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Mechanism of Colchicine-Induced Steroidogenesis in Rat Adrenocortical Cells

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Abstract Conflicting data for the effects of colchicine on cholesterol transport and steroidogenesis raise the question of the role of microtubules in cholesterol transport from the lipid droplet to mitochondria in steroidogenic cells. In this study, using corticosterone radioimmunoassay and immunofluorescence microscopy, we re-evaluated the effects of colchicine on hormone production and morphological changes of lipid droplets' and studied the signaling pathway involved in colchicine-induced steroidogenesis. Colchicine stimulated steroid production in a dose- and time-dependent manner. The structural integrity of both the microtubules and the lipid droplet capsule was destroyed by colchicine treatment. Disruption of the lipid droplet capsule occurred later than microtubule depolymerization. After cessation of colchicine treatment and a 3 h recovery in fresh medium, capsular protein relocated to the droplet surface before the cytoplasmic microtubule network was re-established. β-lumicolchicine, an inactive analogue of colchicine, disrupted the capsule and increased hormone production without affecting microtubular structure. Thus, microtubule depolymerization is not required for the increase in steroid production and capsular disruption. To explore the signaling pathway involved in colchicine-induced steroidogenesis, we measured intracellular cAMP levels. Unlike ACTH, colchicine did not increase cAMP levels, suggesting that the cAMP-PKA system is not involved. Colchicine and ACTH had additive effects on corticosterone production, whereas colchicine and PMA did not, implying that part of the PKC signaling mechanism may be involved in colchicine-induced steroidogenesis. Cycloheximide, a protein synthesis inhibitor, completely inhibited colchicine-induced steroidogenesis and capsular disruption. These results demonstrate that the steroid production and lipid droplet capsule detachment induced by colchicine are both protein neosynthesis-dependent and microtubule-independent. J. Cell. Biochem. 81:162–171, 2001.

Key words: adrenal cell; colchicine; steroidogenesis; signaling pathway; lipid droplet capsule

The cytoskeleton is a highly organized filamentous network that not only supplies the framework to support the cell's shape and anchor the organelles, but also provides paths for cytoplasmic transport. Microtubules and associated motor proteins are thought to play a pivotal role in intracellular movement [Gelfand and Scholey, 1992]. In most endocrine and exocrine cells, various microtubule inhibitors disrupt the microtubule system and block the secretory pathway, resulting in accumulation of secretory vesicles and inhibition of secretory activity [Ravindra and Grosvenor, 1990]. In different types of steroidogenic cells, the same anti-microtubular drugs have the opposite effect on steroid hormone secretion [Feuilloley and Vaudry, 1996], but the mechanism involved has not yet been explained.

Inhibitory effects of anti-microtubular drugs on both basal and cAMP-induced steroidogenesis have been reported for MA-10 mouse Leydig cell lines, bovine adrenocortical cultures, and frog adrenocortical cells [Rainey et al., 1985; Feuilloley et al., 1988; Nagy and Freeman, 1990]. In contrast, in Y-1 mouse adrenal tumor cells, colchicine and vinblastine stimulate steroid hormone secretion in a dose-dependent manner [Temple and Wolff, 1973], without affecting intracellular cAMP levels

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[Sackett and Wolff, 1986]. Stimulatory effects of colchicine and nocodazole on steroidogenesis have also been observed in rat primary granulosa cultures by radioimmunoassay and morphometric measurement [Carnegie et al., 1987]. The authors of this report propose that microtubule depolymerization increases the rate of movement of cholesterol from lipid droplets to mitochondria by reducing the spatial distance travelled.

We have shown that the lipid droplet in rat adrenocortical cells is surrounded by a protein capsule which is immunostained using a monoclonal antibody, A2 [Wang and Fong, 1995]. Most of the intracellular esterified cholesterol is stored in the lipid droplet and is released enzymatically by cholesterol ester hydrolase, the activity of which is regulated by the cAMP-PKA system [Vahouny et al., 1984]. We have shown that, following ACTH stimulation, intracellular cAMP levels increase and hormone secretion increases markedly concomitantly with loss of capsular staining [Fong and Wang, 1997; Wang et al., 1998] and have suggested that loss of capsular integrity may facilitate cholesterol ester hydrolase access to the cholesterol and that the surrounding capsule of the lipid droplet acts as a protein barrier, protecting the internal esterified-cholesterol from hydrolysis [Wang and Fong, 1995].

Although the reduction in distance between lipid droplets and mitochondria as a result of the action of antimicrotubular agents has been proposed to facilitate cholesterol access to the mitochondria and to promote steroidogenesis, other possibilities cannot be excluded; one of these is the intactness of the lipid droplet protein capsule under the action of antimicrotubular agents. In this study, we have therefore investigated the effects of colchicine on both steroid hormone production and the morphology of the lipid droplet capsule in cultured rat adrenocortical cells. We have also used β-lumicolchicine (an inactive isomer of colchicine with no effect on tubulin polymerization) and taxol (which promotes tubulin polymerization) to determine the role of microtubules in these colchicine-induced effects. Since a colchicine-induced increase in intracellular cAMP levels is seen in isolated rat Leydig cells [Saltarelli et al., 1984], but not in Y-1 cells [Sackett and Wolff, 1986], we also studied the signal pathways involved in colchicine-induced steroidogenesis.

