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# Involvement of microtubules in the effect of estradiol on progesterone secretion by hamster corpus luteum

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## Abstract

Involvement of microtubules in progesterone secretion by the corpus luteum is suggested by experiments wherein the inhibitors of microtubule function such as colchicine and vinblastine inhibited progesterone secretion. Lumicolchicine, an inactive isomer of colchicine, was found ineffective, suggesting the specificity of the colchicine effect. The inability of colchicine to affect progesterone secretion by the corpora lutea pretreated with either $D_2O$ or tubulin antibody lends further support to the suggestion of the requirement of the integrity of microtubules for progesterone secretion. Preincubation of corpora lutea either with $D_2O$ or tubulin antibody also abolished the inhibitory effect of estradiol on progesterone secretion, suggesting a role of microtubules in estradiol action.

Key words: Corpus luteum, estradiol, microtubules, hamster.

## 1. Introduction

Recent reports suggest that progesterone release by the corpus luteum might not be due to passive diffusion. A correlation between the presence and concentration of densely staining granules and progesterone secretion has been noted[1,2]. Sawyer *et al*[3], implicating microtubules in the transport and secretion of progesterone, proposed that a progesterone carrier protein could be packaged into secretory granules at the level of Golgi. While Quirk *et al*[4] reported that progesterone is localised within electron-dense granules, a suggestion has been made that these granules are released into extracellular medium by exocytosis. Hall and Robinson[5] have, in fact, observed that colchicine

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inhibited progesterone secretion by luteal cells of pseudopregnant rats. All these reports suggest a role for microtubules in the secretion of progesterone. In the present study the possibility that estradiol might be inhibiting progesterone secretion by interfering with the microtubule assembly has been examined.

## 2. Materials and methods

### 2.1. *Hamsters*

Colony-bred adult female golden hamsters (*Mesocricetus auratus*) were caged with adult males and checked daily for presence of vaginal sperm. Day 1 of pregnancy was designated as that day on which the vaginal smear was sperm-positive. Animals were always sacrificed on the prescribed day between 10 a.m. and 12 noon.

### 2.2. In vitro *incubation*

Corpora lutea obtained from pregnant animals were freed of non-luteal tissue, weighed to the nearest 0·05 mg and incubated in minimal essential medium (MEM) at pH 7·2 and 37° C as described earlier[6]. At the end of incubation, the contents of the incubation flasks were snap-frozen in liquid-$N_2$ and stored until analysis.

### 2.3. *Radio immunoassay of progesterone*

Progesterone was assayed as described earlier[6]. Progesterone was estimated using 1, 2, 6, 7-$^3$H-progesterone and antiserum (1 : 20,000 final dilution) produced in rabbits immunised with progesterone-11-succinyl-BSA (gift of Dr. H. R. Behrman). Cross reaction of the antiserum with 20 $\alpha$-hydroxy progesterone and 17 $\alpha$-hydroxyprogesterone was 7% and 2% respectively. Sensitivity of the assay was 25 pg.

### 2.4. *Lumicolchicine*

Lumicolchicine was prepared by the method of Wilson and Friedkin[7]. Fresh colchicine solution (50 $\mu$g/ml) was exposed to UV light for 30' and the phototransformation of colchicine to lumicolchicine was monitored spectrophotometrically. Absorption maximum of colchicine is at 350 nm whereas that of lumicolchicine is 270 nm.

## 3. Results

### 3.1. *Involvement of microtubules in progesterone secretion*

In order to examine whether the microtubule system is involved in progesterone secretion by the corpus luteum, the effects of colchicine, vinblastine and $D_2O$ on the secretory process have been studied. Colchicine (0·5 $\mu$g/ml) and vinblastine (1 $\mu$g/ml) inhibited progesterone secretion *in vitro* by the corpora lutea of day 8 pregnant hamsters (Fig. 1). Lumicolchicine did not inhibit progesterone secretion (Table I). Preincubation of corpora

lutea with $D_2O$ for 1h and transferring them to medium containing colchicine, resulted in these corpora lutea secreting comparable amounts of progesterone as that in controls (Table II).

