in actin polymerization were accurately measured using a highly sensitive and quantitative rhodamine-phalloidin fluorimetric assay. Ishikawa cells, exposed to 0.1 µM dexamethasone for various time periods up to 24 h, showed a highly significant, rapid, and transient increase in the polymerization of actin starting within 15 min of dexamethasone exposure and lasting 2 h. Treated cells showed a significant (1.79-fold) enhancement of the fluorescent signal compared to untreated cells at 15 min. This value decreased continuously in a time-dependent manner, reaching control levels after 120 min and remained so for the next 24 h. Confocal laser scanning microscopy studies confirmed these findings. Intensive coloration of microfilaments over several scanning sections suggested an enhanced degree of actin polymerization in cells preincubated for 15 min with 0.1 µM dexamethasone. Moreover, actin filaments were more resistant to cytochalasin B. Additionally, quantitative immunoblot analysis showed that the content of total cellular actin remained the same during this period, suggesting that the biosynthesis of actin was unaffected. Northern blot analysis showed that the concentration of the actin transcript was also unaffected. Our data suggest that glucocorticoids induce a fast and self-limited polymerization of actin in human endometrial cells without affecting its synthesis. These findings strengthen the hypothesis that glucocorticoids exert rapid, nongenomic cellular effects and that the actin-based cytoskeleton is an integral part of this pathway, playing an essential role in receiving and mediating steroid signals for the modulation of cellular responses. J. Cell. Biochem. 65:492–500.

Key words: dexamethasone; nongenomic effect; actin assembly; signal transduction; confocal microscopy; total actin; actin transcript

Recent studies suggest that steroid hormones affect cell function not only through genomic events, but also through nongenomic pathways of rapid onset [Wehling, 1994]. Such effects have been suggested for almost every steroid [Edwardson and Bennett, 1974; Hall, 1982; Steiner et al., 1988; Hua and Chen, 1989; Borski et al., 1991; Ffrench-Mullen et al., 1994]. However, the mechanisms by which these nongenomic effects are mediated have not yet been clarified. The actin microfilament system is one of the most likely candidates. The actin cytoskeleton plays a dynamic role in several cellular functions because it is able to change the assembly and organization of its filaments rapidly. Several studies have shown that hor-

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mones regulate the dynamics of actin polymerization and microfilament organization [Rao et al., 1985; Annis and Bader, 1988; Ding et al., 1991; Hesketh and Pryme, 1991; Kiley et al., 1992; Theodoropoulos et al., 1992; Simon et al., 1993]. Thus it appears that rapid changes in the monomer/polymer equilibrium of the actin cytoskeleton may be part of the mechanism by which hormones induce early cellular responses such as secretion, exocytosis, or volume regulation [Annis and Bader, 1988; Kiley et al., 1992; Theodoropoulos et al., 1992].

We have recently reported that glucocorticoids exert rapid nongenomic stimulatory effects on actin assembly in Ishikawa human endometrial cells [Koukouritaki et al., 1996]. Specifically, we found that dexamethasone induces a rapid and dose-dependent decrease of monomeric actin and the G/total-actin ratio. These rapid changes of the polymerization dynamics of actin cytoskeleton were mediated via specific glucocorticoid binding sites. Also, clear stabilization of actin microfilaments was observed by fluorescence microscopy. Finally, our data indicated that these nongenomic effects involved cAMP-dependent regulatory pathways.

Since the nongenomic effect of glucocorticoids appears to be very fast, the aim of the present work was to study precisely the time course of the dynamics of actin polymerization. Levels of rhodamine-phalloidin-labelled F-actin in cells exposed to dexamethasone for variable time periods were measured. In addition, using confocal laser scanning microscopy alterations in organization and stability of actin cytoskeleton induced by dexamethasone were observed. Finally, we examined whether glucocorticoid-induction of actin polymerization is followed by changes of actin gene transcription rate. For the latter, the levels of total actin were compared with actin mRNA, using quantitative immunoblot analysis and Northern blot hybridization, respectively. As our in vitro model we used the Ishikawa human adenocarcinoma cell line, which expresses functional glucocorticoid receptors, responds to glucocorticoids, and proved to be a reliable tool in our initial report.

MATERIALS AND METHODS

Fetal bovine serum (FBS), minimum essential medium (MEM), L-glutamine, penicillin, streptomycin, and Hank's balanced salt solution (HBSS) were from Gibco (Life Technologies, Gaithersburg, MD). Dulbecco's-MEM/Ham's F-12, BSA and cytochalasin B were from Sigma (St. Louis, MO). Rhodamine-phalloidin was from Molecular Probes (Eugene, OR). The monoclonal antibody against β-actin and the ECL Western blotting kit were purchased from Amersham Corp (Arlington Heights, IL). The Gene Screen nylon membranes were from New England Nuclear (Boston, MA). The Bio-Rad protein assay kit II (500-0002) was from Bio-Rad Laboratories (Palto Alto, CA). Actin from rabbit muscle was our own preparation [Faulstich et al., 1984]. All other chemicals were obtained from 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 10 mM L-glutamine, 15 mM HEPES, and 1% antibiotic-antimycotic solution to final concentrations of 100 U/ml penicillin and 100 μg/ml streptomycin. For the determination of the cellular F-actin content, cells were exposed to 0.1 μM dexamethasone for various periods of time (15–120 min, 24 h).

