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Effect of C_2 ceramide on the inositol phospholipid metabolism (uptake of ^{32}P, ^{3}H-serine and ^{3}H-palmitic acid) and apoptosis-related morphological changes in *Tetrahymena*

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Abstract

Sphingomyelin metabolites have significant role in the regulation of many life processes of mammalian cells. In the present experiments the influence of phospholipid turnover and apoptosis related morphologic signs by one of this metabolite, C_2 ceramide was studied, and compared to the control, untreated cells, in the unicellular *Tetrahymena*. The incorporation of phospholipid head group components (serine, phosphorus) show a clear time-dependence; while the incorporation of fatty acid component (palmitic acid) is very fast: no significant alterations were found between 5- and 60-min incubations. C_2 ceramide treatment didn't alter ^{3}H-palmitic acid incorporation into phospholipids, however ^{3}H-serine incorporation was mainly inhibited. The amount of total incorporated ^{32}P was also decreased, on the other hand the lover concentration C_2 ceramide (10 μM) elevated the synthesis of inositol phospholipids. The higher concentration of C_2 ceramide (50 μM) had inhibitory effect on the synthesis of each phospholipids examined. This means that in the presence of the C_2 ceramide the synthesis, recovery and turnover of phospholipids, participating in signal transduction, are altered. However these observations were based the uptake of labeled phospholipid precursors, which gives information on the dynamics of the process, without using lipid mass measurements. C_2 ceramide also caused the rounding off the cells, DNA degradation and nuclear condensation. These latter observations point to morphological signs of apoptosis. The results call attention to the role of sphingomyelin metabolites on signalization of unicellulars, to the cross-talk between the inositol phospholipids and sphingomyelin metabolites, and the role of these molecules in the apoptotic processes at a low evolutionary level.

Keywords: *Tetrahymena*; Ceramide; Inositol phospholipids; Apoptosis; Signal transduction; Second messengers; Evolution

1. Introduction

Signaling networks that use glycerophospholipid metabolites as second messengers have been demonstrated in *Tetrahymena pyriformis* [14–16] and provided evidences for the activity of enzymes as PLA_2 and PLD, which play important role in the generation of such messengers [16,17]. Enzymes has been identified as e.g. PKC, which are activated by glycerophospholipid metabolites [12]. Sphingolipid derived second messengers could also play an important role in signaling in *Tetrahymena*, as it takes place in the higher eukaryotes [9].

The cytokine tumor necrosis factor α (TNFα) has multiple biological activities. TNFα causes a rapid increase of diacylglycerol (DAG) via hydrolysis of phosphatidylcholine (PC) by PC-specific phospholipase C. The generated DAG leads to the activation of sphingomyelinase which causes breakdown of sphingomyelin to produce ceramides [9,11]. Ceramides have been implicated as key mediators in signaling pathways of mammals, with outcomes as diverse as cell proliferation, differentiation, growth arrest and apoptosis, for review see [11].

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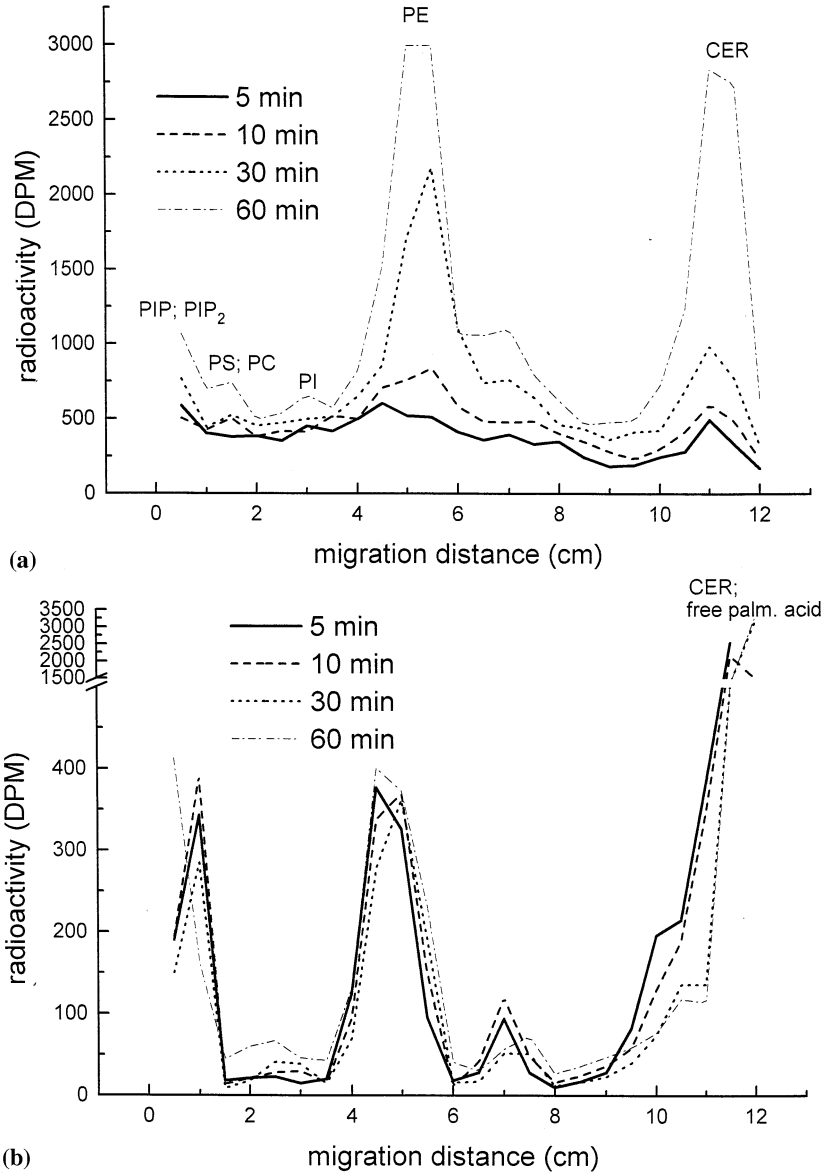

Fig. 1. Time course of the incorporation of ^{3}H-serine (a) and ^{3}H-palmitic acid (b) into the phospholipids of *Tetrahymena*. Total lipids were isolated after Bilgh and Dyer [1]; separated on silica gel G TLC plates (solvent:chloroform:methanol:water = 65:35:2.5 by vol), and the radioactivity was measured by liquid scintillation counter. The experiments were done four times with a representative experiment shown.

