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THE HORMONAL SYSTEM OF THE UNICELLULAR *TETRAHYMENA:* A REVIEW WITH EVOLUTIONARY ASPECTS

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The unicellular ciliate, *Tetrahymena* has receptors for hormones of the higher ranked animals, these hormones (e.g. insulin, triiodothyronine, ACTH, histamine, etc.) are also produced by it and it has signal pathways and second messengers for signal transmission. These components are chemically and functionally very similar to that of mammalian ones. The exogenously given hormones regulate different functions, as movement, phagocytosis, chemotaxis, cell growth, secretion, excretion and the cells' own hormone production. The receptors are extremely sensitive, certain hormones are sensed (and response is provoked) at 10^{-21} M concentration, which makes likely that the function could work by the effect of hormones produced by the *Tetrahymena* itself. The signal reception is selective, it can differentiate between closely related hormones. The review is listing the hormones produced by the *Tetrahymena,* the receptors which can receive signals and the signal pathways and second messengers as well, as the known effects of mammalian hormones to the life functions of *Tetrahymena*. The possible and justified role of hormonal system in the *Tetrahymena* as a single cell and inside the *Tetrahymena* population, as a community is discussed. The unicellular hormonal system and mammalian endocrine system are compared and evolutionary conclusions are drawn.

Keywords: *Tetrahymena,* Protozoan, hormones, hormone receptors, signal transduction, evolution

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Introduction

The investigations on the hormonal system of *Tetrahymena* started at the early seventies of the last century. At first the reaction of *Tetrahymena* to hormones characteristic to the higher vertebrates were observed and on the positive basis of these experiments the hormones of the higher ranked animals as well, as the structure of receptors and the signal transduction pathways were studied. The results of the experiments show that a complete hormonal system is present and working inside the individual *Tetrahymena* and between the members of a *Tetrahymena* population [1–5]. The aim of the present review is to give the detailed analysis of this system and to synthesize the data won by different experiments.

Hormones of the higher ranked animals produced by *Tetrahymena*

Amino acid-type hormones

The presence of serotonin (5-hydroxytryptamine, 5HT) was demonstrated as early, as 1966 [6] in *Tetrahymena*. The presence of the hormone was biochemically as well as immunocytochemically justified [7, 8]. The serotonin metabolite 5-hydroxyindole acetic acid was also found and it was supposed that a serotonergic system is functioning in *Tetrahymena* [7], and serotonin is a chemical mediator [9]. Short and long starvation elevated the level of the hormone [10]. Serotonin in an extremely low concentration can induce the production of other hormones [11]. Monoamino oxydase of *Tetrahymena* has greater affinity to serotonin than to dopamine [12].

Serotonin is the precursor of melatonin. Melatonin was also found in *Tetrahymena* [13], and it is produced, stored and secreted by it. The synthetic pathway is similar to the mammalian one [14]. Prolonged light exposure suppressed melatonin synthesis and secretion. Pretreatment with melatonin (hormonal imprinting) elevated the melatonin content of the cells and their medium alike [15].

Tetrahymena contains histamine, which is produced by the participation of histidine decarboxylase (HDC) enzyme [16]. This enzyme is present in *Tetrahymena* and its gene is similar to the mammalian one, while completely different from the prokaryotic HDC-gene [17]. The hormone also can be taken up

from the medium and this histamine can also be localized in the nucleus of the cell [18, 19].

Triiodothyronine (T_3) or thyroxine (T_4) were not demonstrated by radioimmunoassay technique [20], however, by using immunocytochemical confocal microscopy [10] or flow cytometry [21], the presence of T_3, and the changes of its content were observed. The isotopically labelled T_3 has been incorporated into the nucleus of the cells [19].

The biosynthesis of catecholamines was observed in *Tetrahymena* as early as 1966 [6] and an adrenergic control system was supposed in 1967 [22]. The enzymes related to catecholamine biosynthesis (monoamine oxydase, catechol-O-methyl transferase and GTP cyclohydrolase) were also found [12, 23]. The main catecholamine is dopamine. There is a tyrosine – L-DOPA conversion extracellularly, which is followed by the uptake of this substance by the cells and this is transformed enzymatically to dopamine inside [24]. In addition to dopamine, epinephrine and kinurenine are synthesized and secreted by the cells [25]. Arteficially added dopamine or L-DOPA is toxic to the cells and decreases norepinephrine synthesis in a very low physiological concentration [26, 27].

