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Role of microtubules and microfilaments in polypeptide and steroid hormone secretion: a review

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Abstract

The important part played by the cytoskeleton in the dynamic process of polypeptide and steroid hormone secretion has been examined. Certain specific endocrine tissues have been chosen to analyse the information available regarding hormone secretion. For example, for polypeptide hormones the anterior lobe of the pituitary and the islets of Langerhans have been discussed whereas for the steroid hormones, the ovaries and the adrenals have been chosen for discussion.

Evidence suggesting the involvement of exocytosis in the secretion of the two types of hormones has been discussed. Most of the data regarding the role of the cytoskeleton in hormone secretion has so far been obtained by employing drugs known to affect either microfilaments or microtubules–thereby studying the effects of pharmacological doses of the drugs on hormonal secretion. At these high doses, the specificity of the drug effects is questionable. On the other hand, however, a few investigators have examined the biochemistry of the cytoskeleton during hormone secretion. Sensitive methods have been standardised to study the equilibrium between soluble and polymerised tubulin or actin pools, the assembly of tubulin into microtubules and the GTPase activity of tubulin.

Key words: Secretion, tubulin, actin, hormones.

1. Introduction

Although reviews[1–3] on the role of the cytoskeleton in hormone action were published not too long ago, discoveries made since then have prompted us to attempt this treatise. Although hormone secretion involves components besides cytoskeleton, such as cAMP, Ca^{2+}, calmodulin and phosphoinositides, we have decided to restrict the scope of this paper to the role of microtubules and microfilaments in hormone secretion. We will examine the present understanding of polypeptide and steroid hormone secretion and the studies

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on the role of cytoskeleton therein. We will present evidence to support the theory that secretion of protein as well as steriod hormones follows a similar pattern in that these two types of hormones are packaged into secretory granules and are transported to the plasma membrane by the microtubular system for subsequent exocytosis. We will also examine the reports supporting the hypothesis that steroid hormones interact with the cytoskeleton prior to activating the genome.

2. Cytoskeleton

For brevity, we will include only the important aspects of the three components of the cytoskeleton. For more information regarding these cytoskeletal elements, the reader is referred to detailed works on microtubules[4-8], actin[9-11] and intermediate filaments[12].

2.1. *Microtubules*

Tubulin, the monomeric unit of microtubules, has a molecular weight of about 110,000 daltons and is composed of two non-identical subunits, α and β. Tubulins have remained very stable in evolution, histones apparently being the only class of proteins which have undergone less change since the origin of eukaryotes. Common antigenic determinants in microtubules from mammals, birds, reptiles, teleosts and diptera have been reported[13]. Tubulin is a glycoprotein with approximately 1.2 mole neutral sugar per dimer[14]. The possibility of tubulin being associated with phospholipids was suggested[15] by experiments wherein the addition of phospholipase A to brain extract having the capacity to form microtubules, prevented the assembly at 37°C.

Tubulin has two guanosine nucleotide-binding sites per dimer. One is exchangeable (E), and the other nonexchangeable (N). Although at both sites GTP is bound non-covalently, the N-site can only be removed by denaturing the protein, whereas the E-site GTP is exchangeable with free GTP. The E-site GTP is hydrolysed during tubulin polymerisation[8,16]. Tubulin is accompanied on the electrophoresis columns by several associated proteins of higher molecular weight. The presence of these microtubule-associated proteins (MAPs), often in stoichiometric relation to tubulin, suggests a regulatory role of MAPs in the structure of microtubules[17].

Tubulin from rat brain could be assembled *in vitro*, to form microtubules[18] with an exterior diameter of 24 nm, provided the following conditions are met: 1) concentration of tubulin is sufficiently high, 2) GTP is added, 3) Ca^{2+} is removed using EGTA, and 4) the temperature is 37°C with a pH of 6.8. The assembly reaction is inhibited at cold temperatures. Microtubules generally grow in definite directions from initiation centres such as basal bodies or centrioles. Studies conducted *in vitro* on the directionality of microtubule growth from such initiation sites revealed that microtubules mainly increase their length in one direction. Tubulin assembly appears to be a sequential process, consisting of nucleation, elongation, monomer-polymer equilibrium and length redistribution[8,19-36].