Treatments with TNFα resulted spectacular alterations in the phospholipid metabolism in *Tetrahymena*: the incorporation of ^{32}P into the phospholipids and inositol phospholipids decreased and the ceramide production increased. Moreover these treatments reduced cell growth, altered the morphometric indexes, increased chromatin condensation [20]. Our data led to the conclusion that after TNFα treatment the generated ceramide is the main possible factor responsible for these phenomena. This conclusion is supported by the fact that C_2 ceramide disturbs the actin cytoskeleton and inhibits the normal function of contractile vacuoles of *Tetrahymena* [18]. We focused in the present paper on the effect of C_2 ceramide, a cell permeable analog of naturally produced ceramide, as in our earlier work [20] after TNFα treatments also this compound was, presumably, responsible for the above mentioned alterations.

The main goal of this study was to investigate the effect of exogeneous ceramide on the above mentioned phenomena by using of cell permeable ceramide analogue *N*-acetyl-sphingosine (C_2 ceramide).

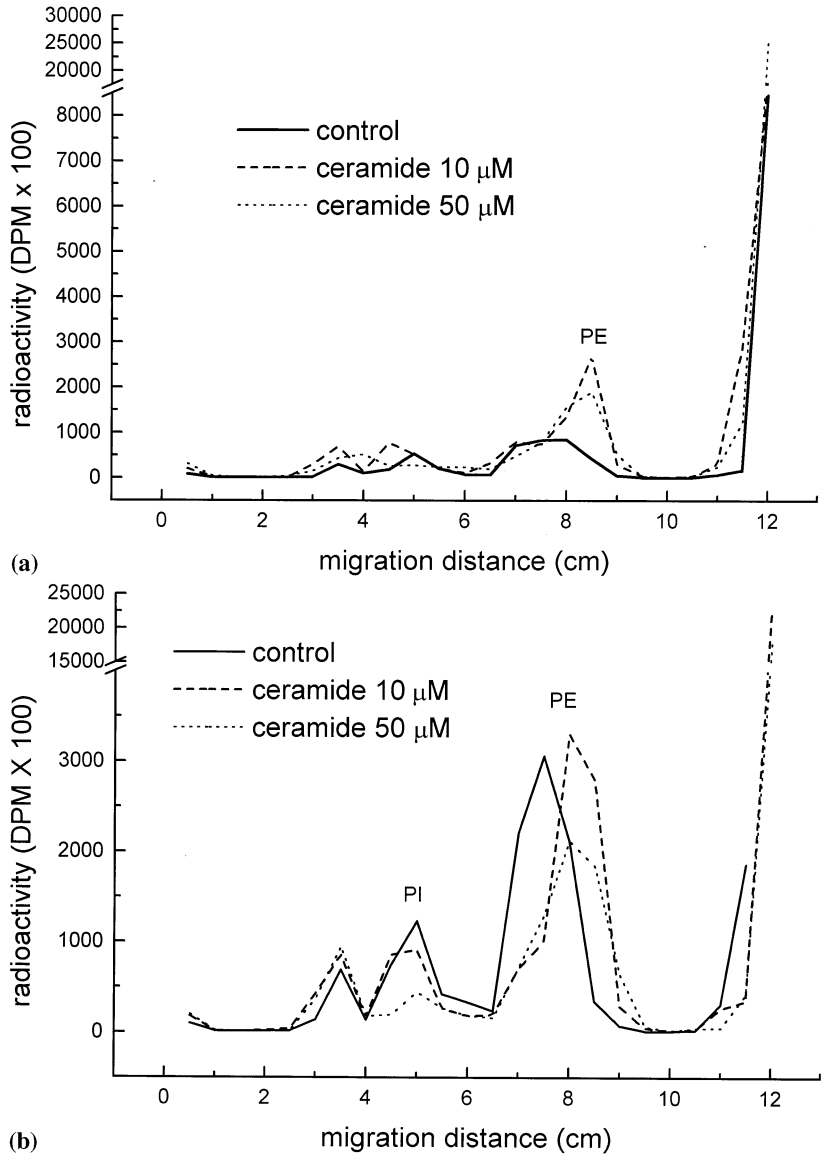

Fig. 2. The effect of C_2 ceramide on the incorporation of ^{3}H-palmitic acid into the phospholipids of *Tetrahymena* after 5 min (a) and 30 min (b) treatment. Lipid separation, analysis as described in Fig. 1. The experiments were done four times with a representative experiment shown.

2. Materials and methods

2.1. Materials

^{32}P Na-orthophosphate (specific activity 7 GBq mM^{-1}) was purchased from Izinta (Budapest, Hungary). [9, 10-^{3}H] palmitic acid (specific activity 2 TBq mM^{-1}) and L-[3-^{3}H] serine (specific activity 962 GBq mM^{-1}) were obtained from Amersham (Buckinghamshire, UK). *N*-acetyl sphingosine (C_2 ceramide), lipid chromatographic standards (PI, PIP, PIP_2, PE, PA, PC, ceramide) and tryptone were obtained from Sigma (St. Louis, MO, USA). Silica gel G TLC aluminium sheets were obtained from Merck (Darmstadt, Germany). Yeast extract was purchased from Oxoid (Unipath, Basingstoke, Hampshire, UK). All other chemicals used were of analytical grade available from commercial sources.

