THE INFLUENCE OF CYTOCHALASIN B ON THE RESPONSE OF ADRENAL TUMOR CELLS TO ACTH AND CYCLIC AMP

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SUMMARY Cytochalasin B inhibits increase in steroid synthesis by mouse adrenal tumor cells (Y-1), produced either by ACTH or cyclic AMP. Basal levels of steroid synthesis are not decreased and the inhibitor acts by decreasing the response of the side-chain cleavage step (cholesterol → pregnenolone) to ACTH. Inhibition is reversible and is seen in medium without glucose. These observations suggest that microfilaments may play a role in the response of adrenal cells to ACTH.

The conversion of cholesterol to secreted steroids begins in mitochondria with conversion of this substrate to pregnenolone* (side-chain cleavage). Cholesterol must reach the mitochondrial side-chain cleavage enzyme from the cytoplasm. Nothing is known of the mechanism by which the intracellular transport of this water-insoluble steroid is brought about (1), although a role for microfilaments and microtubules in this process would be consistent with what is known about the functions of these organelles (2,3). It has been suggested that ACTH may accelerate steroidogenesis by promoting transport of cholesterol to the enzyme system (4-6). The following studies were designed to examine the possible role of microfilaments in the response to ACTH and cyclic AMP in adrenal tumor cells using cytochalasin B which is known to inhibit activity of microfilaments (3).

*pregnenolone: pregn-5-en-3β-ol-20-one

METHODS AND MATERIALS

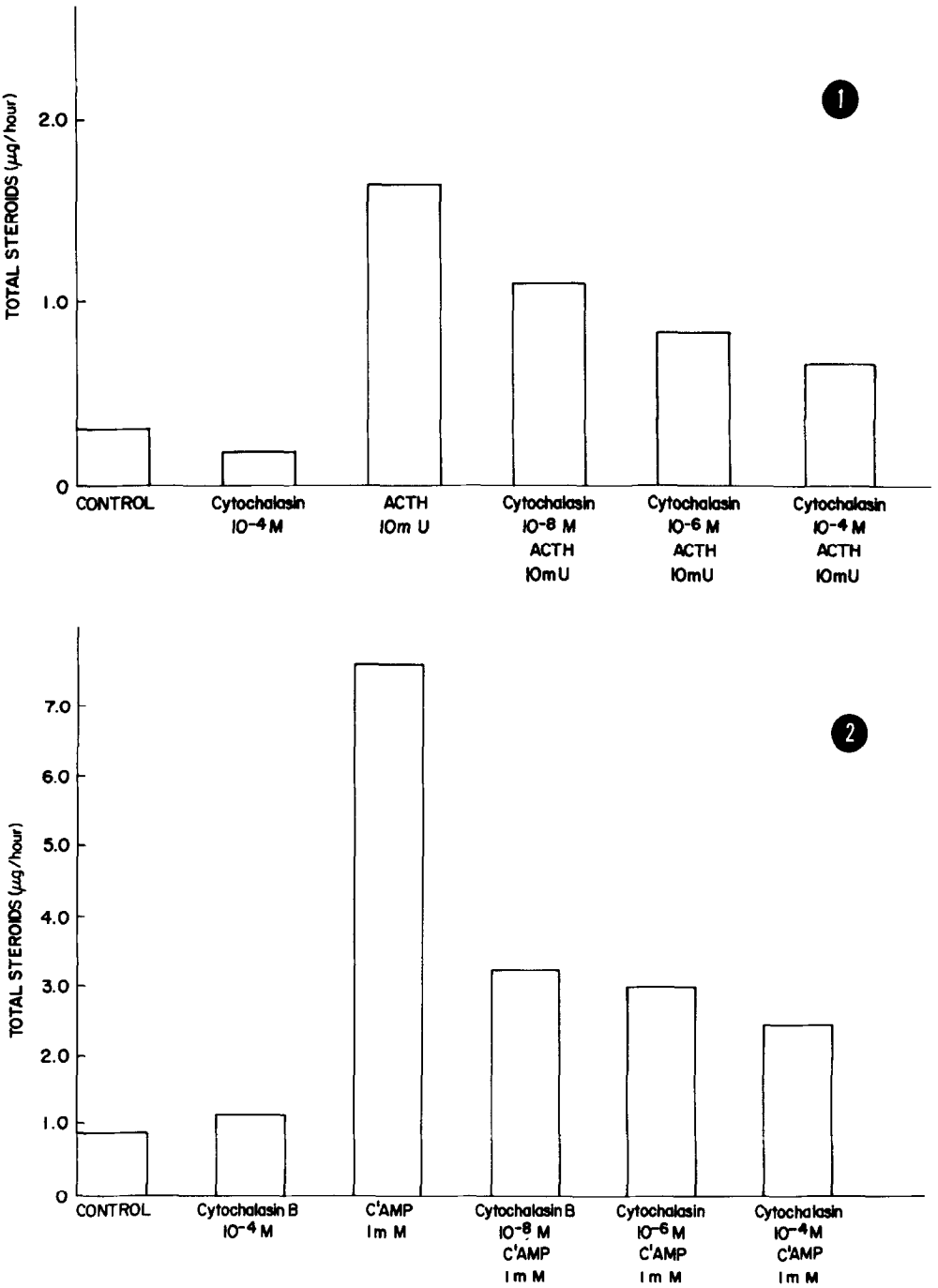
Monolayers of mouse adrenocortical tumor cells (Y-1) obtained from American Type Culture were grown in plastic dishes at 37° to confluence. During these experiments Eagle's minimal essential medium with Earle's salts and no serum was used. At half-hourly intervals medium was removed, cells washed once with normal saline and fresh medium added; incubation was continued at 37°. Inhibitors, hormones and cyclic AMP were added to large batches of medium from which the incubation medium for the various plates was taken; media were kept at 37° prior to use. Cytochalasin B was dissolved in dimethylsulfoxide:saline (1:1); an equal volume of this solvent was added to flasks not containing cytochalasin. The batches of medium removed from cells at each change were extracted twice with ether and total steroid content measured fluorimetrically (7).

