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The Effects of Taxol, a Microtubule-Stabilizing Drug, on Steroidogenic Cells

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The effects of taxol on steroid production and microtubule polymerization were examined using Y-1 adrenocortical tumor cells, MLTC-1 Leydig tumor cells, and primary cultures of bovine adrenocortical cells. Taxol inhibited the following steroidogenic processes within the Y-1 and MLTC-1 cells: (1) hormonal increase of steroid production, (2) dibutyryl cyclic AMP-increased steroid production, and (3) hormone-stimulated pregnenolone production. The inhibitory action of taxol was concentration dependent and also resulted in an increase in cytoplasmic microtubules. In addition, the inhibitory action of taxol on hormone-stimulated steroid production was reversible. Taxol appeared to inhibit cholesterol movement to the mitochondrial site of cholesterol side-chain cleavage enzyme but did not affect overall protein synthesis. Interestingly, taxol did not affect hormone-stimulated steroid production in bovine adrenocortical cells. This lack of inhibition may correspond to the ultrastructural observation that microtubule bundling after taxol treatment was observed in the tumor cells but not in similarly treated bovine adrenal cells. With this conflicting information between cell types, a direct relationship between taxol treatment and inhibition of steroid production has not been established. However, these results suggest that taxol alters the rate of transport of cholesterol to the cholesterol side-chain cleavage enzyme within the steroidogenic tumor cells.

The pathways of steroidogenesis within adrenal cortical cells and Leydig cells of the testis are well established. Using cell culture model systems, steroid production may be stimulated by trophic hormones, cholera toxin, or cAMP (Buonassisi et al., 1962; Yasumura et al., 1966; Wolff et al., 1973; Ascoli and Pruett, 1978). The first step of steroid biosynthesis involves cholesterol conversion to pregnenolone within the mitochondria. This reaction is the rate-limiting step in steroidogenesis and is under hormonal control (Stone and Hechter, 1954; Simpson, 1979). Although it is generally acknowledged that a primary action of pituitary hormonal stimulation is to promote the association of cholesterol with cytochrome P-450 side-chain cleavage (Simpson, 1979), additional effects on the mobilization and redistribution of intracellular cholesterol contribute to the action of hormone stimulation on steroidogenic cells. For example, lipoprotein-cholesterol uptake by adrenocortical cells (Brown et al., 1979; Faust et al., 1977) and the rate of hydrolysis of cholesterol esters (Trzeciak and Boyd, 1974) are increased in response to ACTH. In addition, ACTH promotes the uptake of free cholesterol by the mitochondria (Mahaffee et al., 1974; Crivello and Jefcoate, 1978). Thus, the maintenance of steroid biosynthesis in response to hormonal stimulation requires the continued transfer of intracellular cholesterol to the mitochondria for conversion to pregnenolone.

Several investigators have suggested that microtubules and microfilaments are involved in the movement of cholesterol within steroidogenic cells (Temple and Wolfe, 1973; Mrotek and Hall, 1977; Cortese and Wolff, 1978; Crivello and Jefcoate, 1978; Hall et al., 1979). This evidence has been obtained by examining the effects of drugs which specifically disrupt microfilaments or microtubules. The cytochalasins which affect microfilaments (Tannenbaum, 1978) inhibit hormone-stimulated cholesterol transport in culture Y-1 adrenal cells (Mrotek and Hall, 1977; Hall et al., 1979) but have no effect on steroid production in bovine adrenal cells (Rainey et al., 1984). Colchicine, podophyllotoxin, and vinblastine, which lead to depolymerization of microtubules (Dustin, 1978), stimulate cholesterol transport in Y-1 adrenal tumor cells (Temple and Wolff, 1973), and I-10 Leydig tumor cells (Temple and Wolff, 1973). In addition, the microtubule-disrupting drugs stimulate corticosteroid release by cultured rat adrenal cells (Ray and Strott, 1978). In order to determine if microtubules are involved in steroidogenesis, the previously published studies have used agents that lead to microtubule depolymerization. The ability to act in the opposite manner, i.e., to promote

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or stimulate microtubule polymerization, has recently been made possible by use of taxol. Taxol is a plant alkaloid (Wani et al., 1971) which has proven beneficial in the study of tubulin assembly in vitro (Schiff et al., 1979., Thompson et al., 1981). Though a promoter of microtubule assembly (Schiff and Horwitz, 1980; DeBrabander et al., 1981), taxol inhibits microtubule actions within cells (Schiff and Horwitz, 1980; DeBrabander et al., 1981), suggesting that taxol-induced microtubules may not be functional.

In the present study we report the effects of taxol on steroidogenesis in Y-1 adrenal tumor cells, MLTC-1 Leydig tumor cells, and primary cultures of bovine adrenal cells. In both Y-1 and MLTC-1 culture systems, taxol inhibited steroidogenesis at the rate-limiting step while stimulating microtubule assembly within the cytoplasm. However, no inhibition was observed in bovine adrenal cell steroid production. These results suggest that microtubules or tubulin may be important for steroid production in the tumor cell systems examined but question their importance in bovine adrenal cell steroidogenesis.