Fluorescence Measurements of F-actin Amounts

The effect of dexamethasone on the content of F-actin was measured by a slightly modified method employing the rhodamine-phalloidin labelling of F-actin, as described [Wu et al., 1992]. Briefly, the Ishikawa cells were fixed by adding 0.3 ml of formaldehyde (3.7% in PBS), followed by 15 min incubation at room temperature. After fixation, the cells were 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. After washing with PBS (3×1 ml), the cells were dissolved in 0.5 ml of 0.1 M NaOH. Fluorescence intensities of the samples were measured in a Perkin-Elmer LS 3B fluorimeter using excitation and emission wavelengths of 550 and 580 nm, respectively. Protein concentrations were measured with the Bio-Rad protein determination kit using BSA as a standard.

Confocal Laser Scanning Microscopy

Ishikawa cells were grown for 24 h on 22 × 22 mm^2 coverslips 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 15 min with cytochalasin B (2 μM), dissolved in culture medium. In the appropriate experiments, cells were preincubated for 15 min with 0.1 μM dexamethasone before adding cytochalasin B. The procedure of cell fixation and the direct fluorescence staining of microfilaments by rhodamine-phalloidin was performed exactly as described previously [Koukouritaki et al., 1996]. The coverslips were analysed using a confocal laser scanning modul (Leica Lasertechnik, Heidelberg, Germany), attached to an inverted microscope (Zeiss IM35, Zeiss, Oberkochen, Germany), equipped with an argon-krypton ion laser. Confocal images were aquired using a 63/1.25 oil immersion objective and dedicated CLSM software (Leica Lasertechnik, Heidelberg, Germany). The image data were processed with AVS software (Advanced Visual Systems, Watham, MA) [Sheehan et al., 1996] running on a Silicon Graphics Indigo 2 workstation (Silicon Graphics, Mountain View, CA). Fluorescence images were optimized in brightness and contrast, transferred to a personal computer and printed on a Lexmark Optra C laser printer (Lexmark, Lexington, NY), using Corel Photo Paint 5.0 software (Corel Corp., Ottawa, Canada).

Immunoblot Analysis

In order to measure total actin levels, cells were cultured in the presence and absence of 0.1 μM dexamethasone for 15 min to 24 h as described above. Near confluence, the cells were washed twice and cultured for 12 h in serum stripped of steroids. Cells were rinsed with 1 ml distilled H_2O followed by ultrasound treatment (3 × 10 sec). Equal amounts of protein (10 μg) of the cell extracts were subjected to SDS electrophoresis and the resulting protein-bands were transferred to nitrocellulose membranes, using an LKB electroblot apparatus (LKB, Bromma, Sweden). Nitrocellulose blots were incubated with monoclonal mouse antiactin antibodies, followed by incubation with the appropriate labelled secondary antibody, using the ECL Western blotting kit. Nitrocellulose blots were exposed to Kodak X-omat AR films for variable lengths of time. Band intensities were quantified by PC-based Image Analysis (Ontario, Canada). The actin content in each sample was calculated from a standard curve of increasing concentrations (0.05; 0.1; 0.15; 0.2; 0.3, and 0.4 μg respectively) of isolated pure rabbit muscle actin.

Northern Blot Analysis of Actin mRNAs

Isolation and size-fractionation of total cellular RNA from the Ishikawa cells was as previously described [Antonakis et al., 1991]. After transfer of the RNA to Gene Screen nylon membranes, the filters were prehybridized and then hybridized with cDNA probes γ-^{32}P-labelled by random priming, using oligodeoxynucleotides (specific activity 5×10^7 cpm/μg), according to Feinberg and Vogelstein, [1983]. The β-actin mRNA levels were normalized to the levels of glutamate dehydrogenase mRNA. Hybridizations were carried out as described [Maniatis et al., 1989]. Washed filters were exposed to Kodak XR film at −80°C in the presence of an intensifying screen. Hybridization was quantified by a PC-based Image Analyser (Image Analysis). The probes used were a rat β-actin cDNA Pst I fragment inserted into a pUC 18 plasmid and a human glutamate dehydrogenase cDNA probe [Theodoropoulos et al., 1992].

Statistical Analysis

Results are expressed as mean ± SE (n = number of experiments). The unpaired Student's t-test was used to analyse the F-actin content in treated Ishikawa cells. P values <0.05 were considered significant.

