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Presence in and effects of pineal indoleamines at very low level of phylogeny

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Abstract. The unicellular organism Tetrahymena contains serotonin and is able to take up the hormone from its milieu. The serotonin content of the cell changes as a function of the presence of foreign exogenous hormones. This indicates a possible role of serotonin as a chemical mediator. Exogenous serotonin stimulates the RNA synthesis of Tetrahymena, and it was the only one among the hormones studied which kept the RNA level durably high. Serotonin stimulates phagocytosis and growth of Tetrahymena, and its precursors also stimulate growth. Serotonin can imprint Tetrahymena, and as a consequence of this the effect of the hormone increases in the case of further encounters. Treatment with serotonin-related molecules soon after imprinting can reduce the effect of imprinting. Melatonin can contract the pigment cells of Planaria; however, its precursors serotonin and tryptamine can do this more intensely. Both melatonin and serotonin can influence the regeneration of Planaria, with effects which differ when different phenomena are studied. Evolutionary theories are discussed.
Key words. Serotonin; melatonin; Tetrahymena; Planaria; hormonal imprinting; phylogeny.

Presence of indoleamines in Protozoa

In the living world, only a few amino acids are used for developing signal molecules by chemical transformation of the molecule. Almost all the amino acids play some role in the formation of polypeptide signal molecules, and in these larger molecules the primary structure of the amino acids could play a part in the signal role, as well as the sequence and the secondary or tertiary structure of the protein. However, it is probable that not all amino acids are suitable for transformation into hormones by changes in their chemical structure, rather than incorporation into peptide chains. According to our latest knowledge, tyrosine, tryptophan and histidine are the amino acids which are used as the basis of hormone-like molecules in living systems. The character of the three amino acids is considerably different, as histidine possesses an alkaline side chain, tyrosine has a chargeless polar side chain, and tryptophan has an apolar side chain. Nevertheless, these three amino acids – possibly because of other important features – became the basis for the formation of hormones. From tyrosine, thyroxine and triiodothyronine, noradrenaline and dopamine developed; from tryptophan, serotonin and melatonin; and from histidine, histamine.

Though Protozoa have no endocrine system like higher organisms, they have hormones[22,37,38,45] and receptors[4–7], too. In these unicellular organisms the presence of receptors has a fundamental importance as there are innumerable dissolved materials in the aqueous environment. These include beneficial substances (e.g. food) as well as harmful ones (e.g. toxins). The recognition of these molecules plays an essential role in the life of Protozoa. In contrast, the significance of the hormones synthesized at this unicellular level is not known.

Unicellular organisms contain several hormone-like molecules which resemble the molecules acting in the endocrine regulation of higher organisms. These include the polypeptide hormones insulin, adrenocorticotropin (ACTH), somatostatin and relaxin[37,38,45]; the steroids dihydroepiandrosterone and testosterone[22]; and among the amino acid type of hormones epinephrine, dopamine, histamine and serotonin[1,2,25,29,30,43]. These hormones could be by-products of the intracellular synthetic processes of the unicellular organism or could be a trying-out by Nature of a new, more developed synthesis, and these substances could also be the regulatory elements at this very low level. Since Protozoa have receptors[4–7] and second messengers[35,36,39,40,46], these hormones could influence the physiology of the cells, too. The intracrine, autocrine and paracrine mechanisms for such influence are equally possible, as there is the chance of retroaction to the producer cell, not to mention the intracellular effects. The sensitivity of the receptor or the concentration of the mentioned materials could be so high that the paracrine mode of regulation is also conceivable.