Peptide hormones

Insulin immunoreactivity was observed in *Tetrahymena* and its medium [28–30], and the effect of this hormone was similar to the mammalian insulin in bioassays [31]. The effect of insulin was inhibited by anti-mammalian insulin antibody. In addition to this "standard" insulin, guinea pig insulin was found, which has an unusual structure [31]. Exogenous (^{125}I) insulin is also internalized and this can appear in the nucleus of the cell in a heterochromatic localization [32].

The POMC-hormone, adrenocorticotropine (ACTH) is also produced by *Tetrahymena* as well as endorphin [33–36]. This latter was found mainly in the cortical structures, oral field, cilia and nuclear envelope [37]. Long-lasting starvation increased the production of both POMC hormones [10]. In addition, thyrotropic hormone (TSH) and gonadotropic hormone (FSH and LH) are also present [38].

Relaxin [39], somatostatin [40] and endothelin [41] are also synthesized by it. Epidermal growth factor (EGF) is diffusely localized in the region of cytopharynx [42]. The cytokine, interleukin 6 (IL-6) is localized in the oral apparatus and in the nuclear envelope [43]. Salmon type calcitonin was also demonstrated [29].

Lipid hormones

Tetrahymena does not contain endogenous steroid hormones, however, imprintig with the given hormones can induce the production of dihydroepiandrosterone (DHEA) and DHEA-sulphate in a higher concentration and in lesser concentrations hydrocortisone, testosterone and estradiol [44, 45]. It has a special form of alpha-hydroxysteroid dehydrogenase, which functionally differs from the mammalian and bacterial enzymes [46]. It can transform testosterone and convert progesterone to pregnenolone [47, 48].

Prostaglandin (PGF2) is also present in *Tetrahymena* [20] and it is possibly needed for the growth of *Tetrahymena,* as aspirin, which inhibits prostaglandin synthetase also inhibits the multiplication of it [49].

Endocannabinoids, having a lipid nature are also produced and present in *Tetrahymena,* as well, as the related N-acetylethanolamines and by this, an endocannabinoid system is supposed [50, 51].

The hormone receptors of *Tetrahymena*

The insulin receptor

The first observation on the effect of insulin to *Tetrahymena* was done at 1975 [52]. In this experiments insulin stimulated the glucose uptake of the cell. Later the receptor localization [53, 54] and the nature of the binding sites was also justified, when these were isolated [55] and were compared to the mammalian insulin receptor [56]. The demonstrated receptor was localized in the ciliary membrane [57], however, intracellular insulin binding was also observed [58]. The intracellular localization was found on the nucleus and certain vesicles. The nuclear mebrane specifically binds insulin [59], and insulin has a stronger affinity to nuclear membrane receptors than to that of the plasma membrane [60]. The binding capacity of nuclear membrane receptors diminishes after starvation [61]. Insulin receptor development can be induced by imprinting (insulin pretreatment) [62] or by rat liver receptor antibody [63] and these receptors behave as “classical” insulin receptors. The plasma and nuclear membrane receptor’s specificity is similar [64]. The cells distinguish between insulins according to their amorphous or crystalline form, and their bovine or porcine origin [65]. The insulin binding of plasma membrane receptors can be disturbed by the presence of very low concentrations of other hormones (e.g. endorphin and serotonin in 10^{-18} M) [66, 67] as well as the

lysosomal protein degradation blocker, bacitracin [68]. Starvation also influences the insulin production, binding and uptake of the cells [69]. Higher concentrations (10^{-3}–10^{-5} M) of insulin down regulate the insulin receptors, while lower concentrations (10^{-6}–10^{-18} M) provoke hormonal imprinting [70]. The combinations of different peptide molecules as imprinters do not disturb each other [71].

The histamine receptor

The sensitivity and reaction of *Tetrahymena* to histamine was demonstrated as early as 1973 [72]. Later the localization of these receptors on the cilia was also cleared [73], however, the cilia of the oral field as well as the interciliar membrane regions did not bound histamine. The histamine binding was blocked by histamine itself [74] and histamine antagonists. Structurally different antihistamines did not do this [75]. Concanavalin-A – which is bound by the same receptor – dose-dependently inhibited the histamine effect in phagocytosis test [76].

