# INVOLVEMENT OF MICROTUBULE AND MICROFILAMENT SYSTEM IN THE GnRH-INDUCED RELEASE OF GONADOTROPINS BY RAT ANTERIOR PITUITARY CELLS IN CULTURE

Ashok KHAR, J. KUNERT-RADEK* and Marian JUTISZ
*Laboratoire des Hormones Polypeptidiques, C.N.R.S., 91190 Gif-sur-Yvette, France*

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## 1. Introduction

Microtubules and microfilaments have been shown to be closely associated with the plasma membrane of many types of cells [1–4]. They are probably involved in the control of the redistribution and mobility of the receptors [1,5,6] and therefore they play a key role in the formation of hormone–receptor complexes. On the other hand, microtubules and microfilaments are also involved in the secretion processes of some proteins and polypeptide hormones [7].

Two pharmacological drugs, colchicine which causes disaggregation of microtubules [8] and cytochalasins which impair the function of microfilaments [1,9] have been widely used to study the role of microtubules and microfilaments in the cellular functions.

Microtubules have been attributed a role in the secretion processes of many glands: thyroid gland [10], pancreatic islets [11] and adrenal medulla [12]. Recent work of Zor et al. [13] suggests that microfilaments, but not microtubules, are intimately involved in the process of LH-stimulated ovarian adenylate cyclase activity, while the action of FSH is dependent on both microfilaments and microtubules.

Labrie et al. [14] have shown that deuterium oxide and vincristine, which prevent the action of microtubules, inhibited the basal and induced release of growth hormone and prolactin from rat anterior pituitaries incubated in vitro. Colchicine was found to suppress the high $K^+$ or hypothalamic extract stimulated release of ACTH by rat pituitaries in vitro [15]. In contradiction to the above reported results concerning the inhibitory effect of vincristine and colchicine on pituitary hormone release, others [16] observed enhancement of basal release of growth hormone from anterior pituitaries incubated with colchicine. Sundberg et al. [17] reported that colchicine significantly enhanced the action of hypothalamic extracts on the release of LH and FSH by hemipituitaries in vitro.

We describe here the action of colchicine and cytochalasin B on the basal, GnRH and high potassium stimulated release of gonadotropins by pituitary cell cultures. The possible role of microtubules and microfilaments in this process is discussed.

## 2. Materials and methods

### 2.1. *Chemicals*

Colchicine and cytochalasin B were purchased from Sigma, U.S.A. All other chemicals used were of the reagent grade.

### 2.2. *Cell culture and incubations*

Dispersed rat anterior pituitary cells in monolayer cultures were prepared according to Debeljuk et al. [18]. In the experiments shown in table 1 the incubations were carried out in medium F-10 in the presence of colchicine and cytochalasin B. For the preincuba-

* On leave from Institute of Endocrinology, Medical School of Lodz, Poland.

Send all correspondence to Dr Ashok Khar on the above address.

Table 1
Lack of immediate effect of colchicine and cytochalasin B on the basal and GnRH stimulated release of gonadotropins from pituitary cell cultures[a]

| Group | GnRH (ng/ml) | LH (ng/ml ± SEM) | FSH (ng/ml ± SEM) |
|---|---|---|---|
| Control | – | 8.7 ± 1.70 | 27.7 ± 1.61 |
| Colchicine (3 μg/ml) | – | 10.2 ± 0.60 | 24.3 ± 2.13 |
| Cytochalasin B (3 μg/ml) | – | 9.4 ± 0.65 | 25.8 ± 1.36 |
| GnRH | 9.0 | 68.9 ± 1.52 | 124.2 ± 9.97 |
| Colchicine (3 μg/ml) | 9.0 | 69.0 ± 0.97 | 107.1 ± 6.25 |
| Cytochalasin B (3 μg/ml) | 9.0 | 64.8 ± 2.53 | 105.4 ± 2.91 |

[a] On the 7th day of culture, the cells were incubated for 4 hr in medium F-10. 8 Petri dishes were used per group

tion studies the drugs were added in the culture medium at a given time before the start of the actual incubation on the 7th day of the culture. After washing the cells, the incubations were carried out in the synthetic medium F-10 with or without GnRH for 4 h. For the studies with high $K^+$, the cells were incubated in Krebs-Ringer bicarbonate (KRB) buffer containing 59 mM $K^+$ (instead of $Na^+$), glucose (16 mM) and HEPES (25 mM).