There are quite a few reliable methods[37] to monitor the *in vitro* assembly of tubulin into microtubules: turbidity measurements at 350 nm, electron microscopy, viscosity measure-

ments and sedimentation of the polymer, estimation of the monomers remaining in the supernatant, flow birefringence, dark-field light microscopy, x-ray diffraction, laser light scattering, filtration, calorimetry and immunochemistry. Microtubule function is generally investigated using colchicine[38–41], vinblastine[42], D_2O[43,44] and Taxol[45–48] (see refs 40, 42, 43 for reviews). Colchicine is considered to be a prototype of the class of chemical inhibitors that act on microtubules. It is known that colchicine inhibits the assembly of tubulin monomers into microtubule polymers, resulting finally in the accumulation of tubulin in soluble pools. Addition of vinblastine to cells results in precipitation of tubulin. On the other hand, D_2O and Taxol are known to alter the equilibrium between soluble and polymerised form of tubulin by overstabilising them in their polymerised form.

2.2. *Microfilaments*

Actin is ubiquitous in eukaryotes and has been highly conserved during evolution[49,50]. Actin comprises 15–20% of the total cell protein, and approximately 50% of total actin in non-muscle cells exists as a soluble pool, *i.e.*, not polymerised into F-actin. G-actin is a 42,000 dalton protein with one mol ATP bound per monomer and one mol Ca^{2+} bound to a specific divalent cation-binding site, different from that of the ATP-binding site[51,52]. There are many proteins that form tight complexes with G-actin, thus facilitating an increase in the pool of non-polymerised actin in the cell; there are also proteins in the cell that are known to accelerate the rate of actin depolymerisation[11]. G-actin, under defined *in vitro* conditions, polymerises into F-actin[10]. The microfilament polymer is a two-start, double-stranded, right-handed helix. The filament diameter is approximately 5 to 7 nm. The polymerisation process is similar to that of tubulin, consisting of nucleation, elongation, and monomer-polymer equilibrium. Actin has an intrinsic ATPase activity. G-actin as well as F-actin are both capable of ATP hydrolysis[53,54]. A continued hydrolysis of ATP by F-actin takes place during the interconversion of G-actin to F-actin. This does not mean that ATP hydrolysis is a prerequisite for polymerisation. The rate of polymerisation and the critical concentration of actin are the same in the presence of the non-hydrolysable nucleotide AMPPNP as in the presence of ATP[55]. Cytochalasins, a group of low-molecular weight fungal metabolites, stimulate the ATPase activity of G-actin by 30 fold. Cytochalasin B preferentially blocks elongation of F-actin, and cytochalasins, in general, inhibit the rate of actin polymerisation[56,57]. Methods to monitor the assembly of G-actin to F-actin are similar to those mentioned above for tubulin[58].

2.3. *Intermediate filaments*

In contrast to microtubules and microfilaments, the intermediate filaments are composed of heterogeneous subunit proteins[59–64]. The biochemical and immunological differences in these subunit proteins have resulted in identification of five major subclasses of intermediate filaments: 1) tonofilaments consisting of cyto- or prekeratins (40–68 kDa), 2) neurofilaments composed of triplet proteins (68,160,200 kDa), 3) glial filaments with glial fibrillary acidic proteins (51 kDa), 4) desmin filaments made up of desmin (53 kDa) and 5) vimentin filaments with vimentin (53 kDa). Intermediate filaments are highly insoluble in physiological ionic solutions. The filaments can be solubilised into their constituent subunit

proteins only under denaturing conditions or at very low ionic strength[65]. The denatured subunit proteins assemble into intermediate filaments (inner diameter 7–11 nm); this process is independent of accessory proteins or other cofactors, suggesting that the information to assemble into the polymer is present in the secondary structure of the subunit proteins themselves. A pool of unpolymerised subunit proteins does not seem to exist in the cell. The intermediate filament polymers are extraordinarily stable, and, to date, there is no known specific agent that causes depolymerisation of the intermediate filament polymers[12] The recent finding that the Ca^{2+}-activated proteolytic enzymes are able to degrade the intermediate filaments *in vitro* led to the hypothesis that the changes in intracellular Ca^{2+} concentration could be regulating the assembly and disassembly *in vivo*[12,66–68].

There is a considerable body of evidence that intermediate filaments interact with microtubules and microfilaments[69–76]; the disassembly of microtubules and microfilaments by drugs such as colchicine or cytochalasin is also accompanied by a rearrangement of intermediate filaments[69,77–85]. Microinjection of antibodies against intermediate filament proteins into cells results in a rapid and reversible collapse of all the intermediate filaments into a tight perinuclear cap. However, this dramatic rearrangement of intermediate filaments did not change the cytoplasmic distribution of microtubules and microfilaments, and the microinjected cells retained normal mitotic and cytokinetic properties[86–89].