Other receptors

Receptors for triiodothyronine (T_3), which are confined to cilia and the mouth region were also found [77]. Thyroxine (T_4) was localized on the cilia as well, as in pinocytotic vacuoles and the nucleus [53]. Steroid receptors are not present on or inside the *Tetrahymena,* however, they can be induced by DHEA or dexamethasone pretreatment [44, 78]. Receptors for opiates [79], similar to that of the mammalian brain were also found [80, 81] and benzodiazepine receptors were also present [82]. Many other hormones can influence the behavior of *Tetrahymena* (see later), but the receptors of them were not analyzed. As some lectins bind to hormone receptors, the binding of these was also studied and found [83]. Histamine and histamine antagonists altered lectin binding [75].

Signal transduction and second messengers of *Tetrahymena*

Adenylyl- and guanylyl-cyclases [84–87] as well, as their products, cAMP and cGMP are present in *Tetrahymena*. The adenylyl cyclase seems to be a highly unique subtype of this enzyme group, which is restricted to ciliates [88]. The cyclic AMP formation by the enzyme is influenced by the Ca^{2+} and K^{+} content [89,

90] and by biogenic amines [91], natural amino acids [92], and by hormones, e.g. epidermal growth factor [93], epinephrine, insulin, glucagon, as well as the cPDE blocker theophylline [94, 95]. Some non-hormone agents, e.g. adrenergic agonist isoproterenol also can influence cAMP synthesis [96]. Cyclic AMP and theophylline are influencing the function of the cell in the same direction, increasing phagocytosis, while sugar uptake is decreased [95, 97]. Adenylate cyclase activity was demonstrated in/on pinocytotic vesicles, while in case of growth stimulation it appears in associaton with the plasma membrane and inside many dense bodies [98].

The activity of guanylyl cyclase is calcium regulated [99] as it is in vertebrates. A hormone (insulin) treatment causes its localization to cilia and near the plasma membrane [86].

Protein kinase is also present in *Tetrahymena* and in the presence of Ca^{2+} it is activated [100]. Protein kinase C activity helps cell survival and proliferation [101]. Calcium dependent calmodulin was also found and there are proteins interacting with calmodulin [102]. The calcium-calmodulin system regulates guanylate cyclases in the ciliary membrane [103].

Inositol phospholipids are present in *Tetrahymena* forming a functional signaling system [104], similar to that of the higher eukaryotes [105–107]. The phospholipases are coded by five genes, two of them are similar to bacterial PLC-genes and three are similar to metazoan PLC genes [108]. The phospholipases (PLA2, PLC and PLD) are active in *Tetrahymena,* participating in many signal transduction systems [105, 109–111]. The co-operation of the enzymes (e.g. PLC and PLD) could rescue *Tetrahymena* from "low density death" [112]. Hormones, as insulin or vasopressin influence the synthesis of phosphoinositides [113–115], which effect is similar to that of vertebrates. There is a cross-talk between the metabolites of phospholipids and sphingomyelin [116]. The phosphoinositol system seems to be participating in the mechanism of hormonal imprinting [117].

Effect of hormones on *Tetrahymena*

Insulin

Insulin has a very strong and heterogeneous effect on *Tetrahymena,* as it can be bound by the receptors of the cells [118]. The hormone stimulates glucose uptake [52] and utilization [119]. Cell growth was also enhanced [120, 121] as well as ciliary regeneration [122]. At the same time it decreases the phagocytotic

capacity [123], and movement behavior was also influenced [124]. At very low (10^{-21} M) concentration it increased the histamine level (production) of the cells [125]. The hormone reduced the activity of mitochondrial dehydrogenases in six taxa of *Tetrahymena* [126]. Under the effect of the hormone a lectin-like molecule was discharged from the mucocysts [127]. A single treatment with insulin caused a quantitative decrease in fast movements and an increase in slow *movement* [128]. The hormone has a positive chemoattractant effect on *Tetrahymena* [129] in contrast to *Blepharisma* [130]. The effect of insulin was influenced by the milieu in which the cells were treated or the presence of other hormones [131]. The uridine intake and incorporation was decreased by the hormone [132, 133].