Applying either chemical or immunocytochemical techniques, the presence of serotonin (5 HT) is detectable in Tetrahymena[1,2,25]. The amount of serotonin is about 0.3 µg/g wet weight in *Tetrahymena pyriformis*[25]. After addition of serotonin to the culture medium the insulin content of Tetrahymena increases, which means that the cells do not only produce and contain serotonin, but they are able to take it up from their environment[23]. Serotonin synthesis is increased in Tetrahymena cells and in their offspring generations when they have taken up serotonin from the medium earlier. The above-mentioned phenomenon is proved by the increase of the amount of intracellular serotonin after one day (6–8 new

generations) and one week (about 50 new generations). Gramine, a relative of serotonin, and precursors of serotonin, like tryptophan or 5 HTP, decrease the level of intracellular serotonin just after they have been taken up from the medium. After 24 h the intracellular serotonin level significantly increases, and then there is a permanent decrease (this is present also after one week). Thus the precursors and related molecules do not have the same action with respect to the induction of serotonin synthesis in Tetrahymena cells.

Tetrahymena contains the enzyme monoamine oxidase (MAO) which is required for the decomposition of serotonin[28]. The enzyme has a higher affinity to serotonin than tryptamine, and its specificity is shown by the higher affinity to tryptamine than to dopamine. The activity of the enzyme is increased by an increase of the pH, as is the case with liver MAO, but its affinity to tryptamine is less than that of the mouse liver enzyme. Harmine, clorgyline and tranylcypramine have an inhibitory effect on the activity of MAO in Tetrahymena.

Effect of indoleamines on Protozoa

The general index

The study of the activity of hormones of higher organisms in Protozoa by considering any special function is very difficult, since the protozoon is a single cell and therefore has no division of labour, in contrast to higher organisms where special hormones influence special target cells. That is why a general index – like the monitoring of RNA synthesis, which reflects to the increase of synthetic functions of the cell – seems to be best. Serotonin and other biogenic amines (histamine, epinephrine), the hormones of the thyroid (T3, T4), and the steroids (prednisolone) influence the RNA synthesis of Tetrahymena in a similar manner[18]. There is an increase after only a short period, which turns into a significant decrease after 15 min; after 30 min there is a huge increase, which is followed by a fall to the control level in most cases at 60 min, and a significant decrease to below the control level at 120 min (fig. 1).

Serotonin acts in the same way as the other hormones for the first 30 min of the experiment, and produces more than a tenfold increase of RNA in this time. After this the effect diverges from that of the other hormones as this serotonin effect stays constant and has an eightfold RNA increase at 120 min compared with the other hormones. Thus serotonin surely has an influence on the synthetic processes of Tetrahymena, and this effect is stronger than that of the other hormones. The question is, how can we see this general index of activity reflected in the responses evoked by serotonin in Tetrahymena?

Effect of serotonin on the phagocytosis of Tetrahymena

Serotonin, just like histamine and the other biogenic amines, has the ability to increase phagocytotic activity in higher organisms. In Tetrahymena, histamine has the strongest potency in the activation of phagocytosis[8], and this effect is present even at very low concentrations (10^{-10} M). Serotonin is able to increase significantly the phagocytosis of Tetrahymena in 10^{-7}–10^{-8} M concentrations (fig. 2). The binding site of serotonin proved to

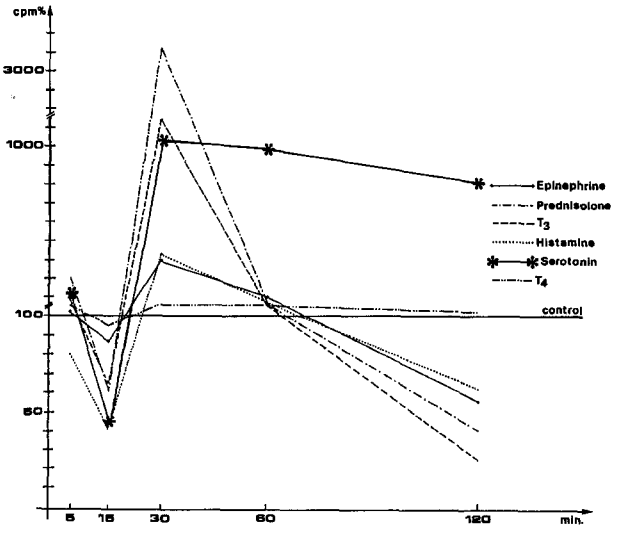

Figure 1. Incorporation of ^{3}H-uridine by RNA in *Tetrahymena pyriformis* exposed to different hormones. Very high elevation is provoked by prednisolone, triiodothyroninne (T_3) and serotonin at 30 min; however, only the effect of serotonin is prolonged (Csaba and Ubornyák[18]).

