A Depolymerizing Effect of the Luteinizing Hormone and Follicle Stimulating Hormone on the Neurotubules of the Hypothalamus-Hypophysial Tract in the Rat

Cytoplasmic microtubules are made up of protein subunits which have the ability to change from isolated monomers to fibrous microtubules and vice versa. Much interest is being placed on the structural stability of microtubules. It is known that microtubules in neurons (neurotubules) are relatively more stable than those of other cell types. Disorganization of neurotubules have been reported after in vitro treatments with antimitotic drugs[1], or elevated temperatures[2]. Low temperatures, on the other hand, are effective to depolymerize microtubules in a variety of cells including amphibian neurons[3].

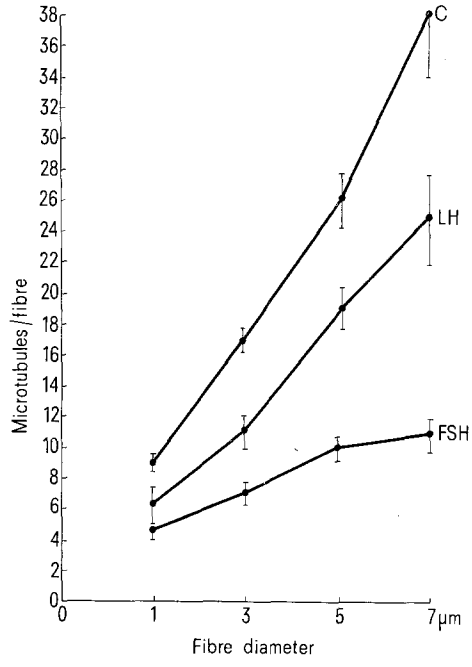

Fig. 1. Number of microtubules per cross sectioned fibres of the hypothalamus-hypophysial tract (means ± standard error of the means). Each value was obtained by counting the microtubules of over 1,300 fibres. Probability between Control (C) and LH treated fibres (LH) $< 2\%$. Probability between C and FSH treated fibres (FSH) $< 3\%$.

In the present account we report a disorganization of neurotubules in nerve fibers of the hypothalamus-hypophysial tract of the rat after in vitro treatments with follicle stimulating hormone (FSH), or luteinizing hormone (LH).

Male rats (120–130 g) were anesthetized with ether, decapitated and their hypothalami dissected out. Each hypothalamus was incubated in 1 ml of medium 199 (Difco). In the control group 0.1 ml of saline was added to the medium. The experimentals were additioned with 0.1 ml of LH (100 μg/ml) or FSH (100 μg/ml) in saline solution. The incubation was performed in a shaker in an atmosphere of 95% O_2, 5% CO_2 at 37°C for 6 h, according to WATANABE et al.[4]. The hypothalami were fixed in KARNOVSKY[5], post fixed in OsO_4 (overnight), dehydrated in ethanol and embedded in Epon. Sections were stained with uranyl-lead.

The amount of microtubules per fibre was found to depend on the fibre diameter. On the basis of this observation, the cross sectioned axons of the hypothalamus-hypophysial tract were classified into 3 groups: 1. 1–3 μm; 2. 3–5 μm; 3. 5–7 μm. Counts of microtubules were performed in each group and the standart errors were small.

Both FSH and LH were found to produce a significant reduction of microtubules in all groups of fibres so far analyzed (Figure 1). FSH was more active (60% of reduction) than LH (30%). High power views of control fibres revealed that most of their microtubules kept the normal spatial pattern of subunits up to the 6th h of incubation (Figure 2). In the material incubated with LH (Figure 3) or FSH (Figure 4), most of the resistent microtubules were expressed by irregular aggregations of subunits (Figures 3 and 4). Images of '8' shaped microtubules were frequently seen in preparations treated with LH but not in the ones incubated with FSH (Figure 3).

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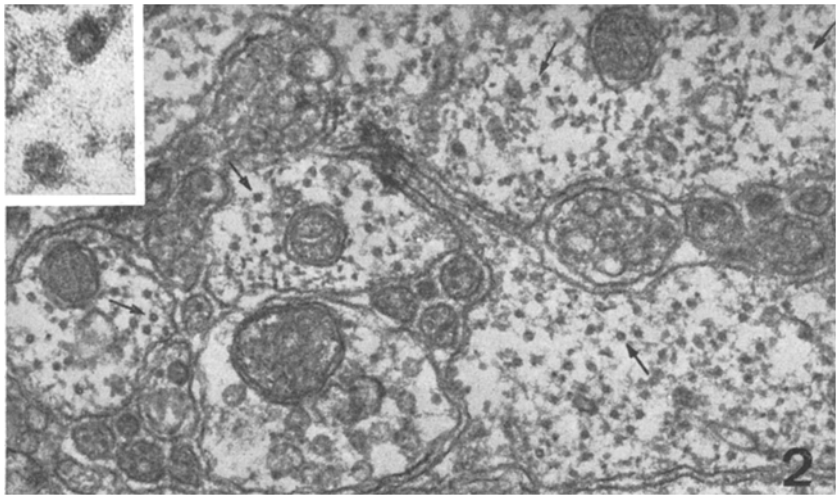

Fig. 2. Hypothalamus-hypophysial tract incubated in medium 199 (control fibres). Microtubules (arrows) are abundant. The inset illustrates 2 microtubules at high magnification. ×40,000. Inset: ×240,000.

