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# MODIFICATION OF LIPID COMPOSITION OF NEUROBLASTOMA C1300 N18 CELLS WITH LIPOSOMES ALTERS THE CHOLESTEROL CONTENT

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**Abstract**—Cell incubation with lecithin–cholesterol liposomes (1:1 mol/mol) caused enhancement of the cholesterol content. The level of cholesterol esters of total and phospholipid unsaturated fatty acids increased in cholesterol enriched cells. Simultaneously the amount of saturated fatty acids decreased and lysophosphatidylcholine appeared in the cells.

On the contrary, cell incubation with lecithin liposomes resulted in cholesterol depletion. This effect was accompanied by a decrease of cholesterol esters, of total and phospholipid unsaturated fatty acids. The content of saturated fatty acids was raised in cells with reduced amount of cholesterol. The quantity of *N*-acylphosphatidylethanolamine and *N*-acylethanolamine, lipids newly found in neuroblastoma cells, also changed in cells with modified content of cholesterol.

The physiological role of cell response to the changes of cholesterol level is discussed.

It is well known that lipid constituents play a significant role in the structure and physiological function of biological membranes.[19,22] Many attempts have been undertaken in order to modify lipid structure of the biological membranes. These investigations were aimed at trying to understand the relationship of some membrane-related properties in relation to lipids.[16,20,21,32]

Recently we have obtained neuroblastoma C1300 N18 cells enriched in cholesterol by cultivation of cells in the presence of lecithin–cholesterol liposomes. It has also been shown that cell incubation with lecithin liposomes causes a sharp decrease of the cholesterol content. The preliminary data have been published elsewhere.[11,30]

The function of many integral membrane proteins and the interaction of some ligands with the specific membrane receptors is now of general interest. It is well known that these functions depend on the lipid composition of the membrane. A great variety of lipids present in membranes and the specificity of lipid composition in different types of membranes suggests that many of these lipids play a unique role in the membrane structure and function. Recently we have shown that the function of fast sodium channels and insulin receptors changed in neuroblastoma cells with modified quantity of cholesterol.[9,13]

High levels of cholesterol in cells changes cholesterol/phospholipid ratio and reduces cell membrane fluidity.[23,33] Besides cholesterol/phospholipid ratio, other factors can also affect the microviscosity and other membrane properties. The most prominent are: a degree of unsaturation and the length of the phospholipid acyl chains, the level of sphingomyeline and the presence of neutral lipids like triglycerides.[4,7,15,23,24,26]

The aim of this work was to study the changes in fatty acyl chains of lipid fractions loosely and tightly (covalently) bound with proteins of neuroblastoma C1300 N18 cells depending on modified cholesterol content. The amount of cholesterol esters, lysophospholipids, *N*-acylphosphatidylethanolamine, *N*-acylethanolamine, phospholipid fatty acyl chains were also analysed.

## EXPERIMENTAL PROCEDURES

### *Cell culture*

Experiments were performed on neuroblastoma C1300 N18 cells cultured in the presence of 10% bovine serum at 37 C in Eagles medium. Differentiation of cells was induced by addition of 5′-bromodeoxyuridine at final concentration 40 $\mu$mol/l. Culture medium was discarded from flasks, cells were washed three times in Eagles medium. Three millilitres of liposome suspension in Eagles medium were added to each flask and cells were incubated for 60 min at 37 C. After incubation the liposome suspension was discarded, cells were washed three times in Eagles medium and then used for experiments.

### *Liposomes*

Lecithin and lecithin–cholesterol liposomes were prepared from a thin layer of egg yolk lecithin or from a mixture of lecithin with cholesterol (2:1 w/w), obtained in a rotor evaporator. The lipid layer was suspended for 20 min in Eagles medium and then sonicated at 22 KGc at 4 C on disintegrator UD-11 (Poland). The obtained suspension was centrifuged for 1 h at 105,000 ***g***. Supernatants were diluted to obtain 5 $\mu$mol of each lipid per 1 ml. They served as lecithin or lecithin–cholesterol (1:1 mol/mol) liposome suspensions.