MATERIALS AND METHODS

Cell culture

Mouse Y-1 adrenal tumor cells were obtained from the American Type Culture Collection (Rockville, MD). Mouse Leydig tumor cells (MLTC-1) were generously supplied by Dr. V. Rebois (National Institutes of Health, Bethesda, MD). Cells were maintained in an equal mixture (v/v) of Ham's F-12 medium and Dulbecco's modified Eagle medium containing fetal calf serum (10.0%). Stock cultures were grown at 37°C on 75-cm^2 tissue culture plates (Costar, Cambridge, Ma) in a humidified atmosphere of air (5 liters/min) supplemented with carbon dioxide (0.2 liters/min). For experiments, cells were subcultured and, after 48 hours, washed and placed in fresh serum-free medium and treated with taxol and ACTH (for adrenal cells) or hCG (for Leydig cells). Taxol was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD), and was dissolved in dimethyl sulfoxide (DMSO). A similar volume of DMSO was added to all incubation media, its final concentration not exceeding 0.5% (v/v). Cortrosyn (ACTH 1–24) was obtained from Organon (West Orange, NJ). Human chorionic gonadotropin (hCG) was obtained from Sigma Chemical Co. (St. Louis, MO).

Bovine adrenocortical cells were isolated from bovine adrenal cortices by treatment with collagenase (3.3 mg $\times$ ml^{-1}, from *Clostridium histolyticus*) and DNase (0.1 mg $\times$ ml^{-1}) in Hank's balanced salt solution. (The collagenase and DNase were obtained from Boehringer Mannheim, Indianapolis, IN.) The cells were harvested, washed, and placed in growth medium composed of Ham's F-12 and Dulbecco's modified Eagle's media with fetal calf serum (10.0%), penicillin-streptomycin solution (1%) (GIBCO, Santa Clara, CA), gentamycin sulfate (0.01%), and HEPES (10 mM). The cells were grown in culture dishes (Costar, Cambridge, MA) in a humidified 5% carbon dioxide/95% air atmosphere at 37°C and maintained in culture for 4–5 days.

Steroid measurement

The total steroid content of the medium was assayed by high-performance liquid chromatography (HPLC) using a C18-μBondapak reverse-phase column (Waters Associates, Milford, MA). Steroids were extracted from the incubation media containing dexamethasone (500 ng) as an internal standard with chloroform (10 ml). The extracts were evaporated to dryness under nitrogen. Steroids were then dissolved in methanol and separated by HPLC in an isocratic system of methanol-water (60/40, v/v) flowing at 1.5 ml per min. Absorbance at 254 nm was used to detect Δ4-3-ketosteroids which were quantified by comparison of absorbance peak areas with those of authentic standards. Standards included 11β,20α-dihydroxypregn-4-en-3-one (generously provided by the Medical Research Council Steroid Reference Collection), 11β-hydroxyprogesterone, 20α-hydroxypregn-4-en-3-one, and progesterone. Steroids, except as noted, were obtained from Sigma Chemical Co. (St. Louis, MO). Pregnenolone was quantitated in the media collected from cells incubated in the presence of 15 μM trilostane (Sterling Winthrop, Inc., Rensselaer, NY) to inhibit Δ^5-3β-hydroxysteroid dehydrogenase/isomerase activity and 19 μM Su-10603 (Ciba-Geigy Inc., Summit, NJ), an inhibitor of steroid 17α-hydroxylase activity. Pregnenolone, progesterone, and corticosterone/cortisol concentrations were determined by radioimmunoassay as previously described (Mason and Robidoux, 1978; Mason et al., 1983), using commercial antisera from Pantex (Santa Monica, CA) and Radioassay Systems Laboratories (Carson, CA). Cells were dissolved in NaOH (0.5 N) and, using bovine serum albumin as a standard, total cellular protein was quantified by the method of Bradford (1976).

Microscopy

For thin-section electron microscopy, cells were grown on Lux Permanox culture dishes (La Jolla, CA), experimentally treated, washed, and fixed in a glutaraldehyde (2%) solution in Hank's balanced salt solution (Grand Island Biological Co., New York, NY). The cells were then postfixed in osmium tetroxide (1%), dehydrated in acetone, and embedded in Epon 812. Sections were made and observed using a Philips 300 transmission electron microscope.

Measurements of protein synthesis

Cells were experimentally treated for 2 hours, washed, and then placed in methionine-free media for 30 min; [^{35}S]methionine (5 μCi) was then added to the methionine-free experimental media for an additional 30 min. Incorporation was determined by measuring the radioactivity in trichloroacetic-acid-precipitable material.