Intermediate filaments as they normally occur in the cell are considered to play a structural role in reinforcing the cytoskeleton[90]. Recent reports[91–94], however, suggest that the subunit proteins of the intermediate filaments could act as transmitters of signals from the cell periphery to the nucleus. The subunit proteins are known to bind to nucleic acids[91]. Purified vimentin binds to different fractions of avian erythrocyte membranes through two distinct domains[93,94]. Sites located at the carboxy-terminal end of the vimentin molecule bind specifically to nuclear envelopes in a cooperative fashion; the plasma membrane fraction interacts in a saturable manner with the amino-terminal head of the vimentin molecule.

3. Mechanism of hormone secretion

The general pathway of polypeptide hormone secretion in vertebrates is well documented[95–100]. From their site of synthesis in the rough endoplasmic reticulum, the proteins are transported to the Golgi apparatus, where glycosylation and packaging into secretory granules take place. From the Golgi apparatus the secretory granules are transported to the cell membrane where exocytosis occurs. Exocytosis is a process by which cells release materials into the extracellular milieu. Upon appropriate stimulus, the secretory granules migrate to the luminal plasma membrane, fuse with the membrane, and the hormones are then secreted.

On the other hand, it was believed that steroid hormones, after being synthesised in the steroidogenic tissue, are released into the extracellular milieu by simple diffusion[101,102]. However, recent reports[103–109] suggest that steroid hormone secretion by the corpus luteum as well as the adrenals could be an active process related to or identical with exocytosis. A correlation between the presence and concentration of densely staining granules in the corpus luteum and progesterone secretion has been noted[103]. Quirk *et al*[105]

reported that progesterone is localised within electron-dense granules and suggested that these granules are released into extracellular medium by exocytosis. Sawyer *et al*[104] proposed that a progesterone-carrier protein (without progesterone) was packaged into secretory granules at the level of the Golgi apparatus; these granules pick up progesterone from the tubular elements of the agranular endoplasmic reticulum as they move towards the plasma membrane; finally the granules are exocytosed.

Although it is generally accepted that cytoskeleton plays a major role in the transport of secretory granules from the Golgi apparatus to the plasma membrane, the exact mechanism of this transport is yet to be delineated. Most of the work regarding the involvement of cytoskeleton in hormone secretion was performed using drugs such as colchicine, vinblastine or cytochalasin. These agents are known to perturb either the microtubules or microfilaments, and the effects these compounds exert on hormone secretion have been related to the cytoskeleton. However, more often than not, these agents were employed at high doses and at these concentrations the drugs are known to affect other cellular functions as well[4,110–115], besides disrupting the cytoskeleton. Therefore, the results obtained with the drugs should be viewed with caution.

The very heterogeneous nature of intermediate filaments—there being at least five protein subunits—and the unavailability of a drug known to affect their function, have made it difficult for the endocrinologist to study the role of the intermediate filaments during hormone secretion. However, the three components of the cytoskeleton are known to interact with each other, and any stimulus affecting one of the three could also affect the other two.

3.1. *Anterior pituitary lobe*

Anti-cytoskeletal drugs were employed to demonstrate the involvement of microtubules and microfilaments in the secretion of gonadotropins by the anterior pituitary lobe in response to GnRH[116–121]. Colchicine was observed to: inhibit the secretion of ACTH[122] in the rat *in vitro*; accumulate secretory granules and cause disappearance of microtubules in somatotrophic cells[123]; inhibit PRL and GH secretion[124–131]. In only one case colchicine was shown to stimulate[132] the release of LH, FSH, and TSH. Vincristine, another drug which interacts with the microtubules, was shown to inhibit PRL and GH release[128]. All these studies[116–132] employed high concentrations of the drugs, and the specificity of their effects on the microtubules was not evaluated with the use of lumicolchicine, an inactive isomer of colchicine which does not affect the microtubule function.

Shino *et al* employed electron microscopy to correlate PRL secretion to the microtubules in the anterior pituitary lobe[133]. Although they visualised the microtubules, they could not observe changes in the tubulin during increased PRL secretion. Sheterline *et al*, using morphometric techniques, demonstrated that stimulation of GH secretion from bovine somatotrophs by non-physiological secretagogues, was accompanied by a decrease in the polymerised microtubules[126]. Holck *et al*, employing immunofluorescent methods, observed more intense tubulin staining of gonadotrophs in castrated rats[134]. This finding was later confirmed by Valenti *et al* by direct measurements of total tubulin by the colchicine binding assay[135,136]. Using morphological techniques, it was demonstrated that in human FSH-

producing adenomas, microtubules were often conspicuous and might increase in number in the cytoplasm of the adenoma cell[137]; the gonadotrophic cells of the non-tumorous pituitary contained only a few microtubules[138]. Niwa *et al* demonstrated that *in vitro* treatment of rat prolactinoma cells with bromocriptine resulted in changing the fine reticular networks of microtubules into coarse aggregates, thus implicating microtubules in PRL secretion[139].