MATERIALS AND METHODS

Cell Culture

Adult female Wistar rats (8–12 weeks of age) were used. Animals were killed with an i.p. injection of 7% chloral hydrate (6 ml/kg), and primary cultures of adrenocortical cells were prepared as previously described [Wang et al., 1998]. Briefly, decapsulated adrenal gland fragments were incubated for 15 min in a water bath at 37°C in serum-free Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) containing 1 mg/ml of type II collagenase (C-6885, Sigma, St Louis, MO), then the reaction was stopped by addition of ice-cold culture medium (1:1 v/v mixture of Ham's F12 medium and DMEM, supplemented with 25 mM HEPES, 1.2 g/l of $NaHCO_3$, 5% horse serum, 2.5% fetal bovine serum, and 1% penicillin and streptomycin). The dissociated cells were collected by centrifugation at 1,000*g* for 10 min, resuspended, and seeded on coverslips (for immunostaining) or in 24-well plates (for radioimmunoassay) and maintained for 3 days at 37°C in a 95% air/5% CO_2 atmosphere.

Drug Treatments

To study the role of microtubules in steroidogenesis and capsular morphology, various concentrations of colchicine (C-9754, Sigma) or β-lumicolchicine (L-0512, Sigma) were added to the culture medium for 3–12 h. To examine the reversibility of colchicine effects, cells were incubated in 30 μM colchicine for 6 h, then in colchicine-free medium for a further 3 h. Taxol (Sigma) was used as a colchicine antagonist. ACTH (A-6303, Sigma) and phorbol-12-myristate-13-acetate (PMA; Calbiochem, San Diego, CA), a pan-PKC activator, were used as positive controls to stimulate adrenocortical steroidogenesis. Cycloheximide (C-7698, Sigma), which inhibits mRNA translation, was used to examine the role of protein synthesis in colchicine-induced steroidogenesis. 2 μM of cycloheximide has been reported to be able to inhibit over 80% of the protein synthesis [Temple and Wolff, 1973].

Corticosterone Radioimmunoassay

Following drug treatment of cells in 24-well plates, the medium was collected and stored at −20°C until assayed. For the test, 5 μl of the medium was added to 95 μl of assay buffer (0.05 M Tris–HCl, pH 8.0, containing 0.1 M NaCl,

0.1% NaN_3, and 0.1% BSA), then mixed with 500 μl of diluted anti-corticosterone antiserum (C-8784, Sigma). After 30 min at room temperature, 100 μl of [1,2,6,7-^{3}H] corticosterone (TRK 406, 10^6 cpm/ml, Amersham, Buckinghamshire, UK) in assay buffer was added to each assay tube and the mixture incubated at room temperature for 1 h, then at 4°C for 1 h. Free hormones were adsorbed by incubation for 10 min at 4°C with 300 μl of cold dextran-coated charcoal (0.5% dextran and 1.25% charcoal in assay buffer), then the mixture was centrifuged at 13,000*g* for 10 min to separate the antibody-bound and free hormone. A 0.7 ml aliquot of the supernatant was then transferred to a counting vial containing 3 ml of scintillant (LS-275, Ecoscient H) and the radioactivity in each vial counted on a β-counter (LS600IC; Beckman, Fullerton, CA; program number 1). A standard curve was prepared using a corticosterone standard (C-2505, Sigma). Corticosterone production was calculated with respect to the total cellular protein concentration, determined using a BSA standard (A-9647, Sigma) and the Bio-Rad protein assay system. Amounts of corticosterone are given as the mean ± SD of triplicates and expressed as ng/mg protein.

Immunofluorescence

Cultured adrenocortical cells were fixed in 0.15% glutaraldehyde in phosphate buffered saline (PBS), pH 7.4, for 5 min at room temperature and permeabilized with 0.15% Triton X-100 in PBS for 10 min. To reduce free aldehyde groups, the cells were then treated with $NaBH_4$ (1 mg/ml) for 30 min at room temperature. After washes in PBS, the cells were either single-labeled with mouse monoclonal antibody A2 (IgM) (1:200 dilution of ascites in PBS), which labels the lipid droplet capsule [Wang and Fong, 1995], or double-labeled with a mixture of A2 and mouse anti-tyrosinated tubulin antibody (IgG) (T-9028, Sigma). After incubation at 37°C for 2 h, the cells were washed with PBS (3 × 5 min), then reacted with FITC-conjugated goat anti-mouse IgG (whole molecule, F-9006, Sigma) for single labeling or with a mixture of Texas Red-conjugated goat anti-mouse IgM (μ-chain specific; Vector, Burlingame, CA) and FITC-conjugated goat anti-mouse IgG (γ-chain specific; F-8264, Sigma) for double labeling. After incubation at 37°C for 1 h, the cells were washed extensively with PBS, mounted using 3% *n*-propyl gallate and 50% glycerol in PBS, and examined using a Leica fluorescence microscope (Polyvar, Leica, Vienna).

cAMP Assay

Cells were incubated for 60 min at 37°C with various concentrations of colchicine (0–150 μM) or 0.01 IU of ACTH in the presence of 500 μM 3-isobutyl-1-methylxanthine (I-5879, Sigma), which inhibits phosphodiesterase, then washed with PBS and treated overnight at 4°C with 0.01 N HCl to extract the cAMP. The total contents of each well were collected, neutralized with 1 N NaOH, and centrifuged at 13,000*g* for 5 min at 4°C, then a 10 μl aliquot of the supernatant was used to measure intracellular cAMP levels using the Amersham [^{3}H]-cAMP assay system (TRK 432). A standard curve was established using a cAMP standard. The intracellular cAMP concentration was calculated with respect to total cellular protein as pmol/mg protein.