A very important effect of insulin that it saves the life of the *Tetrahymena* population when the cell density is very low [134–136], as in this case adequate nutrition alone is not enough for life and proliferation: growth factors are needed [137–139]. The 22–30 fragment of the B chain have this important role. A very low, pico- or femtomolar concentration of the hormone is enough for rescuing the cells [140] and this can be produced and secreted by the mass of the cells [141].

ACTH and TSH

Adrenocorticotropic hormone (ACTH) decreased the phagocytic capacity [123], as well, as the uridine incorporation [132] of *Tetrahymena*. It was able to stimulate the multiplication of the cells [142].

Thyrotropin (TSH) regulates triiodothyronine (T_3) production of the cells [21], however, epinephrine is regulated by it [143]. TSH also influences chemotaxis [144].

Epidermal growth factor (EGF)

EGF influences cell growth of *Tetrahymena* [121, 144], increasing the activity of different kinases which participate in the initiation of cell division [93]. It also enhances DNA, RNA and protein synthesis [145].

Opioids

Nanomolar concentration of opiates inhibits phagocytosis and this effect is antagonized by naloxone [146, 147].

Vasopressin and oxytocin

Oxytocin, which is chemically related to vasopressin (antidiuretic hormone in mammals) influenced the time interval between two contractions of contractile vacuole [148], however, vasopressin itself did not it. Vasopressin decreased the phagocytotic activity [149] in contrast to oxytocin, which – without some pretreatment – was not effective. However, its analogue, isotocin was very effective [150]. These hormones have a negative chemotactic effect on *Tetrahymena* [151].

Steroid hormones

Steroid hormones are able to decrease the growth of *Tetrahymena* [152, 153]. The phagocytotic capacity is reduced under the effect of deoxycorticosterone, while dexamethasone and prednisolne stimulated it [154]. Testosterone, progesterone and dexamethasone are concentration dependently chemoattractant, while hydrocortisone and estradiol are chemorepellent [155]. A suppression of the fatty acyl coenzyme A desaturase system by dexamethasone was observed [156, 157].

Biogenic amines

Serotonin (5HT) influences phagocytosis [72, 158], cell growth [159] and ciliary regeneration [160, 161]. Histamine also regulates phagocytosis through the H_1 receptor [162]. Concanavalin A counteract with the effect of histamine [76]. Histidine, the basic amino acid and histamine similarly influence phagocytosis [163]. Presence of histamine or serotonin enormously decreases insulin binding [69]. Treatment with histamine or serotonin elevates EGF content of the cells [164]. Histamine as well, as serotonin in low concentrations significantly enhanced the synthesis of a steroid, digoxin [165]. The effect of hormones is different in tryptone-yeast medium or in salt solution [131].

Epinephrine influences the glucose metabolism of *Tetrahymena* [166], the phagocytotic capacity [167] and an adrenergic system is supposed [22, 23].

Other hormones

Atrial natriuretic peptide (ANP) can induce the discharge of sodium ions and it has a chemoattractant effect [168]. Human cytokines interleukin 3 and 6 in-

crease the multiplication of cells and also their insulin binding [169]. Endothelins (ET) were chemorepellent (ET2, ET3) or chemoattractant (ET1) and bradikinin was also chemoattractant [170]. Cytokines have a concentration-dependent chemotactic effect [171]. Tumor necrosis factor alpha (TNF alpha) influenced phospholipid metabolism [172]. Melatonin influenced cell division, phagocytosis and chemotaxis [173]. Thyroxine and its precursors increase the phagocytosis, however, their effects are less than the effect of histamine [167]. Thyroxine and its precursors also enhance the multiplication of the cells [174]. Lectins – some of which are bound to hormone receptors [175] – influence chemotactic selection and imprinting [176].

The tetrapeptide hormone tuftsin, which is an activator of thyrotropin releasing hormone and thyrotropin secretion [177] is also a natural activator of phagocytes [178]. It stimulates the phagocytic activity of *Tetrahymena* [179] and influences chemotaxis [180, 181].