In our attempts to examine the anterior pituitary lobe microtubules, we have chosen the lactating rat as our model and stimulated PRL secretion by suckling the mothers with the pups. Instead of using drugs which are known to disrupt the microtubule system and then examining hormone secretion, we have applied a physiological stimulus to release PRL from the anterior pituitary lobe and then studied the pituitary tubulin status. Using the ^{3}H-colchicine-binding assay, we have initially observed that suckling resulted in a significant elevation in tubulin levels in the anterior pituitary lobe[140]. Next we have standardised the buffer conditions to estimate the soluble and polymerised tubulin pools in the anterior pituitary lobe and reported that suckling resulted in a shift in the equilibrium between soluble and polymerised tubulin pools in the anterior pituitary[141]. The total amount of the two tubulin pools did not change appreciably during suckling (fig. 1). In another study we have assayed the GTPase activity of the soluble and polymerised tubulin pools and observed (fig. 2) that the GTPase activities of the two tubulin pools during suckling also appeared to be in a dynamic flux[142]. We followed these observations with the standardization of a method to monitor the *in vitro* assembly of anterior pituitary lobe tubulin into

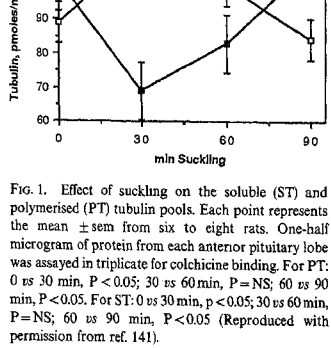

FIG. 1. Effect of suckling on the soluble (ST) and polymerised (PT) tubulin pools. Each point represents the mean ± sem from six to eight rats. One-half microgram of protein from each anterior pituitary lobe was assayed in triplicate for colchicine binding. For PT: 0 *vs* 30 min, $P < 0.05$; 30 *vs* 60 min, P = NS; 60 *vs* 90 min, $P < 0.05$. For ST: 0 *vs* 30 min, $p < 0.05$; 30 *vs* 60 min, P = NS; 60 *vs* 90 min, $P < 0.05$ (Reproduced with permission from ref. 141).

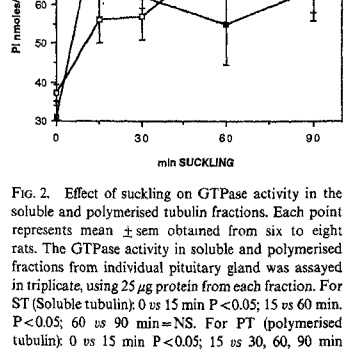

FIG. 2. Effect of suckling on GTPase activity in the soluble and polymerised tubulin fractions. Each point represents mean ± sem obtained from six to eight rats. The GTPase activity in soluble and polymerised fractions from individual pituitary gland was assayed in triplicate, using 25 μg protein from each fraction. For ST (Soluble tubulin): 0 *vs* 15 min $P < 0.05$; 15 *vs* 60 min. $P < 0.05$; 60 *vs* 90 min = NS. For PT (polymerised tubulin): 0 *vs* 15 min $P < 0.05$; 15 *vs* 30, 60, 90 min $P < 0.05$ (Ravindra and Grosvenor, unpublished data).

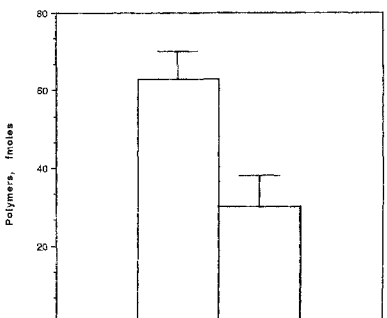

FIG. 3. Effect of suckling on tubulin assembly. The anterior pituitary glands from six suckled (for 30 min) and six nonsuckled rats were individually processed to obtain the 'tubulin fraction'. Protein obtained from each pituitary gland was incubated in duplicate at a concentration of 0.6 mg/ml in MES buffer, pH 6.8, 37°C, GTP 2 mM for one min. The results are expressed as mean ± sem and the suckled group (hatched bar) is significantly different ($P < 0.05$) from the nonsuckled group (open bar) (Figure reproduced with permission from reference 143).

