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Colchicine, Colcemide and Cytochalasin B do not Affect Translocation of the Glucocorticoid Hormone-Receptor in Rat Thymocytes or Ehrlich Ascites Cells

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The involvement of intracellular cytoskeletal elements in the translocation of the dexamethasone-receptor complex from the cytoplasm to the nucleus was studied using the cytoskeleton-disrupting agents colcemide, colchicine and cytochalasin B. These compounds did not affect the translocation of the hormone-receptor complex. We conclude that microfilaments and microtubules do not play a role in the translocation of the glucocorticoid hormone-receptor complex from the cytoplasm to the nucleus.

KEY WORDS: glucocorticoid hormone-receptor; translocation; colcemide; colchicine; cytochalasin B; cytoskeleton.

ABBREVIATIONS: EAT-cells: Ehrlich Ascites Tumor Cells; MEM: Minimum Essential Medium.

INTRODUCTION

A prerequisite for the intranuclear action of glucocorticoids is the translocation of the hormone-receptor complex which is assembled in the cytoplasm into the nucleus (1). Although much has been learned during the last few years on various aspects of the cellular and molecular action of glucocorticoids, practically nothing is known about the translocation process. The mechanism of intracellular transport of micro- and macromolecules is now receiving due attention. Implicated in these processes, among others, has been the cytoskeletal network present in eukaryotic cells, to which the microtubules, actomyosin and the intermediate filaments contribute (2, 3). In a first attempt to assess the role of cytoskeletal structures in glucocorticoid receptor translocation, we have followed the effects of

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colchicine, its analogue colcemide, and cytochalasin B, which are agents known to affect microtubules (4, 5) and actin filaments (6, 7), respectively, on the translocation of the dexamethasone-receptor complex from the cytoplasm to the nucleus in rat thymocytes and Ehrlich ascites tumor (EAT) cells.

MATERIALS AND METHODS

Cells

Thymi were excised from 3 month old rats, cleaned of adhering blood clots and washed in cold Tris-saline buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl). The thymi were teased apart with forceps and the freed thymocytes taken up in the same buffer and filtered through three layers of gauze. The cells were washed twice by centrifugation at $800 \times g_{av}$ and 0°C for 5 min and finally resuspended in minimum essential medium (MEM) at a density of 4×10^6 cells/ml. EAT cells were propagated in suspension in 500 ml aliquots of MEM containing 5% fetal calf serum to a density of 10^6 cells/ml. Before use, the cells were centrifuged at $800 \times g_{av}$ and 37°C for 5 min and resuspended in 125 ml of MEM. The wash was repeated once more.

Incubation of Cells with Agents and [^{3}H]Dexamethasone

25 ml of the cell suspension (4×10^6 cells/ml) were incubated at 37°C for 1 h in the presence of 5×10^{-6} M of colcemide, colchicine and cytochalasin B, respectively, all of which were obtained from Sigma (München, FRG). Subsequently, [^{3}H]dexamethasone (50 Ci/mmol, NEN, Boston, MA, USA) was added at a concentration of 5×10^{-8} M, and the cells were further incubated at 37°C for 30–60 min. 2 ml aliquots of the cell suspension were removed at 0, 5, 15 and 30 min and in some experiments after 60 min.

Assay of [^{3}H]Dexamethasone-receptor Complex in the Cytosol and Nuclear Fraction

After addition of 10 ml of cold Tris-saline to the 2 ml aliquots, the cells were centrifuged at $1000 \times g_{av}$ and 0°C for 5 min. The cell pellet was resuspended in 2 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EGTA, 10 mM KCl, 6 mM β-mercaptoethanol) and left at 0°C for 5 min. The suspension was then homogenized with 10 strokes in a Dounce homogenizer and mixed with 0.22 ml of TNM buffer (10 mM Tris-HCl, pH 8.0, 1 mM EGTA, 100 mM NaCl, 30 mM $MgCl_2$, 6 mM β-mercaptoethanol).

The homogenate was centrifuged for 30 sec at $1000 \times g_{av}$ and the resulting pellet washed four times with 2 ml of TNM buffer. The washed pellet was incubated for 30 min with 0.5 ml of Soluene-350 (Packard Instruments Ltd, Inc., Downers Grove, ILL, USA) at 60°C and, after the addition of 10 ml of scintillation cocktail (0.005% w/v POPOP and 0.416% w/v PPO in toluene), counted in a Packard Tri-carb 460 CD liquid scintillation spectrometer.

The remaining supernatant was centrifuged at $35{,}000 \times g_{av}$ for 60 min (cytoplasm) and subjected to dextran-coated charcoal (DCC) treatment (8). 0.3–0.5 ml aliquots of the DCC-treated supernatant were mixed with 10 ml of scintillation cocktail and counted.

RESULTS AND DISCUSSION

In thymocytes most of the glucocorticoid receptor (9) is present in the cytoplasm. Upon incubation with glucocorticoids, the receptor translocates into the nucleus (10). In the present experiments, thymocytes were incubated in the presence of various cytoskeleton-disrupting agents for up to one hour at 37°C, a period sufficient to obtain effective intracellular concentrations of these drugs. After additional incubation in the presence of [^{3}H]dexamethasone, the [^{3}H]dexamethasone-receptor complex formed was quantitated in the cytosol and in the nucleus.