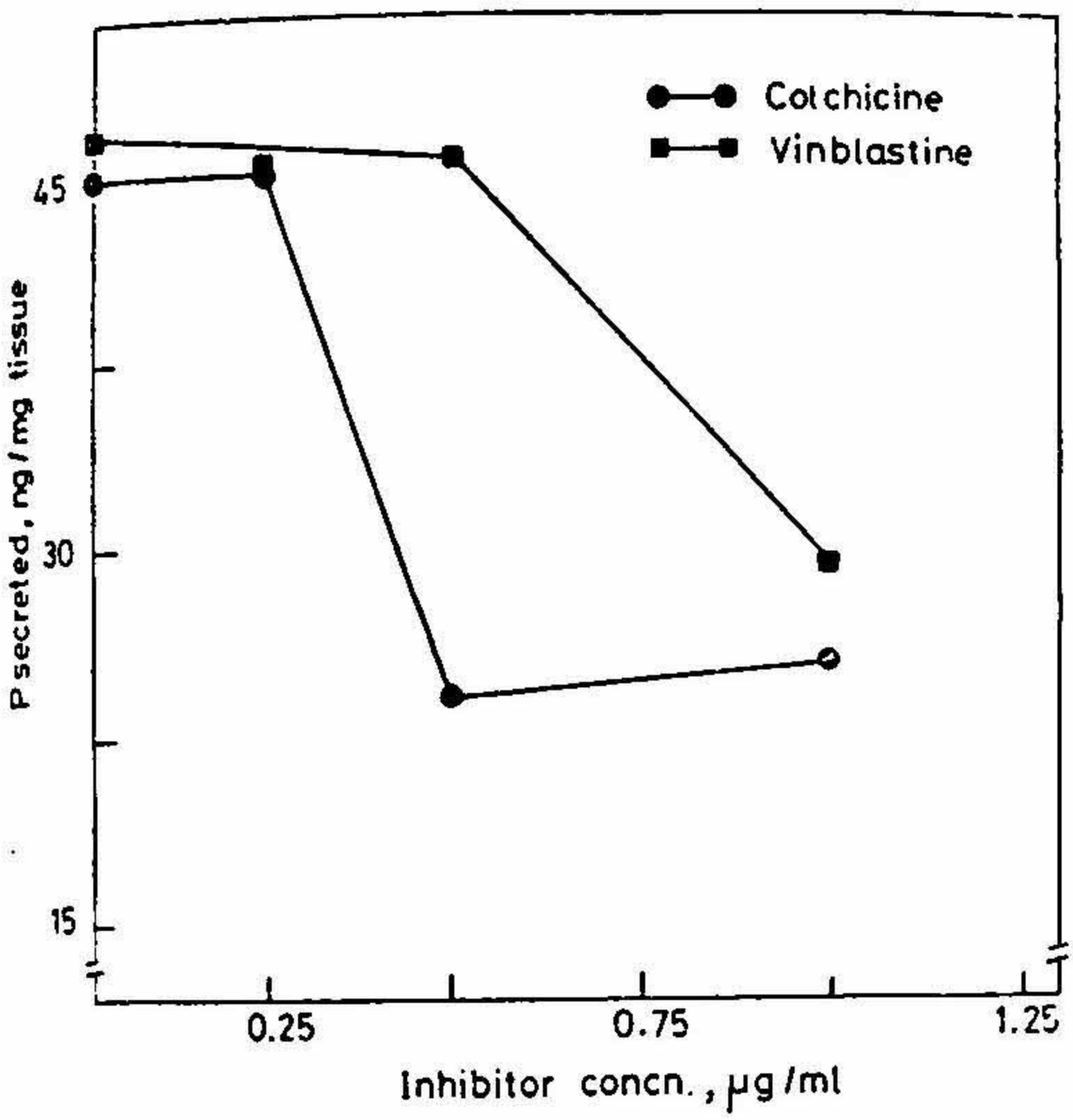


FIG. 1. Effect of colchicine and vinblastine on progesterone secretion *in vitro* by day 8 corpora lutea of pregnant hamster.

Corpora lutea (5–10 mg) were incubated in duplicates in 1 ml MEM buffer, pH 7·2, 37° for 2h. Progesterone was estimated in the medium by RIA. Each point represents the mean of two determinations. Each experiment was repeated at least twice.

**Table I**

***In Vitro* effect of lumicolchicine**

| | P ng/mg tissue/2h* |
|---|---|
| Control | 46·0 ± 4·9[a] |
| Colchicine (1 µg/ml) | 27·7 ±2·8[a] |
| Lumicolchicine (1 µg/ml) | 46·2 ± 3·0 |

* Mean ± S.D. of four determinations.
[a] $p < 0·001$.