RESULTS

Using mouse Leydig tumor cells (MLTC-1), mouse adrenocortical tumor cells (Y-1), and isolated bovine adrenocortical cells in cell culture, we tested taxol's effects on (1) final steroid product release, (2) Bt_2cAMP-stimulated steroid production, (3) pregnenolone synthesis, and (4) cell morphology.

Effect of taxol on steroid production

When steroids released by hCG-stimulated MLTC-1 cells were examined by HPLC, progesterone was observed to be the major product (75% of total UV-detectable steroids). For this reason, we measured progesterone

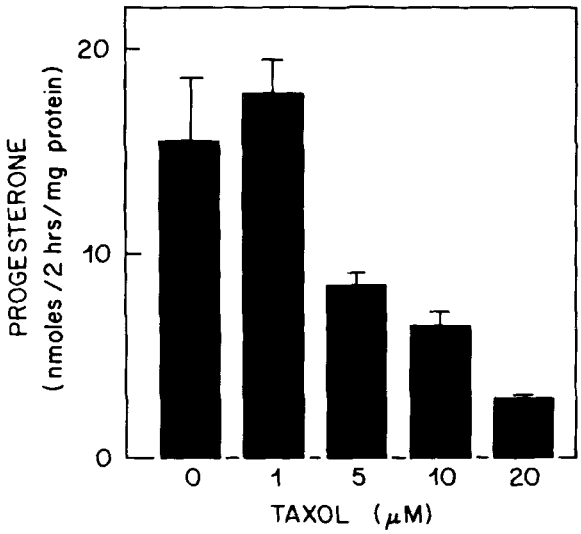

Fig. 1. Influence of taxol on hCG-stimulation of progesterone production in MLTC-1 cells. Incubations were carried out in serum-free media for 2 hours with hCG (100 μU/ml) or hCG plus varying concentrations of taxol (1–20 μM). Following the incubations, the medium was removed and progesterone assayed. Values are expressed as the mean ± the standard deviation.

TABLE 1. The effect of taxol on basal and ACTH-stimulated steroid production by Y-1 adrenal cells[1]

	pmoles Δ^4-3-ketosteroids/mg protein			
	2 hours		6 hours	
Treatment	A	B	A	B
Control	164	114	391	337
Taxol (1 μM)	133	99	340	267
Taxol (10 μM)	93	54	216	179
ACTH (1 μM)	1,120	1,262	1,670	2,273
ACTH + taxol (1 μM)	1,034	916	988	1,270
ACTH + taxol (10 μM)	601	648	847	814

[1]Cells were grown in serum-containing medium until the beginning of the experiment, when they were washed and placed in serum-free medium with no additives (control), taxol (1 μM or 10 μM), ACTH (1 μM), or a combination of ACTH plus taxol (1 or 10 μM). Incubations were carried out in replicates of four. At the indicated time the medium was removed and two replicates were pooled to yield two samples per treatment (A and B). Each sample was extracted, then separated on a C18-μBondapak column, and steroids were quantified by the absorbance at 254 nm as related to standards.

TABLE 2. The effect of taxol on corticosteroid release by primary cultures of bovine adrenal cells[1]

Treatment	nmoles corticosteroid/ mg protein/6 hours
Control	1.4 ± 0.6
Taxol (10 μM)	1.3 ± 0.1
ACTH (1 nM)	12.6 ± 1.7
ACTH (1 nM) + taxol (10 μM)	12.0 ± 1.4

[1]Bovine adrenocortical cells were isolated and cultured as described in Materials and Methods. At the beginning of the experiment cells were washed in serum-free medium followed by addition of taxol (10 μM), ACTH (1 nM), or the combination in serum-free medium. At the end of 6 hours the medium was removed and assayed for corticosterone and cortisol by radioimmunoassay. The values are expressed as the means ± the standard deviation.

by radioimmunoassay in order to determine overall steroid release after experimental treatment. Stimulation of MLTC-1 cells with hCG for 2 hours increased progesterone release from 0.4 to 15.7 nmoles/mg protein (Fig. 1). MLTC-1 cells incubated with hCG and taxol exhibited decreased progesterone release with increasing concentrations of taxol (Fig. 1). At taxol addition (20 μM), an 80% inhibition of hCG-stimulated progesterone release was observed. However, taxol did not decrease cell viability when determined by dye exclusion (Kaltenbach et al., 1958) (data not shown).

When steroid release by Y-1 adrenal cells was examined by HPLC, the major steroid found was 11β,20α-dihydroprogesterone. Because no antiserum was available for radioimmunoassay of this steroid, we used HPLC for the quantification of steroid release. The Y-1 adrenal cells responded to ACTH stimulation with a six- to tenfold increase in total UV-detectable steroid release within 2 hours. Taxol (1 μM), when added with trophic hormone, inhibited steroid release only slightly but taxol (10 μM) inhibited steroid release by 60% (Table 1). Taxol exhibited a time-dependent inhibition on steroid release as shown by the inhibition of taxol (1 μM) at 6 hours. When added alone, taxol inhibited Y-1 basal steroid release in a manner similar to taxol inhibition of ACTH-stimulated steroid release.