Statistical Analysis

All results are expressed as the mean ± SD. Statistical differences between means were assessed using Student's *t*-test, a P value less than 0.05 being considered significant.

RESULTS

Effect of Colchicine on Steroidogenesis

Colchicine induced cultured rat adrenocortical cells to produce corticosterone in a dose-dependent manner (Fig. 1). After 24 h of treatment, 30 μM colchicine had a significant stimulatory effect ($P < 0.05$) and a time-course study showed a significant stimulatory effect of 30 μM colchicine, which was first apparent after 6–8 h of treatment ($P < 0.05$) (Fig. 2). After 24 h of treatment, 150 μM colchicine had a significant increase on steroidogenic activity compared with that of 30 μM colchicine, equivalent to 60% of that of 0.01 IU of ACTH (Fig. 1).

Effect of Colchicine on Microtubules and Capsular Morphology

Figure 3 represents the typical patterns for microtubules and lipid droplet capsule in untreated control cell. It shows the microtubule system radiating out from the microtubule organization center near the nucleus (Fig. 3A) and the ring structure (capsule) around the

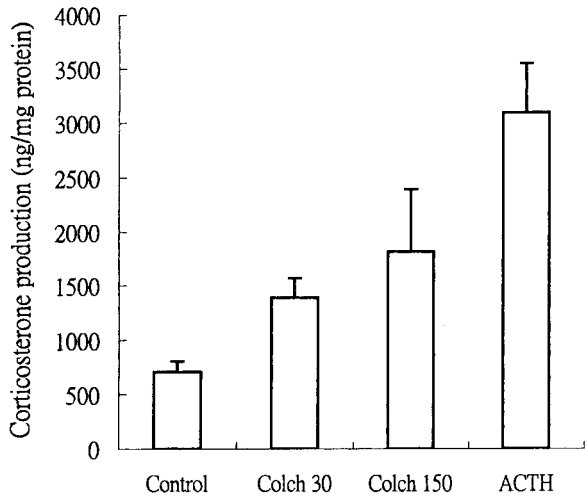

Fig. 1. Colchicine dose-response on steroidogenesis in rat adrenocortical cells. After 24 h of drug treatment, corticosterone production shows a positive correlation with the dose of colchicine. 30 μM colchicine was the minimal effective concentration ($P < 0.05$). Values are the mean ± SD, n = 3.

lipid droplets demonstrated by immunostaining with antibody A2 (arrows in Fig. 3B). From these two images, it is clear that these structures have no association.

The structural integrity of both the microtubule system and the capsule was disrupted

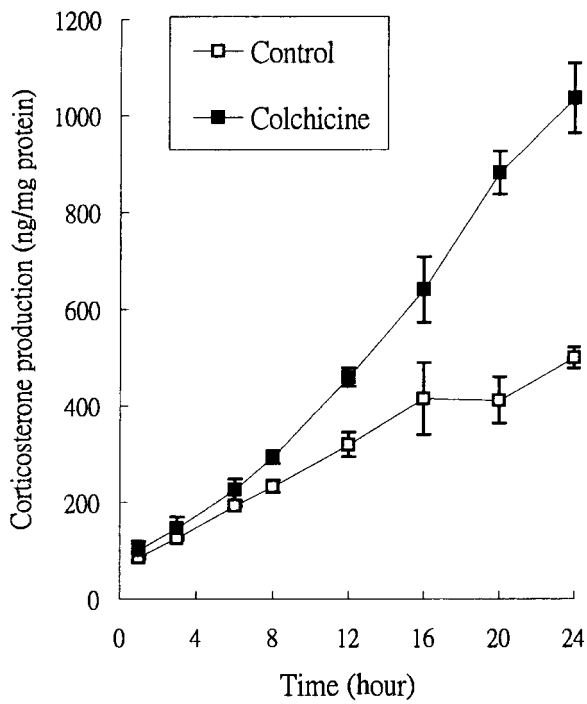

Fig. 2. Time-course study of colchicine-induced steroidogenesis. 30 μM colchicine increases corticosterone production after 8 h of treatment ($P < 0.05$) and this effect is maintained for up to 24 h. Values are the mean ± SD, n = 3.