### *Assay methods*

Lipids were extracted by the method of Bligh and Dyer.[3] The fractions of lipids loosely and tightly bound

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with protein were obtained and determined as described elsewhere.[12]

Microthin layer chromatography and gas liquid chromatography were performed as previously described.[12] Phospholipids were analysed by the methods of Vaskovsky.[25,27,28] Determination and identification of *N*-acylphosphatidylethanolamine and *N*-acylethanolamine was done in special chromatographic solvent systems.[10,29] Protein content was determined by the method of Lowry *et al.*[14] Membrane isolation and analysis of the purity of membrane fractions were performed as described.[5]

In this work we used the criteria of cell differentiation described in our previous report.[12]

## RESULTS

The data presented in Table 1 shows that in cells treated by lecithin liposomes the quantity of cholesterol per mg of protein decreased by 50%. In cells treated with lecithin–cholesterol liposomes the level of cholesterol increased. In this case undifferentiated cells were enriched in cholesterol by 52%, while in differentiated cells the level of cholesterol increased by only 37%.

It was found that the amount of cholesterol tightly bound with protein did not depend on liposome treatment.

In plasma membrane of differentiated cells the amount of cholesterol increased after 10–20 min of incubation with lecithin–cholesterol liposomes and remained constant during the experiment (up to 90 min) (Table 2). Simultaneously, the quantity of cholesterol esters and lysophosphatidylcholine increased by about 15 times.

In microsomal fraction the quantity of cholesterol and cholesterol esters grew to maximal values after 1 h of incubation with lecithin–cholesterol liposomes. While the amount of lysolecithin remained constant, no changes in the level of phospholipids were found either in plasma membranes or in microsomal fraction.

The dynamics of lipid changes after cell incubation with lecithin liposomes differs from that observed after treatment of cells by lecithin–cholesterol liposomes. The minimal levels of cholesterol, cholesterol esters and lysolecithin in plasma membrane were determined after 30 min of incubation with lecithin liposomes.

In microsomal fraction the minimal amounts of cholesterol and cholesterol esters were found after 60 min of incubation. The quantity of lysolecithin did not change in this case. The level of phospholipids in both membrane fractions remained constant after incubation with lecithin liposomes (Table 2).

Though the total amount of cell phospholipids did not change after treating with liposomes, the level of phosphatidylethanolamine decreased in plasma membrane and microsomal fraction of cells incubated with lecithin–cholesterol liposomes. Some changes were also found in the zone of phospholipids with high chromatographic mobility (Table 3). One of these phospholipids was identified as *N*-acylphos-

Table 1. Changes of loosely and tightly bound cholesterol in neuroblastoma C1300 N18 cells treated by lecithin and lecithin–cholesterol liposomes

| Experimental conditions | Undifferentiated cells | | | | Differentiated cells | | | |
|---|---|---|---|---|---|---|---|---|
| | Loosely bound | | Tightly bound | | Loosely bound | | Tightly bound | |
| | nmol of cholesterol per: | | | | nmol of cholesterol per: | | | |
| | mg of protein | $10^6$ cells | mg of protein | $10^6$ cells | mg of protein | $10^6$ cells | mg of protein | $10^6$ cells |
| Intact cells | $51.14 \pm 5.05$ | $8.03 \pm 0.36$ | $3.62 \pm 0.54$ | $0.50 \pm 0.20$ | $70.46 \pm 2.46$ | $17.35 \pm 0.62$ | $4.40 \pm 1.58$ | $1.88 \pm 0.44$ |
| Cells treated by lecithin liposomes | $29.33 \pm 0.52$*** | $4.42 \pm 0.08$*** | $3.89 \pm 0.14$ | $0.59 \pm 0.02$ | $35.94 \pm 0.96$*** | $8.86 \pm 0.24$*** | $3.70 \pm 0.14$ | $0.92 \pm 0.04$ |
| Cells treated by lecithin cholesterol liposomes | $81.68 \pm 0.90$*** | $12.30 \pm 0.14$*** | $4.08 \pm 0.25$ | $0.62 \pm 0.04$ | $95.96 \pm 0.74$*** | $23.69 \pm 0.18$*** | $5.20 \pm 0.10$ | $1.28 \pm 0.02$ |

