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A MATHEMATICAL MODEL OF TUBULIN PULSE GENERATOR

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This article focuses on the mathematical model of tubulin polymerization and depolymerization which is a generator for the high frequency oscillations in the pituitary cells.

1. Introduction. The hypothalamus controls the secretion of gonadotropins by the pulsatile secretion of gonadotropin-releasing hormone (GnRH) into the portal circulation of the pituitary. The major physiological action of GnRH is expressed through activation of G-protein-coupled GnRH receptors in the plasma membrane of gonadotrophs and the resulting changes in cytosolic Ca^{2+} levels [1] leading to the stimulation of the exocytotic release of both luteinizing hormone (LH) and follicle – stimulating hormone (FSH) [8] from the pituitary gonadotrophs.

Pulsatile secretion in a plasma LH concentration was first reported in ovariectomized rhesus monkeys [2]. During the last years high frequency oscillations (high frequency pulses) were observed in the secretion of LH [4], [5] and FSH release [5]. Iranmanesh et al., [3] have proposed that intensive sampling procedure increases the probability for detection of high frequency pulses. The protein tubulin is found in every cell of all eukaryotes. $\alpha - \beta$ tubulin dimers form long, rigid polymers called microtubules (MTs). Experimental evidence of oscillations *in vitro* with period of 1 to 3 min (high frequency oscllatons) in the process of microtubule assembling were found by Mandelkow et al., [6], but not in the pituitary cells. All biological oscillators exhibit a common feature of essentially nonlinear systems. In this context the theoretical description of rhythmic phenomena is based on the theory of self-sustained oscillator as a basis for the description of intracellular tubulin oscillator.

This article focuses on the hypothesis of tubulin polymerization and depolymerization as a generator of high frequency oscillations in pituitary cells.

2. Mathematical modelling of high frequency oscillations in LH. A quantitative mathematical model [9] is developed using the experimental data of [1], [2], [8], etc. The last four equations in this model describe the oscillatory mechanism of LH release from the pituitary cell:

$$\frac{dY_1}{dt} = a_1(b_1 - Y_1)[b_2 + U_1(t)]Y_2 - a_2U_2(t) - a_3Y_1 - a_4 \tag{1}$$

$$\frac{dY_2}{dt} = -c_1\frac{dY_1}{dt} + a_5U_1(t) - a_6Y_2 - a_7Y_1 - a_8 \tag{2}$$

$$\frac{dY_3}{dt} = (b_3 + a_9U_3(t) - Y_3)a_{10}Y_1 \tag{3}$$

$$\frac{dY_4}{dt} = c_2\frac{dY_3}{dt} - a_{11}Y_4 \tag{4}$$

Equations (1) and (2) are concerned with the oscillations (of 1-3 min period) of the tubulin polymerization in the Golgi region, while equations (3) and (4) describe the LH release through the membrane in blood serum. The notations in the equations are as follows: t stands for the time, Y_1 is the concentration of polymerized tubulin, Y_2 is the concentration of Mg^{2+} ions in the region of polymerization, Y_3 is the concentration of the LH molecules which are expulsed through the cell membrane, Y_4 is the concentration of the LH in blood serum. The input functions $U_1(t), U_2(t)$ and $U_3(t)$ link the system equations (1) – (4) with the rest of equations of the mathematical model [9]. $U_1(t)$ is the concentration of proteins phosphorylated by PKC or CaM products in the zone of microtubules, $U_2(t)$ is the free concentration of Ca^{2+} in the cytoplasm, $U_3(t)$ is the concentration of synthesised LH. a_i – rate constants, where $i = 1, \ldots, 11$, bj – concentration constants, where $j = 1, 2, 3$ and c_q – proportional coefficients, $q = 1$ or 2. All variables are given in concentration scale ng/ml.