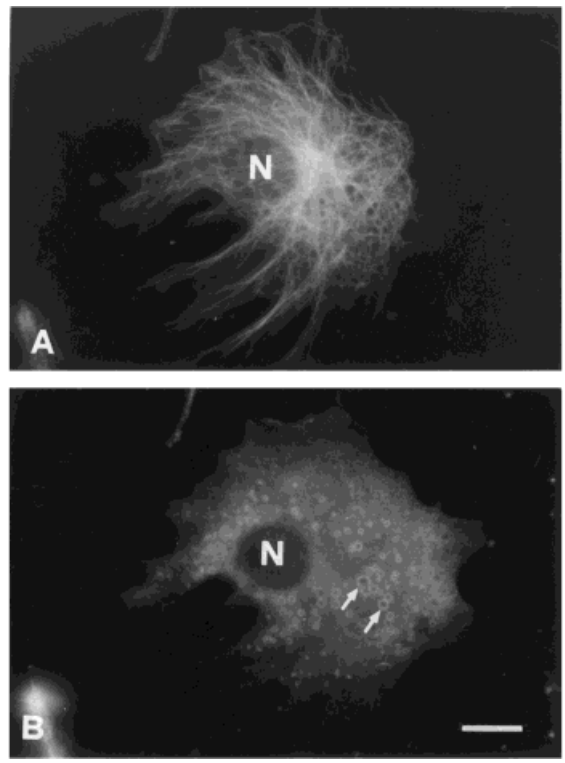

Fig. 3. Double immunostaining for microtubules and the lipid droplet capsule in a non-treated normal cell. The microtubule system is revealed by mouse anti-tubulin antibody (**A**) and the lipid droplet capsule is seen as a ring structure around the surface of lipid droplets stained with antibody A2 (arrows in **B**). N, Nucleus. Scale bar = 10 μm.

by colchicine treatment, but with different time-courses. In the presence of 30 μM colchicine, the microtubule system was completely disassembled after 3 h (arrows in Fig. 4A; control Fig. 3A), while the lipid droplet capsule remained intact (Fig. 4B; control Fig. 3B), capsular staining not lost until after 6 h (Fig. 4C,D). When colchicine-treated cells were allowed to recover in fresh medium, almost all of the A2-positive capsules reappeared within 3 h (Fig. 4F) in the absence of microtubule reassembly (Fig. 4E), again suggesting independence of capsule structure and microtubule integrity.

Lack of Relationship Between Microtubule Integrity and Capsular Morphology

In previous studies, the facilitation of cholesterol transport to the mitochondria by microtubule depolymerization was reversed by microtubule-stabilizing agents [Temple and Wolff, 1973; Sackett and Wolff, 1986]. We therefore used taxol as a colchicine antagonist