The inhibitory actions of taxol on steroid production were not observed in primary cultures of bovine adrenocortical cells (Table 2). No significant change was observed in basal or ACTH-stimulated corticosteroid release when taxol (10 μM) was used for 6 hours (Table 2).

Bt_2cAMP-stimulated steroid release

The addition of Bt_2cAMP (250 μM), which bypasses hormone binding and adenylate cyclase production of cAMP, caused a 25-fold increase in progesterone release from the MLTC-1 cells (Table 3). Taxol (10 μM) inhibited progesterone release by 60% (Table 3). Bt_2cAMP stimulation of progesterone release was not decreased by taxol (1 μM).

Effect of taxol on pregnenolone synthesis

The production of pregnenolone from cholesterol is the initial step in steroidogenesis. In order to determine the effect of taxol on pregnenolone production, trilostane and SU-10603 (inhibitors of pregnenolone metabolism) were added to the incubation media. When MLTC-1 cells were incubated in this medium and hCG for 1–8 hours, a 20-fold increase in pregnenolone release was detected (Fig. 2). Taxol (5–20 μM) was observed to inhibit the steroidogenic response of MLTC-1 cells to hCG stimulation at all time points examined (Fig. 2). The inhibitory action of the drug was concentration-dependent, with higher concentrations acting with greater inhibitory potency (Fig. 2). In addition to inhibition of pregnenolone released into the medium, taxol (10 μM) inhibited pregnenolone which was retained within the cells (Table 4). This was observed when cells were extracted and pregnenolone assayed following experimental treatment. Taxol (10 μM) inhibited hCG-stimulated accumulation of intracellular pregnenolone by 50% of the levels observed within cells treated with hCG in the absence of taxol for 2 hours (Table 4). Y-1 adrenal cells showed a similar pattern of taxol inhibition of hormone-stimu-

TABLE 3. Effect of taxol on the steroidogenic response of MLTC-1 cells to Bt_2 cAMP[1]

Treatment	nmoles progesterone/ mg protein/2 hours
Control	0.64 ± 0.37
Taxol (10 μM)	0.27 ± .04
Bt_2 cAMP	14.3 ± 1.2
Bt_2 cAMP + taxol (10 μM)	5.7 ± 0.7

[1]Cells were grown in serum-containing medium until the beginning of the experiment, when they were washed and placed in serum-free medium with no additives (control), taxol (10 μM), bt_2 cAMP (250 μM), or a combination of bt_2 cAMP plus taxol (10 μM). Incubations were carried out for 2 hours, at which time the media were removed and assayed for progesterone. The values are expressed as the mean ± the standard deviation.

TABLE 4. The effect of taxol on MLTC-1 intracellular levels of pregnenolone and pregnenolone released into the medium[1]

Treatment	nmoles pregnenolone/ mg protein/2 hours		
	Release into media	Cellular levels	Total
Control	0.6 ± 0.3	0.5 ± 0.4	1.1 ± 0.2
Taxol (1 μM)	0.9 ± 0.1	0.6 ± 0.9	1.3 ± 0.1
Taxol (10 μM)	0.7 ± 0.3	0.6 ± 0.1	1.1 ± 0.3
hCG (100 μU/ml)	6.2 ± 0.2	3.7 ± 0.4	9.8 ± 0.5
hCG + taxol (1 μM)	5.5 ± 0.3	3.1 ± 0.2	8.5 ± 0.2
hCG + taxol (10 μM)	2.65 ± 0.7	1.7 ± 0.4	4.1 ± 0.8

[1]Cells were maintained in growth medium until the onset of the experiment, at which time they were washed in serum-free medium. The various treatments were added to the cells in serum-free media containing trilostane (15 μM) and SU-10603 (19 μM). At the end of 2 hours the media were removed and cells were washed and extracted with ethanol. Pregnenolone was then measured by RIA. The values are expressed as the mean ± the standard deviation.

