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Serotonin in *Tetrahymena* – How Does It Work?

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Summary. Presence, uptake and production of serotonin and its effect on the production of other hormones were studied using immunocytochemical flow cytometric method. In a serotonin (10^{-12} M) containing medium up to 15 min. serotonin level does not elevate in the cells, but after 30 min. there is a significant elevation which remains till 4 h. In cells starved in salt solution the elevation is higher which calls attention to the effect of (starvation) stress. Using four enzyme blockers tryptophane hydroxylase inhibitor PCPA decreased (in serotonin-containing medium) and MAO B blocker deprenyl increased serotonin content, while serotonin reuptake inhibitor fluoxetine and MAO-A blocker clorgyline were ineffective. Extremely low concentrations of serotonin (10^{-15} M in case of histamine and 10^{-18} M in case of ACTH and T_3) in the milieu was sufficient for increasing hormone (ACTH, T_3, histamine) levels inside the cells. In conclusion; serotonin can be taken up by the cells and can be produced by induction, as *Tetrahymena* has enzymes for building it up and decomposing it. For synthesizing serotonin; basic molecules from outside are not needed. Serotonin in a minute amount can induce production of different hormones.

Key words: *Tetrahymena*, serotonin, Protozoan hormones, enzyme blockers.

INTRODUCTION

More than thirty years ago we observed for the first time that the unicellular *Tetrahymena* can react to the hormones of higher-ranked animals, such as serotonin, histamine, insulin, thyroxin and precursors (Csaba and Lantos 1973, 1975; Csaba and Németh 1980). Motivated by this observation; many hormones, characteristic to vertebrates were found in *Tetrahymena* (Lenard 1992; LeRoith *et al*. 1980, 1981, 1982a, b, 1987). Signal pathways (Kuno *et al*. 1979; Kőhidai *et al*. 1992; Kovács and Csaba 1987, 1990, 1997a and b, Hassenzahl *et al*. 2001; Bartholomew *et al*. 2008) similar to the ones present in higher-ranked animals were also demonstrated in it. The *Tetrahymena* receptors were similar to those of the vertebrates (O'Neill *et al*. 1988, Zipser *et al*. 1988, Christopher and Sundermann 1995, Kuruvilla and Hennessey 1998, Mace *et al*. 2000, Rosner *et al*. 2003, Robinette *et al*. 2008; Christensen *et al*. 2003, Leick *et al*. 2001) and the phenomenon of hormonal imprinting was found at first in Tetrahymena and only later in phylogenetically more developed animals (Csaba 1980, 1981, 1985, 1994, 2008). One of the hormones, the specific effect of which was demonstrated, was serotonin. This amino acid-type hormone (5-hydroxytryptamine, 5-HT, a neurotransmitter) is present in *Tetrahymena* produced by itself, and can influence many of its physiological functions (Csaba 1993). *Tetrahymena* has enzymes not

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only for the preparation of the serotonin molecule (and its relative, melatonin), but for the decomposition of it (monoamino oxydase, MAO) (Iwata *et al*. 1971, Feldman *et al*. 1977). Serotonin can influence the growth, endocytosis, ciliary regeneration of *Tetrahymena* as well as the production of other hormones (Csaba *et al*. 1979, Quinones-Maldonado and Renaud 1987, Castrodad *et al*. 1988, Csaba and Pállinger 2008a). It seems to be a very important physiological factor at this low level of phylogeny and Essman (1987) hypothesizes a serotonergic system in Tetrahymena. However, some simple and important problems remain unsolved. In the present experiments we search answers to the following questions:

1. When serotonin is present in the milieu of *Tetrahymena* and it is bound by receptors, is it also taken up by the cell, or not?
2. If it is taken up, is there a rapid decomposition or is it stored inside the cell?
3. The enzymes which influence the metabolism of serotonin in the cells of higher organisms are present in *Tetrahymena* and work similarly, or not?
4. What is the minimal concentration of serotonin which can influence *Tetrahymena*?