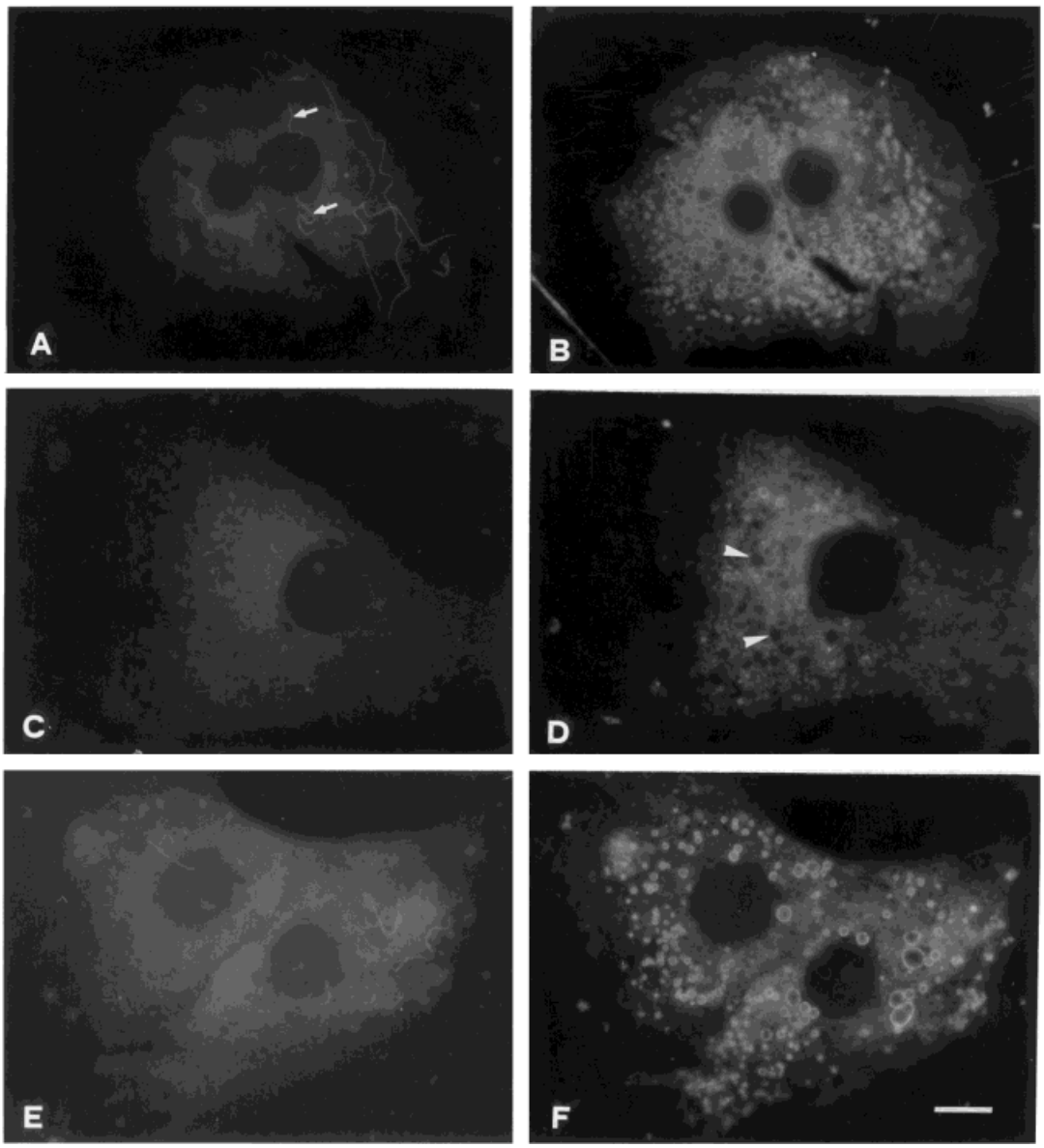

Fig. 4. Effect of colchicine on the microtubule system and the lipid droplet capsule in rat adrenocortical cells. Cells were double-labeled with anti-tubulin antibody (**A**, **C**, **E**) and antibody A2 (**B**, **D**, **F**). After 3 h treatment with 30 μM colchicine, the microtubule system is partially disrupted (arrows in A), but the lipid droplet capsule is still intact (B). After 6 h of treatment, capsular staining is either discontinuous or absent (arrowheads in D), and the microtubules are completely depolymerized (C). When colchicine-treated cells are placed in colchicine-free culture medium for 3 h, capsular staining reappears earlier (F) than the microtubules (E). Scale bar = 10 μm.

to test whether a microtubule-stabilizing agent could prevent colchicine-induced decapsulation. Application of colchicine at a high dose (150 μM) shortened the period required for the decapsulation of lipid droplets to 3 h. This dosage of colchicine resulted in disruption of microtubules (Fig. 5A; control Fig. 3A) and capsules of lipid droplets (Fig. 5B; control Fig. 3B) after 3 h of treatment; in the presence of taxol (1 μM), the microtubule-disruption effect of colchicine was blocked (Fig. 5C), but colchicine-induced decapsulation was unaffected (arrows in Fig. 5D). β-lumicolchicine (150 μM), although structurally similar to colchicine, does not bind to and disrupt microtubules [Rudd et al., 1981]; in its presence, the lipid droplet capsule was disrupted (arrows in Fig. 5F), while microtubule organization was not affected (Fig. 5E). β-lumicolchicine stimulated steroidogenesis in a dose-dependent