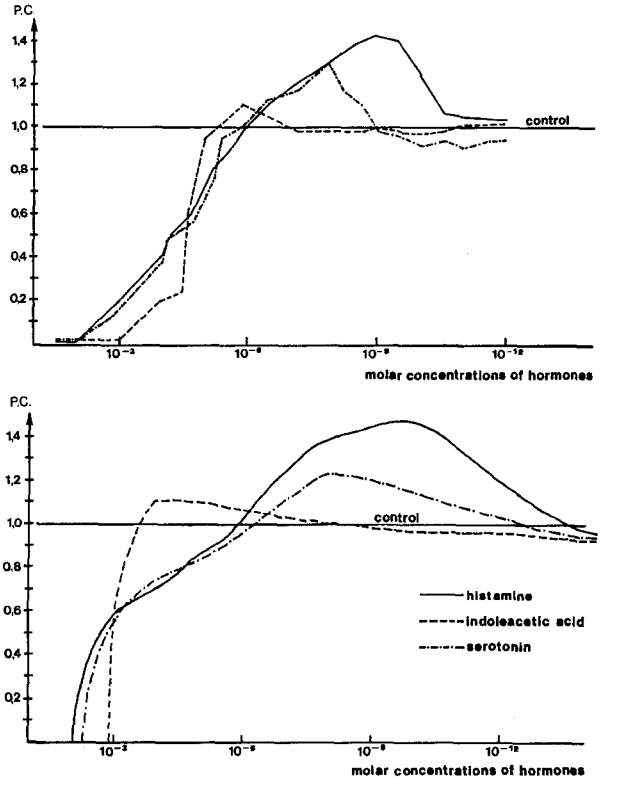

Figure 2. Effect of histamine on the phagocytic coefficient (P.C.) of Tetrahymena (top) and frog (bottom). The sequence of hormonal effects is similar, with minimal differences in top concentrations. The maximal effective dose of serotonin is 10^{-8} M in both cases (Csaba and Lantos[8]).

be specific in Tetrahymena, as the serotonin-related molecule indoleacetic acid (a plant hormone) could not increase phagocytosis even in higher concentrations. It is not clear what mechanism serves to increase phagocytosis, but experiments[33] demonstrate that both biogenic amines increase the protein (e.g. bovine serum albumin) binding of the plasma membrane, in contrast to hormones which have no or only a moderate effect on phagocytosis in Tetrahymena. So it is possible that an increase of adsorption causes the higher level of phagocytosis. At the same time it is probable that a more complex mechanism is also involved, since on the one hand serotonin itself could have a role as an intracellular mediator[23], and on the other hand the adenylate cyclase-cyclic-AMP system could also be influenced by this molecule[11,12,36,39,40,46].

It seems that the effect of serotonin on phagocytosis – as an essential cell-physiological event – remains constant during evolution. Comparing the results of experiments in which serotonin, histamine and indoleacetic acid were applied to frog macrophages, the order of activity of the molecules is found to be the same[10]; there are hardly any differences even in the effective concentrations (fig. 2).

When the concentration of hormones is increased, a toxic-inhibitory effect develops with respect to phagocytosis. This demonstrates clearly that these amino acid-type hormones are not influencing phagocytosis simply by functioning as consumed food substances.

The specificity of binding sites for biogenic amines is also demonstrated by the abolition of the phagocytosis-stimulating effect after minimal changes of molecular structure of the hormone[43].