RESULTS

Stimulatory Effect of Dexamethasone on Actin Assembly Is Rapid in Onset and of Short Duration

Figure 1 depicts the changes of F-actin levels in the Ishikawa cells. The cells were incubated with 0.1 μM dexamethasone for various times ranging from 15–120 min. A rapid and significant 1.79-fold fluorescence enhancement, indicating polymerization of actin, was evident within the first 15 min of exposure to dexamethasone (29.9 ± 2.7 units/mg protein) compared to parallel untreated controls (16.7 ± 2.3 units/mg protein). This increase was short lived; 120 min later, it returned to control levels (16.9 ± 1.4 and 19.5 ± 1.5 units/mg protein for untreated and dexamethasone-treated cells respectively) (Fig. 1) and remained so for the next 24 h (not shown). These findings suggest that glucocorticoids induce a rapid and transient polymer-

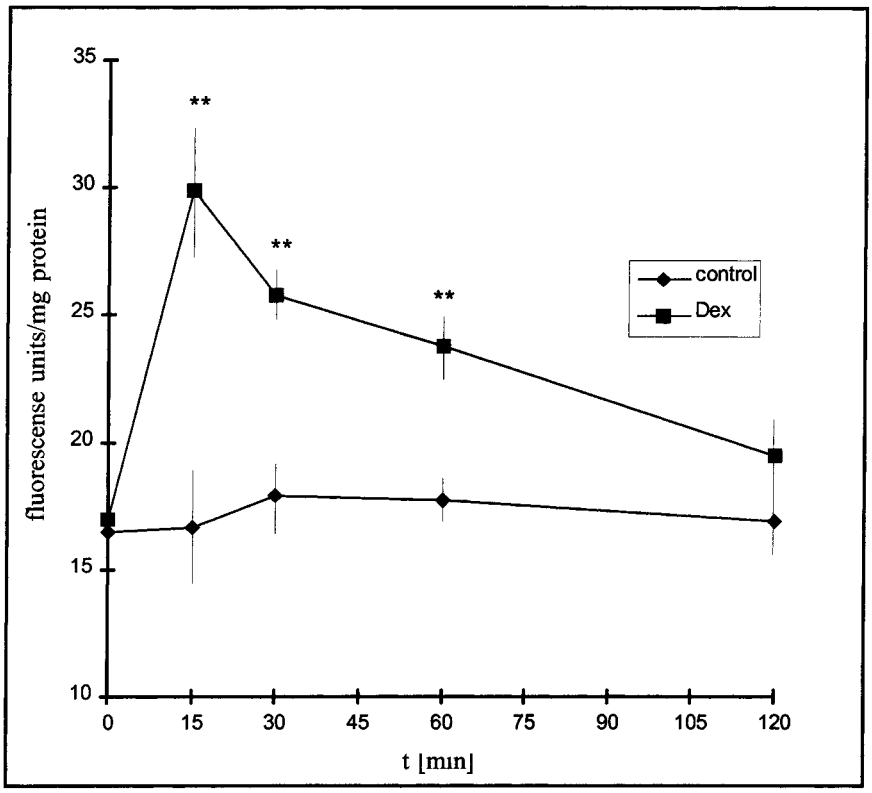

Fig. 1. Time-course of F-actin amounts in untreated (—◆—) and dexamethasone-treated (0.1 µM, —■—) Ishikawa cells. Results are expressed as fluorescence units of rhodamine-phalloidin labelled F-actin per mg of Triton insoluble protein and represent mean values ± SE, from n = 6 (t = 15 min) and n = 8 (t = 30, 60, and 120 min, respectively) distinct experiments. **denotes $P < 0.01$ between dexamethasone-treated and untreated cells.

ization of monomeric G-actin to filamentous F-actin.

Dexamethasone Alters Rapidly Microfilament Organization and Stability

The acute effect of glucocorticoids on the arrangement of actin microfilaments was examined by confocal laser scanning microscopy of Ishikawa cells preincubated for 15 min with 0.1 µM dexamethasone. In control cells, microfilaments were observed near the attachment sites of cells to the substratum (up to 0.5 µm, Fig. 2A, micrographs 3–4). Microfilaments could not be recognized at higher scanning levels (Fig. 2A, micrographs 1–2). However, in cells preincubated with dexamethasone, actin microfilaments stained intensively, from the attachment sites into higher scanning levels towards the upper cytoplasmic regions (up to 1.5 µm, Fig. 2B, micrographs 1–4). These observations suggest a major reorganization of actin filaments, confirming the rapid increase of polymerized actin shown in dexamethasone-treated Ishikawa cells (Fig. 1). The above findings are consistent with studies of actin filament stabilization in the presence of cytochalasin B. Untreated and dexamethasone-treated cells were incubated with a concentration of cytochalasin B (2µM), which has been shown to induce an almost complete disappearance of the characteristic network of microfilaments at these cells [Koukouritaki et al., 1996]. Incubation of untreated cells with cytochalasin B induced, as expected, a marked actin filament shortening and an almost complete disappearance of intact microfilaments (Fig. 2C, micrographs 1–4). In contrast, in dexamethasone-treated Ishikawa cells intact microfilamentous structures were visualized throughout several scanning sections (Fig. 2D, micrographs 1–4).