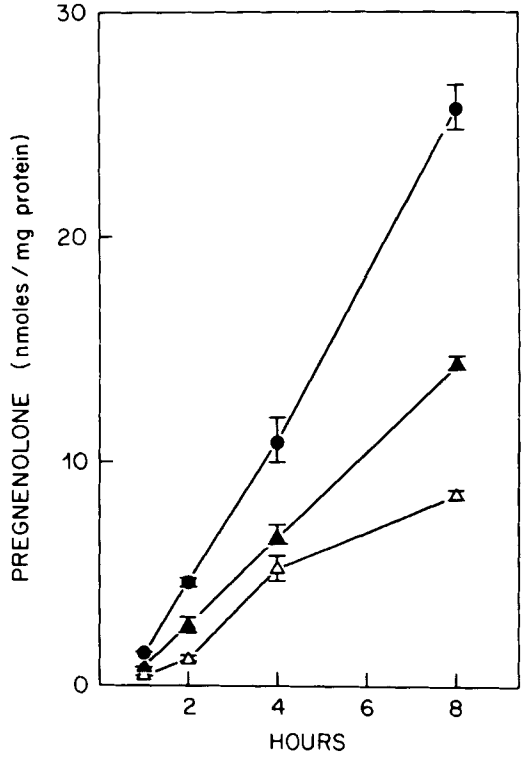

Fig. 2. The effect of taxol on hCG-stimulated pregnenolone production by MLTC-1 cells. Incubations were carried out in serum-free medium containing trilostane (15 μM) and SU-10603 (19 μM). This medium containing hCG (-○-), hCG plus 10 μM taxol (-▲-), or hCG plus 20 μM taxol (-△-) was removed from the cells after the indicated time and assayed for pregnenolone. Values are expressed as the mean ± the standard deviation.

lated pregnenolone production (Fig. 3). Taxol (10 μM) decreased basal and ACTH-stimulated Y-1 cell production of pregnenolone by 50% (Fig. 3).

In order to further test the method by which taxol inhibited pregnenolone production we examined the reversibility of the inhibition. When either Y-1 or MLTC-1 cells were treated with trophic hormone plus taxol for 5 hours, rates of pregnenolone production were inhibited by 50–60%. If taxol was removed after 2 hours the level of hormone-stimulated pregnenolone production was elevated to that observed with trophic hormone alone (Table 5). In addition, the inhibition of taxol on hormone-stimulated pregnenolone synthesis was not due to inhibition of protein synthesis. This was determined by examining the effect of taxol on [^{35}S]methionine incorporation into TCA-precipitable proteins (Table 6). Taxol did not decrease affect overall protein synthesis as determined using this method.

Effect of taxol on microtubules of steroidogenic cells

Electron microscopy was used to observe the effects of taxol on cellular microtubules. We found that non-treated MLTC-1 cells contained few microtubules (Fig. 4A). Treatment of MLTC-1 cells with taxol (10 μM) alone or taxol plus hCG for 2 hours resulted in an increase in cytoplasmic microtubules above levels observed within controls (Fig. 4B). The microtubules formed bundles which could be seen within the cell. Similar results were observed within Y-1 adrenal cells. Control cells had few microtubules (Fig. 4C) which were increased to the extent of bundling after taxol (10 μM) treatment for 6 hours (Fig. 4D).

As opposed to the tumor systems, microtubules were often seen in non-treated bovine adrenocortical cells (Fig. 4E). Taxol increased cellular microtubules but no bundling was observed in 2- or 6-hour incubations (Fig. 4F).

DISCUSSION

In recent years taxol has become widely used as a pharmacological probe for studying microtubule function. As opposed to colchicine, colcemid, and nacodazole, which cause a breakdown of microtubules, taxol enhances the extent of microtubule assembly within cells. Thus cellular functions that rely on the breakdown of microtubles or exchange of tubulin subunits with polymer (treadmilling) are impaired (Schiff and Horwitz, 1980). In order to examine further the involvement of microtubules in steroid production, we studied the effects of taxol on several steps in the steroidogenic pathway using Y-1 adrenal, MLTC-1 Leydig cells, and primary cultures of bovine adrenocortical cells. Previous investigators have used a variety of in vivo and in vitro model systems to evaluate the role of microtubules in steroidogenesis. Though in many cases similar microtubule-disrupting drugs were used as probes, the effects on steroidogenesis have varied depending on the model system used. However, the findings using steroidogenic cell culture model systems have been consistent. These

TABLE 5. The reversibility of taxol inhibition on hormone-stimulated pregnenolone production by Y-1 and MLTC-1 cells[1]

Treatment	nmoles pregnenolone/mg protein			
	Y-1 adrenal cells		MLTC-1 Leydig cells	
	2 hours	5 hours	2 hours	5 hours
Hormone	1.13 ± 0.12	3.76 ± 0.20	7.30 ± 0.62	22.93 ± 1.60
Hormone + taxol (10 μM)	0.39 ± 0.5	2.51 ± 0.10	3.02 ± 0.76	14.90 ± 1.13
Hormone + taxol (10 μM)	0.47 ± 0.5[2]	3.80 ± 0.09	3.39 ± 0.71[2]	21.39 ± 1.59

[1]Cells were maintained in growth medium until the onset of the experiment, at which time they were washed in serum-free medium. The hormones used were ACTH (1 μM) and hCG (100 μU/ml) for Y-1 and MLTC-1 cells, respectively. The hormones plus taxol were incubated with the cells for the indicated time in medium containing trilostane (15 μM) and SU-10603 (19 μM). Where indicated taxol was rinsed from the cells and replaced with the experimental medium with hormone only. Pregnenolone was measured by RIA. The values are expressed as the mean ± the standard deviation.
[2]Taxol removed following this incubation.

data can be summarized as follows: (1) drugs which cause the breakdown of microtubules do not affect hormone-stimulated steroidogenesis (Ray and Strott, 1978) and (2) chronic (>6 hours) treatment of steroidogenic cells with drugs which cause depolymerization of microtubules leads to increases in steroid production (in the absence of hormone) which approaches the steroid released by stimulation via hormone (Temple and Wolff, 1973; Ray and Strott, 1978). These studies suggest that microtubules are not necessary for hormone-stimulated steroidogenesis and indeed may act as a barrier which when removed leads to increased steroid production. Our present findings that taxol, which increases cellular microtubules, can inhibit hormone-stimulated steroid production are consistent with this hypothesis.