Effect of indoleamines on the division of Tetrahymena

In addition to phagocytosis, cell division is the other fundamental physiological index in the case of unicellular organisms. Serotonin is able to influence the growth of Tetrahymena. In a concentration range of 10^{-5}–10^{-10} M a significant agonist effect was observed between 10^{-8} and 10^{-10} M; the maximum value was at 10^{-8} M. The serotonin-related molecules like tryptamine, or 5-hydroxy-tryptophan (the precursor of serotonin) are also able to increase the growth of cells in the mentioned range, but their stimulatory effect is much lower[14,16]. Only the parent amino acid, tryptophan, has an agonist influence on the growth of cells similar to that of serotonin. This amino acid produces a growth-inductive effect at exactly the same concentration (10^{-8} M) as the hormone. In lower concentrations the effect is less, while an increase of concentration further stimulates growth, and it reaches a maximal value at 10^{-5} M which exceeds the effect evoked by serotonin.

Comparing the effect of serotonin and related molecules to that of tryptophan clearly shows that serotonin and its related molecules express hormone-like effects. This means that they are ineffective or have negative effects in low concentrations; there is a peak of the effect they evoke, and a further increase of concentration results in toxic effects. In the case of tryptophan the increasing effect on growth brought about by the concentrations applied makes it probable that it is used as a food (as an amino acid), and therefore an increase of concentration improves the conditions for the growth of Tetrahymena.

It is interesting that, in contrast to the evolutionarily late amino acid-type hormones thyroxine and triiodotyronine, the effect of serotonin is more definite compared with that of its precursor, while among the thyroid hormones and their precursors the phylogenetically ancient diiodotyrosine is the most effective. It is worth mentioning that the growth-kinetic effect of the parent amino acid for thyroid hormones, tyrosine, is similar to the effect of tryptophan, and this supports the above-mentioned tryptophan-serotonin relationship. Serotonin is present at all levels of living systems (at this low level, too), whereas the thyroid hormones are absent at this level. Probably the above-mentioned fact is responsible for the greater effect of serotonin compared with its precursors.

Effect of serotonin on the regeneration of cilia in Tetrahymena

The regeneration of cilia is a morphogenetic process which is influenced by several hormones. Serotonin belongs to this group, as it can accelerate the formation of new cilia in deciliated Tetrahymena[2,24]. This is a dose-dependent effect. Very low (micromolar) concentrations have the capacity to increase the regeneration, and millimolar concentrations are ineffective or have a mild effect[3]. Tetrahymena possesses second messenger systems, and therefore serotonin could influence the regeneration through these systems. cAMP and calcium acting through the second messenger systems have a similar capacity to influence the regeneration of cilia. Both beta-chlorophenylalanine, an inhibitor of serotonin synthesis, and the calcium chelator EGTA are able to inhibit the process, while the addition of serotonin will turn off this inhibition[44]. During regeneration the amount of intracellular cyclic AMP was increased, mainly when the regeneration was stimulated by serotonin. Though the phenomena occur in parallel their relationship cannot yet be proved[44].

Effects of biogenic amines in Blepharisma

To state that serotonin – or hormones in general – have the capacity to express an effect in Protozoa it is obviously essential to investigate other species besides Tetrahymena. Like other ciliated protozoa, *Blepharisma undulans* (Stein) also responds to hormones, and this is not a general response[34]. For example, the growth of Blepharisma is decreased by insulin and increased by

other hormones like biogenic amines (among them serotonin). After the withdrawal of the hormones diiodotyrosine, triiodothyronine or histamine the increased division induced by the hormones continues. The division of serotonin-accustomed cells following the withdrawal of the hormone is slower. This means that most of the hormones bring about a 'durable modification' with respect to division, but the constant presence of serotonin is required for the increased division of cells. Cells that have once been treated with serotonin demand the further presence of serotonin, as the division rate falls below the control level after the withdrawal of serotonin. The repeated addition of serotonin to the population of cells restores the division rate nearly to the control level.