Cytochalasin B was obtained from Serva Feinbiochemica Heidelberg, Germany and ACTH and Cyclic AMP from Sigma Chemical Company.

RESULTS

No effect of cytochalasin B (10^{-4}M) was observed upon the unstimulated production of steroids by the tumor cells (Figures 1 and 2); in 6 similar experiments cytochalasin B was without significant effect on the basal synthesis of steroids. ACTH added to the medium stimulated steroid production in confirmation of findings by other investigators (8). The response to ACTH was inhibited by cytochalasin B; this effect was rapidly and completely reversed when cells were washed and fresh medium containing ACTH but no cytochalasin, was added after previous incubation with both substances (Table). After washout it will be seen that production of steroids returns to levels comparable to those observed with ACTH alone, in contrast to the plates in which cytochalasin was present with ACTH after medium was changed. The same qualitative effect of cytochalasin was observed when synthesis of steroids was stimulated by cyclic AMP (Figure 2).

Inhibition of stimulation by ACTH was observed at low concentrations of cytochalasin B (10^{-8}M). Moreover inhibition by cytochalasin of the response to cyclic AMP was observed in the absence of glucose in the incubation medium, as follows: inhibition of maximal response to cyclic AMP was 60% (glucose present) and



Figures 1 and 2. Production of steroids by mouse adrenal tumor cells. Cells were preincubated in medium for 30 (Figure 1) or 60 minutes (Figure 2); the buffer was then changed and fresh buffer containing the additions shown was added. Incubation was continued for 30 minutes, the reaction was stopped and the steroid content of the medium was measured. The bars represent means of duplicate determinations.

TABLE

Total Steroids (μg/hour)

	1st Incubation	2nd Incubation
Control	0.35 ± 0.15	0.30 ± 0.05
Cytochalasin B	0.35 ± 0.05	0.25 ± 0.05
ACTH	2.70 ± 0.40	1.20 ± 0.10
ACTH + Cytochalasin B	1.60 ± 0.30	0.70 ± 0.30
ACTH + Cytochalasin B	1.55 ± 0.15	*1.25 ± 0.15

Incubations of Y-1 cells were performed as shown (1st incubation) and after 1 hour medium was removed and steroid content was determined. Incubation was continued in fresh medium (2nd incubation) of the same composition as before except that in one case (indicated by *) fresh medium included ACTH and no cytochalasin B.

45% (glucose absent). Finally, we have found that cytochalasin B (10^{-7}-10^{-5}M) does not inhibit side-chain cleavage catalysed by purified cytochrome P-450 (data not shown).

DISCUSSION

Cytochalasin B inhibits the steroidogenic responses to both ACTH and cyclic AMP so that it presumably acts beyond the synthesis of the nucleotide second messenger. It will be shown elsewhere that cytochalasin is without effect on the conversion of pregnenolone to secreted steroids and the present studies show that it does not inhibit purified side-chain cleavage P-450. Moreover the inhibitor does not reduce basal or unstimulated production of steroids so that its effect is confined to the stimulating influences of ACTH and cyclic AMP. Since stimulation by these agents is believed to result from increased side-chain cleavage

(cholesterol → pregnenolone) (9,10), it is likely that this step is specifically involved in inhibition by cytochalasin.

It has been proposed that the rate-limiting step in side-chain cleavage may involve movement of cholesterol to the mitochondrial enzyme system (4-6). Since cytochalasin does not affect the purified side-chain cleavage enzyme, it is reasonable to suggest that the inhibitor acts on this movement of cholesterol and hence that ACTH and cyclic AMP accelerate movement of extramitochondrial cholesterol to the side-chain cleavage enzyme system.

The actions of cytochalasin on various cells include decreased microfilament activity and inhibition of glucose transport (11). Cytochalasin inhibits the response of tumor cells to cyclic AMP in medium without glucose (Results). Moreover, the concentration of glucose in the medium used in these studies (5mM) is sufficient to overcome competitive inhibition of glucose transport by cytochalasin (12). This observation is consistent with results, by other workers, showing that the effect of cytochalasin on cell motility is not the result of decreased glucose transport (12-15). Incidentally, in keeping with experience in other cells, the effect of cytochalasin was readily reversed by washing the tumor cells (12). These observations are most readily explained by proposing that cytochalasin acts on the adrenal cells by inhibiting microfilaments and that ACTH and cyclic AMP act by stimulating the activity of these organelles.

The failure of cytochalasin to inhibit unstimulated production of steroids may be attributable to the presence of sufficient cholesterol in mitochondria for basal but not for stimulated levels of steroid production. This appears to be the case for beef adrenocortical mitochondria (16). The possible role of microfilaments in the mechanism of action of ACTH is under investigation in this laboratory.

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