MATERIALS AND METHODS

Cells and culturing

Tetrahymena pyriformis GL strain was used in the logarithmic phase of growth. The cells were cultured at 28°C in tryptone medium (Sigma, St Louis, USA) containing 0.1% yeast extract, for 24 h. The density of *Tetrahymena* cultures studied was 10^4 cell/ml

Experiments

Experiment No. 1

Serotonin concentrations of the cells were measured (using immunocytochemical flow cytometry) in different time points from one minute to 4 hours. The cells were kept in a serotonin (10^{-12} M) containing tryptone-yeast medium. The medium of some cells were discarded after 1 h and in a similar new medium (without serotonin) the cells were cultivated for further 3 h (Table 1).

Experiment No. 2

The cells were kept in 10^{-12} M serotonin containing tryptone-yeast medium or in 10^{-12} M serotonin containing Losina salt solution ([10 ml 1% NaCl, 10 ml 0.1% MgCl, 10 ml 0.1% CaCl, 10 ml 0.1% KCl, + 950 ml boiled distilled water + 10 ml 0.2% $NaHCO_3$], LSS; Losina-Losinsky 1931) for 30 min. After centrifugation and thorough washings (in he appropriate solutions, with 1500 rpm) the cells were kept in the other medium without serotonin for 1, 2, 3 h, or in the same medium during the whole experiment (Table 2).

Experiment No. 3

Cells (in tryptone-yeast medium) with or without treatment with serotonin (10^{-12} M) received enzyme inhibitors (PCPA methylester, – 0.1 mg/ml, fluoxetine – 0.02 mg/ ml, clorgyline – 0.04 mg/ml, deprenyl – 0.01 mg/ml) and the serotonin content of the cells were measured (Table 3). For solvent of the inhibitors Losina salt solution was used, only in the necessary, equal amount.

Experiment No. 4

Extremely low concentrations of serotonin (10^{-15} – 10^{-21} M) was given for the cells starving in Losina salt solution (for 1 h) and hormone (ACTH [adrenocorticotropin], T_3 [triiodothyronine] and histamine) content was studied after finishing the treatment (Table 4).

The cells were viable in each experiment after the treatments.

Flow cytometric analysis

After the procedure the cells were fixed with 4% paraformaldehyde solution (dissolved in pH 7.2 phosphate buffered saline [PBS]) for 5 min., and then washed twice in wash buffer (0.1% BSA; 20 mM Tris-HCl; 0.9% NaCl; 0.05% Nonidet NP-40; pH 8.2).

To block nonspecific binding of antibodies, the cells were treated with blocking buffer (1% bovine serum albumin [BSA] in PBS) for 30 min. at room temperature. Aliquots from cell suspensions (50 µl) were transferred into tubes, and 50 µl primary antibodies (anti-serotonin, anti-ACTH; anti-histamine; anti-T_3, produced in rabbit [in accordance with the type of the experiment]) purchased from Sigma, StLouis, USA; diluted 1:200 in antibody buffer [1% BSA in wash buffer]) were added for 30 min. at room temperature. Negative controls were carried out with 50 µl PBS containing 10 mg/ml BSA instead of primary antibody. After washing four times with wash buffer to remove excess primary antibody the cells were incubated with FITC-labelled secondary antibody (anti-rabbit IgG; Sigma; dilution 1: 50 with antibody buffer) for 30 min. at room temperature.

For controlling the specificity, the autofluorescence of the cells and unspecificity of the secondary antibodies were checked. This meant that the fluorescence of cells treated only with the secondary antibody (without the specific first antibody) was also measured in the antibody-treated series. The method is suitable for demonstrating the presence of specific hormones inside the cells. The measurement was done in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA), using 5000 cells for each measurement (2 × 5 samples in each subgroup). For the measurement and analysis the CellQuest Pro program was used. The numerical comparison of detected values was done by the comparison of percentage changes of geometric mean channel values (Geo-mean) relative to the appropriate control groups by using the Origin program and Student t-test.

RESULTS AND DISCUSSION

In a 10^{-12} M serotonin-containing milieu the serotonin content of *Tetrahymena* cells does not change up to 15 min. However, at 30 min. there is a significant elevation which remain relatively stable for 4 h. If the cells were thoroughly washed after 1 h and have been studied for further 3 h there was a significant decrease

related to the 1 h value, however the content remained significantly higher than the control value was (Table 1). This could mean that serotonin, which was taken up by the cells in the first half hour, was not decomposed or transformed. The other possibility is that the cells, which are able to produce serotonin, had been induced for serotonin production (hormonal imprinting) (Csaba 1980, 2008). On the basis of these experiments this could not be resolved. A further experiment was done to clarify this problem.