Dexamethasone Does Not Affect Total Actin Content

We further examined if the above mentioned rapid polymerization of actin by dexamethasone was due to synthesis of new actin. Short-time exposure (15 and 60 min) to 0.1 µM dexamethasone had no effect, since the total actin amounts did not change significantly during this time period (Fig. 3A, lanes 1–4). Similarly,

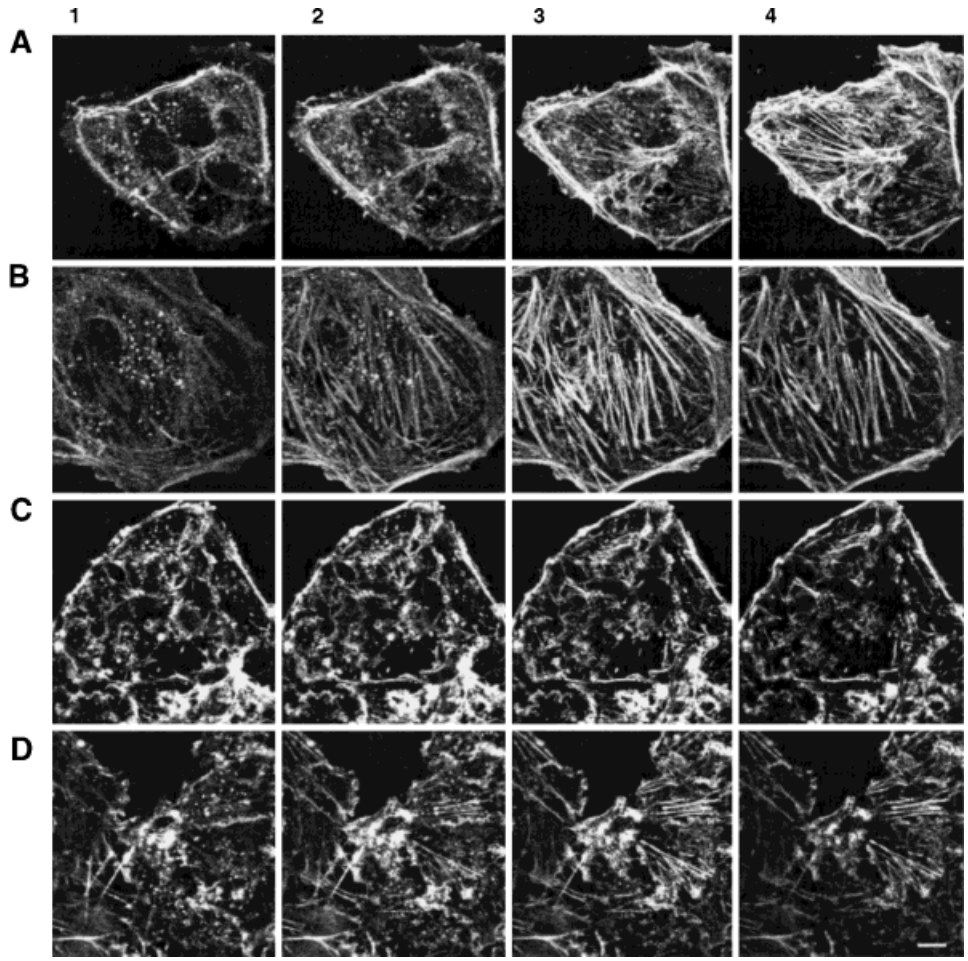

Fig. 2. Confocal laser scanning micrographs of Ishikawa cells stained with rhodamine-phalloidin: **A,** untreated cells; **B,** cells treated with 0.1 μM dexamethasone for 15 min; **C,** cells incubated for 15 min with 2 μM cytochalasin B, **D,** cells treated with 0.1 μM dexamethasone for 15 min and incubated for 15 min with 2 μM cytochalasin B. Micrographs 1–4 represent scanning sections from the upper cytoplasmic region toward the attachment site of the cells. The step size of the optical sections was adjusted to 0.5 μ (A, B) and 0.33 μm (C, D), respectively. Bar: 10 μm.

the total actin content remained unchanged for up to 24 h (Fig. 3B, lanes 1–10). Indeed, by reference to a standard curve (Fig. 3B, lanes 11–16, r = 0.959), the calculated ratios of the relative total actin amounts from control- and treated-cells were very similar (Table I). These data suggest that actin synthesis does not appear to be affected by dexamethasone. Thus the observed transient alterations of actin cytoskeleton dynamics do not seem to involve protein synthesis but changes in the actin polymerization/depolymerization equilibrium.

Dexamethasone Does Not Influence the Transcription Rate of the Actin Gene

Figure 4 depicts the quantitative Northern blot hybridization data. The levels of actin mRNA were unaffected following exposure to 0.1 μM dexamethasone for all tested time periods, up to 24 h. Normalization of the levels of actin mRNAs (Fig. 4A, lanes 1–10) to glutamate dehydrogenase mRNAs (Fig. 4B, lanes 1–10), showed unaltered results (Table I), indicating that the rate of transcription of the actin gene was not affected by dexamethasone and the resulted rapid but transient actin assembly.

