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BIOGENIC AMINES AT A LOW LEVEL OF EVOLUTION: PRODUCTION, FUNCTIONS AND REGULATION IN THE UNICELLULAR TETRAHYMENA

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The unicellular eukaryote Tetrahymena synthesize, store and secrete biogenic amines (histamine, serotonin, epinephrine, dopamine, melatonin) and also can take up amines from the milieu. It also has (G-protein-coupled) receptors (binding sites) for these amines as well, as second messengers. The factors infuencing the mentioned processes are shown. For certain amines the genes and the coded enzymes are demonstrated. The amines influence phagocytosis, cell division, ciliary regeneration, glucose metabolism and chemotaxis. There are interhormone actions between the amines, and between the amines and other hormones produced by Tetrahymena. The critical review discusses the role of amines in the early stages of evolution and compares this to their functions in mammals. It tries to give answer how and why biogenic amines were selected to hormones, and why new functions formed for them in higher ranked animals, preserving also the ancient ones.

Keywords: hormones, neurotransmitters, biogenic amines, phylogeny, Protozoa

The biogenic amines are one or more amine groups-containing molecules, transformed from amino acids. The "classic" biogenic amines are serotonin (from tryptophane), histamine (from histidine), epinephrine or norepinephrine (from tyrosine) and dopamine (from phenylalanine or tyrosine), produced mainly by decarboxylation of the amino acids. It can be mentioned here also melatonin (from tryptophane through serotonin). They can be found in members of the

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whole animal world, in many plants, protozoa and bacteria. It is believed that they, or some members of the biogenic amine group were present in the last two billion years of evolution in all creatures from bacteria to mammals [1, 2]. There are about 10–12 species among bacteria which contain catecholamines and serotonin, however, histamine is present in most species of prokaryotes [3, 4]. Biogenic amines have different – nevertheless very important – functions from the regulation of intra- and intercellular processes in single eukaryotic cells to the role as hormones and neurotransmitters in human beings. Some of them, e.g. dopamine in plants [5] or melatonin in animals [6] are antioxidants.

Although biogenic amine receptors were not studied in bacteria, they react to the presence of the amines [7]. The amines have one or more type of G-protein-coupled receptors on the surface of the cells in mammals. These receptors are structurally determined and classified in mammalian cells, however they are not classified, but are also present in the lower levels of evolution, including Protozoa. This means that here a complete signaling system serves the manifestation of biogenic amine effects.

The unicellular Tetrahymena is a suitable model cell of many mammalian physiological processes, at the same time as a ciliate, it represents a higher form of unicellular existence. In the evolution Ciliates appeared about 700 million years ago and studying the functions and regulation of biogenic amines in Tetrahymena, we can get an overwiew on the evolutionarily ancient role of these molecules.

Synthesis and uptake of the biogenic amines

Histamine, serotonin, epinephrine, dopamine and melatonin are synthesized, stored in and secreted by Tetrahymena [8, 9]. The synthesis of serotonin and histamine is taking place also in nutrient-free physiological medium, even this condition, as a stressor enhances the production of amines [10, 11]. Long-lasting starvation (in nutrient-free medium) elevates serotonin and histamine content of the cells with 50% [12]. However, the cells are also able to take up the biogenic amines from the surrounding medium and localize them in the cytoplasm and intranuclearly [13, 14], predominantly in a heterochromatic localization. There is also the possibility of combined synthesis, which means that e.g. tyrosin is transformed non-enzymatically in the medium to L-DOPA, this is incorporated by the cells and enzymatically transformed to dopamine [15]. Melatonin is also synthesized by the cells, which is influenced by the lighting conditions and previous encounter (hormonal imprinting) [16–19].