In the laboratory, *Tetrahymena* is cultivated in nourishment-rich milieu, in a tryptone-yeast medium. Nevertheless, it can be kept in an inorganic salt solution (LSS), physiological for Tetrahymena, however does not contain nourishment at all. The cells are starved in it, and does not have the possibility to gain basic molecules for hormone production from outside. In this situation the excess serotonin gradually decreases and after 3 hours its level in the cells is similar to the control (Table 2). In a reverse situation serotonin was given in salt solution. After the cells were taking it up, they were placed in medium; we observed a similar situation with a small difference: the serotonin concentration is significantly more related to the medium-LSS arrangement. Maybe, the higher uptake is due to stress-effect caused by the starvation (Csaba *et al*. 2008, Csaba and Pállinger 2009). This is supported by the third situation, when there was an LSS-LSS arrangement and this produced the highest level with a gradual but low decrease and the value after 3 h did not diminished to the control level. These experiments show (if the LSS + 5HT and LSS + 5HT + LSS are compared) that in addition to the uptake, the production of serotonin is induced by the serotonin treatment and for the "manufacturing" of serotonin the presence of the basic molecule (tryptophane) in the milieu is not needed.

In mammals the basic amino acid of serotonin synthesis is tryptophane. The initial and rate limiting enzyme is the tryptophan hydroxylase, which forms 5-hydoxitriptophane. This latter is decarboxylated by 5-hydroxitriptophane decarboxylase resulting in serotonin (5-HT). In the nervous system the synthesized serotonin is secreted by the nerve cells as a neurotransmitter and in certain nerve cells there is also reuptake of the biogenic amine. However, in many not-nervous cells is also serotonin synthesis and secretion. Monoamino-oxydases MAO A and B) decompose serotonin. As serotonin is a neurotransmitter having important role in psychiatric diseases many molecules had been synthesized for influencing its synthesis, decomposition, uptake and secretion. These molecules seemed to be suitable to study what enzymes are participating in the above mentioned processes, if they can be found in *Tetrahymena* at all. Four inhibitors were used. PCPA (p-chlorophenylalanine) inhibits tryptophan hydroxylase, basic enzyme of serotonin synthesis, fluoxetine (Prozac) a serotonin reuptake inhibitor, clorgyline a specific MAO-A enzyme blocker and deprenyl (Jumex), a specific MAO-B enzyme blocker. These substances were used by other

Table 1. Serotonin content of the cells in different time points after the start of the treatment (serotonin, 10^{-12} M).

Time (min)	Geo-mean +/– SD	p < to the control (0)	p < to the previous
0	11.78 +/–0.15		
1	11.62 +/–0.14	n.s.	n.s.
5	11.63 +/–0.53	n.s.	n.s.
15	11.94 +/–0.16	n.s.	0.02
30	13.19 +/–0.22	0.001	0.001
1 h	12.48 +/–0.18	0.001	0.001
2 h	12.38 +/–0.17	0.001	n.s.
3 h	12.12 +/–0.08	0.01	0.02
4 h	12.99 +/–0.24	0.001	0.001
1 h +1 h	12.71 +/–0.08	0.001	0.05 to 1 h
1 h + 2 h	12.83 +/–0.26	0.001	n.s. to 1 h + 1 h
1 h + 3 h	12.19 +/–0.21	0.02	0.01 to 1 h + 2 h

Table 2. Serotonin levels (10^{-12} M) in the cells in different media combinations in Tetrahymena.