microtubules. This technique was found to be sensitive and capable of measuring the assembly in approximately 2 μg of tubulin present in the 100,000 × g supernatants. After extensively validating this protocol we have observed (fig. 3) that suckling affected the extent to which anterior pituitary tubulin of the rat assembled into microtubules[143]. These observations are consistent with the hypothesis that microtubules are being recruited to transport PRL granules from the Golgi apparatus to the plasma membrane. This process could conceivably involve many cycles of polymerisation/depolymerisation. Although our studies suggest that microtubules could be involved in PRL secretion by the anterior pituitary lobe, it was pointed out that the changes we have observed in the microtubules during PRL secretion may not be specifically occurring in the lactotrophs, as we were dealing with the total cell population of the anterior pituitary lobe. In the rat, suckling stimulates the release of TSH[144,145], GH[146] and ACTH/β-endorphin[147] in addition to PRL. However, electron microscopy has revealed that in most species examined, PRL secretory cells increase in number and cytoplasmic volume and become the dominant cell type during lactation[148]. We have attempted to address this question by employing drugs that are known to affect PRL secretion. We have demonstrated that domperidone-elevated anterior pituitary lobe polymerised tubulin levels and plasma PRL concentration comparable to those increases caused by suckling. Bromocriptine blocked the suckling-induced rise in polymerised tubulin and the rise in plasma PRL levels as well[141]. Domperidone was also demonstrated to increase the soluble tubulin GTPase activity comparable with that

at 15 min suckling, and bromocriptine blocked the suckling-induced rise in the polymerised tubulin GTPase activity (Ravindra & Grosvenor, manuscript in preparation). The *in vitro* assembly of anterior pituitary lobe tubulin into microtubules was also affected by domperidone, bromocriptine, and oPRL[149]. Treatment of lactating rats with oPRL prior to suckling was shown to inhibit the release of PRL into plasma[150]; the same dose of oPRL also resulted in a shift in the equilibrium in the two tubulin pools and their GTPase activities (Ravindra & Grosvenor, unpublished results). Furthermore, we have observed that the equilibrium between the two tubulin pools was also affected in the lactotrophs of ovariectomised rats primed with estradiol (Ravindra, Hymer & Grosvenor, preliminary observations). Thus, these results support our contention that the changes seen in the anterior pituitary microtubules in response to a physiological stimulus could be correlated to those occurring in the lactotrophs during PRL secretion.

Sherline *et al* reported that porcine pituitary secretory granules, enriched in GH and PRL, bound to brain microtubules *in vitro*, but not to depolymerised microtubules (*i.e.*, tubulin), suggesting that microtubules might facilitate the cell interior to cell surface movement of PRL secretory granules by providing tracks along which granules could move[151]. Bloom *et al* published a good method to purify tubulin from the bovine anterior pituitary tissue and GH_3 cells which secrete GH and PRL[152]. These methods should stimulate more work regarding the biochemical interaction between the secretory granules and tubulin system in the anterior pituitary lobe.

The role of actin in the pituitary hormone secretion has not been as vigorously pursued as that of tubulin. Benzonana *et al* adapted the DNAse method to the anterior pituitary lobe for estimating the G-actin content[153]. Ostlund *et al*[154] demonstrated that anterior pituitary gland secretory granules bound to G-actin *in vitro*.

3.2. *Islets of Langerhans*

The secretion of insulin has been well studied, using either the whole organ or isolated cells[155–160]. Although the bulk of the work was done using pharmacological doses of drugs known to disrupt the cytoskeleton, some studies combined this approach with morphological examination of the cytoskeleton, while others investigated the biochemistry of the cytoskeleton in these cells. Taken together, these results suggest that insulin secretion is a two-step process, involving both microtubules and microfilaments. The first step involves the transport of the insulin secretory granules from the Golgi apparatus to the periphery of the cell; microtubules appear to be involved in this process. In the second step, the insulin-containing secretory granules which are at the periphery of the cell membrane fuse with the plasma membrane to release insulin into the extracellular milieu; microfilaments are implicated in this step.

The secretion of glucagon has not received much attention, and the reports are rather ambiguous[161,162].

3.3. *Adrenals*

Information regarding adrenal steroid secretion was obtained mainly with the use of drugs

that are known to interact with the cytoskeleton. Vinblastine caused the appearance of tubulin crystals in the rat[163] and stimulated the secretion of adrenal steroid hormones in the mouse[164]. Stimulation of tissue cultures by ACTH and cAMP appeared to increase the number of visible microtubules as seen by immunofluorescence, indicating that the hormone might induce tubulin polymerisation. The microtubules thus formed could help in the translocation of steroid containing granules[165]. Payet *et al* reported that administration of colchicine to rats stimulated aldosterone and corticosterone secretion[166]. Colchicine and other antimicrotubular drugs increased steroid secretion by cultures of Y-1 adrenal tumor cells[167], and in normal rat adrenocortical cells[168,169]. O'Hare, however, found that steroid secretion in the normal rat adrenal cells was inhibited by these drugs[170].