Enzymes and genes related to biogenic amines

Adenylate cyclase and guanylate cyclase were found in Tetrahymena and their activity was influenced by biogenic amines [20–22]. Epinephrine stimulated the enzyme's action [23] while in other experiments adrenergic receptor agonists inhibited the enzyme and epinephrine blockers decreased this effect [24]. Serotonin and histamine provoked 30% increase of the cAMP level and serotonin antagonists reduced the effect of serotonin [25]. Serotonin increased Ca-sensitive adenylyl cyclase [26] activity with the participation of G proteins [27]. The cyclic phosphodiesterase is also present and its blockade by theophylline increases the phagocytic capacity [28]. Under the effect of epinephrine the cAMP level is increasing [29]. The histidine decarboxylase (HDC) enzyme is present in Tetrahymena mainly in an epiplasmic localization [30] influenced by insulin. The HDC-gene was also demonstrated and it was found similar to the human one [31] and different from the prokaryotic HDC-gene. Galactokinase gene was also found, which is regulated by catecholamines [32]. GTP cyclohydrolase, which transforms GTP into unconjugated pteridine derivatives, is also present, influenced by catecholamines, mainly by dopamine [33]. Aromatic L-amino acid decarboxylase, a dopamine synthesizing enzyme was also found [33]. In the medium of Tetrahymena the conversion of tyrosine to L-DOPA takes place extracellularly however, L-DOPA is taken up by the cells, where it is transformed enzymatically to dopamine [34]. Tetrahymena shows monoamino oxidase (MAO) and catechol-O-methyl transferase activity [35] however, this was much lower, than it is usual in mice. The highest affinity of the enzyme was for tryptamine and the less to dopamine. The activity was higher in the stationary phase than in the logarithmic one [36]. MAO blocker deprenyl increased serotonin content, but serotonin reuptake inhibitor fluoxetine and MAO-A blocker clorgyline were ineffective [37].

Reception of biogenic amines

Epinephrine binds to the plasma mebrane which is followed by internalization into cytoplasmic vacuoles and the nucleus [38]. Histamine is bound to the ciliary membrane, except the oral field. There is no binding in the interciliary regions and by the cilia of the oral field. Dopamine D1 receptor was demonstrated, the binding capacity of which was reduced by equimolar concentration of D1 antagonist [39]. The receptor was found also on the endoplasmic reticulum and endosomes. Serotonin, histamine and melatonin also find their receptors, justified by the specific effect of the molecules on different functions. Histamine

binding was blocked by histamine antagonists, however, structurally different antogonists did not do this [40, 41]. There is also hormonal overlap, demonstrated by the effect of femtomolar (10^{-15} M) concentrations of serotonin and histamine, which enormously reduced the insulin binding to its receptor [42, 43]. The same concentration of serotonin (10^{-15} M) was able to elevate histamine level inside the cell [37].

Functions influenced by biogenic amines

Phagocytosis

The biogenic amines influence the phagocytotic capacity of *Tetrahymena pyriformis*. Histamine and serotonin can stimulate phagocytosis and serotonin is differentiated from its close relative, the plant hormone indoleacetic acid [44]. In *T. thermophyla* histamine did not stimulate phagocytosis however, the antihistamine diphenhydramine decreased it. Histamine and serotonin also enhance the adsorption of FITC-labelled bovin serum albumin in a similar manner, as phagocytosis is stimulated [45]. The action of amines is transmitted by the adenylate cyclase – cyclic AMP, cyclic GMP route [46–48] and their effect is dose dependent [49]. The optimal concentrations for the stimulation are 0.1 to 1.0 microMole [50]. The two amines influenced phagocytosis to a similar degree, however, their effect to the lysosomal phosphatase was different: histamine stimulated and serotonin depressed it [51]. Some serotonin antagonists, as spiperone and metergoline also stimulate the process, others, as propranolol, alprenolol and ergocryptine are neutral. The presence of exogenously given histamine did not influence the interstage variation of phagocytic activity [52]. Histidine, the basic molecule of histamine also stimulates phagocytosis, similarly to histamine [53]. Insulin abolishes the phagocytosis stimulating action of histamine, either it is given simultaneously or two days before (however, weaker in this latter case). Histamine + insulin combination does not influence phagocytosis [54]. Microtubules and microfilaments have a role in the phagocytotic ingestion, which is controlled by a cAMP mediated serotonergic and adrenergic system [55]. The endocytosis of dimethylbenzanthracene particles was also stimulated by serotonin, while catecholamines did not influence the ingestion and lysosomal phosphatase release. However, catecholamine antagonists inhibited the process [55].