Treatment	Duration of treatment	Geo-mean +/–SD	Significance to control p <
Medium (untreated)		24.9 4+/–1.05	
Medium + 5HT	30 min.	30.7 +/–0.35	0.01
(Medium + 5HT) + salt	30 min. + 1 h	30.46 +/–1.78	0.01
	30 min. + 2 h	28.32 +/–1.07	0.01
	30 min. + 3 h	24.84 +/–0.49	n.s.
Salt + 5-HT	30 min.	26.64 +/–0.9	0.05
(Salt + 5HT) + medium	30 min. + 1 h	33.86 +/–0.98	0.01
	30 min. + 2 h	30.08 +/–1.17	0.01
	30 min. + 3 h	26.66 +/–1.73	n.s.
(Salt + 5-HT) + salt	30 min. + 1 h	33.94 +/–1.87	0.01
	30 min. + 2 h	32.32 +/–1.69	0.01
	30 min. + 3 h	30.22 +/–2.92	0.01

authors in mammalian cell cultures (except PCPA) in nanomolar to micromolar effective concentrations from 5 minutes to chronic treatments (Xu *et al*. 1999, Shimazu *et al*. 2003, Choi *et al*. 2003, Seymour *et al*. 2003, Stokes *et al*. 2000, Kim *et al*. 2005, Mannerström *et al*. 2006, Abdel-Razaq *et al*. 2007) without destroying the cells (as in our present experiments). However, their concentration dependent results can not be compared to our ones, considering that different cell lines required different effective concentrations and *Tetrahymena* is not only a cell, but a complete unicellular organism.

According to the results, control (untreated) *Tetrahymena* does not react to PCPA, fluoxetine and clorgyline however, deprenyl significantly elevates the serotonin level (Table 3). It is possible that there is not serotonin reuptake and MAO A in *Tetrahymena*, however, tryptophan hydroxylase must function there as without it there is not serotonin synthesis. It is possible that the enzyme of *Tetrahymena* is not sensitive to PCPA or the dose was insufficient for inhibiting it. It was known earlier, that MAO enzyme is present in *Tetrahymena* (Feldman *et al*. 1977, Essman 1987), now it seems to be clear that this enzyme is a MAO-B. In case of serotonin excess (in the medium) PCPA blocks tryptophan hydroxylase and diminish serotonin level, there is not MAO-A and inhibition of reuptake. MAO-B inhibition elevates serotonin level. The role of reuptake is dubious, however, the presence of MAO B is clear again.

The last question is, what concentration of serotonin is needed for influencing *Tetrahymena*. Supposing that serotonin is a signal molecule for *Tetrahymena* – as numerous experiments point to this (Csaba *et al*. 1979, Quinones-Maldonado and Renaud 1987, Castrodad *et al*. 1988, Csaba and Pállinger 2008b, Essman 1987) – in wild conditions these cells are living in a huge amount of water which dilutes these signal molecules. This was the reason why the influences for hormone production (as an index) was studied between10^{-15} and 10^{-21} M. In 10^{-18} M (attomolar concentration) only a few molecules/ml are present and in 10^{-21} M only near to nothing. ACTH and T_3 were highly significantly elevated in the cells under the effect of 10^{-18} M serotonin and only histamine production was less sensitive (Table 4). This means that only the presence of a few serotonin molecules are needed for the effect in this low level of phylogeny and this points to a very sophisticated and sensitive mechanism. As the experiment was done in LLS, the production of hormones was done by self-materials.

Table 3. Presence and uptake of serotonin in Tetrahymena with or without treatment with enzyme inhibitors.

Treatment	Geo-mean +/– SD	Significance to untreated control	Significance to the 5-HT--treated group
Untreated	13.9 +/– 0.77		
PCPA	13.12 +/– 0.28	n.s.	
Fluoxetine	13.7 +/– 0.44	n.s.	
Clorgyline	13.58 +/– 0.46	n.s.	
Deprenyl	15.48 +/–0.31	$p < 0.01$	
5-HT treated	16.26 +/–0.4	$p < 0.01$	
PCPA + 5-HT	15.28 +/–0.39	$p < 0.01$	$p < 0.01$
Fluoxetine + 5-HT	18.24 +/– 0.86	$p < 0.01$	$p < 0.01$
Clorgyline + 5-HT	16.84 +/– 1.39	$p < 0.01$	n.s.
Deprenyl + 5-HT	17.38 +/– 0.08	$p < 0.01$	$p < 0.01$

Table 4. Effect of extremely low doses of serotonin on the hormone production of starving Tetrahymena (Geo-mean +/– SD).