Cytochalasins stimulated Y-1 cell steroidogenesis within an hour of incubation *in vitro*; this effect of the drug was reversible by washing[171]. On the other hand, other studies found that in the Y-1 cells, cytochalasin and anti-actin antibodies inhibited the ACTH- or cAMP-stimulated steroid secretion[2,3,172]. Cytochalasin also inhibited the ACTH-stimulated steroid secretion by normal adrenal cells[173,174]. Employing dispersed bovine adrenal cells, it was reported that actin fibre bundles were distributed transversely in the cytoplasm; after the addition of ACTH or cAMP the microfilaments became inconspicuous with dot-like appearance and their distribution pattern was altered from circular to radial[175]. It was also observed[176] that after ACTH treatment, bovine adrenocortical cells became rounded with the breakdown of microtubules. It is doubtful that there is any correlation between steroidogenesis and cell shape. Using immunoelectron microscopy, Loesser and Malamed[177] showed that in freshly isolated rat adrenocortical cells, ACTH had no effect in actin content in cytoplasm, mitochondria or lipid droplets; ACTH increased the actin concentration in the peripheral cytoplasmic band. These findings are in contradiction to those reported by Cheitlin and Ramachandran[178,179]. These conflicting results suggest that more work needs to be done to clarify the role of cytoskeleton in the secretion of adrenal steroids.

3.4. *Ovary*

Soto *et al*[180] reported that the stimulation of progestins by hCG and hLH in human granulosa cells *in vitro* was accompanied by an alteration in the cell shape, and the changes in cell shape brought about by the hormones were mimicked by treating the cultures with cytochalasin B or D. However, the response of the cells to these agents was much more rapid than the hormones, occurring within 20 min as compared to 4 h with the hormones. Using rat granulosa cell cultures, it was observed[181–184] that colchicine, cytochalasin and Ca^{2+} ionophore-stimulated steroid secretion and was accompanied by a change in cell morphology. Zor *et al*[185] demonstrated that the presence of anti-actin antibodies or cytochalasin B in the culture medium prevented the rat Graafian follicles from responding to LH or FSH; they also reported that colchicine did not impair the response to LH but prevented the stimulatory effect of FSH and PGE_2 on follicular cAMP production.

In bovine luteal cells colcemid, vinblastine and cytochalasin B inhibited the cAMP- or LH-induced morphological changes, suggesting that LH and cAMP could be promoting

the formation of cytoskeleton[186]. Cytochalasin inhibited hCG-induced progesterone production by rat[187] luteal cells *in vitro*; cytochalasin inhibited basal as well as LH-stimulated progesterone production by the bovine[188] luteal tissue. Cytochalasin and colchicine inhibited progesterone production by ovine[189,190] luteal cells *in vitro*. *In vivo* treatment of ewes[191] and rats[192] with pharmacological doses of colchicine resulted in a significant reduction in plasma progesterone. However, anti-microtubule drugs had no effect on either the basal or LH-stimulated steroid production *in vitro* by collagenase-dispersed rat[187] luteal cells and bovine[188] luteal tissue. Using ovine luteal tissue, Sawyer *et al*[104] reported that LH-stimulated progesterone secretion was significantly reduced in the presence of colchicine. It was observed that *in vivo* colchicine treatment inhibited the *in vitro* progesterone production (cells + medium) by collagenase-dispersed rat luteal cells, but did not alter the microtubule content as assessed by quantitative electron microscopy[193]. We know from experience that sensitive biochemical methods are required to monitor the subtle changes in the equilibrium between the soluble and polymerised tubulin pools[140–143,149].