### 2.3. *Radioimmunoassays*

LH and FSH were estimated by the double antibody radioimmunoassay method [19]. Antisera against oLH-β and oFSH were kindly provided by Dr B. Kerdelhué from this laboratory. Rat LH (1.26 × NIH-LH-S1, 0.94–1.69, $p = 0.05$) and FSH (37.60 × NIH-FSH-S1, 26.27–53.96, $p = 0.05$) prepared in this laboratory were used as standard hormones.

## 3. Results

Table 1 shows the effect of colchicine (3 μg/ml) and cytochalasin B (3 μg/ml) on the basal and GnRH stimulated release of LH and FSH from pituitary cells

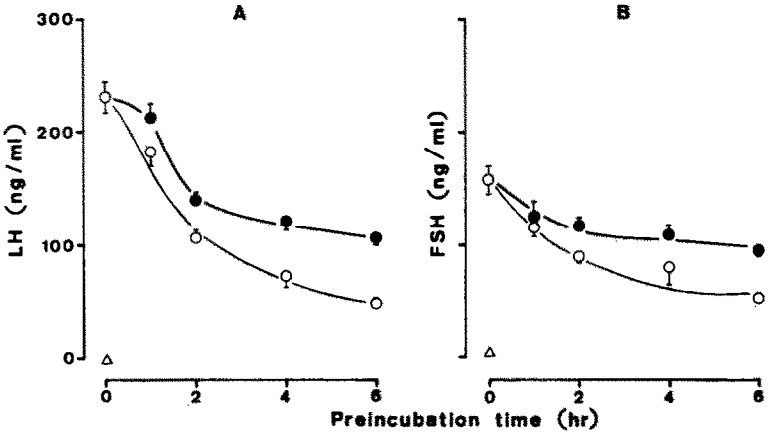


Fig.1. Effect of different time of preincubation with colchicine (3 μl/ml), ○ and cytochalasin B (3 μl/ml), ● on the GnRH (9 ng/ml)-induced release of LH (A) and FSH (B) by pituitary cells in culture. After the preincubation the drugs were removed and the incubations were carried out in F-10 for 4 h. △, denotes the basal release of LH and FSH without any drug treatment after 4 h incubation.

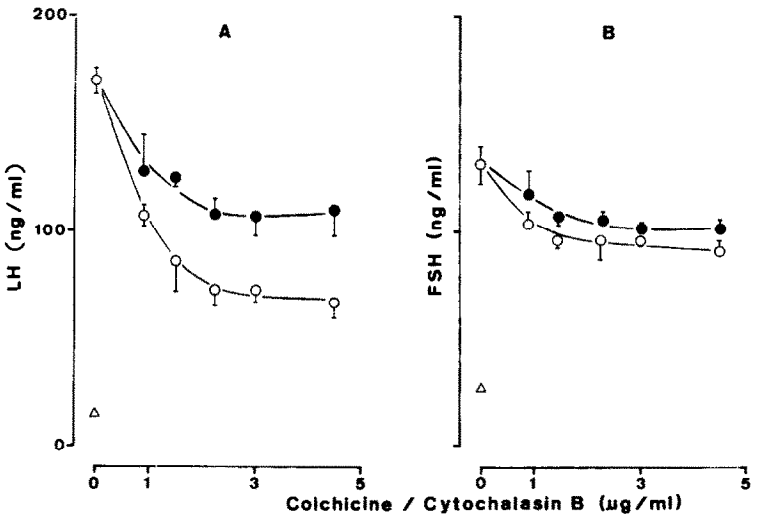


Fig.2. Effect of different concentrations of colchicine, ○ and cytochalasin B, ● on the GnRH (9 ng/ml)-induced release of LH (A) and FSH (B) by pituitary cells in culture. Preincubation with drugs was done for 2 h and, after washing the cells the incubations were carried out in F-10 for 4 h in the presence of GnRH (9 ng/ml). The basal release of LH and FSH without any treatment is denoted by △.