DISCUSSION

In a previous study [Koukouritaki et al., 1996], we have shown that one of the mechanisms by which glucocorticoids induce rapid, nongenomic cellular events involves change in the dynamics of actin polymerization. We found that exposure of Ishikawa human endometrial cells to dexamethasone induces a decrease in the monomeric to total actin ratio [Koukouri-

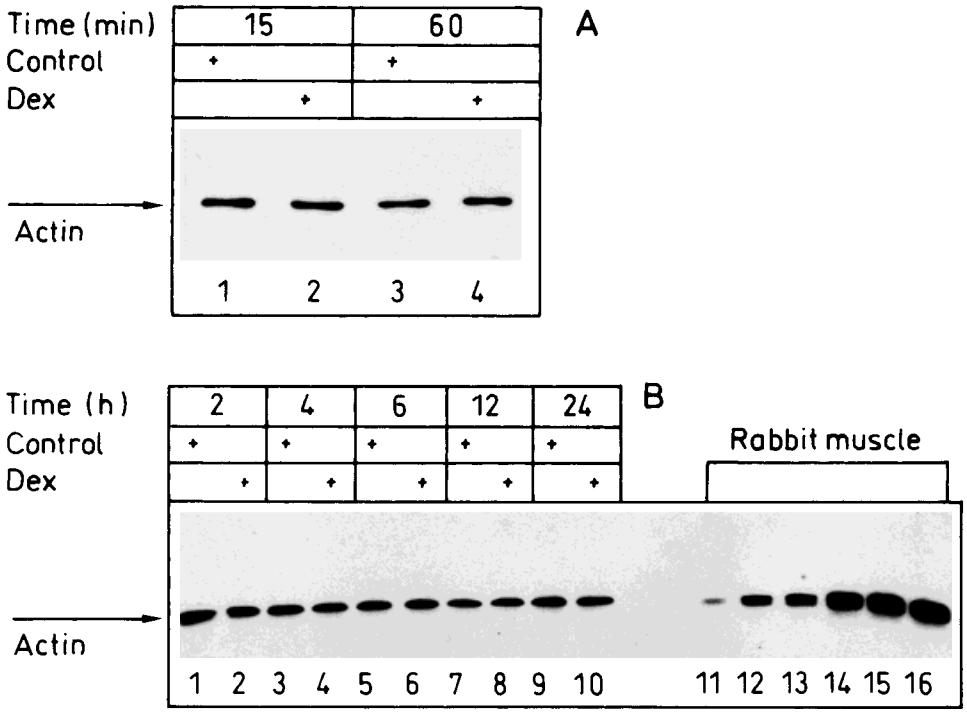

Fig. 3. Quantitative Western blot analysis (representative of two experiments) depicting amounts of total cellular actin in Ishikawa cells treated with 0.1 μM dexamethasone for various time periods. **A.** *Lanes 1,3:* amounts of total actin in 10 μg cellular aliquots from untreated Ishikawa cells after 15 and 60 min, respectively. *Lanes 2,4:* amounts of total actin in 10 μg cellular aliquots from Ishikawa cells treated with 0.1 μM dexamethasone for 15 and 60 min, respectively. **B.** *Lanes 1, 3, 5, 7, 9:* amounts of total actin in 10 μg cellular aliquots from untreated Ishikawa cells after 2, 4, 6, 12, and 24 h, respectively. *Lanes 2, 4, 6, 8, 10:* amounts of total actin in 10 μg cellular aliquots from Ishikawa cells treated with 0.1 μM dexamethasone for 2, 4, 6, 12, and 24 h, respectively. *Lanes 11–16:* standard curve of isolated rabbit muscle actin (0.05; 0.1; 0.15; 0.2; 0.3; 0.4 μg), respectively.

TABLE I. Relative Total Actin Amounts and β-actin mRNA Levels respectively, in Ishikawa Cells Treated With 0.1 μM Dexamethasone (Dex)*

Time (h)	Relative total actin (Dex/control)[a]	Relative β-actin mRNA levels (Dex/control)[b]
0.5	n.d.	1.2
2	0.83	1.4
4	0.82	n.d.
6	0.97	0.7
12	1.02	0.7
24	0.88	0.8

*For various time periods from 30 min to 24 h

[a]Relative total actin amounts are expressed as the ratios of dexamethasone-treated (Dex) versus untreated (control) cells, calculated from the values of μg of total actin per mg total cellular protein corresponding to Figure 3B.

[b]Relative values for β-actin mRNA levels were normalized for glutamate dehydrogenase mRNA levels in dexamethasone-treated vs. untreated cells, corresponding to Figure 4A,B.

n.d. = no data.

taki et al., 1996]. In this study, we have examined the time course of dexamethasone action on actin cytoskeleton, using the highly sensitive fluorimetric method for F-actin quantitations with rhodamine-phalloidin. This represents a novel experimental approach for accurate, direct, and quantitative determinations of cellular F-actin levels [Wu et al., 1992; Cable et al., 1995; Cox et al., 1996; Stournaras et al., 1996]. Our data show that dexamethasone induced actin assembly within 15 min and that it ended by 120 min. The stimulatory effect of dexamethasone on actin polymerization is reversible and of short duration. This finding supports the hypothesis that rapid changes in the actin polymerization dynamics play an essential regulatory role on the way by which several stimuli induce early cellular responses. Such a relationship has been established for cellular events, such as the control of transport proteins [Jessen and Hoffmann, 1992], ion channels [Cantiello et al., 1991, 1993; Wu et al., 1992; Papakonstanti et al., 1996], water trans-

