

## CYTOCHALASIN B

### Effect on Hormone-Mediated Responses in Cultured Cells

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#### INTRODUCTION

Cytochalasin B, a mold metabolite, has been shown to inhibit cytokinesis and motility (3), morphogenic development (18), endocytosis (5), and the transport of sugars (8–10, 12). It has also been demonstrated that this drug inhibits the stimulated elaboration of extracellular substances from glands—the release of thyroxine from the thyroid gland after stimulation by thyroid-stimulating hormone (TSH) (19), and the release of  $\alpha$ -amylase from the parotid gland after stimulation by epinephrine (1).

To date, the effects of cytochalasin B on other aspects of hormone-mediated responses have not been reported. Therefore, studies were initiated to

study the effects of the drug on another hormone-modified system—the induction of tyrosine aminotransferase (E.C. 2.6.1.5) by insulin, cortisol, or N<sup>6</sup>-2'-O-dibutyryl adenosine-3'-5'-cyclic monophosphate. These studies were carried out with Reuber hepatoma (H-35) cells grown in culture.

#### MATERIALS AND METHODS

Reuber hepatoma (H-4-II-E, also referred to as H-35) cells were cultured in Swim's 77 modified medium as a nonconfluent monolayer according to the previously published procedure (2). The H-35 cells were used in these experiments when they were in a mid-log phase of growth. The cells were incubated with insulin (0.5  $\mu$ g/ml), cortisol (50  $\mu$ M final concentration), or dibutyryl cyclic AMP (0.5 mM

final concentration), dissolved in either Swim's 77 or 0.16 M NaCl for 6 or 4 h before their harvest and the preparation of cell extracts (2).

In all experiments, the H-35 cells were incubated with dimethyl sulfoxide (DMSO) or cytochalasin B, dissolved in a corresponding amount of DMSO, for 30 min at 37°C in Swim's 77 medium before the addition of the hormones or cyclic nucleotide.

Tyrosine aminotransferase (TAT) was assayed by the procedure of Diamondstone (6). 1 U of TAT is equivalent to the formation of 1  $\mu$ mole of *p*-hydroxyphenylpyruvate per hour by 1 mg of extract protein. Protein was determined by the procedure of Lowry et al. (11).

Cytochalasin B was a gift from Dr. S. B. Carter of the Imperial Chemical Co. (Los Angeles, Calif.) and was made up to 5 mg/ml in dimethyl sulfoxide (DMSO). Insulin was donated by Dr. W. R. Kirtley of the Eli Lilly & Co., Indianapolis, Ind. Cortisol was from the Upjohn Co., Kalamazoo, Mich. N<sup>6</sup>-2'-O-dibutyryl adenosine-3'-5'-cyclic monophosphate was purchased from Schwarz/Mann, Orangeburg, N. Y. The [<sup>14</sup>C]cortisol and [<sup>3</sup>H]dibutyryl cyclic AMP were purchased from the New England Nuclear Corp.

(Boston, Mass.). [<sup>125</sup>I]insulin was obtained Amersham/Searle Corp., Arlington Heights, Ill.

## RESULTS AND DISCUSSION

### Induction of Tyrosine Aminotransferase

Cytochalasin B (50  $\mu$ g/ml of culture medium) completely inhibited the induction of TAT by either insulin or cortisol (Table I). At a concentration of 10  $\mu$ g/ml, the drug inhibited the hormone-induced effect to a small but significant extent. At a concentration of 1.0  $\mu$ g/ml, cytochalasin B was without effect on the hormone-inducible levels of TAT.

Dibutyryl cyclic AMP, at a concentration of 500  $\mu$ M, induced the formation of TAT (Table I). However, unlike its inhibition of the insulin and cortisol induction of TAT, cytochalasin B had no effect on the cyclic AMP inducing system.