Hormonal imprinting in Tetrahymena

The accidentally-persisting binding sites on the plasma membrane of Protozoa are sufficient to enter into a relationship with hormones in the environment and, because of the presence of a second-messenger system, a response will develop. The first encounter with the signal molecule, the hormone, gives rise to *hormonal imprinting*, and as a result working receptors in the cell membrane are developed[4,6]. These receptors have the capacity to bind the hormone in a more specific manner, and the responsiveness of the cell will be changed for hundreds of generations: in most cases it is enhanced, more rarely depressed. Each hormone which is able to bind to the cell membrane has the ability to lead to imprinting. Serotonin and its related molecules also have this characteristic[15,17].

As described above, serotonin has a significant agonist effect on the growth of Tetrahymena in relatively low concentrations (10^{-8}–10^{-10} M), and higher concentrations reduce it. The effect of serotonin is more intensive than is that of its relatives. If Tetrahymena cells have already encountered serotonin, they are imprinted, and the inductive effect of serotonin is expressed more after cell division. This imprinting could be perturbed by synchronous application of other hormones after development of imprinting, but it is not possible to quench the developed imprinting in this way[19].

Gramine, a chemical relative of serotonin, is able to develop imprinting like serotonin, but the intensity of this imprinting is much lower. When the imprinting is developed by serotonin and the effect is checked by applying gramine, the gramine effect will appear. When gramine is the substance used for the imprinting and serotonin is the indicator applied to measure the developed effects, again the gramine effect will arise. If the inserted perturbing agent is diiodotyrosine, this will not, or only slightly, influence the imprinting developed by serotonin[20]. This means that the related molecule is able to perturb the imprinting more than a non-related molecule which also has an amino acid structure.

The selectivity of the imprinting-related receptor is clearly shown by the fact that a 72-h treatment with analogues of serotonin (indole acetic acid, gramine, methyl-triptamine) at such low concentrations as 10^{-9} M is able to decrease the rate of division, but serotonin has not such potency, and moreover it will develop imprinting. This is demonstrated by a moderate increase of the division rate to the control level, at least when analogues of serotonin are applied for the second time, when there is a significant increase in the case of serotonin treatment. Serotonin is able to induce a very significant self-imprinting which persists after division and reaches twice the value of the control. When imprinting was carried out using simultaneous treatment with serotonin and other hormone-like substances, this positive effect of serotonin was not observed. In the same manner, the imprinting induced by serotonin is present only at a low level, when serotonin accompanied by other hormone-like substances (gramine, diiodotyrosine) at the second encounter with the cells. This means that serotonin and its related molecule gramine, each of which is able to affect the division rate of Tetrahymena alone, are both able to neutralize the effect of the other. This observation is completed by the fact that each of these substances is able to reduce if not quench the effect of the other when they are applied one after the other and not together[15,17,20] (table).

Effects of hormones on the level of serotonin in Tetrahymena (serotonergic system)

It was demonstrated before that several biogenic amines like serotonin, dopamine and epinephrine are present in Tetrahymena. These substances are neurotransmitters, tissue hormones or hormones in higher organisms. In Tetrahymena, which is a unicellular organism, the first two functions are not probable. At most, analogous functions are conceivable. This can be proved only when, after the introduction of hormones and other materials which are signals, or are able to bind to Tetrahymena, the changes in serotonin level are consistent.