Melatonin in concentrations between 10^{-6} and 10^{-10} M stimulated the *E. coli* phagocytosis by Tetrahymena between 10^{-6} and 10^{-10} concentrations [56].

Cell growth

Adrenergic [57] and serotonergic [58] mechanisms are present in Tetrahymena. Epinephrine influences the cell growth [59]. Serotonin enhanced the growth and gramine, a chemically related plant alkaloid also did it [60]. The second encounter with the hormone caused a more expressed enhancement [61]. On the contrary, serotonin analogues decreased the reproduction rate [62]. A time and concentration dependence was observed in the case of histamine, as at 10^{-5} M concentration decreased the reproduction at 3, 5, 7 and 24 h, however, at 10^{-6} M concentration there was not difference and at 10^{-7} M concentration the reproduction was increased, nevertheless only up to 5 h [63]. The conditions of growth strongly influence the cells' biogenic amine synthesis (content). When dopamine was measured in the cells it was found in stationary and logarithmic growth phase alike, however, in this latter phase the dopamine content was half that of the stationary phase cells [64]. A similar situation was observed in the case of serotonin, when its level was maximal in the stationary phase and declined in the logarithmic one [65]. Melatonin in 10^{-6} to 10^{-10} M concentrations decreased the rate of cell division [56], which was also suppressed by dopamine [66]. The circadian rhytm – light and darkness, regulated by melatonin – strongly influences the cell division and the production of hydrolytic enzymes [67].

Chemotaxis

In chemotaxis experiments serotonin showed a negative (repellent) effect, while histamine a positive (attractant) one [68]. Their effects were also different after pretreatment (hormonal imprinting), when serotonin caused an effect similar to which was in the first occasion, however, histamine's effect was more moderate [69]. Melatonin between 10^{-6} and 10^{-10} M concentrations has a chemotactic effect depending on the lighting conditions: it was chemoattractant in light and chemorepellent in darkness [56].

Cilia regeneration

The ciliary regeneration of deciliated Tetrahymena was strongly stimulated by serotonin treatment [70]. The same was done also by catecholamines [71], the effect is dose dependent and specific. Micromolar concentrations are

stimulatory, as millimolar concentrations have less effect or are neutral. The serotonin synthesis blocker p-chlorophenylalanine or EGTA inhibited the process, however, a serotonin treatment overcomes the inhibition [72].

Glucose metabolism

Epinephrine enhances the glucose utilization (from the medium) by Tetrahymena [73]. Addition of glucose to the medium can cause a 8-fold increase of intracellular cAMP within 1 h [74]. If this elevation is arteficially blocked, the blockade can be reversed by epinephrine. The increase of cAMP caused by epinephrine or glucose is counteracted by beta-adrenergic inhibitor. Epinephrine decreases the exogenously given sugar in each region of the cell [75]. Histamine also influences glucose metabolism by stimulating glucose utilization and without interfering with the effect of insulin [76]. The glycogen content of Tetrahymena was also influenced by histamine and this was done also by the H-antagonists depending on their structure and receptor recognition. The H2 receptor antagonists were more effective than histamine itself, while H1 antagonist phenindamine was ineffective [77]. Epinephrine, as well as glucose can regulate transcription of the galactokinase gene [78].

Interhormone relationships

The hormones, which are also produced by Tetrahymena, can influence each other. Insulin at a picomolar concentration consequently elevates the level of other hormones, among them histamine and serotonin inside the cells [79]. Combined treatment with elevating hormones produced no summation [80]. Thyrotropin or gonadotropin decreased the intracellular level of serotonin [81]. Stress (higher or lower than optimal temperature, formaldehyde or salt treatment, etc.) almost doubled the serotonin concentration inside the cells [82]. This is right also to epinephrine and histamine, however, the latter was the less reactive [83]. Heat stress for 1 h increased the serotonin and histamine level for two weeks [84]. Epidermal growth factor (EGF) production was stimulated by histamine and serotonin [85]. Serotonin related molecules (tryptophane, 5-hydroxytryptophane) immediately after treatments diminish the serotonin content of the cells and histamine increase it [86, 87]. The Tetrahymena hormone receptors are rather sensitive. Serotonin down to 10^{-21} M decreased histamine level and insulin increased it in the same concentration [88]. At the same time, histamine influenced insulin level down to 10^{-6} M concentration. Heat, salt, formaldehyde or ethanol stress elevated (some almost doubled) serotonin level inside the cells [89].