3. System of differential equations for tubulin pulse generator. Certain conditions and factors are necessary for the polymerization of tubulin. GnRH, for example, depends on secondary mediators like microtubule-associated proteins (MAPs), Ca^{2+}, etc. [7]. Apart from this the polymerization is carried out in the presence of magnesium ions Mg^{2+}, guanosine triphosphate (GTP), MAPs or a high level of protein concentration. Oscillations are generated by interplay between microtubule stabilzers and destabilizers. Microtubule stabilizers are GTP, glycerol, Mg^{2+}, MAPs, or high protein concentration. Destabilizers include GDP, Ca^{2+}, elevated ionic strength, and a range of drugs. It has been established that the proteins phosphorylated by PKC participate in the process of tubulin polymerization and depolymerization by MAPs phosphorylation. It is supposed that there is a linear dependence between the MAP concentration C_{MAP} and $U_1(t)$, i.e. $C_{MAP} \sim b_2 + U_1(t)$, where $U_1(t)$ is the concentration of proteins phosphorylated by PKC or CaM products in the area of microtubules, and the concentration constant b_2 is the basic level of $U_1(t)$. According to the mathematical model [9] the velocity of formation of the polymerized tubulin is equal to:

$$(v_p) = a_1(b_1 - Y_1)[b_2 + U_1(t)]Y_2 + a_4 \tag{5}$$

where b_2 is the initial concentration of the nonpolymerized tubulin; $(b_1 - Y_1)$ the concentration of the nonpolymerized tubulin; a_4 is the additive constant of (v_p) indicative of its constant level, regardless of the factors mentioned above. According to Zengbush [7] the most significant factors that facilitate the disassembly of highmolecule monomeric compounds, i.e. the depolymerization, are Ca^{2+} ions, low temperature, pressure, etc. It is assumed that there is a linear dependence between the disassembly velocity (v_n) of the polymerized tubulin Y_1 and two negative feedbacks:

$$(v_n) = -a_2U_2(t) - a_3Y_1 \tag{6}$$

The inhibitive effect of the Ca^{2+} ions can be expressed by the negative feedback $-a_2U_2(t)$. The velocity constant $-a_2$ expresses the size (dimension) of this effect, and $U_2(t)$ is the free concentration of Ca^{2+} ions in the citoplasm. The other negative feedback $-a_3Y_1$ expresses the velocity of the disassembly of the polymerized tubulin. The velocity of alteration of the polymerized tubulin concentration, i.e. Y_1 is equal to:

$$\frac{Y_1}{dt} = (v_p) - (v_n) \tag{7}$$

If (5) and (6) are substituted in (7) is obtained:

$$\frac{Y_1}{dt} = a_1(b_1 - Y_1)[b_2 + U_1(t)]Y_2 - a_2U_2(t) - a_3Y_1 - a_4 \tag{8}$$

The equation depicts the velocity of formation of the polymerized tubulin. The velocity of alteration of dY_2/dt of the concentration Y_2 of Mg^{2+} ions decreases proportionally to dY_2/dt, i.e. it is $-c_1(dY1/dt)]$ and expresses the spontaneous neutralization of the Mg^{2+} ions, which is proportional to the concentrations Y_2 and Y_1 (the latter is necessary for support of the already formed tubulin molecules). The member $(-a_7Y_1)$ reveals the concentration of the tubulin polymers, and $(-a_6Y_2)$ reflects the process of eliminating Mg^{2+} ions from the system. The process of tubulin polymerization and depolymerization is quasi periodical. This process is not likely to occur at a constant concentration of the Mg^{2+} ions. It is highly probable that the factors, stimulating the polymerization process will assist in the depolymerization process, so that a oscillating process will be obtained. It is generally assumed that there is a linear dependence between the Mg^{2+} ions and the concentration $U_1(t)$ of proteins phosphorylated by PKC or CaMK products the microtubuline area. The latter gear the processes of decomposition (disintegration), i.e. $Mg^{2+} = a_5U_1(t)$. Taking into consideration what is stated above concerning Y_2, if can be written in a linear proximation:

$$\frac{Y_2}{dt} = -c_1\frac{Y_1}{dt} + a_5U_1(t) - a_6Y_2 - a_7Y_1 - a_8 \tag{9}$$