Using antibodies to serotonin it was demonstrated that exogenously applied serotonin increases the endogenous serotonin level, and this is detectable also after a week[23]. During a week there are about 50 new generations of cells and this suggests that the treatment with serotonin sets the content of intracellular serotonin to a higher level, or that at the time of imprinting, the excess amount of serotonin not only turned the binding sites into receptors, but also changed the production of serotonin, perhaps acting at the gene level. The chemical relatives of serotonin (tryptophan, 5-hydroxy-tryptophan or gramine) do not bring about the above-mentioned changes. The action of these related molecules is a prompt and universal fall in the serotonin level, which could be explained by their antagonistic

Table. Effect of combined and repeated hormone treatments on the growth of Tetrahymena (Csaba and Németh[19])

Time of treatment (day)	Experimental groups 1	2	3	4	5	6	7	8	9
1	T_2	Gramine	5HT	Gramine, 5HT, T_2	Gramine, 5HT, T_2	Gramine	T_2	5HT	Control
3	5HT	5HT	T_2	–	Gramine, 5HT, T_2	Gramine	T_2	5HT	Control
5	Gramine	T_2	Gramine	–	Gramine, 5HT, T_2	Gramine	T_2	5HT	Control
14	Gramine, 5HT, T_2	Gramine, 5HT, T_2	Gramine, 5HT, T_2	Gramine, 5HT, T_2	Gramine, 5HT, T_2	Gramine	T_2	5HT	Control
Mean cell counts	13.5	$15.50^{\triangle\square\bullet}$	$16.25^{\triangle\square\bullet}$	13.55	13.60°	$14.45^{\triangle}$	$21.00^{\triangle\square\bullet}$	$23.65^{\triangle\square\bullet}$	12.55

Significance vs control (group 9): ○, $p < 0.05$; △, $p < 0.01$.
Significance vs group 1: □, $p < 0.01$.
Significance vs group 4: ●, $p < 0.01$.

effect. After 24 h – in the 6th–8th offspring generation – these molecules increase the serotonin level significantly, but this is not a permanent effect as a repeated significant decrease is characteristic after one week. It is not certain that the response is an antagonistic effect. It is possible that it is a real hormonal effect mediated by alteration of the serotonin level; a similar phenomenon was observed in experiments with epinephrine and dopamine, too.

From the above observations we could conclude that this is characteristic for the effects of amino acid type hormones or hormone-like substances. However, the action of histamine is different. Treatment with this hormone leads to a significant increase in the serotonin level immediately after the treatment, which is followed by a decrease to the control level after 24 h and also after one week. Considering the effect of histamine or other hormones, it looks as though the serotonin level alters as a result of the hormone-binding to the receptors of the Tetrahymena membrane. This suggests that serotonin may be the mediator of these hormonal actions. Here, the outstanding problem is the effect of thyroxine and its precursors (thyroxine series) on the changes in serotonin level. That is, diiodotyrosine was the only molecule which increased the serotonin level far above the control level after 24 h, and also after one week. Previous experiments showed that this molecule of the tyrosine series had striking functional effects, too. This would suggest a mediator role of serotonin.

It is worth mentioning that after the development of imprinting with these molecules, histamine treatment results in the highest serotonin level after the second encounter. As histamine can influence the phagocytosis of Tetrahymena, and this function is also increased by imprinting (following the repeated encounter with histamine) it can be presumed that serotonin plays a role in it as a mediator.

Effect of indoleamines on Planaria

Effect of indoleamines on the pigment cells

Melatonin is a hormone influencing the pigment cells and in the frog it causes the contraction of these cells. This effect is observed in Planaria, too. There is a 30–46% reduction of the size of the pigment cells after treatment in vitro for 5, 10, and 20 min at concentrations from 10^{-6} M. The serotonin effect is higher, and tryptamine results in more intense contraction of pigment cells[13] (fig. 3). Increasing the concentration of hormone did not result in further reduction. The melatonin effect in Planaria demonstrates that a characteris-