General hormonal effects (stress)

Tetrahymena is very sensitive to different stress factors which influences – in men – the whole endocrine system [182]. In *Tetrahymena* this changes not only membrane lipids [183], histone phosphorilation [184] as well, as gene expression [185, 186], but also the hormonal system of it, is activated by stress. Acute stress caused by heat, formaldehyde, ethanol or higher salt concentration elevated hormone (ACTH, endorphin, serotonin and triiodothyronine) concentration in the cells [187]. Long-lasting starvation increases its hormone (endorphin, ACTH, insulin, serotonin, histamine and triiodothyronine) levels [10]. Insulin binding is also touched by stress [36, 69].

Conclusions

The hormonal system of Tetrahymena

It seems to be clear that the unicellular *Tetrahymena* has a complete hormonal system. Hormones are produced by it, hormone receptors are present in the ciliary and nuclear membrane and second messengers as well, as signal pathways are functioning [3, 4]. The question is, what is the function of this system inside the cell or between the cells, whether it is needed for the life at this unicellular

level, or it is only the result of sophisticated methods which can be used for demonstrating these elements. The completeness of the hormonal system and the complexity of it allows to surmise, that this system is necessary for the life of *Tetrahymena*.

Tetrahymena is a single cell and at the same time it is an organism [188–190]. It has all of the constituents which are characteristic to an organism, in a single cell. However, the presence of hormonal system in it is very interesting, considering the complexity of the hormonal system in multicellulars, where one of the cell types produce the signal molecule and others accept this. This is impossible in a single celled organism. However, there is such a possibility, if we consider the *Tetrahymena* population as an organism, where could be members which produce a hormone and others react to it. However, the receiver of the hormone in one occasion could be the producer of the same hormone in other occasion or this situation also could be at the same time. This means that two possibilities are at our disposal: 1. the hormones produced by the cell effect the same cell or 2. the hormone produced by a cell influences an (or more) other cells. In higher ranked organisms autocrine regulation is a well-known notion and it functions in a closed community, however, in *Tetrahymena* which is living in a broad watery milieu this seems to be meaningless. So, we have to suppose the second variation: the *Tetrahymena* population is the community which is adequate to a cell community of a higher ranked organism and the hormonal regulation is working inside it. In this case one or more members of the given population are the senders (regulators, which produce and secrete the hormone) and other ones are the receivers, the receptors of which bind the hormone and as a consequence, the receptor bearing cell reacts to the signal. These receptors are extremely sensitive, a hormone in 10^{-21} M concentration can activate the cell's machinery, and in this concentration only a few molecule are present in the neighborhood of the cell [125].

The acceptance of intercellular communication's theory is supported by the presence, secretion and functions of pheromones. These are extracted from Euplotes and *Blepharisma* and were thoroughly studied. They have very important role in the growth and chemoattraction of these cells [188–190] and can act also to *Tetrahymena* [191]. There is a possibility that the hormones found in *Tetrahymena* also have pheromone-like role.

The exogenously given hormones influence many different functions of *Tetrahymena*. These functions are the movement (swimming), chemotaxis, phagocytosis, excretion, ciliary regeneration, hormone production, and cell division etc., what shows that practically all important life functions studied are touched by hormonal treatments. In addition one of the hormones (insulin) has a

justified life saving function, and it is not known what other hormones bear this capacity as this was not studied till now. This allows the supposition that hormones have a very important role in the life of *Tetrahymena*. As a free-living organism, *Tetrahymena* is exposed to stress frequently and this influences its hormon-household (production and sensitivity) similar to the stress-effects in higher ranked animals [192]. It is feasible that hormones produced by stress in an altered quality and amount, alarm the *Tetrahymena* population (other cells) for escape, which saves the life of the population as a whole.

In *Tetrahymena*, almost all of the vertebrate hormones were found, which were searched at all. Superficially studied it could mean that the unicell "knows" more, than an endocrine cell of a higher organism. However, it is not right. An endocrine cell, e.g. an insulin producing cell of a mammal also have the genes for producing each hormone, but these have been blocked during the ontogenetic development and only the "specific one" is in function. This is well demonstarted by studies, when insulin was found in a lot of non-pancreatic cells [192–194]. However, in multicellulars, the distribution of functions requires the repression of genes in different cell types. In *Tetrahymena* the hormone-genes are not closed and they can instruct the machinery for producing each hormone.