2.2. Tetrahymena cultures

In the experiments *Tetrahymena pyriformis* GL strain was tested in the logarithmic phase of growth. The cells were cultivated at 28°C in 0.1% yeast extract containing 1% trypton medium. Before the experiments the cells were washed with fresh culture medium, and were resuspended at a concentration of 5×10^5 cells ml^{-1}.

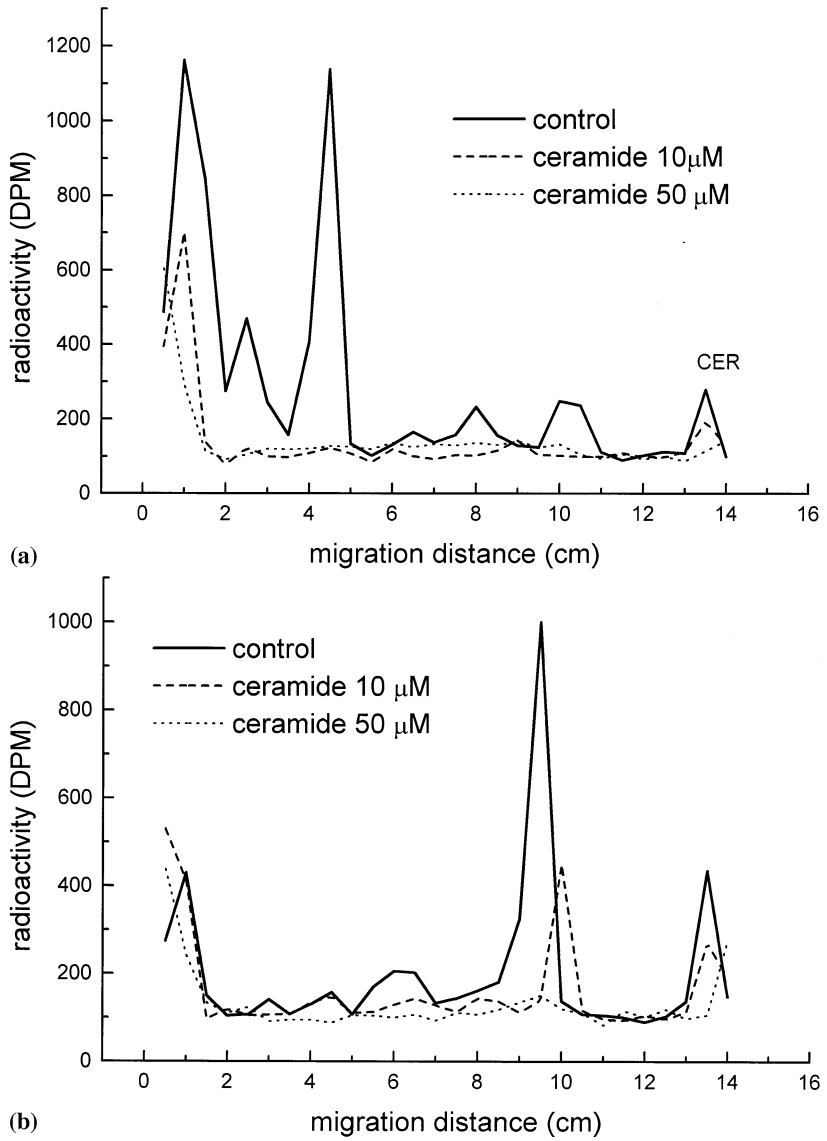

Fig. 3. The effect of C_2 ceramide on the incorporation of ^{3}H-serine into the phospholipids of *Tetrahymena*. Lipid separation, analysis as described in Fig. 1. The experiments were done four times with a representative experiment shown.

2.3. Time-course of the incorporation of ^{3}H-palmitic acid and ^{3}H-serine into the phospholipids of Tetrahymena

Tetrahymena cultures were treated with: (a) 1 μCi ml^{-1} = 37 KBq ml^{-1} ^{3}H-palmitic acid or (b) 1 μCi ml^{-1} ^{3}H-serine. Samples of 5 ml were taken after 5, 10, 30 and 60 min. The incubation was stopped by rapid chilling the cells on ice. After centrifugation the lipid content of the cell pellet was separated according to the method of Bligh and Dyer [1]. The lipids were separated on silica gel G TLC aluminium sheets with chloroform:methanol:water = 65:35:2.5 (by volume) solvent system. After development 0.5 cm strips were cut from the chromatogram into scintillation vials, and the radioactivity was measured by liquid scintillation counter. The individual lipids were identified by parallel run of authentic standards.

2.4. Time course of the incorporation of ^{32}P into the phospholipids of Tetrahymena

Tetrahymena cultures were treated with 0.6 MBq ml^{-1} ^{32}P Na-orthophosphate. Samples were taken after 1, 5, 15, 30 and 60 min. The phospholipids were separated according the method of Suchard et al. [25]. The phospholipid samples were analyzed on silica gel G TLC plates; solvent system: chloroform: acetone: methanol:acetic acid:water = 45:15:13:12:8 (by volume). The chromatograms were covered by Kodak TMG X-ray film. After exposure (~18 h) and development of radiograms, the radioactivity of individual phospholipid spots were analyzed by laser densitometer (Ultroscan XL, Pharmacia-LKB, Uppsala, Sweden). Comparative data are given by the laser densitometric measurements on radiograms of TLC-separated indi-

Table 1
Time-course of the ^{32}P incorporation into the phospholipids of *Tetrahymena pyriformis*

	1 min	5 min	15 min	30 min	60 min
PI	454 ± 67	666 ± 55	1090 ± 63	7210 ± 325	31 023 ± 880
PIP	—	—	449 ± 37	2247 ± 138	14 486 ± 1025
PIP_2	—	651 ± 67	2301 ± 72	3256 ± 221	9293 ± 600
PC + PS	—	800 ± 95	4706 ± 250	18 154 ± 922	38 266 ± 1220
PE	—	4226 ± 182	22 148 ± 975	60 696 ± 3840	82 000 ± 3200
PA	—	964 ± 54	1986 ± 142	6363 ± 355	10 933 ± 775

The phospholipid spots from chromatogram were scraped into scintillation vials, and the radioactivity (DPM) measured with liquid scintillation counter.
The data represent means of four independent experiments ± S.D.

vidual phospholipids expressed as percent of total incorporated ^{32}P as 100%. To get absolute values from incorporated radioactivity of phospholipids, after the radiographic exposure the spots were scraped into scintillation vials and the activities were measured by liquid scintillation counter.