where a_5, a_6, a_7 are velocity constants, c_1 is the proportionality coefficient and a_8 is the additive constant. High frequency oscillations are generated by an interplay between microtubule stabilizers and destabilizers. By appropriately chosen constants in equations (1) and (2) the solution Y_1 describing a high frequency process in the Golgi region was obtained. The values of parameters of the mathematical equations (1) – (4) are as follows:
– rate constants: $a_1 = 2.2 \times 10^{-3}(\text{ng/ml})^{-2}\text{h}^{-1}$, $a_2 = 1665 \times 10^3\text{h}^{-1}$, $a_3 = 6 \times 10^{-3}\text{h}^{-1}$, $a_4 = 0$, $a_5 = 187 \times 10^3\text{h}^{-1}$, $a_6 = 4 \times 10^{-2}\text{h}^{-1}$, $a_7 = 13.6\text{h}^{-1}$, $a_8 = 17 \times 10^2(\text{ng/ml.h})$, $a_9 = 1.4 \cdot 10^3\text{h}^{-1}$, $a_10 = 2 \cdot 10^{-5}\text{h}^{-1}$, $a_11 = 4.10^2\text{h}^{-1}$;
– concentration constants (ng/ml): $b_1 = 5.10^6$, $b_2 = 3.33 \cdot 10^{-4}$, $b_3 = 8 \cdot 10^4$;
– proportional coefficients: $c_1 = 0.0034$, $c_2 = 2$;
– initial conditions (ng/ml): $U(0) = 0.0001$, $V(0) = 0.01$, $Y_1(0) = 25$, $Y_2(0) = 1700$, $Y_3(0) = 0.005$, $Y_4(0) = 0.0001$.

The model meanings of the constants have been chosen adequately: 1) all of them are positive, 2) express actual biochemical and biophysical dimensions, selected on the basis of the existing knowledge of the processes taking place in the pituitary and in accordance