It seems not to be likely that the very broad palettes of hormones which can be produced by the *Tetrahymena* are used in the intercellular communication. However, it is not known what are important for its life indeed. Solely insulin is known as a life-saving factor however, we do not know why and how it is. It is known what hormones influence physiological processes, but it is not known whether these are needed for them, or they only do it, if we arteficially (exogenously) give them to the cells. This exactly means that *Tetrahymena* have the capacity for producing and receiving all of the hormones as well as to react to them and this is very important from the point of view of hormone and receptor evolution. It was supposed earlier that hormones and receptors appear earlier than the endocrine system itself [195]. However, it is not only a possibility that *Tetrahymena* utilizes the hormonal system physiologically, as there are facts which support it. These are:

1. *The high sensitivity of hormone receptors.* These receptors are very sensitive [190], they are able to sense such a low hormone concentration, as 10^{-21} M [125], when only few molecules are present around the cell. Considering that the mammalian hormone receptors bind hormones in 10^{-6}–10^{-8} M concentrations, it makes likely that the *Tetrahymena* receptors are "constructed" for an open, watery life-mode. The dilution of the hormone which is produced by a *Tetrahymena* pop-

ulation in natural conditions is very high, which requires the also very high sensitivity of receptors.

2. *The justified life-saving effect of insulin*. This hormone, which is produced, stored and secreted by *Tetrahymena,* helps its survival when the concentration of cells is extremely low. If the cell number can decide between life and death in a fluid milieu, which contains food enough, the significance of growth factors must be supposed. These factors could be found among the hormones and these are insulin and perhaps other hormones.

3. *The elevation of hormone production during stress.* If the life of a single *Tetrahymena,* or the life of the population is treatened by stress, some factors are needed which helps the sustenance of life by escape or proliferation. Insulin is known as life saving factor, however, the role of other hormones is also not excluded, as some of these hormones help the "survival", because T_3 enhances growth, histamine and serotonin increase phagocytosis etc.

4. *The hormonal imprinting.* When an exogenously given hormone meets *Tetrahymena* at first occasion hormonal imprinting develops. The cell memorizes the encounter and this memory is transmitted to hundreds of progeny generations [1, 3]. Some permanent epigenetic change happens, possibly influencing DNA-methylation and this transgenerationally affects the receptor's binding capacity [196]. This epigenetic alteration is unthinkable without supposing the use of the hormonal machinery.

5. *The hormonal network.* The hormones, which can be produced by the *Tetrahymena,* influence the binding and production of each other in picomolar concentrations [197]. This for itself strongly supports the utilization of hormones by the unicell. However, the presence of trop-hormone – target hormone pairing is more justifiing. In higher ranked animals the hypophyseal regulation of target hormones' production is a rather sophisticated function. It is very surprising that *Tetrahymena* has this function. Exogenously given thyrotopic hormone (TSH) regulates triiodothyronine (T_3) synthesis in *Tetrahymena*. At the same time, chorionic gonadotropin, the other (chemically related) pituitary hormone mimics this function, as it is done in vertebrates [198].

6. *The presence of a complete signal mechanism in the cell*. This means that not only hormones or hormone receptors or signal pathways are present in a cell, but hormones + receptors + signal pathways which are interconnected. This authorized the researchers to surmise adrenergic [22], serotonergic [7] and opioid [199] mechanisms in *Tetrahymena*.

Unicell-induced thoughts on the evolution of endocrine system

For human beings the "hormone" is a well-defined molecule, a regulator of certain well defined organ or function, which is working and transported in a closed system (e.g. blood circulation). However, for the *Tetrahymena* the exogenously given hormone is a molecule, one of the huge amount of molecules around it, which can bound to a receptor. The recognition of these molecules is very important for the cell, as they could be useful (e.g. nourishments) or harmful (toxic substances), which endanger the life of the cell. The recognition of a hormone is the recognition of one of such a molecules, however, this can induce certain changes of the cell, which produce specific reactions. At this unicellular level hormones must have been selected from the oceans of molecules, as a molecule which is suitable for provoking specific reactions of the cell. It is likely that in the beginning of the evolutionary process, membrane molecules and to be hormones would be independent from each other and later, when their suitability for connecting – and the useful effect of this – was cleared, became the membrane structure to receptor and the to be hormone, to hormone. However, the today *Tetrahymena* bears preformed receptors for hormones, as it was shown in the case of insulin, which can specifically recognize the hormone, and the receptor as well, as the hormone have a very similar character to the vertebrate ones. It is not known whether the other hormones (also produced by *Tetrahymena*) have preformed receptors or not.