2.5. Effect of C_2 ceramide on the incorporation of ^3H-palmitic acid; ^3H-serine and ^{32}P into the phospholipids of Tetrahymena

Tetrahymena cultures were treated (a) 10 μM or (b) 50 μM C_2 ceramide. After 20 min 37 KBq ml^{-1} ^3H-palmitic acid; 37 KBq ml^{-1} ^3H-serine or 0.6 MBq ml^{-1} ^{32}P were added to the cultures. Non C_2 ceramide treated cells served as controls. Samples were taken after 5 and 30 min (in case of ^{32}P after 1, 5, 15, 30 and 60 min). The lipid extraction and analysis were done after the methods described above.

2.6. Effect of C_2 ceramide on the chromatin condensation of Tetrahymena

Washed and in fresh culture medium resuspended *Tetrahymena* cultures were divided into three experimental groups: (a) control group; (b) 10 μM C_2 ceramide treated group and (c) 50 μM C_2 ceramide treated group. Samples were taken after 10 and 60 min. The cells were fixed in 4% paraformaldehyde solution (in pH 7.2 PBS), washed in two changes of PBS and Feulgen reaction for DNA was done [8]. The duration of hydrolysis with *n*HCl at 60°C was 30 min. The colour intensity of Feulgen reaction (transparence, ϑ) was assessed with a Zeiss Amplival cytophotometer at 546 nm. The cytophotometer measures the light permeability (transparence): the greater chromatin concentration per volume (condensation) results a decrease in the transparence, thus the lower cytophotometric values mean higher condensations. 100 cells were evaluated in each group.

2.7. Effect of C_2 ceramide on the nucleosomal fragmentation of Tetrahymena

Tetrahymena cultures were treated with 10 or 50 μM C_2 ceramide for 60 min. Non treated cultures served as controls. After treatments the cells were lysed, and the DNA was extracted and submitted to agarose gel electrophoresis as described in [28]. The samples were electrophoresed at 40 V for 6 h through a 0.9% agarose gel in Tris–borate buffer. DNA bands were visualized under UV light after staining with ethidium bromide. Pharmacia 100 Base-pair Ladder (Pharmacia Biotech, Uppsala, Sweden) was used as molecular size marker.

2.8. Morphometric analysis of the Tetrahymena cells

Tetrahymena cultures were treated 10 or 50 μM C_2 ceramide for 60 min. Non treated cells served as controls. Before the analysis the samples were fixed in 4% paraformaldehyde solution and after washing stained with 0.1% toluidin blue for 2 min. The gently washed cells were applied onto microscopic slides and were photographed in light microscope; magnification: 800 ×. The prints were scanned by Hewlett Packard HP-Scan Jet II scanner and the data obtained were analyzed by a computer programme (Biomorph 1.1). The interactive morphometry programme provided us to determine area and ratio of the shortest and longest axis (W/L) of cells. 100 cells were analyzed in each group.

2.9. Analysis of the viability of the cells

After each treatment the viability of the cells was analyzed by trypan blue exclusion. In the further measurements only the unlabeled (viable) cell populations were used.

2.10. Statistical treatment of data

In each experiment, the experimental data represent the means of quadruplicate experiments. Student's *t*-

Table 2
Effect of C_2 ceramide on the ^{32}P incorporation into the phospholipids of *Tetrahymena*

Time (min)	PI	PIP	PIP_2	PC + PS	PE	PA
Control cultures						
1	3.6 ± 0.11	—	—	—	—	—
5	1.45 ± 0.07	—	1.9 ± 0.05	1.0 ± 0.05	2.01 ± 0.14	2.3 ± 0.12
15	4.1 ± 0.1	0.9 ± 0.04	3.55 ± 0.14	4.1 ± 0.1	17.4 ± 1.35	5.05 ± 0.38
30	8.0 ± 0.32	3.05 ± 0.13	3.35 ± 0.14	5.95 ± 0.16	21.15 ± 1.36	5.6 ± 0.26
60	11.3 ± 0.26	5.3 ± 0.22	3.4 ± 0.1	15.15 ± 0.9	32.51 ± 1.97	3.4 ± 0.23
10 μM C_2 ceramide treated cultures						
1	—	—	—	—	—	—
5	2.7 ± 0.13*	—	—	—	4.8 ± 0.27**	2.0 ± 0.13
15	7.55 ± 0.29*	1.2 ± 0.1	9.8 ± 0.32*	1.2 ± 0.09*	14.3 ± 0.9**	2.85 ± 0.2**
30	7.7 ± 0.87	1.9 ± 0.09*	6.5 ± 0.17*	4.3 ± 0.22**	22.3 ± 1.2	6.05 ± 0.5
60	11.1 ± 0.66	1.5 ± 0.1*	6.6 ± 0.26*	7.7 ± 0.63*	26.5 ± 1.09*	10.6 ± 0.58*
50 μM C_2 ceramide treated cultures						
1	—	—	—	—	—	—
5	—	—	—	—	—	—
15	3.7 ± 0.2***	—	8.6 ± 0.4****	1.3 ± 0.08*	—	—
30	3.0 ± 0.1*,***	—	2.2 ± 0.1***	—	1.7 ± 0.1*,***	—
60	6.4 ± 0.3*,***	1.4 ± 0.07*	6.3 ± 0.22*	4.0 ± 0.1*,***	5.2 ± 0.2*,***	1.4 ± 0.09*,***