In the present study we found that taxol significantly decreased both the acute hCG- stimulation of progesterone production in the MLTC-1 cells and UV-detectable steroids released after ACTH stimulation of the Y-1 adrenal tumor cells. The inhibitory effect on steroid production occurred with an increase of microtubules within the cytoplasm, as observed with electron microscopy. A similar increase in microtubule number was observed in cultured ovarian granulosa cells after treatment with taxol (Herman and Langevin, 1983). Though a detailed morphological study was conducted by these investigators, the effects of taxol on steroid production were not reported. The effects of D_2O, another compound thought to stabilize microtubules within cells (Carolan et al., 1966), have been examined in cultured steroidogenic cells. Though cellular microtubule content was not analyzed, D_2O was found to inhibit both hormone and Bt_2cAMP-stimulated steroidogenesis in Y-1 adrenal and I-10 Leydig cells (Temple and Wolff, 1973). The present study concurs with this observation that drugs which increase intracellular microtubules inhibit steroid production. Taxol, however, did not affect steroid production in primary cultures of bovine adrenocortical cells. The bovine system has previously been observed to differ in its morphological response to ACTH stimulation (Rainey et al., 1983) as well as its response to cytochalasin treatment (Rainey et al., 1984) from other steroidogenic model systems. Cytochalasin D, which inhibits steroid production in mouse adrenal tumor cells (Mattson and Kowal, 1983; Mrotek and Hall, 1977), has no effect on steroid production by primary cultures of bovine adrenal cells. The lack of a taxol inhibition on bovine adrenal cell steroid production may correspond to the observation that microtubules within these cells did not undergo bundling after taxol treatment for 6 hours, as was observed in the Y-1 and MLTC-1 cells. The difference in steroidogenic and morphologic responses to taxol may relate to the fact that the Y-1 adrenal cells and MLTC-1 Leydig cells are tumor cell systems. In

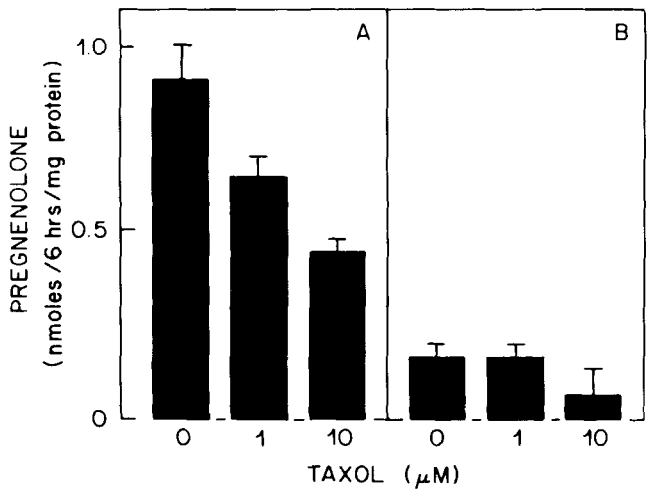

Fig. 3. The effect of taxol on pregnenolone production by Y-1 adrenal cells. Y-1 cells were incubated for 6 hours in serum-free medium containing trilostane (15 μM) and SU-10603 (19 μM). At the end of 6 hours the media were removed and pregnenolone assayed. The effects of taxol on ACTH- stimulated pregnenolone production are seen in A. B represents the influence of taxol on basal pregnenolone production. The values are expressed as the mean ± the standard deviation.

TABLE 6. The effect of taxol on [^{35}S]methionine incorporation into protein[1]

Treatment	Radioactivity incorporated (CPM × 10^{-3}/μg protein/30 min)	
	Y-1 adrenal cells	MLTC-1 Leydig cells
Control	1,318 ± 182	2,071 ± 268
Hormone	1,380 ± 272	1,933 ± 250
Taxol (10 μM)	1,075 ± 146	1,755 ± 255
Taxol + hormone	1,281 ± 75	2,056 ± 420

[1]Cells were maintained in growth medium until the onset of the experiment, at which time they were washed in serum-free medium. The hormones used were ACTH (1 μM) and hCG (100 μU/ml) for Y-1 and MLTC-1 cells, respectively. Following 2 hours of incubation in various experimental media, cells were washed and placed in methionine-free medium for 30 min; 5 μCi of [^{35}S]methionine was then added to each well for an additional 30 min. Incorporation is an expression of [^{35}S]methionine found in TCA-precipitable protein.