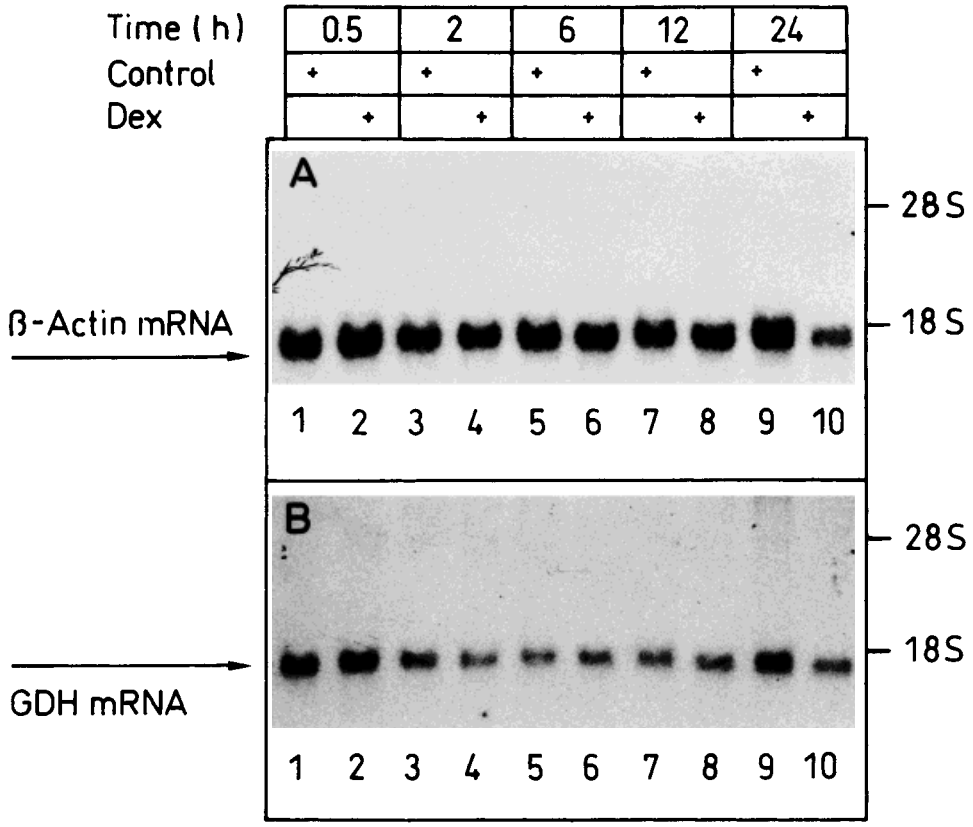

Fig. 4. Northern blot analysis of β-actin mRNA **(A)** and glutamate dehydrogenase (GDH) mRNA **(B)** from untreated Ishikawa cells (*lanes 1, 3, 5, 7, 9*), and cells exposed to 0.1 μM dexamethasone (*lanes 2, 4, 6, 8, 10*) for the following time periods: 30 min (lanes 1, 2); 2 h (lanes 3, 4); 6 h (lanes 5, 6); 12 h (lanes 7, 8); and 24 h (lanes 9, 10). 10 μg of total RNA was loaded to each lane.

port [Ding et al., 1991; Simon et al., 1993], and other transmembrane transport processes [Mils et al., 1994; Mils and Mandel, 1994]. In addition, the rapid nongenomic effect of dexamethasone on actin polymerization involves cAMP regulation [Koukouritaki et al., 1996]. Furthermore, a link has been described between mitogenic signal transduction mediated by G-proteins of the *ras* superfamily and the organization of the actin-based cytoskeleton [Dartsch et al., 1994; Symons, 1996; Machesky and Hall, 1996]. Therefore, our findings support the assumption that rapid and transient modification of the dynamics of actin cytoskeleton may act as a sensor and/or mediator of signal transduction cascades regulating various cellular events.

The transient nature of actin polymerization induced by dexamethasone implies modifications of the architecture of actin filaments in Ishikawa cells. These alterations were studied by confocal laser scanning microscopy, since direct fluorescence microscopy represents an experimental approach with limited resolution capacity for such kind of observations. Confocal micrographs showed enhanced coloration of microfilaments in treated cells through several scanning sections, compared to untreated cells. These observations indicate important and rapid reorganization of actin filaments in the cytoplasmic region near the attachment sites of the cells. Additionally, short incubation with dexamethasone increased the resistance of microfilaments to the shortening action of cytochalasin B. These findings are in line with our previous observations of glucocorticoid-induced stabilization of actin filaments [Koukouritaki et al., 1996].

Several agents, mostly toxins, can modify the dynamics of actin polymerization, altering the rate of actin biosynthesis via regulation of actin gene transcription. In cultured rat hepatocytes, modifications of the cellular content of G-actin, or the ratio of monomeric to filamentous actin and the resulting prolonged reorganization of actin cytoskeleton [Reuner et al., 1995, 1996] were found to induce autoregulatory control of

actin synthesis. Indeed, such a control has been documented in studies of actin mRNA levels and monomer to polymer actin ratios after exposure to actin-stabilizing agents such as clostridium botulinum C2 toxin and the mycotoxin phalloidin [Reuner et al., 1995]. Furthermore, the increase of actin mRNA following treatment of rat hepatocytes with insulin, glutamine, or hypotonic conditions provided additional evidence that the prolonged change of the G/total-actin ratio is accompanied by changes in the rate of actin gene transcription [Theodoropoulos et al., 1992, Stournaras et al., 1994]. To examine whether the rapid effect of dexamethasone on the polymerization dynamics of actin involves changes of the transcription rate of actin gene, we measured both the actin transcript and its final protein product. Our quantitative immunoblot analysis data showed that the amount of total actin did not change significantly during exposure to dexamethasone for at least 24 h. This finding provides strong evidence that actin synthesis was not affected. Also, quantitative Northern blot hybridization showed that the concentration of actin mRNA was not affected by dexamethasone. Together, these data suggest that the rapid and transient effect of dexamethasone does not involve regulation of actin biosynthesis. Accordingly, these observations are distinct from the prolonged reorganization of the actin cytoskeleton induced by cell swelling and various stimuli, such as toxins, which have been reported to involve autoregulatory control of actin biosynthesis [Theodoropoulos et al., 1992; Reuner et al., 1995, 1996].