The results shown are the mean (± S.D.) of four separate experiments and expressed as percent of total incorporated ^{32}P as 100%.
Changes in comparative values were monitored by laser densitometric measurements of radiograms of TLC-separated phospholipids.
* $p < 0.01$ to the control;
** $p < 0.05$ to the control;
*** $p < 0.01$ to the 10 μM C_2 ceramide treated;
**** $p < 0.05$ to the 10 μM C_2 ceramide treated.

test was used for calculations, with $p < 0.05$ accepted as the level of statistical significance.

3. Results

The incorporation of ^3H-serine into the phospholipids of *Tetrahymena* show time-dependence (Fig. 1(a)), while the incorporation of ^3H-palmitic acid is extremely quick: no significant alterations were found between the 5- and 60-min incubations (Fig. 1(b)).

Treatments with C_2 ceramide didn't alter significantly the incorporation of ^3H-palmitic acid into the *Tetrahymena* phospholipids (Fig. 2(a) and (b)): 5-min treatments with 10 and 50 μM C_2 ceramide resulted in the PE higher incorporation compared to the control values; while after 30-min treatments the higher concentration (50 μM) C_2 ceramide resulted in a decrease of radioactivity in the PE and PI (Fig. 2(a) and (b)).

The incorporation of ^3H-serine into the phospholipids was inhibited by both 10 and 50 μM C_2 ceramide at 5- and 30-min incubations, except into the PIP and PIP_2 after 30-min treatments (Fig. 3(a) and (b)).

The incorporation of ^{32}P into the phospholipids of *Tetrahymena* was time dependent (Table 1) and it was significantly influenced by C_2 ceramide. Partly the amount of incorporated ^{32}P decreased significantly (186000 ± 1400 DPM into the controls; 81012 ± 375 DPM into the 10 μM C_2 ceramide-treated cultures and 7992 ± 116 DPM into the 50 μM C_2 ceramide-treated cultures after 60-min treatments); partly the comparative values between the individual phospholipids were altered (Table 2). It was remarkable that in case of 50 μM C_2 ceramide the ^{32}P labeling appeared only after 15 min incubation with radioactive phosphorus. The lower C_2 ceramide concentration (10 μM) caused in each inositol phospholipid higher absolute values in the radioactivity after 15 min treatments compared to the controls measured the radioactivity of individual phospholipid spots on TLC by liquid scintillation counter. These values were always significantly under the control level in the case of 50 μM C_2 ceramide.

Treatments with 50 μM C_2 ceramide altered significantly the transparence (increased the condensation) of chromatin both in the case of 10 and 60 min incubation revealed by cytophotometry at 546 nm of Feulgen-reaction product, while the 10 μM C_2 ceramide treatments showed effect only after 60 min incubation (Fig. 4). Likewise the nucleosomal degradation refers to the effect of C_2 ceramide on the nuclear structure: treatment with 50 μM C_2 ceramide resulted in the production of small (200–400 kB) DNA fragments a significant elevation, while the 60-min treatment with 10 μM C_2 ceramide showed no alteration compared to the controls (Fig. 5).

Morphometric characters were effectively influenced by 50 μM C_2 ceramide concentration: both the area and W/L ratio altered: the size of cells decreased signifi-

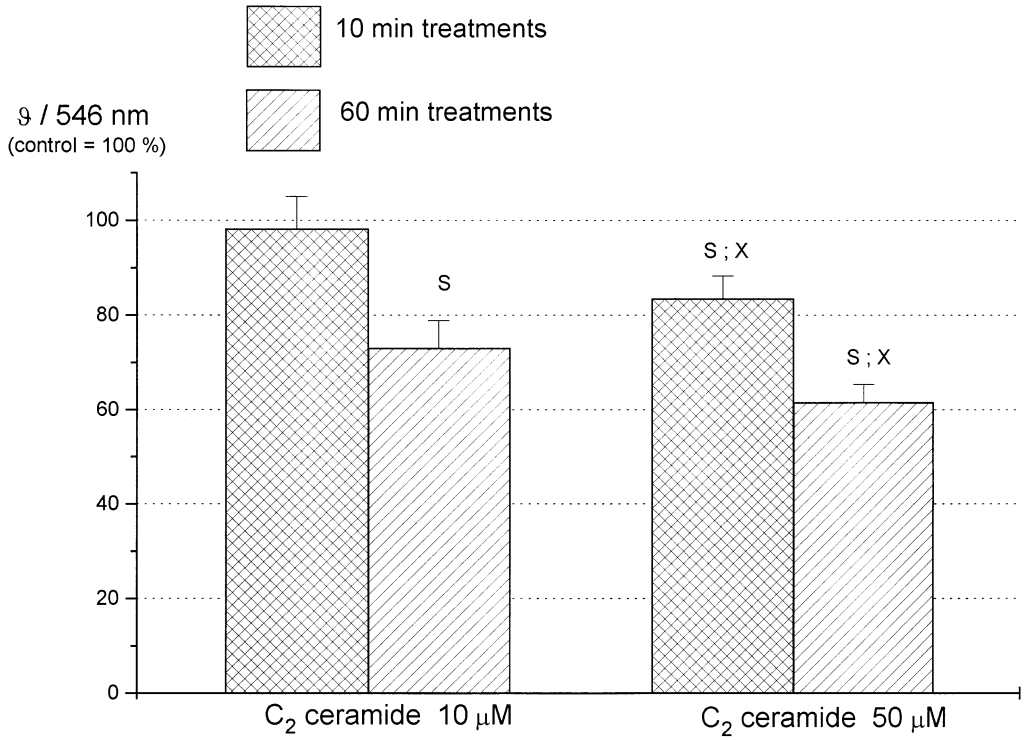

Fig. 4. The effect of C_2 ceramide on the transparence (ϑ) of Feulgen reaction product (chromatin condensation) in the nuclei of *Tetrahymena*. The data represent the mean ($\pm$S.D.) derived from independent experiments performed in quadruplicate. $s = p < 0.01$ to the control; $x = p < 0.01$ to the 10 min treated.

cantly and the elevation of W/L ratio indicated the rounding off the cells (Fig. 6).