The synthesis of new enzyme is implicit in the hormone and cyclic AMP induction of tyrosine aminotransferase. However, since we have found a

TABLE I  
The Effect of Cytochalasin B on the Induction of Tyrosine Aminotransferase by Insulin, Cortisol, or Dibutyryl Cyclic AMP in Reuber H-35 Hepatoma Cells

Additions	Untreated	Units of tyrosine aminotransferase		
		Insulin	Cortisol	DBC AMP
Experiment 1				
None	1.29 $\pm$ 0.1	3.31 $\pm$ 0.07	4.85 $\pm$ 0.10	—
DMSO (1.0%)	1.35 $\pm$ 0.07	3.45 $\pm$ 0.11	4.70 $\pm$ 0.30	—
Cytochalasin B (50 $\mu$ g/ml)	1.23 $\pm$ 0.07	1.55 $\pm$ 0.09	1.42 $\pm$ 0.15	—
Experiment 2				
DMSO (0.5%)	1.51 $\pm$ 0.01	3.15 $\pm$ 0.25	3.25 $\pm$ 0.05	—
Cytochalasin B (1 $\mu$ g/ml)	1.40 $\pm$ 0.12	3.00 $\pm$ 0.30	3.30 $\pm$ 0.10	—
Cytochalasin B (10 $\mu$ g/ml)	1.35 $\pm$ 0.06	2.20 $\pm$ 0.01	2.60 $\pm$ 0.10	—
Experiment 3				
DMSO (0.4%)	1.40 $\pm$ 0.03	3.03 $\pm$ 0.02	—	2.40 $\pm$ 0.01
Cytochalasin B (10 $\mu$ g/ml)	1.31 $\pm$ 0.03	2.33 $\pm$ 0.06	—	2.36 $\pm$ 0.06
Cytochalasin B (20 $\mu$ g/ml)	1.30 $\pm$ 0.02	2.02 $\pm$ 0.02	—	2.72 $\pm$ 0.04

H-35 hepatoma cells were incubated for 30 min at 37°C in Swim's 77 medium containing DMSO, 0.4–1.0% (vol/vol), and varying concentrations of cytochalasin B dissolved in a corresponding amount of DMSO. At the end of the preincubation period, insulin at 0.5  $\mu$ g/ml, cortisol to a concentration of 50  $\mu$ M, or dibutyryl cyclic AMP (DBC-AMP) to a concentration of 0.5 mM was added, and the hepatoma cells were incubated for 6 h (experiment 1) or 4 h (experiments 2 and 3). After incubation, the plates were rinsed with Swim's 77 medium, and a cell extract was prepared. The results are the mean  $\pm$  SE of three to four plates per point.

TABLE II  
*The Effect of Cytochalasin B on the Binding and Uptake of Insulin, Cortisol, and Dibutyryl Cyclic AMP by Reuber H-35 Hepatoma Cells*

Additions	Quantity of bound hormone or dibutyryl cyclic AMP		
	Insulin (ng/mg protein)	Cortisol (nmoles/plate)	DBC-AMP (nmoles/plate)
Experiment 1			
None	1.48 ± 0.13	—	—
DMSO (0.2%)	1.30 ± 0.10	—	—
Cytochalasin B (1 µg/ml)	1.44 ± 0.19	—	—
Cytochalasin B (10 µg/ml)	1.30 ± 0.05	—	—
Experiment 2			
DMSO (0.5%)	—	1.2 ± 0.1	4.4 ± 0.1
Cytochalasin B (20 µg/ml)	—	1.3 ± 0.1	4.4 ± 0.1

H-35 cells were incubated for 30 min at 37°C in 2 ml of Swim's 77 medium containing cytochalasin B, 1–20 µg/ml, and a corresponding quantity of DMSO. The pretreated plates were incubated with radiolabeled hormone or dibutyryl cyclic AMP (DBC-AMP) for designated periods of time: [<sup>125</sup>I]insulin (1 µg, sp act 36 nCi/µg) for 3.5 h, a time at which the cell-associated radioactivity was at equilibrium; [<sup>14</sup>C]cortisol (final concentration, 50 µM and 25 nCi) for 1 h, a time at which the cell-associated radioactivity was at equilibrium; and [<sup>3</sup>H]dibutyryl cyclic AMP (final concentration, 0.5 mM and 0.75 µCi) for 1 h. The uptake of cyclic AMP was linear out to 3 h. After incubation, the plates were washed in several volumes of 0.154 M NaCl, the cells digested in 0.2 N NaOH, and portions taken for the determination of protein and for the determination of radioactivity. The results are expressed as the mean ± SE of four plates.

differential inhibition by cytochalasin B on the induction of this enzyme, it would seem unlikely that the failure to induce the formation of enzyme is the result of an inhibition of protein synthesis. In support of this conclusion, it was found that incubation of H-35 cells for 3.5 h with cytochalasin B at a concentration (20 µg/ml, experiment 3 in Table I) that produced a significant inhibition of enzyme induction did not inhibit the incorporation of a 30 min pulse of radiolabeled leucine (data not presented). This observation is in agreement with those of others (7, 15) using different cell lines, but does not agree with the data of Springer and Perdue<sup>1</sup>, who found a 20% decrease in amino acid incorporation during a 1 h incubation with the drug in cultured chick embryo fibroblasts. Another possible explanation for the effect of cytochalasin B on hormone versus cyclic AMP induction of tyrosine aminotransferase could be through a differential uptake or binding of insulin or cortisol compared with that of the nucleotide.