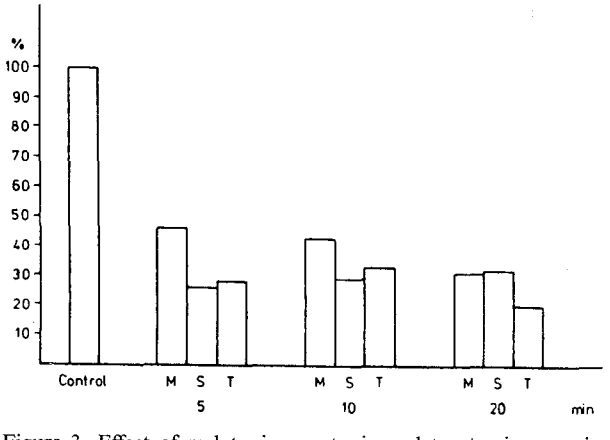

Figure 3. Effect of melatonin, serotonin and tryptamine on pigment cell contraction at 2.5 µg/ml concentration over 5, 10 and 20 min periods of exposure, expressed as a percentage of the untreated control. All of the differences are significant; however, the effects of serotonin and especially tryptamine are stronger (Csaba et al.[13]).

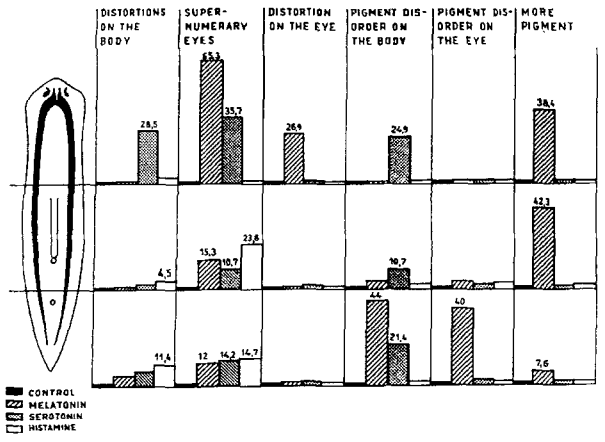

Figure 4. Effect of hormones on the regeneration of the three fragments of Planaria. Supernumerary eyes and pigment disorders of the body were caused first of all by melatonin and serotonin; pigment excess and eye pigment disorder were provoked exclusively by melatonin (Csaba and Bierbauer[9]).

tic response to this hormone is present at a very low level of phylogeny. The fact that serotonin is a precursor of melatonin, and that the parent molecule tryptamine induces a more intensive effect than melatonin, suggests that the receptor is not properly selective. The other possibility is that at this low level the hormone precursor acts more like a hormone than does the molecule which works as a hormone at the higher levels of evolution.

Effects on regeneration

Both melatonin and serotonin are able to influence regeneration in Planaria[9,26,27] (fig. 4). During the regeneration of Planaria only melatonin has the ability to induce excess of pigment in the three fragments (oral, body and tail regions) of the cut organism[9]. Serotonin cannot influence the amount of pigment in any fragment, and only melatonin results in aberration of pigment (loss of the pigment-calyx) first in the tail region. Abnormalities of appearance of pigment, with unusual localization (aggregates of pigment) are caused by both melatonin and serotonin. However, serotonin produces this effect in the head and body regions and melatonin effects are localized to the region of the tail. Supernumerary eyes are produced by both melatonin and serotonin (by melatonin to a larger extent), but this is also caused by the control substance histamine, to a small extent. Distortions of the head pole (tumor-like formations, broad shoulders) are caused only by serotonin.

The facts presented above mean that indoleamines influence the regeneration of Planaria. The specific effect of melatonin on the pigment is more pronounced than the serotonin effect, though the latter is not absolutely negligible. Melatonin and serotonin are both able to induce a pathological change in regeneration.

Inhibitors of serotonin prevent the regeneration of Planaria[26], which points to the necessity of the presence of serotonin for regeneration. This is established by the fact that serotonin supplementation to inhibited Planaria restores the normal rate of the regeneration. This effect of serotonin is mediated by the adenylate cyclase – cAMP system.

P-chlorophenylalanine (pCpa) alters the speed of locomotion and type of movement of Planaria[31]. This is a transient and quickly terminated effect. There is an exception when Planarias are cut into two parts immediately after the treatment, and the head fragments start to regenerate. In this case the characteristic pCpa changes in the movement are present even 6 or 11 days after the treatment. It is supposed that a lasting depletion of serotonin from the head fragment is responsible for this looping effect.