4. Discussion

Tetrahymena has receptor, hormone, second messenger systems, which are working seemingly similar to vertebrate ones [6,13]. Receptor activated cyclic-AMP [7], cyclic-GMP [21], calmodulin-dependent guanylate cyclase [19], and inositol phospholipids [14] have very important role in the regulation of the cellular functions of *Tetrahymena*.

The sphingomyelin metabolites as ceramide, sphingosine, sphingosine-1-phosphate have been shown to play an important role in such fundamental biological processes as cell proliferation, oncogenesis and apoptosis, for review see [10]. The role of these sphingomyelin breakdown products are analogous to the role of glycerolipid derived metabolites such as DAG and PA. There is a significant interaction and 'cross-talk' between the sphingolipid and glycerolipid pathways that modifies signal transduction participating in the control of some cellular functions as cell division, apoptosis, etc., for review see [2].

Since *Tetrahymena* contains sphingomyelin [26], and TNFα treatments generated ceramide also in this organism [20], it was presumable that this sphingomyelin metabolite plays an important role in regulation of certain cellular functions also in *Tetrahymena*. The results of present experiments corroborate this premise.

In our experiments the phospholipid metabolism has been detected by the use of ^{3}H-labeled serine and palmitic acid as well as ^{32}P, giving information on the dynamics of the processes, without using lipid mass measurements. The incorporation of palmitic acid into the phospholipids of *Tetrahymena* is very fast, and its intensity was not inhibited significantly by C_2 ceramide treatments. Whereas the incorporation of ^{32}P and serine, the components of phospholipid head groups, showed a very clear time-dependence, and their activity were hindered by C_2 ceramide treatments, although the incorporation of ^{3}H serine into PIP and PIP_2 was slightly increased.

Incorporation of serine is an essential step in the synthesis of glycerophospholipids and sphingolipids alike. Serine is incorporated directly into the head group of PS. The metabolism of phospholipid head groups, e.g. in case of PS, converts these molecules into different phospholipids as PE (by decarboxylation) or PC (by methylation of PE). These metabolic steps take up a certain time, thus the time-dependence of serine incorporation into different phospholipids is explainable in this way.

Treatments with C_2 ceramide inhibit significantly the incorporation of serine into the most phospholipids,

nevertheless in presence of both C_2 ceramide concentration for 5 or 30 min didn't abolish the incorporation of serine into the PIP and PIP_2. It presumable that the breakdown of PIP_2 and the phosphorylation of PIP were hindered, and these effects inhibited also the formation of DAG, PA and later CDP.DAG from these phospholipids, and thus the common turnover of inositol phospholipids.

The observations in the course of ^{32}P incorporation demonstrated similar phenomena. In case of ^{32}P incorporation the first radioactively labeled phospholipid is the PI; after that the PE contains the greatest part of radioactivity. The C_2 ceramide treatments retarded this labeling, but in case of lower concentration (10 μM) at 15 min we measured higher labeled PI, PIP and PIP_2 concentration, that in the controls. These findings indicate that in the presence of C_2 ceramide the synthesis, recovery and turnover of phospholipids are inhibited. Because of the inositol phospholipids play a very important role in signalization, it is presumable that the inhibition of the normal function and the turnover of this system lead to numerous signalization problems.

Phosphoinositides have been reported to be important in the regulation of actin polymerization [5]. After C_2 ceramide treatment the labeling with F-actin-specific phalloidin in the epiplasmic area of *Tetrahymena* was disappeared, and also the function of contractile vacuoles were inhibited [18]. Depolymerization of F-actin during the TNFα-induced apoptosis occurs after the generation of ceramide in mammalian cells [22]. The morphometric alterations of *Tetrahymena*, the rounding off the cells (elevation of the W/L ratio) indicates the possible actin skeleton-disturbing effect of C_2 ceramide; indicate the induction of apoptotic alterations also in these cells. These facts suggest a connection between the inositol phospholipid turnover, C_2 ceramide treatments and actin polymerization in *Tetrahymena*.

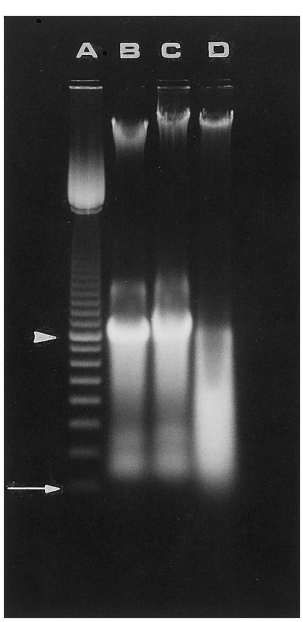

Fig. 5. Effect of C_2 ceramide on the nucleosomal fragmentation of *Tetrahymena*. DNA was extracted and electrophoresed in 0.9% agarose gel at 40 V for 6 h. DNA bands were visualized under UV light after staining with ethidium bromide. Lane A, molecular size markers (arrowhead, 800 KB; arrow, 100 KB); Lane B, control; Lane C, 10 μM C_2 ceramide treated cultures; Lane D, 50 μM C_2 ceramide treated cultures.