in culture. When the drugs are introduced at the start of the incubation along with GnRH, under these conditions the two drugs had no significant effect neither on the basal nor on the GnRH stimulated release of gonadotropins. There was about 8-fold release of LH and about 4.5-fold release of FSH in the presence of GnRH (9 ng/ml) alone.

When the cells were preincubated with colchicine

Table 2
Lack of effect of colchicine and cytochalasin B on the high $K^+$ induced release of LH and FSH by cultured pituitary cells[a]

| Group | Preincubation time (h) with drugs | LH (ng/ml ± SEM) | FSH (ng/ml ± SEM) |
|---|---|---|---|
| Control | – | 7.5 ± 1.06 | 34.0 ± 2.81 |
| 59 mM $K^+$ | – | 59.1 ± 3.57 | 183.3 ± 7.29 |
| Colchicine (3 μg/ml) + 59 mM $K^+$ | 2 | 67.6 ± 3.75 | 193.9 ± 11.53 |
| Cytochalasin B (3 μg/ml) + 59 mM $K^+$ | 2 | 65.8 ± 3.39 | 201.2 ± 12.94 |
| Colchicine (3 μg/ml) + 59 mM $K^+$ | 4 | 68.6 ± 2.24 | 180.1 ± 17.12 |
| Cytochalasin B (3 μg/ml) + 59 mM $K^+$ | 4 | 57.4 ± 3.00 | 205.8 ± 13.95 |

[a] Cells (6 Petri dishes per group) were preincubated with drugs in the culture medium for a given time, then, after washing the cells, the medium was replaced by KRB-buffer containing 59 mM $K^+$ (instead of $Na^+$), glucose (16 mM) and HEPES (25 mM). In the control group the concentration of $K^+$ was 5.9 mM

or cytochalasin B before the actual incubation with GnRH (9 ng/ml), the release of LH and FSH was significantly inhibited. Figure 1 shows the effect of different preincubation times (2 to 6 h) on the GnRH-induced release of LH and FSH by pituitary cells. There was about 53% and 40% inhibition of the release of LH after 2 h preincubation with colchicine and cytochalasin B respectively (fig.1A). The inhibition increased with time of incubation. Colchicine and cytochalasin B, respectively (fig.1A). The inhibi- release of FSH, which was after 2 h preincubation of the order of 46% and 28%, respectively (fig.1B). As for LH, the inhibition of the FSH release increased with time of incubation in the presence of the drugs.

Figure 2 shows the effect of different concentrations (0.75 to 4.5 $\mu$g) of colchicine and cytochalasin B on the release of LH (fig.2A) and FSH (fig.2B) after 2 h preincubation of the cells with the drug. There was about 57% inhibition of the release of LH and 28% of that of FSH with 3 $\mu$g/ml (7.5 $\mu$M) colchicine. Similarly cytochalasin B inhibited the release of LH (27%) and that of FSH (25%) at the concentration of 3 $\mu$g/ml (6.3 $\mu$M).

The effect of colchicine (3 $\mu$g/ml) and cytochalasin B (3 $\mu$g/ml) on the release of gonadotropins in the presence of high $K^+$ (59 mM) is shown in table 2. There was no significant inhibition of the release of LH and FSH by colchicine or cytochalasin B when the cells were incubated in high $K^+$ KRB, although the release of gonadotropins was significant in the presence of high $K^+$.