Corpora lutea were incubated in 1 ml MEM buffer pH 7·2, 37°. Progesterone was estimated in the medium by RIA.

**Table II**

**Effect of colchicine on corpora lutea pretreteated with $D_2O$ *in vitro***

| | P ng/mg tissue/2h* |
|---|---|
| Control | $45{\cdot}4 \pm 3{\cdot}7^a$ |
| $D_2O$ | $44{\cdot}5 \pm 3{\cdot}5$ |
| Colchicine (1 $\mu$g) | $25{\cdot}7 \pm 5{\cdot}6^a$ |
| $D_2O$ + colchicine (1 $\mu$g) | $43{\cdot}5 \pm 3{\cdot}9$ |

* Mean ± S.D. of four determinations.

$p^a < 0{\cdot}002$.

Corpora lutea were incubated in 1 ml MEM buffer containing 5% $D_2O$ at pH 7·2, 37° for 1h or in plain buffer as indicated, after which they were extensively washed, transferred to flasks containing fresh MEM buffer (1 ml) with or without colchicine and incubated for 2 h. Progesterone was estimated in medium by RIA.

### 3.2. *Effect of tubulin a/s on progesterone secretion*

It was of interest to study the effect of tubulin a/s on progesterone secretion by the corpora lutea. Tubulin a/s did not affect basal or LH-stimulated progesterone secretion by the corpora lutea (data not presented). But, pretreatment of corpora lutea with tubulin antibody prevented the inhibitory effect of colchicine on progesterone secretion (Table III).

**Table III**

**Effect of colchicine on *in vitro* progesterone secretion by corpora lutea of day 8 pregnant hamsters preincubated with tubulin a/s**

| | P ng/mg tissue/2h* |
|---|---|
| Control | $47{\cdot}8 \pm 9{\cdot}3$ |
| Colchicine (1 $\mu$g) | $30{\cdot}9 \pm 1{\cdot}6$ |
| NRG + Colchicine (27 $\mu$g) (1 $\mu$g) | $32{\cdot}9 \pm 0{\cdot}6$ |
| Tubulin a/s + Colchicine (27 $\mu$g) (1 $\mu$g) | $41{\cdot}7 \pm 2{\cdot}4$ |

* Mean ± S.D. of three determinations.

NRG—Normal Gamma Globulin.

Corpora lutea were incubated in 1 ml MEM buffer at pH 7·2, 37° for 1h with NRG or tubulin a/s or MEM buffer, after which they were washed extensively, transferred to flasks containing MEM buffer with or without colchicine. The subsequent incubation was also conducted in 1 ml MEM for 2h. Progesterone was estimated in the medium by RIA.

### 3.3. *Mechanism of estradiol action*

In view of the fact that progesterone secretion involves microtubules, it was thought worthwhile to examine the possibility of the involvement of microtubules in estradiol action on the corpus luteum.

At a concentration of 250 ng/ml neither estradiol nor colchicine influenced progesterone secretion to an appreciable extent. These two compounds, when used together at sub-optimal doses, exhibited a synergistic effect (Table IV). Pretreatment of corpora lutea with $D_2O$ prevented the inhibitory effect of estradiol (Table V). It was also found that estradiol did not inhibit progesterone secretion by the corpora lutea pretreated with tubulin a/s (Table VI).

**Table IV**

**Effect of submaximal concentrations of colchicine and estradiol on *in vitro* progesterone secretion by 8 day corpus luteum**

| | P ng/mg tissue/2h* |
|---|---|
| Control | 47·8 |
| E (250 ng/ml) | 43·7 |
| Colchicine (250 ng/ml) | 41·7 |
| E (250 ng/ml) + Colchicine (250 ng/ml) | 27·0 |

* Mean of duplicate incubations.
Corpora lutea were incubated in 1 ml MEM buffer at pH 7·2, 37°. Progesterone was estimated in medium by RIA.

**Table V**

**Preincubation with $D_2O$ : Subsequent effect of estradiol on progesterone secretion *in vitro***

| | P ng/mg tissue/2 h* |
|---|---|
| Control | 46·3 ± 4·4[a] |
| $D_2O$ | 44·7 ± 3·0 |
| Estradiol (1 μg) | 25·5 ± 4·4[a] |
| $D_2O$ + Estradiol (1 μg) | 46·5 ± 3·0 |

* Mean ± S.D. of four determinations.
[a] $p < 0·001$

Corpora lutea were incubated in 1 ml MEM buffer containing 5% $D_2O$ at pH 7·2, 37° for 1h or in plain buffer as indicated, after which they were extensively washed, transferred to flasks containing fresh MEM buffer (1 ml) with or without estradiol and incubated for 2h. Progesterone was estimated in medium by RIA. This experiment was repeated thrice.