The use of collagenase or trypsin to prepare cells from the luteal tissue could affect their function, and this might complicate matters when the cells are immediately incubated with cytoskeletal inhibitors. Carnegie *et al* have, in fact, demonstrated[184] that when rat granulosa cells were grown on collagen gels, they secreted almost three fold more progesterone than cells cultured in minimal essential medium alone. Also, it is a general practice to keep the luteal tissue on ice until the required amount of tissue is dissected out of the animals. It is known[8] that *in vitro* microtubules (polymerised from purified brain tubulin) depolymerise when exposed to cold temperatures and can be readily repolymerised by incubating at 37°C. However, we do not have extensive knowledge of the behaviour of microtubules present in a tissue. In one study using the hamster corpora lutea the tissue was not exposed to either enzymes or cold temperatures[194]. The tissue was kept in minimal essential medium at room temperature for not more than 15 min and then was incubated at 37°C. Vinblastine and colchicine inhibited progesterone secretion *in vitro* by the corpora lutea; the effect of colchicine was observed at about 30 min and was significant at 60 min, indicating that these drugs act rapidly. In an attempt to demonstrate the specificity of colchicine binding and relate its effect to the disassembly of microtubules, experiments were conducted with lumicolchicine, an isomer of colchicine that has no effect on microtubule assembly. Lumicolchicine did not inhibit progesterone secretion by the hamster corpora lutea. Moreover, colchicine inhibition of progesterone secretion could be overcome by preincubating the corpora lutea with D_2O. D_2O is known to alter the equilibrium between soluble and polymerised form of tubulin[43,44]. Preincubation of corpora lutea with anti-tubulin antiodies prevented the inhibitory effect of colchicine on progesterone secretion; anti-tubulin antibodies did not affect basal or LH-stimulated progesterone secretion. This observation suggested that the antibody bound to cell surface of the luteal tissue and prevented the effects of colchicine. The presence of a plasma membrane-associated tubulin has been demonstrated in the brain of a few species[195,196]. The presence of plasma membrane-associated tubulin in the hamster corpus luteum might explain these effects of anti-tubulin antibodies. Zor *et al* also observed that the presence of antibodies to tubulin in the medium did not inhibit the stimulatory effect of LH on the rat Graafian follicles[185].

4. Steroid-cytoskeleton interaction

It was observed that estradiol inhibited the progesterone secretion by the hamster corpus luteum by interfering with microtubule function of the luteal cell[194,197–199]. Sub-optimal concentrations of estradiol and colchicine added together resulted in a maximal inhibition of steroid secretion. Preincubating the corpora lutea with either anti-tubulin antibodies or D_2O prevented the inhibitory effect of estradiol on steroid secretion, suggesting that the hormone could be initially binding to a membrane component[194]. The concept that steroid hormones interact with membrane components is relatively new[200–209]. It was assumed until recently[210] that steroid hormones passively diffuse to bind to the cytoplasmic receptors. The hormone-receptor complexes thus formed provoke increased transcription of specific genes, leading to the accumulation of specific mRNAs[211–213]. The initial localisation of the receptor in the absence of hormone is not known. It is suggested that the receptor may be attached to, or a component of, the plasma membrane from which it is quite easily detached during homogenisation and hence obtained in the cytoplasm[214,215]. A marked saturability and temperature dependence of steroid hormone entry, which cannot be attributed to the function of cytoplasmic receptors[216–218], has been noted. Rat endometrial cells exposed to estradiol for a brief period of time exhibited pronounced altered membrane functions[201,202] and micropinocytotic vacuolation of plasmalemma[219].

Reports[219–223] from the Szego laboratory suggest that estradiol interacts with components of biological membranes and may enter cells by a membrane-mediated process. Szego and coworkers demonstrated specific, saturable and temperature-dependent cell surface-binding sites on endometrial cells to estradiol-BSA conjugate immobilised to nylon fibres[203]. Employing carefully controlled homogenisation and isolation procedures that are different from the methods generally used[224], Pietras and Szego[223] demonstrated that approximately 27% of receptor component with high affinity and ligand specificity for binding estradiol-17β is concentrated in plasma membranes purified from isolated uterine cells of ovariectomised rats. This is in contrast to the widely reported occurrence of estradiol receptors in cytosolic fractions[214]. High-affinity and low-capacity receptors for estradiol have also been reported for hepatocyte plasma membrane fractions[205,206]. Estradiol[225] was demonstrated to alter the morphology and arrest mitosis in a Chinese hamster cell line.

There is also evidence that the membrane surface is involved in the progesterone-induced meiosis in *Xenopus laevis* oocytes[226] and LHRH secretion *in vitro* by mediobasal hypothalamic slices of rats[227]. It is known that in endometrial cell suspensions obtained from uteri of ovariectomised rats, estradiol increases Ca^{2+} uptake to a large extent within 30 min[201]. In light of the reports that Ca^{2+} depolymerises microtubules[228,229], it is possible that Ca^{2+} may be mediating the effect of estradiol on progesterone secretion[194,197–199]. Based on their experiments with CHO-K1 cell lines and the effect of testosterone on the cell morphology, Hsie and Puck[230] postulated that the effect of testosterone was mediated *via* the microtubules. Using Drosophila melanogaster Kc cells, Sobrier *et al* demonstrated that the insect-moulting hormone, 20-hydroxyecdysone affected actin and tubulin function as well as their biosynthesis[231]. It was reported that the synthetic estrogen diethystilbestrol inhibited the *in vitro* assembly of brain tubulin[232–234]. Sato *et al* briefly mentioned that

estradiol had no effect on tubulin assembly, but did not give details regarding their experimental conditions[232].