Treatment	ACTH	T3	Histamine
Untreated	93.96 +/–6.3	46.68 +/–1.02	31.76 +/–1.63
Serotonin 10^{-15} M	121.72 +!–9.66**	56.44 +/–1.62**	35.18 +/–2.49*
Serotonin 10^{-18} M	112.04 +/–5.86**	49.82 +/–1.17**	32.08 +/–2.31
Serotonin 10^{-21} M	94.7 +/–2.54	47.68 +/–3.14	31.94 +/–1.7

Significance to untreated control: *= $p < 0.05$; **= $p < 0.01$.

Summarizing the results: serotonin seems to be an important signal molecule in *Tetrahymena* which can act at extremely low concentrations. This earlier statement of us (Csaba *et al*. 2007) is strongly supported by the present results. Serotonin stimulates the production of other hormones (e.g. ACTH, T_3 and histamine) from self-materials. Serotonin is taken up by the cells, however, the cell can be stimulated for formation of serotonin. Starvation (stress) increases serotonin formation by the cells which is done using endogeneous basic molecules. Tryptophane hydroxylase and MAO B are present and function in the cells.

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REFERENCES

Abdel-Razaq W., Bates T. E., Kendall D. A. (2007) The effects of antidepressants on cyclic AMP-response element-driven gene transcription in a model cell system. *Biochem. Pharmacol.* **73:** 1995–2003

Bartholomew J., Reichart J., Mundy R., Recktenwald J., Keyser S., Riddle M., Kuruvilla H. (2008) GTP avoidance in *Tetrahymena thermophila* requires tyrosin kinase activity, intracellulat calcium, NOS, and guanylyl cyclase. *Purinergic Signal* **4:** 171–181

Castrodad F. A., Renaud F. L., Ortiz J., Phillips D. M. (1988) Biogenic amines stimulate regeneration of cilia in *Tetrahymena thermophila*. *J. Protozool.* **35:** 260–264

Choi J. S., Choi B. H., Ahn H. S., Kim M. J., Yoon S. H., Min D. S., Jo Y. H., Kim M. S., Sung K. W., Hahn S. J. (2003) Mechanism of block by fluoxetine of 5-hydroxytriptamine3(5-HT3)-mediated currents in NCB-20 neuroblastoma cells. *Biochem. Pharmacol.* **66:** 2125–2132

Christensen S. T., Guerra C. F., Awan A., Wheatley D. N., Satir P. (2003) Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Curr. Biol*. **13:** R50–2

Christopher G. K., Sundermann C. H. (1995) Isolation and partial characterisation of the insulin binding site of *Tetrahymena pyriformis*. *Biochem, Biophys. Res. Com.* **212:** 515–523

Csaba G. (1980) Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. *Biol. Rev.* **55:** 47–63

Csaba G. (1981) Ontogeny and phylogeny of hormone receptors. Karger, Basel

Csaba G. (1985) The unicellular *Tetrahymen*a as a model cell for receptor research. *Int. Rev. Cytol.* **95:** 327–377

Csaba G.(1993) Presence in and effects of pineal indoleamines at very low level of phylogeny. *Experientia* **49:** 627–634

Csaba G. (1994) Phylogeny and ontogeny of hormone receptors: origin and development of hormone receptors. *Int. Rev. Cytol.* **155:** 1–48

Csaba G. (2008) Hormonal imprinting: phylogeny, ontogeny diseases and possible role in present-day human evolution. *Cell Biochem. Funct.* **26:** 1–10

Csaba G., Lantos T. (1973) Effect of hormones on *Protozoa*. Studies on the phagocytotic effect of histamine, 5-hydroxytryptamine and indoleacetic acid in *Tetrahymena pyriformis*. *Cytobiologie* **7:** 361–365

Csaba G., Lantos T. (1975) Effect of insulin on glucose uptake of *Protozoa*. *Experientia* **31:** 1097–1098

Csaba G., Németh G., Prohászka J. (1979) Effect of hormones and related compounds on the multiplication of *Tetrahymena*. *Exp. Cell. Biol.* **47:** 307–311

Csaba G., Németh G. (1980) Effect of hormones and their precursors on protozoa – the selective responsiveness of *Tetrahymena*. *Comp. Biochem. Physiol.* **65B:** 387–390