The molecular mechanism of dexamethasone-actin interaction is not yet clear. However, the alterations of the dynamics of actin cytoskeleton by dexamethasone were shown to be closely associated with rapid changes of cellular cAMP levels [Koukouritaki et al., 1996]. Additionally, activation of proteins interacting with the actin-based cytoskeleton by several kinases also have been implicated in such processes (Burridge et al., 1992; Tidball and Spencer, 1993; Chrzanowska-Wodnicka and Burridge, 1994). Accordingly, these findings may indicate that activation of specific proteins, controlled by cAMP-dependent, or other signal transduction cascades may be part of the mechanisms regulating the alterations of actin microfilament dynamics following dexamethasone treatment. Further studies are now in progress to address this issue.

In summary, our data indicate that the effect of dexamethasone on actin assembly is transient, rapid in onset, and short in duration. It does not involve actin synthesis. These results strengthen the hypothesis that some cellular events following glucocorticoid action do not involve gene transcription and that the rapid effect of dexamethasone on the polymerization of actin falls squarely in this category. Moreover, they are compatible with the recently proposed dual model for steroid action, which addresses both the rapid nongenomic and the genomic-mediated effects [Wehling, 1994; Koukouritaki et al., 1996]. Finally, they support the hypothesis that the polymerization dynamics of actin cytoskeleton represent a fast and flexible system that could act as a sensor as well as a mediator of steroid signals that regulate cellular functions.

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REFERENCES

Annis D, Bader M-F (1988): The cytoskeleton as a barrier to exocytosis in secretory cells. J Exp Biol 139:253–266.

Antonakis N, Markogiannakis E, Theodoropoulou M, Georgoulias V, Stournaras C, Gravanis A (1991): The antiglucocorticoid RU486 downregulates the expression of interleukin-2 receptors in normal human lymphocytes. J Steroid Biochem Mol Biol 39:929–935.

Borski RJ, Helms LMH, Harold Richman N, Gordon Grau E (1991): Cortisol rapidly reduces prolactin release and cAMP and $^{45}Ca^{2+}$ accumulation in the cichlid fish pituitary in vitro. Proc Natl Acad Sci USA 88:2758–2762.

Burridge K, Turner CE, Romer LH (1992): Tyrosine phosphorylation of paxillin and $pp125^{FAK}$ accompanies cell adhesion to extracellular matrix: A role in cytoskeletal assembly. J Cell Biol 119:893–903.

Cable HC, El-Mansoury A, Morgan NG (1995): Activation of alpha-2-adrenoceptors results in an increase in F-actin formation in HIT-T15 pancreatic B-cells. Biochem J 307: 169–174.

Cantiello HF, Stow JL, Prat AG, Ausiello DA (1991): Actin filaments regulate epithelial Na^+ channel activity. Am J Physiol 261:C882–C888.

Cantiello HF, Prat AG, Bonventre JV, Cunningham CC, Hartwig JH, Ausiello DA (1993): Actin binding protein contributes to cell volume regulatory ion channel activation in melanoma cells. J Biol Chem 268:4596–4599.

Chrzanowska-Wodnicka M, Burridge K (1994): Tyrosine phosphorylation is involved in reorganization of the actin cytoskeleton in response to serum or LPA stimulation. J Cell Sci 107:3643–3654.

Cox D, Chang P, Kurosaki T, Greenberg S (1996): Syk tyrosine kinase is required for immunoreceptor tyrosine activation motif-dependent actin assembly. J Biol Chem 271:16597–16602.

Dartsch PC, Ritter M, Häussinger D, Lang F (1994): Cytoskeletal reorganization in NIH 3T3 fibroblasts expressing the ras oncogene. Eur J Cell Biol 63:316–325.

Ding G, Franki N, Condelis J, Hays RM (1991): Vasopressin depolymerizes F-actin in toad bladder epithelial cells. Am J Physiol 260:C9–C16.

Edwardson JA, Bennett GW (1974): Modulation of corticotropin-releasing factor release from hypothalamic synaptosomes. Nature 251:425–427.

Faulstich H, Merkler I, Blackholm H, Stournaras C (1984): Nucleotide in monomeric actin regulates the reactivity of the thiol groups. Biochemistry 23:1608–1612.

Feinberg AP, Vogelstein B (1983): A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–17.

Ffrench-Mullen JMH, Danks P, Spence KT (1994): Neurosteroids modulate calcium currents in hippocampal CA1 neurons via pertussis toxin-sensitive G-protein-coupled mechanism. J Neurosci 14:1963–1977.