## 4. Discussion

Although the cellular mechanism of action of GnRH is not yet clear the first step of GnRH action, i.e., binding to the receptors present on the plasma membrane of gonadotropic cells is well established [20,21]. The binding step is probably followed, through a mechanism which still has to be clarified, by the transport of LH- and FSH-containing granules to the plasma membrane and the exocytosis of the hormones.

Our results show that colchicine and cytochalasin B, when introduced directly into the cell incubation media, do not inhibit the basal or the GnRH-induced release of LH and FSH. The inhibition is only observed when the cell cultures are preincubated with the drugs for at least 2 h. Therefore, it seems that the drugs need some time to exert a significant effect. Similar observations were made with other cell types where inhibition was observed only after the preincubation of cells in the presence of drugs for at least 2–3 h [14,15].

Our results are in contradiction with those of Sundberg et al. [17] who have shown a stimulatory effect of colchicine on the hypothalamic extract-mediated and high $K^+$ mediated release of LH and FSH by rat anterior pituitaries in vitro. The discrepancy between our results and those of Sundberg et al. may be due to the different experimental material used by the two groups. The latter authors employed halved rat anterior pituitaries and crude rat hypothalamic extracts, while in our work pituitary cell cultures and synthetic GnRH were used. It is well known that methods using incubation of pituitary halves give less precise results than those employing cell cultures [22]. On the other hand, hypothalamic extracts contain, in addition to GnRH, many other substances, some of which could interfere with colchicine. The discrepancy between the results of Sundberg et al. [17] with colchicine and high $K^+$ medium and our data is however surprising. Moreover, it should be noted that the latter authors reported the results of one single experiment.

Our results concerning the inhibitory action of colchicine on the secretory processes are in agreement with the observations of several authors [8,13,23,24].

However, in our work colchicine and cytochalasin B had no significant effect on the high $K^+$-induced release of LH and FSH after 2 and 4 h preincubation of cells in the presence of drugs (table 2). Therefore it seems that the mechanism governing the releasing process in a high $K^+$ medium may be different from that governing the hormone-induced release. In high $K^+$ medium, a proportion of the cells of the adenohypophysis will depolarize [25] and this depolarization, which would be expected to decrease the transmembrane potential of the cells [15], will probably lead to an alteration in the permeability characteristics of the plasma membrane. One could postulate that releasing of several anterior pituitary hormones in a high $K^+$ medium, but not in the case of the hormone-induced release, results from the alteration of the permeability of the plasma membrane.

Colchicine and cytochalasin B had no effect on the incorporation of $^{14}$C-labelled amino acids into total cellular proteins (data not shown) suggesting thereby that the biosynthesis of proteins is not effected by these drugs. Similar results have been observed by Chambaut-Guérin et al. [23] in the case of rat lacrimal glands.

The drugs colchicine and cytochalasin B seem quite specific in their action [12]. Cytochalasin B might inhibit the binding of GnRH to its receptor sites on the cell membrane and/or impair the mobility of the receptor within the membrane since this drug has been shown to inhibit the binding of insulin and growth hormone to their receptor sites [26]. Colchicine on the other hand may impair the mobility of GnRH-receptor complex or secretory granules through the microtubular system in the cell since aggregates comprised of the microtubular material have been observed in the cells treated with colchicine [24].

Under our experimental conditions, a cytotoxic effect of the drug at the doses used can be ruled out since the cells in consideration (gonadotrophs) still responded fully to high $K^+$ and partially to the stimulation with GnRH. Routine microscopic examination of the cells after trypan blue staining demonstrated the viability of most of the cells in the Petri dishes. On the other hand, after the preincubation with colchicine or cytochalasin B, the cells were still attached to the bottom of the dish which is a normal characteristic of the living cells in culture.

From the data presented here, it seems that the microtubule-microfilament system is involved in the action of GnRH but not in that of high $K^+$ on gonadotropic cells. However, further work would be necessary to know the mechanism by which this system intervenes in the process of gonadotropin secretion.

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