The rounding off the cells in *Tetrahymena* is a sign of prospective cell death. Moreover, as it is cleared by the present experiments, after C_2 ceramide treatment another apoptotic phenomena can develop. The nuclear condensation revealed by photometric measurements of Feulgen reaction-product or the nucleosomal degradation indicated by appearance of small (200–400 kB) DNA fragments refer to apoptotic-like alterations: the results of morphometry and cytochemistry supporting each other and point to the apoptotic effect of C_2 ceramide. The rounding off the cells indicates also the non-apoptotic cell death (necrosis), however in the case of necrosis *Tetrahymena* does not show an oligonucleosomal pattern on agarose gel [23]. The results of trypan blue exclusion also strengthen our supposition about the apoptotic phenomenon: after the treatments we found no labeling with trypan blue.

Raff suggested that cytokines or inadequate amounts of growth factors induce cell elimination; all metazoan cells are genetically programmed to kill themselves, only constant support from neighbouring cells keeps them alive [24]. Similar phenomenon is in the case of unicellulars [4]: *Tetrahymena thermophila* cells die rapidly when inoculated at low initial densities into chemically defined medium. Cell free, conditioned medium from high density cultures prevents cell death and activates proliferation [3]. Taken together, these results indicate that *Tetrahymena* cells produce and release growth factors which in appropriate concentration stimulate the cells to survive and proliferate. These factors act in a variety of different ways which directly or indirectly activate second messenger pathways necessary to promote cell survival and proliferation [27]. Suppression of cell death seems to be dependent on guanylate cyclase activation and increased Ca^{2+} concentration; absence of growth factors, rather than a direct apoptotic signal, give the starting signal for apoptotic processes [4].

It can be argued on the basis of our data presented here that C_2 ceramide (a) inhibits the precise function of the signaling system (e.g. inositol phospholipid, IP3 and by this the Ca^{2+} concentration); or (b) C_2 ceramide inhibits the synthesis and release the growth factors necessary to avoid of apoptotic processes; or (c) can serve as an apoptosis mediating second messenger downstream signal molecule (by activating protein kinases, phosphatases, c-myc, nuclear factor κ B, etc.), similarly to the higher eukaryotes [11], although the

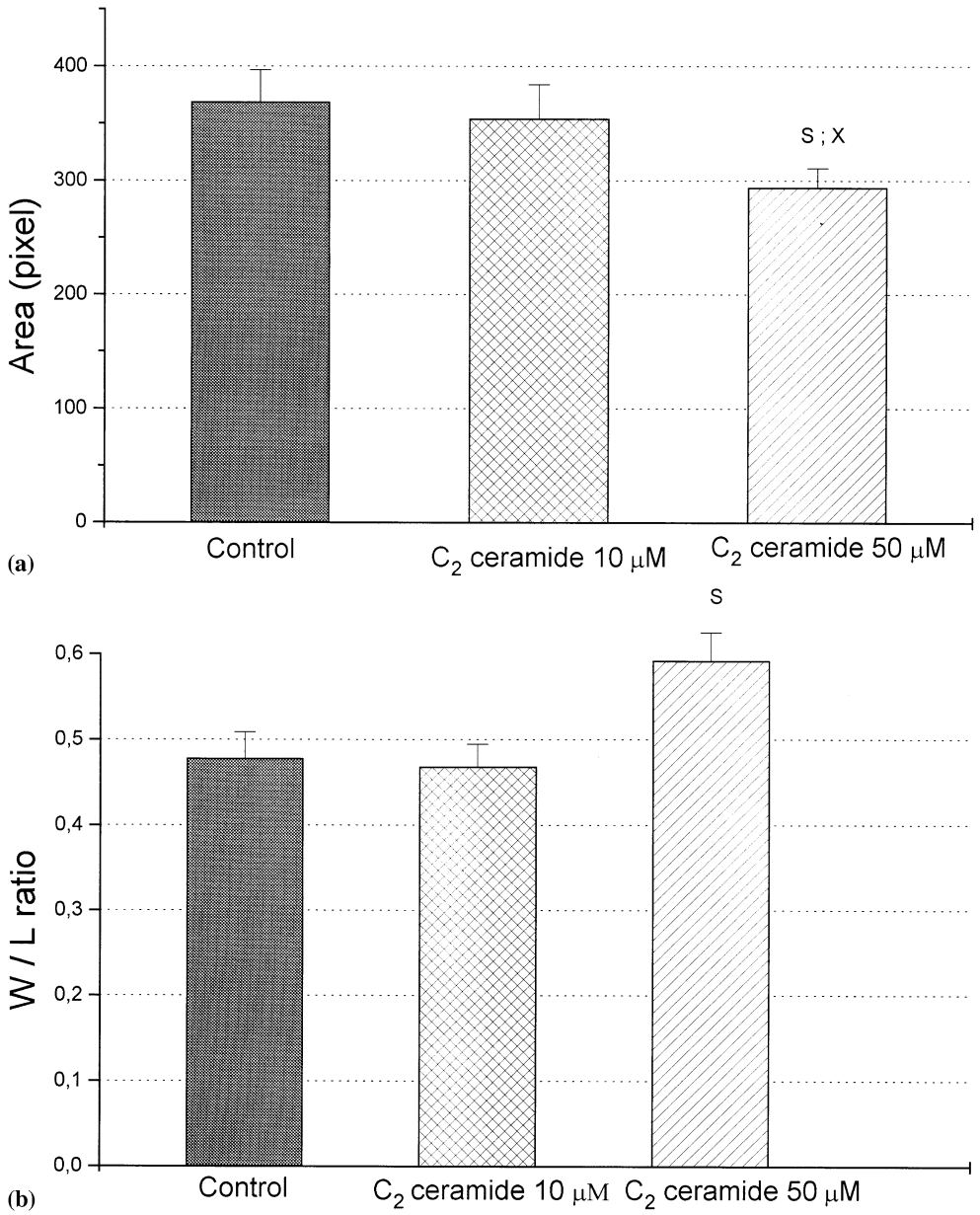

Fig. 6. Effect of C_2 ceramide on the size (area) (a) and the ratio of shortest and longest axis (W/L) (b) of *Tetrahymena*. The data represent averages ($\pm$S.D.) of four separate experiments. $s = p < 0.01$ to the control.

presence and function of these latter factors still have not been elucidated in *Tetrahymena*.