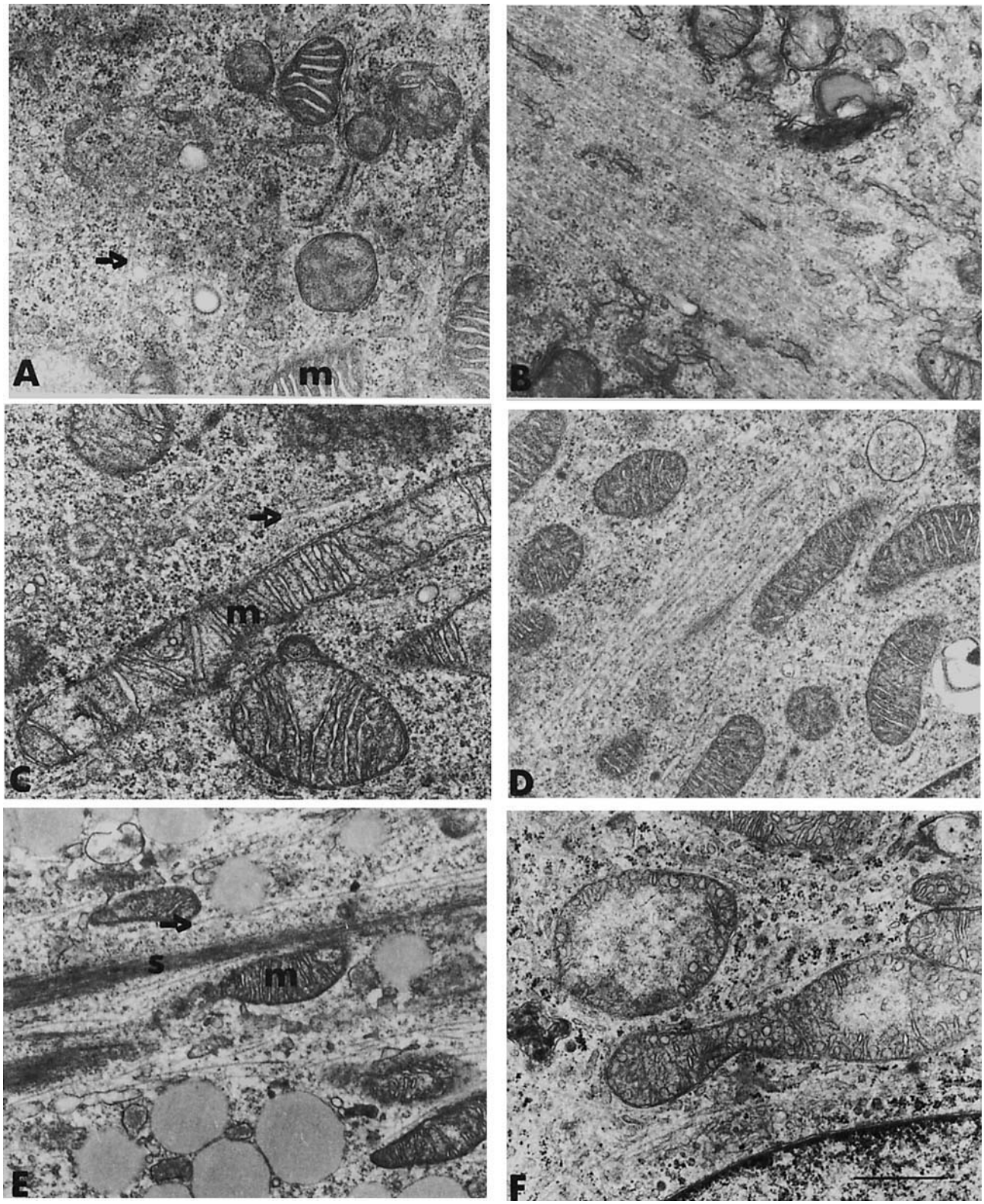

Fig. 4. Electron microscopic observation of steroidogenic cells. A. Control Y-1 adrenal tumor cell with microtubules (arrow) and mitochondria (m). B. Y-1 adrenal cells treated with taxol (10 μM) and ACTH (1 μM) for 6 hours exhibiting microtubule bundling. C. Control MLTC-1 cells with microtubules (arrows) and mitochondria (m). D. MLTC-1 cells treated with taxol (10 μM) and hCG (100 μU/ml) for 6 hours. E. The basal region of a control bovine adrenocortical cell with microtubules (arrows), stress fibers (s), and mitochondria (m). F. Bovine adrenocortical cell after taxol (10 μM) and ACTH) (1 nm) treatment for 6 hours. Bar = 500 nm.

some cases normal cells have been shown to differ in their cytoplasmic microtubules from tumor cells, which usually have fewer microtubules (Brinkley et al., 1975). This was also seen when the MLTC-1 and Y-1 cells were compared to normal bovine adrenal cells. Thus, a drug such as taxol, which stimulates polymerization of tubulin, may have a greater effect in the steroidogenic tumor cells, which contain fewer microtubules.