In an attempt to check whether the effect of estradiol on progesterone secretion by the hamster corpus luteum was due to the direct action of this steroid hormone on the tubulin system, rat brain tubulin was purified by phosphocellulose chromatography and the effects of estradiol on tubulin GTPase activity and assembly were monitored. Brain tubulin was used based on the fact that it is a highly conserved protein and the differences between tubulin from hamster corpus luteum and rat brain could be minimal; also, rat brain is a rich source of this protein, whereas too many hamsters had to be sacrificed to purify a few mg of protein from the corpus luteum. Estradiol inhibited tubulin GTPase activity in a dose-dependent manner, and this inhibition could be overcome by excess GTP, suggesting that the effect of this steroid on tubulin function is reversible. Estradiol also inhibited the assembly in a dose-dependent manner, as monitored by turbidimetric measurements and electron microscopy[199]. Taken together, these results[194,197–199] suggest that estradiol inhibited progesterone secretion by the hamster corpora lutea by interfering with the equilibrium between the soluble and polymerised tubulin pools. It is essential to maintain the equilibrium between soluble and polymerised forms of tubulin in cells, and regulatory mechanisms must exist to prevent all cytoplasmic tubulin from assembling into microtubules. It is tempting to speculate that estradiol may be one such molecule which has a role in regulating tubulin function *in vivo*.

5. Perspective

Recent work on the interaction of synaptic vesicles and microtubules provided some interesting data regarding the transport of vesicles on microtubules and could very well inspire similar work on hormone secretion *via* the cytoskeleton (see 235 and 236 for references). In an elegant paper, Gray[235] observed that microtubules are in contact with presynaptic dense projections of the central nervous system leading to the suggestion that microtubules translocated the synaptic vesicles. Subsequently, Baines and Bennett[236] demonstrated that synapsin I, a synaptic vesicle protein from calf brains, bound saturably to microtubules *in vitro*. Crosslinking of microtubules by synapsin I was observed by electron microscopy. Thus, synapsin I could play a role in mediating the synaptic vesicle–microtubule interaction. Other models of cell motility in metazoa are actomyosin-based system in muscle[237], and the dynein-based system in the axonemes of flagella and cilia[238]. Another 'motor' system has been recently described in the chick brain[239], squid giant axons and bovine brain[240]. Subsequently this protein was demonstrated to be widely distributed among organisms and cell cultures[241]. Brady[239] and Vale *et al*[240], working independently, have reported that this protein is distinct in molecular weight and enzymatic behaviour from myosin or dynein. Vale *et al*[240] proposed the name Kinesin (from the Greek *Kinein*, to move). In gel filtration columns, both the squid and bovine translocators elute with an apparent molecular weight of 600 kDa. The quaternary structure of kinesin is yet to be described. Stoichiometry studies by gel densitometry of the polypeptides in highly purified kinesin preparations indicate that kinesin is a complex of two or three different polypeptides. Addition of kinesin to a mixture of highly purified microtubules (free of microtubule-

associated proteins) and membrane organelles from squid axoplasm resulted in the translocation of the organelle on the microtubule as visualised with video-enhanced differential interference contrast microscopy. Trypsinisation of the organelles blocked their movement, suggesting that the binding of the organelle may be protein mediated. Latex beads adsorbed to kinesin could also be translocated along microtubules. Moreover, the movement of microtubules was noted in the presence of kinesin. ATP facilitated the initial interaction between kinesin and microtubules, but the intrinsic ATPase activity of kinesin caused ATP hydrolysis and eventual dissociation of kinesin and microtubules. In the presence of AMP-PNP, a non-hydrolysable analog, kinesin binding to microtubules was enhanced.

Paschal and Vallee[242] reported that MAP1C, one of the five high molecular-mass microtubule-associated proteins, has the ability to translocate microtubules. When microtubules were placed on a glass microscope slide coated with MAP1C, microtubule gliding occurred in a continuous, unidirectional manner. MAP1C was demonstrated to be a soluble form of dynein. It was also shown to be a retrograde translocator *i.e.*, movement from the cell periphery to the cell centre suggesting a role for MAP1C in endocytosis. Kinesin, on the other hand, was observed to operate in the opposite direction, anterograde, *i.e.*, movement from the cell centre to the cell periphery. Thus kinesin appears to be candidate for exocytosis. Future work should reveal if similar 'motor' systems could be characterised in endocrine glands.

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