Csaba G., Kovács P, Pállinger É. (2007) How does the unicellular *Tetrahymena* utilise the hormones that it produces? Paying a visit in the realm of atto- and zeptomolar concentrations. *Cell Tissue Res.* **327:** 199–203

Csaba G., Pállinger É. (2008a) Is there a hormonal network in *Tetrahymena*? A systematic investigation of hormonal effects on the hormone content. *Cell Biochem. Funct.* **26:** 303–308

Csaba G., Pállinger É. (2008b) A general response to stressors by the unicellular *Tetrahymena*: effect of stress on the hormone levels. *Cell Biochem. Funct.* **26:** 797–800

Csaba G., Kovács P., Pállinger É. (2008) Comparison of the insulin binding, uptake and endogeneous insulin content in long- and short-term starvation in *Tetrahymena*. *Cell Biochem. Funct.* **26:** 64–69

Csaba G., Pállinger É. (2009) How applicable is the general adaptation syndrome to the unicellular *Tetrahymena*? *Cell Biochem. Funct.* **27:** 12–15

Essman E. J. (1987) The serotonergic system in *Tetrahymena pyriformis*. *Ric. Clin. Lab*. **17:** 77–82

Feldman J. M., Roche J. M., Blum J. J. (1977) Monoamine oxidase and catechol-O-methyl transferase activity in *Tetrahymena. J. Protozool.* **24:** 459–462

Hassenzahl D. L., Yorgey N. K., Keedy M. D., Price A. R., Hall J. A., Myzcka C. C., Kuruvilla H. G. (2001) Chemorepellent signaling through the PACAP/lysozyme receptor is mediated through cAMP abd PKC in *Tetrahymena thermophila*. *J. Comp. Physiol. A* **187:** 171–176

Iwata H., Kariya K., Okamoto H. (1971) Amine oxydative system in *Tetrahymena pyriformis* W. *Experientia* **27:** 388–389

Kim H. J., Choi J. S., Lee Y. M., Shim E. Y., Hong S. H., Kim M. J., Min D. S., Rhie D. J., Kim M. S., Jo Y. H., Hahn S. J., Yoon S. H. (2005) Fluoxetine inhibits ATP-induced [Ca(2+)] (i) increase in PC12 cells by inhibiting both extracellular Ca (2+) influx and Ca (2+) release from intracelluilar stores. *Neuropharmacology* **49:** 265–274

Kovács P., Csaba G. (1987) The role of Ca^{2+} in hormonal imprinting of the *Tetrahymena*. *Acta Physiol. Hung.* **69:** 167–169

Kovács P., Csaba G. (1990) Influence of the phosphoinositol (PI) system in the mechanism of hormonal imprinting. *Biochem. Biophys. Res. Com.* **170:** 119–126

Kovács P., Csaba G. (1997) PLA_2 activity in *Tetrahymena pyriformis*. Effects of inhibitors and stimulators. *J. Lipid Mediators Cell Signaling* **15:** 233–247

Kovács P., Csaba G. (1997) Indomethacin alters phospholipid and arachidonate metabolism in *Tetrahymena pyriformis*. *Comp. Biochem. Physiol.* **117C:** 311–315

Köhidai L., Barsony J., Roth J., Marx S. J. (1992) Rapid effects of insulin on cyclic GMP location in an intact protozoan. *Experientia* **48:** 476–481

Kuno T., Yoshida N., Tanaka C. (1979) Immunocytochemical localization of cyclic AMP and cyclic GMP in synchronously dividing *Tetrahymena*. *Acta Histochem. Cytochem.* **12:** 563

Kuruvilla H. G., Hennessey T. M. (1998) Purification and characterisation of a novell chemorepellent receptor from *Tetrahymena thermophila*. *J. Membr. Biol.* **162:** 51–57

Leick V., Bog-Hansen T. C., Juhl H. A. (2001) Insulin/FGF-binding ciliary membrane glycoprotein from Tetrahymena. J. Membr. Biol. **18:** 47–53

Lenard J. (1992) Mammalian hormones in microbial cells. *Trends Biochem. Sci.* **17:** 147–150

LeRoith D., Schiloach J., Roth J., Lesniak M. A. (1980) Evolutionary origins of vertebrate hormones: substances similar to mammalian insulin are native to unicellular eukaryotes. *Proc. Natl. Acad. Sci. USA* **77:** 6184–6186