It is generally believed that trophic hormones act acutely via cAMP within steroidogenic cells (Schimmer, 1980). Colchicine has been observed to partially inhibit hormone-stimulated cAMP production in dispersed ovarian granulosa cells (Zor et al., 1972) and dispersed rat adrenal cells (Bisgaier, 1981). To insure that the inhibitory effects of taxol were not through action on the cells' adenylate cyclase system, taxol and Bt_2cAMP (added in place of hormone) were incubated with MLTC-1 cells. We observed that taxol exhibited an inhibition of Bt_2cAMP-stimulated steroid production similar to the effect seen on hCG stimulation.. This result suggests that the effect of taxol is not due to action on the adenylate cyclase system within the cells.

The inhibitory action of taxol was further investigated by examining pregnenolone production. As previously noted, the conversion of cholesterol to pregnenolone occurs within the mitochondria and is considered the rate-limiting step for steroidogenesis (Simpson, 1979; Stone and Hechter, 1954). Taxol inhibited hCG-increased pregnenolone production in MLTC-1 cells, as well as ACTH-stimulated pregnenolone production in Y-1 cells. This inhibition was probably not due to a direct effect on the cholesterol side-chain cleavage enzyme since taxol did not decrease hormone stimulation of steroidogenesis in bovine adrenocortical cells. Thus the inhibition of taxol observed within the tumor cells occurred prior to pregnenolone synthesis.

Several investigators have suggested that microtubules may be important for steroid export from cells (Gemmell and Stacy, 1977; Nussdorfer et al., 1978; Basset and Pollard, 1980). Nussdorfer et al. (1978) suggested that after injection of vinblastine, rat adrenal steroids were retained rather than released from the cell. In order to determine if taxol's inhibition of steroidogenesis was due to cellular retention of steroids we compared hCG-stimulated pregnenolone secretion into the medium with pregnenolone retained by the cells. Both were decreased when taxol was present in the incubation medium. These results indicate that taxol inhibition of steroidogenesis was not due to lack of export of steroids.

The polymerization of microtubules may affect the steroidogenic system in several ways. We have previously observed that microtubules increased in cellular processes after ACTH or Bt_2cAMP stimulation (Clark and Shay, 1981). Taxol also increases microtubules but the functional abilities of these microtubules have been questioned. Previous reports showed that microtubules produced by taxol treatment were unable to function in chromosome migration (Schiff et al., 1980; De-Brabander et al., 1981) or in cell migration (Schiff and Horwitz, 1980). Thus taxol-polymerized microtubules may not operate in the steroidogenic processes. Taxol treatment also affects the cellular arrangement of intermediate filaments (Herman and Langevin, 1983). Under taxol treatment these filaments rearrange near the nucleus. At present, no studies have been done concerning the involvement of intermediate filaments in steroid production; however, colchicine, which also causes aggregation of intermediate filaments around the nucleus, does not inhibit hormone-stimulated steroid production in cultured steroidogenic cells (Ray and Strott, 1978). In addition, steroidogenic cells, like many other cells, may rely on microtubules for maintenance of mitochondrial organization (Ball and Singer, 1982; Hirokawa, 1982; Summerhayse et al., 1983). Thus, changing the microtubule organization within the steroidogenic cells could decrease cholesterol movement to the mitochondria. Finally, it has been reported (Cervera et al., 1981) that the cytoskeleton may play a role in localization of mRNA, ribosomes, and translation initiation factors within cells. Acute stimulation in steroidogenic cells has been suggested to rely on a labile protein produced after hormone stimulation (Garren et al., 1971). This protein is involved in the transport of cholesterol from the outer mitochondrial membrane to the side-chain cleavage complex within the inner mitochondrial membrane (Ohno et al., 1983). Though taxol did not alter overall protein synthesis, there could be an affect on the cytoskeleton which alters specific protein synthesis or addition of newly synthesized protein to mitochondria.

Our present observations of the effect of taxol on steroid production show that taxol blocks acute steroid production within the Y-1 adrenal and MLTC-1 Leydig cell culture systems but does not affect steroid production by bovine adrenocortical cells. The action of taxol on this process may correspond to the large increase of microtubules within the Y-1 and MLTC-1 cell cytoplasm. These data are suggestive that alterations in microtubules or tubulin may be important for steroid production within the MLTC-1 and Y-1 steroidogenic model systems. The reasons for bovine adrenal cell resistance to taxol or cytochalasin inhibition of hormone-stimulated steroidogenesis are not known. However, the differences between the bovine adrenal cell cytoskeleton and that within the MLTC-1 and Y-1 adrenal cells are presently being analyzed.

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