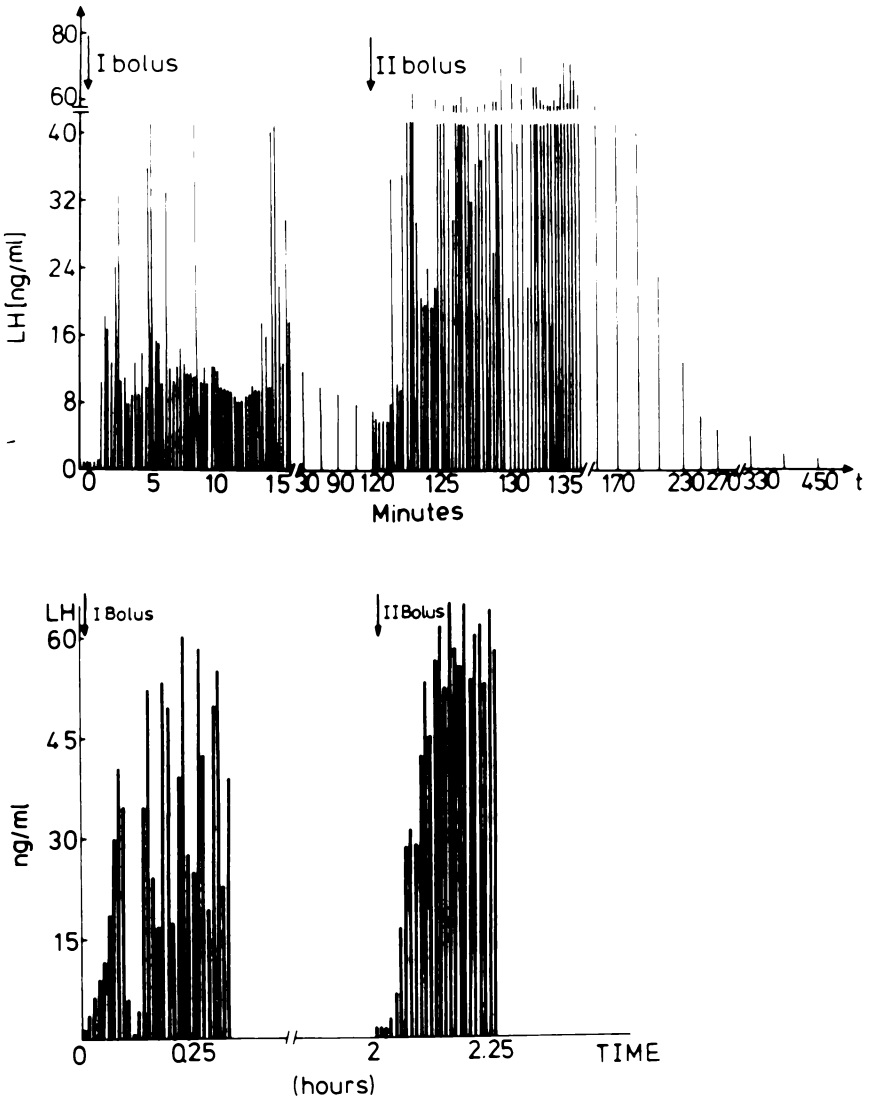

Fig. 1 Comparison between the experimental records (upper panel) and model predictions (bottom panel). Typical pattern of LH release (ng/ml) (upper panel) after double injection of 1 μg GnRH to an intact anoestrous ewe sampled every 15 second. Arrows indicates the times of GnRH injections. The model predictions of LH release (ng/ml) (bottom panel).

with the principles that are observed. Taking into account the meanings of the constants included in the differential equations, the solution of Y_1 is obtained as a high-frequency process in the Golgi apparatus are called a generator of high frequency oscillations or tubulin pulse generator (TPG). This TPG is the reason for the high frequency of response LH from the pituitary. In Yanev [10] the relation between function $Y_1(t)$ and $Y_4(t)$ from the parameters of the mathematical model [9] is derived i.e.

$$\frac{Y_4(t)}{Y_1(t)} \approx \mu = const \tag{10}$$

From (10) follows that amplitudes A_{tub} and A_{LH} differ only by coeffcient of proportionality μ, i.e.

$$A_{tub} = \mu A_{LH} \tag{11}$$

while the frequency and the damping coefficent are equal:

$$\Omega_{tub} = \Omega_{LH} = \frac{2\pi}{T_{LH}} \tag{12}$$

$$\lambda_{tub} = \lambda_{LH} \tag{13}$$

where $\lambda_{tub}, \Omega_{tub}, A_{tub}$ and $\lambda_{LH}, \Omega_{LH}, A_{LH}$ are the damping coefficient, the frequency and the amplitude of polymerized tubulin and LH hormone, respectively. The numerical values of the mathematical model are chosen so that a satisfactory agreement with respect to the form and the amplitude is obtained between the model output $Y_4(t)$ (Figure 1) and the evidence for the experimentally recorded LH(t) concentrations in the blood serum of ewes (Figure 1), as well as for some concentration and rate constants.

The agreement shows that the ratios between the values of the mathematical model parameters are properly chosen but this is not valid with certainty for their absolute numerical values. The comparison of the periods obtained from the experimental data of LH [6], the experimental results for the tubulin oscillations [9] and the model results [12] are in good agreement and confirm the correctness of the rate constant options.

Conclusion. In conclusion, this study expands our knowledge for the processes of tubulin polymerization and depolymerization in the zone of microtubules in the gonadotrophs where granulated LH and FSH are concentrated. The tubulin polymerization and depolymerization is the last step of the processes leading to the high frequency release of LH and FSH from the pituitary cell. It is suggested that equations (1 and 2) from the mathematical model describe the high frequency oscillations in the LH response thus defining a generator of high frequency oscillations or the sp called tubulin pulse generator.

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МАТЕМАТИЧЕН МОДЕЛ НА ТУБУЛИНОВ ИМПУЛСЕН ГЕНЕРАТОР

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В тази статия се предлага математически модел за тубулиновата полимеризация и деполимеризация, която е генератор за високочестотните осцилации в хипофизните клетки.