Conclusions

Some estimates show that the unicellular ciliates have been present on the earth for 2 billion years[41,42]. This means that the receptor-type structures for recognition and the signal molecules – hormones – which bind to these receptors must have appeared at the same time. Among these hormones there was 5-hydroxy-tryptamine (serotonin), and as a result of the process of evolution, this was used partly as a neurotransmitter, partly as a tissue hormone in multicellular organisms. Simultaneously, the hormone of the pineal body, melatonin, appeared as the result of an enzymatic transformation.

The Protozoa have the capacity to take up amino acids or use them as food, but they do not transform all of them into hormone-like molecules, and they do not build up all of the possible hormones from them. From tyrosine, which is the parent substance of many hormones in higher organisms, Protozoa form catecholamines but not thyroxine. Catecholamines, like serotonin, can be formed by relatively simple transformations of the parent substance, whereas the formation of thyroxine is more complex. Presumably, only these possibilities of transformation are available at this level of evolution. This hypothesis does not solve the question of why, exactly, serotonin and not another amino acid was suitable to fulfil the role of a neurotransmitter or a tissue hormone, or why it was better for a further 'development'. The fact is, the substances which originate from tryptophan are 'selected' substances for influencing Protozoa, and it is also probable that they function as intracellular mediators.

Our way of thinking is adapted to the higher ranks of evolution. It is difficult for us to conceive that a molecule which possesses hormonal functions in higher animals functions otherwise than as a hormone, even at lower evolutionary levels. We know that during evolution hormonal functions have undergone very large changes, with respect to both the target organs (cells) or the functions. Nevertheless, we tend to look for a hor-

monal function for hormone or hormone-like substances even at the lowest level. In Blepharisma there is a tryptophan-related substance, blepharismone (gamone 2), and this specifically forms an active complex with blepharmone (gamone 1) localized in the membrane[42]. In view of this, it is supposed that serotonin also has a gamone-like function. The gamone-like function of the molecules might establish the evolutionary basis for development both in the direction of hormones – for example towards melatonin, and towards an intracellular mediator role, leading to a neurotransmitter function at a higher level of phylogeny. Of course, the serotonin effects on unicellular organisms are not limited to the gamone functions, since it has an influence on the division rate and on phagocytosis, too. In addition, the eightfold increase of RNA synthesis leads us to suppose that there are other functions, which may be even more important than those mentioned.

The relationship between hormone evolution and receptor evolution is remarkable. At a given level of phylogeny the effective hormone (or precursor) is the one which can combine with the receptor, and not necessarily the top product of the higher evolutionary level from which it comes. This means that the effect of a higher rank hormone on the lower level organism may be due to an overlap at the receptor level, caused by the similarity of the molecules. It is not an indication of an ideal state, indicating an identity of the hormones at the two evolutionary levels. This proposition is not contradicted even if the hormone substance characteristic of the higher level is actually present at the lower level too, as it is not certain that the functions performed are the same.

Protozoa are able to react to the presence of serotonin, and the ability to synthesize this molecule is also present in these cells. There are binding sites showing some specificity for this molecule. In the presence of the hormone, these binding sites become specialized. As serotonin acts through G-protein transduction, and the adenylate cyclase cAMP system, and these components are also present in Tetrahymena, it is likely that the functional responses – which occur in a controllable manner – are influenced by this means.

During evolution, the well-proved mechanisms and the substances they depend upon survive and become perfect, or adjust to the function which is required by the new evolutionary level. We are probably witnesses of this in the transformation of serotonin to melatonin, and its gaining a new function as a consequence. It is also presumable that the receptor of melatonin is formed by a transformation of the serotonin receptor (probably following a gene-level multiplication and mutation). This is supported by the overlap of the action of the two hormones in the pigment cells of Planaria.

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