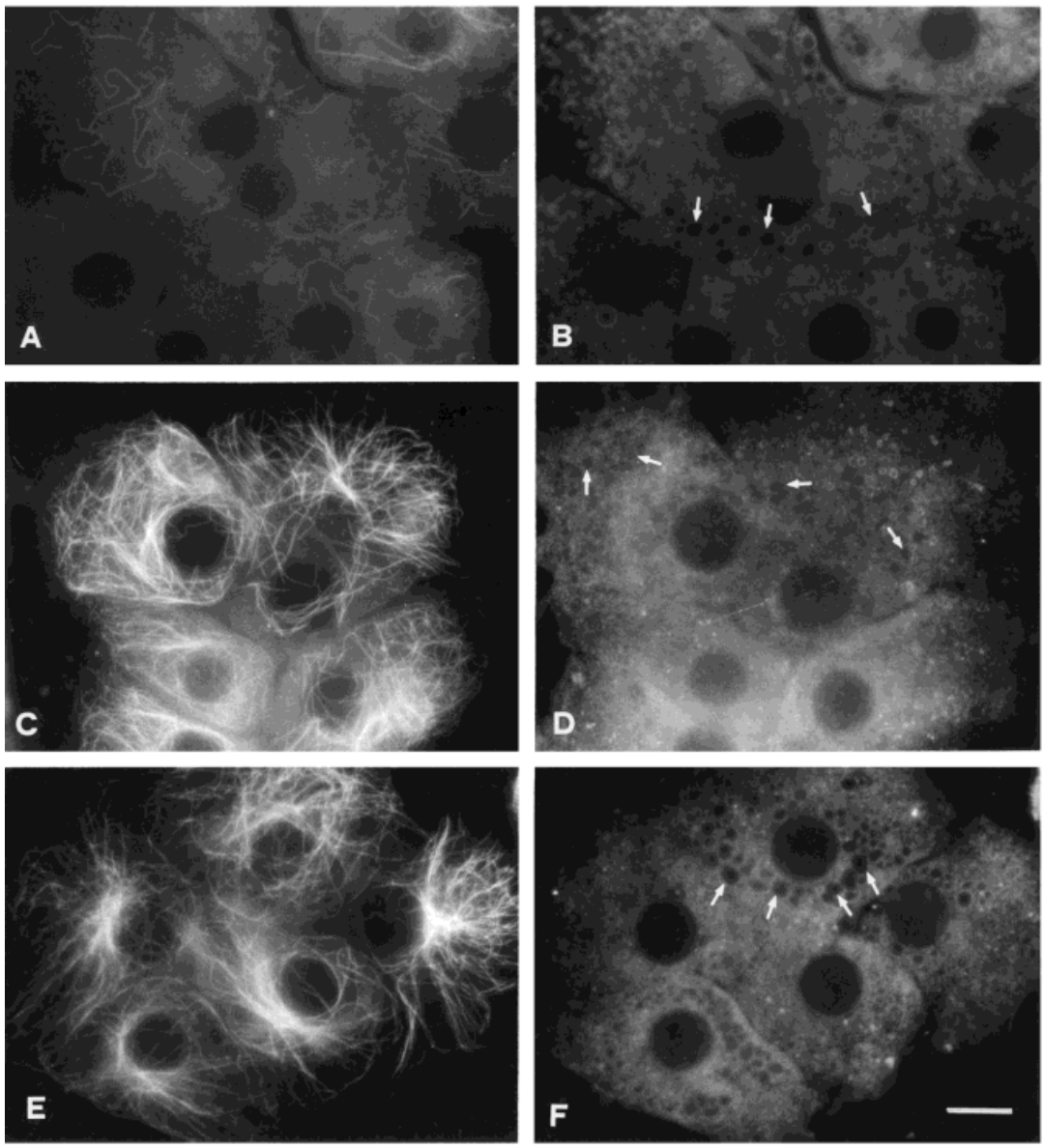

Fig. 5. Independence of microtubule integrity and capsular staining. All cells were labeled with anti-tubulin antibody (**A**, **C**, **E**) and antibody A2 (**B**, **D**, **F**). A, B: Cells treated with 150 μM colchicine for 3 h. The microtubule system is disrupted (A), and the lipid droplet capsule is incomplete (arrows in B). C, D: Cotreatment with taxol (1 μM) and 150 μM colchicine for 3 h. Microtubules are partially disrupted (**C**), but capsular staining is lost (arrows in D). E, F: Treatment with 150 μM β-lumicolchicine for 3 h. The microtubule system is not affected (E), while the lipid droplet capsule is absent (arrows, F). Scale bar = 10 μm.

manner and was as effective as colchicine itself (Fig. 6, compared with Fig. 1). We therefore propose that the colchicine-induced decapsulation of lipid droplets is independent of the microtubule system.

Mechanism of Colchicine-Induced Steroidogenesis and Capsule Disruption

Under physiological conditions, most steroidogenic cells respond to trophic stimuli (ACTH or LH) and produce steroid hormones via a cAMP-PKA-dependent signaling mechanism [Boyd et al., 1983], with cAMP acting as the second messenger to activate the enzyme cascade system. Since Saltarelli et al. [1984] reported that in isolated rat Leydig cells, colchicine stimulates testosterone secretion by increasing intracellular cAMP levels, we examined whether intracellular cAMP levels were increased by colchicine treatment in our sys-

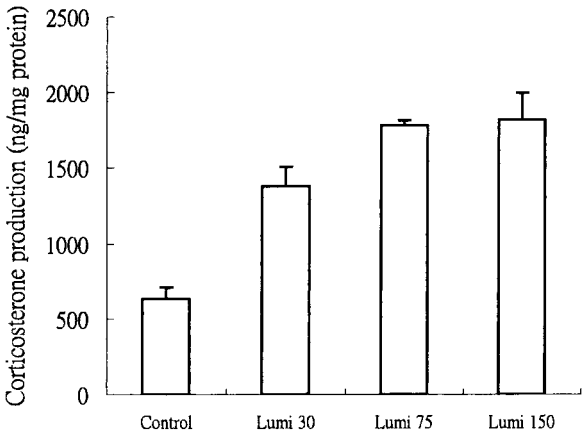

Fig. 6. Dose-dependent effect of β-lumicolchicine on steroidogenesis in rat adrenocortical cells. After treatment with various concentrations of β-lumicolchicine for 24 h, corticosterone production increased with the dose of β-lumicolchicine. 30 μM β-lumicolchicine was the minimal effective steroidogenic dose ($P < 0.05$). Values are the mean ± SD, n = 3.

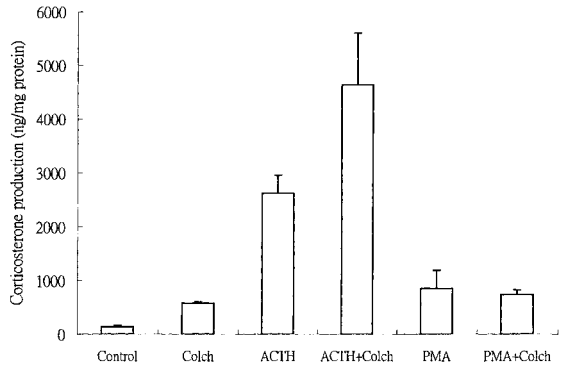

Fig. 8. Effect of ACTH or PMA on colchicine-induced steroidogenesis. Cells were treated with 0.01 IU of ACTH or 100 nM PMA, with or without 150 μM colchicine, for 24 h, and the culture medium collected for corticosterone radioimmunoassay. In the control group, 150 μM colchicine induces a significant fourfold increase in steroidogenesis. In the ACTH plus colchicine group, an additive effect is seen. PMA alone increases corticosterone production, but cotreatment with colchicine does not increase hormone production. Values are the mean ± SD, n = 3.

tem and found that, at colchicine concentrations of 2–150 μM, this was not the case (Fig. 7). In order to study the effect of ACTH-colchicine cotreatment, we first determined the saturating concentrations for these two reagents. At 150 μM colchicine, corticosterone production of adrenal cells was maximal without causing cell detachment from the culture substratum. In a series of dose-response studies of ACTH, we found that 0.01 U/ml is the saturating concentration to induce maximal corticosterone secretion of adrenal cells, since there was no significant difference between the doses of 0.01 to 0.3 U/ml. The results showed that the effects of ACTH and colchicine on corticosterone production were additive (Fig. 8), suggesting that these two stimulants act on the steroidogenic pathway by different mechanisms and that the cAMP-PKA system is not involved in colchicine-induced steroidogenesis.