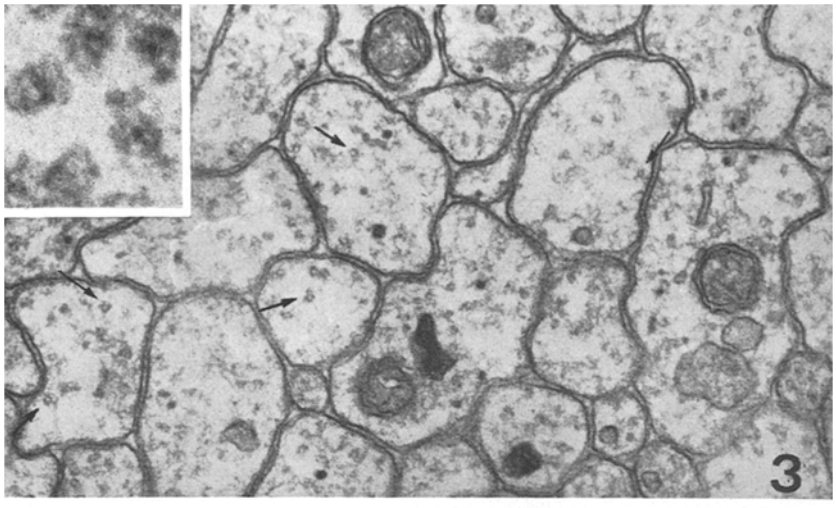

Fig. 3. LH treated hypothalamus. The fibres of hypothalamus-hypophysial tract contain scanty microtubules (arrows). At high magnification disorganization of the microtubules subunits is apparent. ×40,000. Inset: ×240,000.

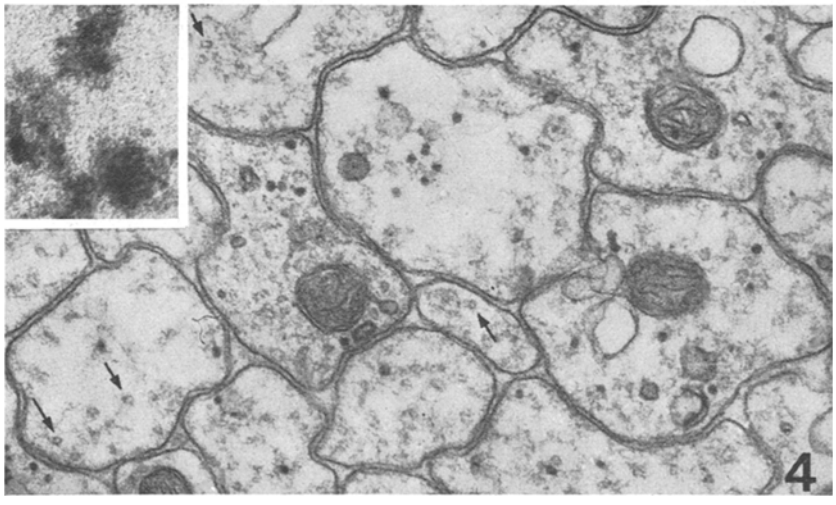

Fig. 4. FSH treated hypothalamus. Some FSH resistant microtubulus are indicated by the arrows. The inset illustrates aggregation of microtubules subunits in zones presumably occupied by the pretreatment tubules. ×40,000. Inset: ×240,000.

It is known that tubulins (MW 120,000), the microtubule protein, polymerize into 12 or 13 subunits or protofibrils. Tubulins contain 2 mols of guanine nucleotides per 120,000 daltons[6]. The attractive interaction of the monomeric tubulins may be due to binding by hydrophobic residues[7]. Calcium ions are also essential for tubulin to polymerized into tubular structures[8].

Several mechanisms have been proposed for microtubule depolymerization. Hydrostatic pressure and coldness would operate on the hydrophobic bounds joining the adjacent monomers[7]. Antimitotic drugs[9], and divalent ions, e.g., nickel sulfate or copper sulfate, would replace a guanine nucleotide of tubulins dealing with a product which can be pelleted by ultracentrifugation[10]. Certainly, little can be said at present on the mechanism of microtubule disintegration by FSH or LH. However, gonadotrophins might act upon sulphidril groups of microtubules. The results are of interest, in view of the probable function of microtubules in translocating axoplasmic particulates such as neurosecretory granules[11] and synaptic vesicles[12].

In vivo experiments on various types of nerve fibres are to be done before conclusions can be reached on the significance of the reported lability of neuronal microtubules to gonadotrophins.

Résumé. Les neurotubules des fibres nerveuses du tract hypothalamo-hypophysaire du rat sont désorganisées après des traitments in vitro avec l'hormone lutéinisante et l'hormone folliculaire stimulante. Le méchanisme de dépolymérisation est discuté.

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