In control cells, the [^{3}H]dexamethasone-receptor complex was rapidly formed (Fig. 1). Time zero denotes cells to which [^{3}H]dexamethasone was added and the cells were then immediately centrifuged and subsequently lysed. Even during this period, detectable [^{3}H]dexamethasone-receptor complex was produced. Within 15 min of incubation of the cells with [^{3}H]dexamethasone, the amount of [^{3}H]dexamethasone-receptor complex formed reached a plateau due to

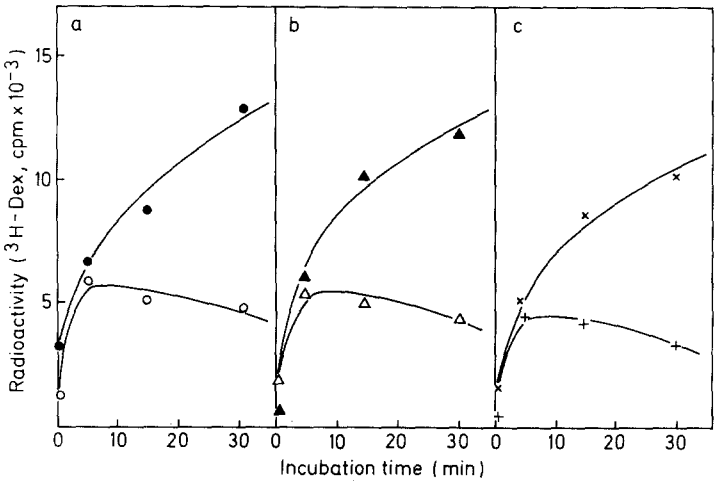

Fig. 1. Effect of the cytoskeleton-disrupting agents colchicine and cytochalasin B on the translocation of [^{3}H]dexamethasone-receptor complex from the cytoplasm to the nucleus in rat thymocytes. (a) Control: macromolecular-bound radioactivity found in the cytoplasmic fraction (○); radioactivity in the nuclear fraction (●). (b) Colchicine-treated cells: macromolecular-bound radioactivity found in the cytoplasmic fraction (△); radioactivity in the nuclear fraction (▲). (c) Cytochalasin B-treated cells: macromolecular-bound radioactivity found in the cytoplasmic fraction (+); radioactivity in the nuclear fraction (×). For experimental details see Materials and Methods.

continuous translocation of the complex into the nucleus. Translocation continued for 30–60 min (Figs. 1, 2). No measurements were taken after this time period.

Cells incubated in the presence of colchicine, colcemide or cytochalasin B did not differ either in their capacity to form the [^{3}H]dexamethasone-receptor complex in the cytosol nor in their ability to translocate the complex to the nucleus (Fig. 1). It is well known that colchicine and colcemide, at the concentrations applied, bind tightly to tubulin dimers preventing tubulin polymerization and, due to the dynamics of microtubules, cause microtubule depolymerization and disruption (4–7). Cytochalasin B, on the other hand, binds to microfilament ends thus inhibiting the addition of actin molecules and, due to the dynamic equilibrium existing between actin filaments and free actin molecules, causes depolymerization of the filaments.

Although the thymocytes' morphology was affected by the agents (in phase contrast microscopy the cytoplasm of the cells had a granular appearance), in the presence of cytochalasin B translocation of the [^{3}H]dexamethasone-receptor complex continued as in control cells. These results exclude the possibility that the microtubular and actin systems contribute to receptor translocation.

We repeated the above experiments using EAT cells which possess cytoplasmic glucocorticoid receptors as shown by us in preliminary experiments. The agents tested showed no effect on glucocorticoid receptor translocation. Figure 2 depicts the effect of colcemide on receptor translocation in EAT cells.

Unfortunately, there is no agent which directly interacts with the intermediate filaments and could be used to explore their role in receptor translocation, as

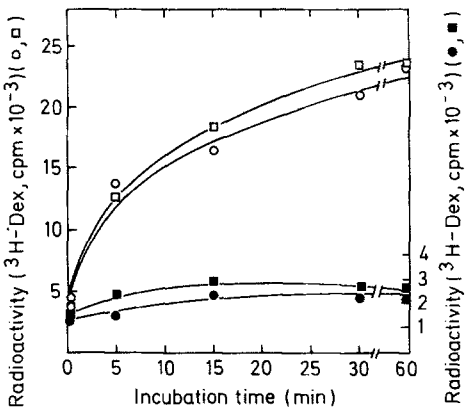

Fig. 2. Effect of the cytoskeleton-disrupting agent colcemide on the translocation of [^{3}H]dexamethasone-receptor complex from the cytoplasm to the nucleus in EAT cells. Macromolecular-bound radioactivity found in the cytoplasm (●) and in the nucleus (○) of control EAT cells; macromolecular-bound radioactivity found in the cytoplasm (■) and in the nucleus (□) of colcemide-treated EAT cells. For experimental details see Materials and Methods.

is the case for the other two cytoskeletal structures. One possibility which could be considered in this respect is the use of antibodies directed against intermediate filament proteins; this, however, would necessitate another type of experimental approach (11). A further approach could be to use vanadate (12), cycloheximide, diphtheria toxin or exotoxin A, which are all drugs known to alter vimentin filament organization (13); although the inhibition of protein synthesis caused by these agents would probably complicate the interpretation of the results eventually obtained. Perhaps longer incubation experiments with colchicine, which is known to lead not only to microtubule destruction but also to damage of the intermediate filament network, could assist in assessing the role of intermediate filaments in the translocation process.

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