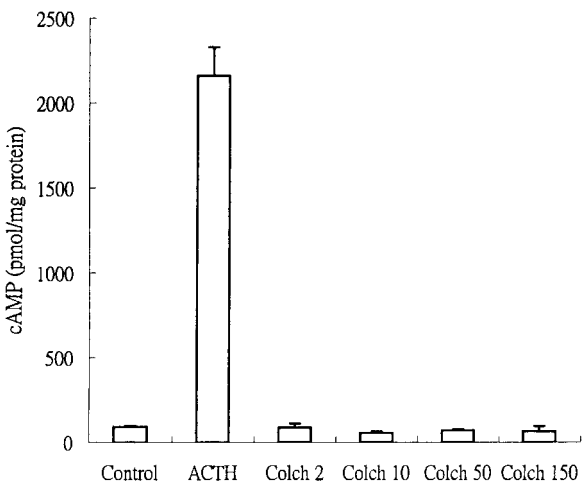

Fig. 7. Intracellular cAMP levels in colchicine- or ACTH-treated cells. Adrenocortical cells were incubated with various concentrations of colchicine or 0.01 IU of ACTH (positive control) for 1 h prior to the assay. The intracellular cAMP levels in all colchicine-treated groups are comparable to the control value, whereas ACTH induces a significant increase in cAMP levels ($P < 0.01$).

PMA, which mimics the effect of diacyl glycerol in activating the protein kinase C (PKC) system [Castagna et al., 1982], also induced steroidogenesis, but the effect was not additive with that of colchicine (Fig. 8), implying that part of the PKC signaling pathway may be used in colchicine-induced steroidogenesis.

To test whether protein synthesis is required for colchicine-induced steroid hormone production and capsular detachment, cycloheximide, a protein synthesis inhibitor, was applied to colchicine-treated cells. In its presence, basal corticosterone production was unaffected, whereas colchicine-induced corticosterone secretion was almost completely blocked (Fig. 9). In an immunofluorescence study, 3 h treatment with 150 μM colchicine disrupted the lipid droplet protein capsule (Fig. 10A). Cycloheximide alone did not affect capsule integrity (Fig. 10B), while the colchicine-induced decapsulation of lipid droplets was blocked by

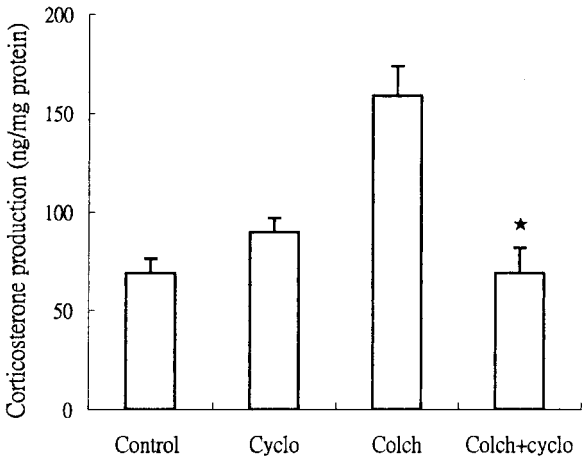

Fig. 9. Effect of a protein synthesis inhibitor on colchicine-induced steroidogenesis. 2 μM cycloheximide was applied, with or without 150 μM colchicine, for 6 h. Cycloheximide alone slightly reduces corticosterone production and cycloheximide has no effect on colchicine-induced steroidogenesis. Values are the mean ± SD, n = 3 (*$P < 0.05$, vs. colchicine treatment).

cycloheximide (Fig. 10C). These results demonstrate that both the steroid production and lipid droplet capsule detachment induced by colchicine require new protein synthesis.

DISCUSSION

In this study, we have demonstrated that colchicine stimulated corticosterone secretion, and also disrupted the lipid droplet capsule in rat adrenocortical cell cultures. We have also provided evidence that microtubule depolymerization is not required for either of these effects. The cAMP-PKA system was not activated by colchicine treatment, but part of the PKC-associated signaling mechanism may be involved. Both steroidogenesis and lipid droplet decapsulation required new protein synthesis; therefore gene expression and/or mRNA translation might be involved. Our results are consistent with the observations that colchicine promotes steroidogenesis in Y-1 adrenal tumor cells [Temple and Wolff, 1973; Sackett and Wolff, 1986], isolated rat adrenocortical cells [Ray and Strott, 1978], isolated rat Leydig cells [Saltarelli et al., 1984], and rat granulosa cell cultures [Carnegie et al., 1987].

Several proteins are found on the surface of lipid droplets in both adipocytes and steroidogenic cells; these include perilipins [Greenberg et al., 1991], adipophilin [Heid et al., 1996], and