It is not clear how the membrane patterns, which are suitable for being receptors are selected during the evolution. However, it was absolutely needed, as receptors are the main prerequisite to the adaptation to the environment. Koch's theory [200], combined with the hormonal imprinting [1, 3] can give some explanation on the development of the receptor–hormone connection. According to Koch, in the unicells' plasma membrane there is a continuous change of molecules, always other molecules are building in and submerged and this gives the possibility for selection. Among these molecules are not complete receptors but there are parts of them, which can be combined in the plasma membrane by chance. If such a combination is taking place in the presence of an exogenous hormone this could be fixed epigenetically, by methylation of DNA [201, 202]. This event could explain the mechanism of selection of membrane structures for receptors and molecules for hormones. However, only the fixation by methylation – during imprinting – is justified, the others are suppositions.

The absence of steroid hormones from the repertory of *Tetrahymena* hormones is understandable [44, 45]. These hormones are not soluble in water, so

Tetrahymena cannot use them for transmitting information intercellularly. However, the hormone as well, as receptor formation can be provoked by hormonal imprinting. This means that *Tetrahymena* has the capacity for producing steroid hormones, but it is not needed in normal conditions. The induced steroid receptors are present in the plasma membrane, what makes probable that at the beginning of the evolution of the endocrine system the membrane perception was the characteristic (or only) form and later some membrane receptors have been engulfed into the cytoplasm and finally into the nucleus.

It has to be supposed that the present-day *Tetrahymena* is not identical with the ancient one, consequently it is not sure that in the ancient conditions the hormonal system was also present and it is not a result of the evolution of *Tetrahymena*. However, the similarity of hormones and receptors (as well as signal pathways) of the present-day *Tetrahymena* to that of mammals, makes likely that the multicellular evolution used the unicellular hormonal system as a model for that of multicellulars [138]. The present-day mammals are not originated from present-day unicellulars, however, their hormonal systems are very similar. Another possibility is that the hormonal evolution of *Tetrahymena* used the same way as that of the evolution of multicellulars, reaching to the same level, however, this is not likely.

While *Tetrahymena* can differentiate between related hormones – as e.g. serotonin and 5-hydroxyindoleacetic acid –, it is not able to differentiate well between the amino acid hormone and the basic amino acid [3]. This could mean that the hormone receptors developed from the amino acid (nourishment) receptors and could explain why all of the amino-acid and peptide hormones studied have receptors in *Tetrahymena* [203]. However, there are more important amino acids from the point of view of receptor development as e.g. proline [204]. In addition, studying thyroxine and its prescursors [174] it was cleared that the precursor more intensely promoted growth than the vertebrate hormones T_3 or T_4. This makes likely that there is a hormone evolution and at lower levels of phylogeny the hormone-precursors are more effective. However, the hormone character is decisive as the effect of diiodotyrosine was stronger, than that of monoidotyrosine. This seems to be right in the case of thyroxin however, it is not right in the case of serotonin, which is not only a vertebrate, but a universal hormone.

In *Tetrahymena* the "urinary organ" is the contractile vacuole. This is sensitive for oxytocin. In mammals the oxytocin-related vasopressin is the antidiuretic hormone. It seems to be likely, that oxytocin (in mammals) was more suitable for regulating other functions (e.g. delivery) and vasopressin was undertaking the supervision of kidney.

If there is a hormonal network between the members of the *Tetrahymena* population, this could be the base of the hormonal network in higher ranked animals [196]. If all of the components of a hormonal system are present at an open unicellular level [1, 3, 204], the only requirement has been during the further evolution to combine these elements in a closed condition.

Histamine and its (H_1) receptor is present in *Tetrahymena* and this hormone can influence different indexes. Histidine decarboxylase (HDC) is the enzyme which synthesize histamine from the amino acid, histidine. The HDC gene is present in *Tetrahymena* and its base sequence is similar to that of mammals. In addition, this sequence is rather similar to that of the men, than to that of rat. Considering the evolution of unicells, *Tetrahymena,* as a ciliate is a top-product, as it is the human being in the mammalian evolution. It would be difficult to conclude to a parallel evolution of unicells and multicells, however, the similarities are interesting.

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