Conclusions

As the above-mentioned data shows, the role of biogenic amines in Tetrahymena was not systematically studied. However, many conclusions can be drawn, which demonstrate the importance of these molecules.

The first conclusion is that all of the biogenic amines can be found in Tetrahymena, they are synthesized, stored and secreted by the cells. Because of the sporadic studies this is only a generalization, as in the case of one amine the gene was found, while in other case the enzyme, etc. However, though Tetrahymena is able to take up complete biogenic amines or their components from the watery surroundings, it seems to be right that it also can synthesize them. It is also justified that it has (G-protein coupled) receptors for amines and also has second messengers (cAMP, cGMP) for transmitting the information, and the system is working. This means that a complete hormonal system is present to receive information given by amines and also for sending commands by them. These commands given by the amines can effect intra- or intercellularly alike.

The biogenic amines can influence many essential life-processes in different directions. In the case of Tetrahymena, as it is a unicellular animal, the life functions of a higher organism are represented in one cell. This means that the biogenic amine synchronously influences each function inside the cell, and the positive or negative effect is dependent on the reactivity of the substrate of the given function. However, the intracellular concentration of the amine also could be a regulator of the effect, as it was shown in the effect of histamine to cell division.

When the biogenic amines are used as effectors or intercellular communicators the situation is clear. They have hormonal effects mostly similar to the mammalian ones. Histamine increases phagocytosis, epinephrine influences glucose metabolism and some antagonists inhibit the hormonal effect or stimulate functions depending on the similarity to the basic molecule. It is obvious that the similarities between Tetrahymena and higher organisms can be considered only in the case of basic functions, which can be observed in each step of phylogeny; neurotransmitter function cannot be expected in Tetrahymena.

Why amino acid derived hormones and among them mainly biogenic amines are in the service of the intra- and intercellular regulation of Tetrahymena? The question is important, however, it is not completely answered. Amino acids are the oldest organic molecules in the prebiotic evolution [90] and they are very suitable for simple transformation by decarboxylation, or amination. However, protein synthesis is also an ordinary process in unicellular animals and polypeptide hormones can also be synthesized in them. The explanation could be in the relationship between the hormones and their receptors. According to the

theory of Lenhoff [91] the amino acids are foods for Tetrahymena (for unicellulars in general), and for the recogniton of them before ingestion, specified membrane patterns are needed on the surface of the cell. These patterns (binding sites) are present or develop in the presence of the amino acid in the watery milieu of the cell [92–94]. If this receptor has a transmissional background, the amino acid can be considered as a signal for any cellular processes, such as chemotaxis [95], which helps to approach the nourishment. However, it seems impossible that each encounter with an amino acid (as food) cause a signal for some other cell function. So, the cell is forced to transform the amino acid receptor for better recognizing an amino acid derived, but transformed molecule, which will have the hormonal function. Indeed, there are overlaps between the effects of an amino acid and its hormone derivate (e.g. histidine-histamine). However, at the same time, the receptor's recognition is so sofisticated that can differentiate between the L and D variants of amino acids [96].

The outstanding suitability of amino acids for becoming hormones is supported also by other facts:

As it was told, peptide and protein hormones are also synthesized by Tetrahymena and have regulatory functions. However, these hormones are composed of many, some of them from more than hundred, amino acids. The Tetrahymena in natural condition is living in a milieu which is poor in food components. This means that the preparation of a hormone from one amino acid is more economical.

As the biogenic amines are tiny hydrophylic molecules, they are very easily dissolved. At the same time in experimental conditions in a very low concentration they are effective: 10^{-15} M, sometimes 10^{-21} M concentrations are enough for provoking an answer. In natural conditions the dissolution of molecules is extremely high and only those molecules (such as biogenic amines) could be effective as hormones, which can act in very low concentrations.