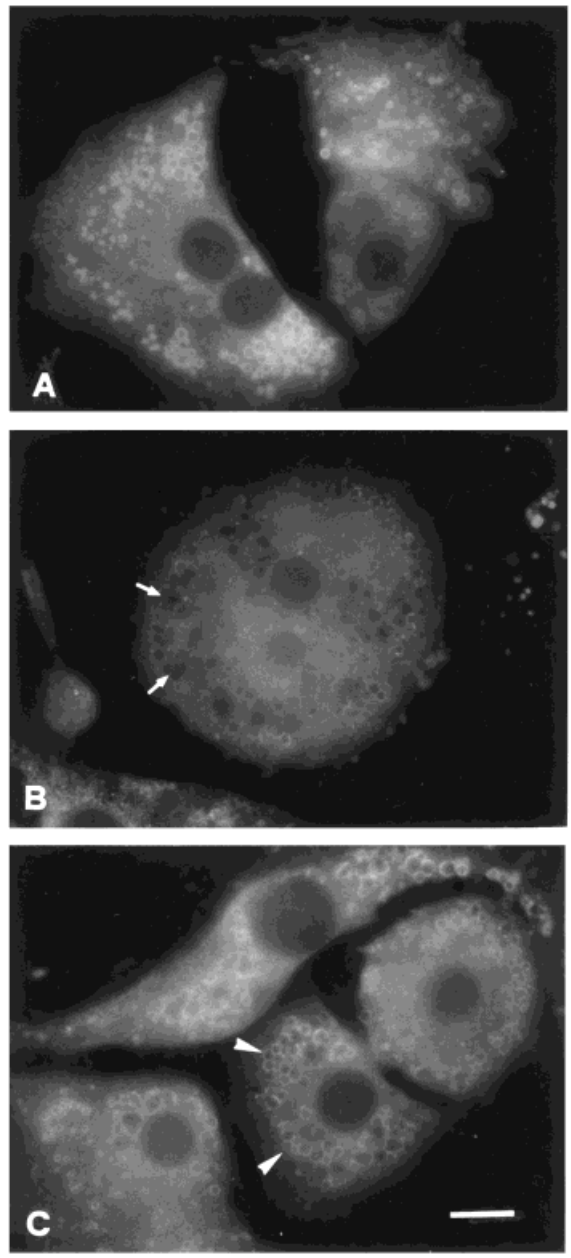

Fig. 10. Effect of cycloheximide and colchicine treatment on the capsular morphology of adrenocortical cells. **A**: Cells treated with 2 μM cycloheximide for 3 h; the antibody A2-positive capsule is intact. **B**: Cells treated with 150 μM colchicine for 3 h; colchicine disrupts the lipid droplet capsule (arrows in B). **C**: Cells cotreated with colchicine and cycloheximide for 3 h; most lipid droplets have complete antibody A2–positive capsules (arrowheads in C). Scale bar = 10 μm.

the 160 kDa protein recognized by antibody A2 [Wang and Fong, 1995]. Our previous unpublished work has shown that, following appropriate stimuli (such as ACTH or db-cAMP), a significant increase in corticosterone secretion

is closely related to the disruption of the antibody A2-positive capsule in rat adrenocortical cells. We hypothesized that the surrounding lipid droplet capsule acts as a barrier which protects the internal esterified cholesterol from hydrolysis. In the Y-1 cell model, microtubules are suggested to act as a barrier, restricting the movement of lipid droplets to the mitochondria [Clark and Shay, 1981], and microtubule disruption would remove this barrier, allowing cholesterol to reach the mitochondrial site for side-chain cleavage [Sackett and Wolff, 1986]. However, the present study provides evidence that, following colchicine treatment, the barrier removed is actually the protein layer. Removal of the barrier, especially the antibody A2-positive capsule, may facilitate cholesterol ester hydrolase to access the lipid droplet surface, thereby promoting cholesterol release for steroid hormone synthesis. In the present study, the time-courses of the colchicine-induced corticosterone increase and capsule disruption were very similar, providing further support for our hypothesis.

Cycloheximide completely inhibited colchicine-induced steroidogenesis, suggesting that activation of pre-existing cytoplasmic enzyme systems plays either a minor, or no, role and that new protein synthesis is important. Furthermore, it also prevented colchicine-induced capsule disruption, implying that some newly synthesized protein(s) might regulate capsular morphology. In adrenal Y-1 cells, there is a time lag of 6–9 h between colchicine application and a significant increase in steroid production [Temple and Wolff, 1973], similar to that of 6–8 h seen in the present study. Several hours may be needed for new synthesis of proteins required for lipid droplet decapsulation and regulation of steroid synthesis. It should be noted that a significant increase in steroidogenic activity only occurred after decapsulation and this may explain the ineffectiveness of short-term colchicine treatment [Carnegie et al., 1987].

Under physiological conditions, trophic hormones (ACTH or LH) might upregulate the expression of many steroidogenesis-associated proteins, including the key protein(s) involved in regulation of capsular integrity. Colchicine treatment may activate or induce these key proteins and this approach may help in understanding the mechanism of capsule detachment. Using two-dimensional gel electrophoresis analysis, we intend to compare the protein patterns of normal and colchicine-treated cells to try to identify the key proteins involved in the regulation of capsule integrity.

The facts that colchicine did not cause an increase in intracellular cAMP levels and that the effects of ACTH and colchicine were additive demonstrate that the cAMP-PKA system is not involved in colchicine-induced steroidogenesis. In bovine adrenocortical cells, in addition to the cAMP-PKA system, activation of the PKC system also promotes steroidogenesis [Culty et al., 1984]. In our previous studies, PMA-induced activation of the PKC system promoted corticosterone production without causing capsule detachment in rat adrenocortical cells [Fong and Wang, 1997; Wang et al., 1998]. Since the effects of colchicine and PMA were not additive, we suggest that the action of colchicine might be mediated by the PKC-associated signaling pathway.

In this study, we have re-examined the steroidogenic effect of colchicine in cultured rat adrenocortical cells and hypothesize that colchicine may directly modify an intracellular signaling pathway and affect steroidogenesis by regulating the integrity of the protein capsule, which consequently promotes steroid hormone synthesis.

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