The experiments call attention to the interrelations between disturbances of signaling and apoptosis, especially in case of ceramid-mediated signal transduction in this unicellular organism. The experiments add new data on the signal transduction mechanism of *Tetrahymena* and demonstrate the role of a factor (ceramide), newly recognized from this aspect in mammals, at a low evolutionary level. This observation further support the theory that the origin of endocrine system can be deduced to the unicellular level of evolution [6].

References

[1] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.

[2] Brindley DN, Abousalham A, Kikuchi Y, Wang C-N, Waggoner DW. 'Cross talk' between the bioactive glycerolipids and sphingolipids in signal transduction. Biochem Cell Biol 1996;74:469–76.

[3] Christensen ST, Rasmussen L. Evidence for growth factors which control cell multiplication in *Tetrahymena thermophila*. Acta Protozool 1992;31:215–9.

[4] Christensen ST, Wheatley DN, Rasmussen MI, Rasmussen L. Mechanisms controlling death, survival and proliferation in a model unicellular eukaryote *Tetrahymena thermophila*. Cell Death Diff 1995;2:301–8.

[5] Cooper JA. The roles of actin polymerization in cell motility. Annu Rev Physiol 1991;53:585–605.
[6] Csaba G. Phylogeny and ontogeny of chemical signaling: origin and development of hormone receptors. Int Rev Cytol 1994;155:1–48.
[7] Csaba G, Nagy SU, Lantos T. Are biogenic amines acting on *Tetrahymena* through a cyclic AMP mechanism? Acta Biol Med Germ 1976;35:259–61.
[8] Feulgen R, Rosenbeck H. Mikroskopischer–chemischer Nachweis einer Nucleinsäure von Typus der Thymonucleinsäure und auf die darauf beruhende elektive Färbung in mikroskopischen Preparaten. Z Physiol Chem 1924;135:103–48.
[9] Hannun YA. The sphingomyelin cycles and the second messenger function of ceramide. J Biol Chem 1994;269:3125–8.
[10] Hannun YA, Bell RM. Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. Science 1989;243:500–7.
[11] Hannun YA, Obeid LM. Ceramide: an intracellular signal for apoptosis. TIBS 1995;20:73–7.
[12] Hegyesi H, Csaba G. Calcium-dependent protein kinase is present in *Tetrahymena*. Cell Biochem Funct 1994;12:221–6.
[13] Kovács P. The mechanism of receptor development as implied by hormonal imprinting studies on unicellular organisms. Experientia 1986;42:770–5.
[14] Kovács P, Csaba G. Involvement of phosphoinositol (PI) system in the mechanism of hormonal imprinting. Biochem Biophys Res Commun 1990;170:119–26.
[15] Kovács P, Csaba G. Effect of phorbol 12-myristate 13-acetate (PMA) on the phosphoinositol (PI) system in *Tetrahymena*. Study of the ^{32}P incorporation and breakdown of phospholipids. Cell Biochem Funct 1995;13:85–9.
[16] Kovács P, Csaba G. PLA_2 activity in *Tetrahymena pyriformis*. Effects of inhibitors and stimulators. J Lipid Mediat Cell Signal 1997;15:223–47.
[17] Kovács P, Csaba G. Phospholipase D activity in the *Tetrahymena pyriformis* GL. Cell Biochem Funct 1997;15:53–60.
[18] Kovács P, Csaba G. Effect of ceramide-analogues on the actin cytoskeleton of Tetrahymena pyriformis GL. A confocal microscopic analysis. Acta Protozool, 1997; in press.
[19] Kovács P, Csaba G, Nagao S, Nozawa Y. The regulatory role of calmodulin-dependent guanylate cyclase in association with hormonal imprinting in *Tetrahymena*. Microbios 1989;59:123–8.
[20] Kovács P, Köhidai L, Csaba G. Effect of tumor necrosis factor α (TNFα) on the phospholipid metabolism of *Tetrahymena*. Cell Biochem Funct, 1997; in press.
[21] Köhidai L, Bársony J, Roth J, Marx SJ. Rapid effects of insulin on cyclic GMP location in an intact protozoan. Experientia 1992;48:476–81.
[22] Laster SM, Mackenzie JMJ. Bleb formation and F-actin distribution during mitosis and tumor necrosis factor-induced apoptosis. Microsc Res Tech 1996;34:272–80.
[23] Mpoke S, Wolfe J. Differential staining of apoptotic nuclei in living cells: application to macronuclear elimination in Tetrahymena. J Histochem Cytochem 1997;45:675–83.
[24] Raff MC. Social controls on cell survival and cell death. Nature 1992;356:397–400.
[25] Suchard SJ, Rhoads DE, Kaneshiro ES. The inositol lipids of *Paramecium tetraurelia* and preliminary characterizations of phosphoinositide kinase activity in the ciliary membrane. J Protozool 1989;36:185–90.
[26] Viswanathan CV, Rosenberg H. Unequivocal identification of sphingomyelin in the lipids of *Tetrahymena pyriformis*. J Chromatogr 1973;86:250–3.
[27] Wheatley DN, Rasmussen L, Tiedtke A. *Tetrahymena*: a model for growth, cell cycle and nuritional studies, with biotechnological potential. BioEssays 1994;16:367–72.
[28] Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980;284:555–8.