Table VI

**Effect of estradiol on *in vitro* progesterone secretion by corpora lutea preincubated with tubulin a/s**

| | P ng/mg tissue/2h* |
|---|---|
| Control | $45 \cdot 9 \pm 2 \cdot 0$ |
| Estradiol (1 $\mu$g) | $28 \cdot 6 \pm 1 \cdot 6^a$ |
| NRG (27 $\mu$g) + Estradiol (1 $\mu$g) | $28 \cdot 1 \pm 2 \cdot 0^a$ |
| Tubulin a/s (27 $\mu$g) + Estradiol (1 $\mu$g) | $46 \cdot 0 \pm 2 \cdot 8$ |

* Mean ± S.D. of three determinations.

[a] Significantly different from the control group ($p < 0 \cdot 05$).

NRG—Normal Gamma Globulin.

Corpora lutea were incubated in 1 ml MEM buffer at pH 7·2, 37° for 1 h with NRG or tubulin a/s or MEM buffer, after which they were washed extensively, transferred to flasks containing MEM with or without estradiol. The subsequent incubation was conducted for 2 h. Progesterone was estimated in the medium by RIA.

## 4. Discussion

Microtubule function is generally investigated using colchicine, vinblastine and $D_2O$. It is known that colchicine inhibits the assembly of tubulin monomers into the polymerised microtubules[8]. Addition of vinblastine to cells results is precipitation of tubulin[8]. On the other hand, $D_2O$ is known to alter the equilibrium between soluble and polymerised form of tubulin by overstabilising them reversibly in their polymerised form[9].

The involvement of microtubules in progesterone secretion by the corpus luteum is suggested by the observations that colchicine and vinblastine inhibited progesterone secretion. Similar results have been obtained by other workers using ovine corpora lutea and rat luteal cells[1–5]. Further, the present studies reveal that lumicolchicine, an inactive isomer of colchicine, does not affect progesterone secretion by the corpora lutea. Lumicolchicine was shown not to inhibit microtubule function[7]. Moreover, preincubation of corpora lutea with $D_2O$ and later exposing them to colchicine did not result in decreased progesterone secretion. All these observations strongly suggest that the integrity of microtubules is a prerequisite for progesterone secretion by the corpus luteum.

Assuming that estradiol inhibits progesterone secretion by interfering with microtubule function, the effect of sub-optimal concentrations of estradiol and colchicine added together was checked. The synergistic effect thus obtained suggested a possibility that estradiol might be acting *via* the tubulin system.

Earlier observations suggested that agents which are known to disrupt the tubulin-microtubule system could cause impaired progesterone secretion. However, tubulin a/s, which is known to disrupt the microtubule system, is ineffective in altering the secretory levels of progesterone, suggesting that the antibody may not be entering the luteal cell. Moreover, the responsiveness to LH of the tubulin a/s treated corpora lutea did not alter compared to untreated controls, giving further support to the suggestion that the antibody does not enter the cell (data not presented). However, pretreatment of corpora lutea with tubulin a/s prevented colchicine from exerting its inhibitory effect on progesterone secretion. This suggests that the antibody binds to cell surface of the luteal tissue and prevents the entry of colchicine into the cell. The presence of a plasma membrane associated tubulin has been demonstrated in the brain of a variety of organisms[8]. The presence of a similar membrane associated tubulin in the corpus luteum can probably explain these effects of tubulin a/s as well.

The tubulin a/s also prevents the action of estradiol in inhibiting progesterone secretion. This may also mean that the a/s is preventing the binding of estradiol to membrane components of the luteal cell. The ability of $D_2O$ treatment to block the action of estradiol also suggests that the hormone could be initially binding to the membrane component. Moreover, specific, saturable and temperature-dependent cell surface binding sites on endometrial cells to estradiol-BSA conjugate immobilised to nylon fibers were earlier demonstrated[10]. The results of the present study suggest that estradiol action on the corpus luteum of pregnant hamster could be mediated *via* the tubulin system.

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