#### *Binding and Uptake of Insulin, Cortisol, and Cyclic AMP*

Cytochalasin B, at concentrations of 1–20 µg/ml, did not inhibit the binding and subsequent uptake

<sup>1</sup> Springer, A., and J. F. Perdue. Manuscript submitted for publication.

of insulin, cortisol, or cyclic AMP (Table II). The cells incubated in dimethyl sulfoxide had levels of hormone binding which were comparable to that observed for untreated cells (Table II). No attempt was made to determine whether the radioactivity taken up was still in the form of the parent compound.

The active transport of sugars across the cell membrane represents one plasma membrane-associated system which is very sensitive to environmental conditions, such as pH, serum levels, and the degree of cell confluency (4, 14). This system is also sensitive to cytochalasin B. Kletzien et al. (9) and Kletzien and Perdue (10) found that at a concentration of 70 ng/ml, 50 percent of 2-deoxy-D-glucose uptake was inhibited. At this concentration of metabolite, there were no detectable changes in cell shape.

It may be inferred from this work (9, 10) that minor perturbations of the membrane-cell cortex regions brought about by local depolymerization of microfilaments or microtubules could influence plasma membrane functions. In this regard, Ukena and Berlin (16) have found that treating polymorphonuclear leukocytes with colchicine or vinblastine, drugs which depolymerize microtubules, disrupted the geographically separated plasma membrane functions of phagocytosis and adenine and lysine transport. However, the hy-

pothesis of drug-initiated depolymerizations of elements of the cytoskeleton influencing cell membrane functions cannot be generalized for all cell processes. In Chang liver cells, cytochalasin B altered their shape but it had no inhibiting effect on the basal or fluoride or isoproterenol stimulated levels of adenylyl cyclase (data not presented). Furthermore, cytochalasin B, although a potent and competitive inhibitor of sugar transport, was only weakly inhibitory toward the uptake of amino acids and thymidine (12)<sup>1</sup> or of inorganic phosphate<sup>2</sup>, and had no effect on choline transport (8).

One explanation for the differential cytochalasin B inhibition of tyrosine aminotransferase induction by the hormones versus cyclic AMP takes into account the role of microfilaments and microtubules in maintaining cell membrane architecture and cell shape. Puck et al. (13) have shown that treating Chinese hamster ovary cells with cytochalasin B or Colcemid resulted in violent extensions and retractions of the cell surface. The surface membrane effects were reversed by dibutyl cyclic 3'-5'-AMP; the plasma membrane became smooth and tranquil. The cells assumed a fusiform-like morphology with longer periods of culture in the presence of the cyclic nucleotide. The shapes of Reuber H-35 cells are also modified by cyclic nucleotides. Van Wijk et al. (17) have shown that the dibutyl and two 8-thio derivatives of cyclic 3'-5'-AMP inhibited DNA synthesis and the rate of growth of the H-35 cells. During this treatment, the cells became larger and developed long narrow processes; these effects were reversed with the removal of the inhibitors. The shapes of the cultured Reuber hepatoma cells are also altered by cytochalasin B. The treated cells are round with a few narrow processes that remain attached to the culture dish.

It is conceivable that cytochalasin B prevents the induction of tyrosine aminotransferase by the hormones through a mechanism of microfilament depolymerization with concomitant membrane perturbations and rearrangements. Cyclic AMP promotes the assembly of microtubules and possibly microfilaments. As such, exogenous levels of this compound could become antagonists to the effects of cytochalasin B on depolymerization and thereby permit the nucleotide to function in its

<sup>2</sup> Warner, D., and J. F. Perdue. Unpublished observations.

other roles, including the induction of tyrosine aminotransferase.

#### SUMMARY

Cytochalasin B inhibited the induction of tyrosine aminotransferase (E.C. 2.6.1.5) by insulin and cortisol in Reuber H-35 hepatoma cells. The induction of this enzyme by dibutyl cyclic AMP was not inhibited by cytochalasin B.

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