LeRoith D., Schiloach J., Roth J., Lesniak M. A. (1981) Evidence that material very similar to insulin is native to *E. coli*. *J. Biol. Chem*. **256:** 6533–6536

LeRoith D., Liotta A. H., Roth J., Schiloach J., Lewis M. E., Pert C. B., Krieger D. T. (1982) ACTH and beta endorphin like materials are native to unicellular organisms. *Proc. Natl. Acad. Sci. USA* **79:** 2086–2090

LeRoith D., Schiloach J., Berelowitz M., Frohman L. A., Krieger B. T., Roth J. (1982) Are messenger molecules in microbes the ancestors of the vertebrate hormones and tissue factors? *Fed. Proc.* **42:** 2602–2607

LeRoith D., Roberts C Jr., Lesniak M. A., Roth J. (1987) Receptor for intercellular messenger molecules in microbes: similarity to vertebrate receptors and possible implications for diseases in man. In: Development of hormone receptors, (Ed. G. Csaba). Birkhauser, Basel

Losina-Losinsky L. K. (1931) Zur Ernährungsphysiologie der Infusorien. Unterschungen über die Nahrungsmittel und Vermehrung bei *Paramecium caudatum*, *Arch. Protistenk.* **74:** 18–20

Mace S. R., Dean J. G., Murphy J. R., Rhodes J. L., Kuruvilla H. G. (2000) PACAP-38 is a chemorepellent and an agonist for the lysozyme receptor in *tetrahymena thermophila*. *J. Comp. Physiol. A* **186:** 39–43

Mannerström M., Toimela T., Ylikomi T., Tahti H. (2006) The combined use of human neural and liver cell lines and mouse hepatocytes improves the predictability of the neurotoxicity of selected drugs. *Toxicolol. Lett.* **165:** 195–202

O'Neill J. B., Pert C. B., Ruff M. R., Smith C. C., Higgins W. J., Zipser B. (1988) Identification and characterization of the opiate receptor in the ciliated protozoan *Tetrahymena*. *Brain Res.* **450:** 303–315

Quinones-Maldonado V., Renaud F. L. (1987) Effect of biogenic amines on the phagocytosis in *Tetrahymena thermophila*. *J. Protozool.* **34:** 435–438

Robinette E. G., Gulley K. T., Cassity K. J., King E. E., Nielsen A. J., Rozelle C. L., Warren T. J., Morrow J. M., Kuruvilla H. G. (2008) A comparison of the polycation receptors of *Paramecium tetraaurelia* and *Tetrahymena thermophila J. Eukaryot. Microbiol.* **55:** 86–90

Rosner B. N., Bartholomew J. N., Gaines C. D., Riddle M. L., Everett H. A., Rulapaugh K. G., Nickerson L. E., Marshall M. R., Kuruvilla H. G. (2003) Biochemical evidence for a P2Y-like receptor in *Tetrahymena thermophila J. Comp. Physiol. A.* **189:** 781–789

Seymour C. B., Mothersill C., Mooney R., Moriarty M., Tipton K. F. (2003) Monoamone oxydase inhibitors l-deprenyl and clorgyline protect nonmalignant human cells from ionising radiation and chemotherapy toxicity. *Br. J. Cancer.* **89:** 1979–1986

Shimazu S., Tanigawa A., Sato N., Yoneda F., Hayashi K., Knoll J. (2003) Enhancer substances: selegiline and R-(-)-benzofuran-2-yl)-2-propylaminopentane [(-)-BPAP] enhance the neurotrophic factor synthesison cultured mouse astrocytes. *Life Sci.* **72:** 2785–2792

Xu L., Seigel G. M., Ma J. X. (1999) l-Deprenyl, blocking apoptosis and regulating gene expression in cultured retinal neurons. *Biochem. Pharmacol.* **58:** 1183–1190

Zipser B., Ruff M. R., O'Neill J. B., Smith C. C., Higgins W. J., Pert C. B. (1988) The opiate receptor: a single 111 kDa recognition molecule appears to be conserved in *Tetrahymena*, leech and rat. *Brain Res.* **463:** 296–304

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