In case of protein hormones, after the polypeptide synthesis a posttranslational modification used to be needed. In the case of a biogenic amine, the hormone is ready for function after decarboxylation of the amino acid.

Tetrahymena synthesize not only biogenic amines, but other hormones characteristic to higher ranked animals [94, 97]. These are peptides or polipeptides (proteins) as insulin, ACTH, TSH and others. These hormones also act to Tetrahymena, having receptors in its plasma membrane [98–100]. Some of them, as insulin, have life saving function in critical conditions [101]. Exogenously given to the medium of Tetrahymena regulate important physiological processes (as sugar metabolism by insulin). This means that Tetrahymena has a complete endocrine system – however, without feed-back mechanism [102] –

and in the frame of it have the biogenic amines an important, nevertheless not exclusive role.

The effect of exogenously given biogenic amines reports that some function is imaginable however, the effect could be caused by the physicochemical properties of the molecule or its food-nature, etc. In addition, the presence (endogenous synthesis) of a hormone or a hormone-like molecule does not mean that it has a functional role. It can be a side-product of different syntheses, a trial of Nature, etc. However, the biogenic amines in Tetrahymena have such effects, which are provoked by natural conditions and are manifested in basic life functions. It must be considered at first the effect of stress factors, which elevate the hormone production, protecting the life prospects of the unicellular. Stress situations could be very frequent in the animal's life in natural conditions and the protection against it seems to be vital. However, very interesting is the duration of stress effects (two weeks, after one hour exposition), which shows the transmission of the response to the – almost lethal – traumatization across many generations. Also, important evidence is the necessity of amines in the synthesis and effect of melatonin. Melatonin is formed not only by the decarboxylation of tryptophan, to serotonin, but the transformation of serotonin, the primary biogenic amine, to melatonin. And melatonin synthesis is influenced chronobiotically, by the rhytm of light and darkness already in Tetrahymena. This rhythmicity influences the velocity of cell division as well as nourishment (phagocytosis). In addition, this rhythmicity could be the basis of circadian rhytm in higher ranked animals. This means that at unicellular level there are some primitive physiological mechanisms, which developing further during the evolution, creates the functions of the very complicated organisms, and biogenic amines have a rather important role in this process.

During the evolution some of the biogenic amines are changing their functions. In Tetrahymena there is not neural system and there are no data on the effect of biogenic amines on the sensitivity of cells. However, in higher ranked animals the nervous system appears and most biogenic amines became to neurotransmitters. Nevertheless, they or some of them preserve the ancient function, as histamine, which is a phagocytosis promoting hormone in Tetrahymena and man alike. This means that new functions not always requires new executor, but the old hormone (hormone-like molecule) wins new or supplementary function. The hormones which are produced in the same cell in case of Tetrahymena, are synthesized by cells of different organs in the higher ranked animals. However, the "common production" is preserved and manifested in the cells of the immune system, which produces, stores and secrets each hormone of the ancient Tetrahymena and also can transport the materials to the place of needs [103–105].

As it was mentioned, the plasma membrane of Tetrahymena binds biogenic amines. However, the receptor or receptors are not classified, except the dopamine receptor [39], which was found to D1 type. It seems to be likely, that only one type of bioamine receptors is present at this level, in contrast to the classified multiple receptor types (e.g. 5HT-1-4, D1-4 + D1A-D) which are manifested in different organs of mammals [106], while the Tetrahymena is a one-celled organism. The diversification of receptors was required by the diversificaton into organs, which needed the regulation by the same biogenic amine for different functions. It is supposed that the serotonin proto-receptors appeared more than 700 million years ago and after that also the dopaminergic and adrenergic receptor systems, which explains, why so many biogenic amine receptors exist today [106]. The multiple receptor subtypes already can be found in molluscs and arthropods [107] and the second diversification was about 400 million years ago, when the cephalization of the neural system was taking place [108]. However, these receptors (binding sites) without diversification were present already in Tetrahymena at a very low level of phylogeny, though their structures have not been studied in details.

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