Gravanis A, Gurpide E (1986): Effects of estradiol on DNA polymerase a activity in a human endometrial adenocarcinoma cell line (Ishikawa cells). J Clin Endocrinol Metab 63:356–359.

Hall ED (1982): Glucocorticoid effects on central nervous excitability and synaptic transmission. Neurobiology 23: 165–195.

Hesketh JE, Pryme IF (1991): Interaction between mRNA, ribosomes and the cytoskeleton. Biochem J 277:1–10.

Hua S-Y, Chen Y-Z (1989): Membrane receptor-mediated electrophysiological effects of glucocorticoid on mammalian neurons. Endocrinology 124:687–691.

Jessen F, Hoffmann EK (1992): Activation of the $Na^+/K^+/Cl^-$ cotransport system by reorganization of the actin filaments in Ehrlich ascites tumor cells. Biochim Biophys Acta 1100:199–201.

Koukouritaki SB, Theodoropoulos PA, Margioris AN, Gravanis A, Stournaras C (1996): Dexamethasone alters rapidly actin polymerization dynamics in human endometrial cells: Evidence for nongenomic action involving cAMP turnover. J Cell Biochem 62:251–261.

Kiley SC, Parker PJ, Fabbro D, Jaken S (1992): Hormone- and phorbol ester-activated protein kinase C isozymes mediate a reorganization of the actin cytoskeleton associated with prolactin secretion on GH_4C_1 cells. Mol Endocrinol 6:120–131.

Machesky LM, Hall A (1996): Rho: A connection between membrane receptor signalling and the cytoskeleton. Trends Cell Biol 6:304–310.

Makrigiannakis A, Margioris AN, Markogiannakis E, Stournaras C, Gravanis A (1992): Steroid hormones regulate the release of immunoreactive β-endorphin from the Ishikawa human endometrial cell line. J Clin Endocrinol Metab 75:584–589.

Maniatis T, Fritsch EF, Sambrook J (1989): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Mils JW, Schwiebert EM, Stanton BA (1994): The cytoskeleton and membrane transport. Current Op Nephr Hyper 3:529–534.

Mils JW, Mandel LJ (1994): Cytoskeleton regulation of membrane transport events. FASEB J 8:1161–1165.

Papakonstanti EA, Emmanouel DS, Gravanis A, Stournaras C (1996): Na^+/Pi co-transport alters rapidly cytoskeletal protein polymerization dynamics in opossum kidney cells. Biochem J 315:241–247.

Rao KMK, Betschart JM, Virji MA (1985): Hormone-induced actin polymerization in rat hepatoma cells and human leucocytes. Biochem J 230:709–714.

Reuner KH, Wiederhold M, Dunker P, Just I, Bohle RM, Kröger M, Katz N (1995): Autoregulation of actin synthesis in hepatocytes by transcriptional and posttranscriptional mechanisms. Eur J Biochem 230:32–37.

Reuner KH, Dunker P, van der Does A, Wiederhold M, Just I, Aktories K, Katz N (1996): Regulation of actin synthesis in rat hepatocytes by cytoskeletal rearrangements. Eur J Cell Biol 69:189–196.

Sheehan B, Fuller SD, Pique ME, Yeager M (1996): AVS software for visualization in molecular microscopy. J Struct Biol 116:99–106.

Simon H, Gao Y, Franki N, Hays RM (1993): Vasopressin depolymerizes apical F-actin in rat inner medullary collecting duct. Am J Physiol 265:C757–C762.

Steiner A, Vogt E, Locher R, Vetter W (1988): Stimulation of the phosphoinisitide signalling system as a possible mechanism for glucocorticoid action in blood pressure control. J Hypertens 6:S366–S368.

Stournaras C, Theodoropoulos PA, Stoll B, Häussinger D (1994): Altered cytoskeletal protein function and gene expression during cell volume changes in rat hepatocytes. J Hepatol 21:19.

Stournaras C, Stiakaki E, Koukouritaki SB, Theodoropoulos PA, Kalmanti M, Fostinis Y, Gravanis A (1996): Altered actin polymerization dynamics in various malignant cell types: Evidence for differetial sensitivity to cytochalasin B. Biochem Pharmacol 52:1339–1346.

Symons M (1996): Rho family GTPases: The cytoskeleton and beyong. Trends Biochem Sci 21:178–181.

Theodoropoulos PA, Stournaras C, Stoll B, Markogiannakis E, Lang F, Gravanis A, Häussinger D (1992): Hepatocyte swelling leads to rapid decrease of the G/total-actin ratio and increases actin mRNA levels. FEBS Lett 311:241–245.

Tidball JG, Spencer MJ (1993): PDGF stimulation induces phosphorylation of talin and cytoskeletal reorganization in skeletal muscle. J Cell Biol 123:627–635.

Wehling M (1994): Nongenomic actions of steroid hormones. TEM 5:347–353.

Wu YN, Yang YC, Wagner PD (1992): Modification of chromaffin cells with pertussis toxin or N-ethylmaleimide lowers cytoskeletal F-actin and enhances Ca^{2+}-dependent secretion. J Biol Chem 267:8396–8403.