***$P < 0.001$ compared with intact cells.
M ± m; $n = 7$.

Table 2. The content of cholesterol, cholesterol esters and phospholipids (nmol/mg protein) in plasma membrane and microsomal fraction of neuroblastoma C1300 N18 cells treated by lecithin and lecithin–cholesterol liposomes (5 $\mu$mol of each lipid per ml of liposomes)

| Time of incubation (min) | Plasma membrane | | | | Microsomal fraction | | | |
|---|---|---|---|---|---|---|---|---|
| | Cholesterol | Cholesterol esters | Phospholipids | Lysophosphatidyl choline | Cholesterol | Cholesterol esters | Phospholipids | Lysophosphatidyl choline |
| 0 (intact cells) | 271 ± 9 | 1.75 ± 0.01 | 433 ± 17 | 1.61 ± 0.30 | 114 ± 6 | 3.18 ± 0.02 | 312 ± 26 | 2.03 ± 0.44 |
| | | | | Lecithin–cholesterol liposomes | | | | |
| 10 | 354 ± 17* | 17 ± 2** | 420 ± 24 | 19 ± 6* | 109 ± 11 | 2.97 ± 0.09 | 321 ± 30 | 2.29 ± 0.38 |
| 20 | 390 ± 26* | 26 ± 3** | 442 ± 21 | 24 ± 4** | 128 ± 16 | 3.29 ± 0.11 | 336 ± 28 | 2.76 ± 0.63 |
| 30 | 381 ± 20** | 25 ± 7* | 430 ± 26 | 27 ± 4** | 121 ± 20 | 3.41 ± 0.33 | 319 ± 17 | 2.13 ± 0.27 |
| 45 | 376 ± 18** | 22 ± 4** | 438 ± 19 | 25 ± 3** | 189 ± 14** | 27 ± 6* | 307 ± 21 | 2.20 ± 0.31 |
| 60 | 371 ± 10** | 24 ± 3** | 427 ± 15 | 24 ± 2*** | 256 ± 21** | 77 ± 9** | 311 ± 14 | 2.53 ± 0.50 |
| 75 | 409 ± 23** | 26 ± 6* | 438 ± 20 | 27 ± 8* | 278 ± 18** | 94 ± 7*** | 324 ± 26 | 3.07 ± 0.34 |
| 90 | 385 ± 19** | 23 ± 5* | 446 ± 24 | 25 ± 6* | 262 ± 26** | 93 ± 12** | 301 ± 28 | 3.19 ± 0.46 |
| | | | | Lecithin liposomes | | | | |
| 15 | 115 ± 11*** | 0.32 ± 0.01*** | 439 ± 17 | 0.29 ± 0.01** | 116 ± 9 | 2.96 ± 0.05 | 306 ± 14 | 2.56 ± 0.32 |
| 30 | 71 ± 4*** | 0.01 ± 0.001*** | 421 ± 18 | trace*** | 108 ± 11 | 3.26 ± 0.05 | 331 ± 28 | 3.11 ± 0.47 |
| 45 | 66 ± 8*** | — | 420 ± 26 | — | 89 ± 7 | 0.48 ± 0.01*** | 320 ± 24 | 2.17 ± 0.25 |
| 60 | 74 ± 7*** | — | 438 ± 19 | — | 43 ± 8** | 0.01 ± 0.002*** | 307 ± 17 | 2.24 ± 0.31 |
| 75 | 78 ± 6*** | — | 440 ± 21 | — | 41 ± 2*** | — | 319 ± 26 | 1.48 ± 0.48 |
| 90 | 74 ± 7*** | — | 437 ± 28 | — | 40 ± 4*** | — | 309 ± 22 | 1.08 ± 0.23* |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with 0 min (intact cells).
M ± m; $n = 3$.

Table 3. The content of individual phospholipids and *N*-acylethanolamine in plasma membrane and microsomal fraction of neuroblastoma C1300 N18 cells after 1 h incubation with lecithin and lecithin–cholesterol liposomes (5 $\mu$mol of each lipid per ml of liposomes)

| Phospholipids and lipids | Plasma membrane Incubation with: | | | Microsomal fraction Incubation with: | | |
|---|---|---|---|---|---|---|
| | Control | Lecithin Liposomes | Lecithin–cholesterol Liposomes | Control | Lecithin Liposomes | Lecithin–cholesterol Liposomes |
| Phosphatidylcholine | 195.3 ± 14.7 | 199.8 ± 12.6 | 172.8 ± 12.2 | 96.3 ± 11.0 | 101.5 ± 7.0 | 100.0 ± 5.3 |
| Lysophosphatidylcholine | 1.6 ± 0.3 | — | 23.7 ± 1.9*** | 2.0 ± 0.4 | 0.8 ± 0.1* | 2.5 ± 0.2 |
| Phosphatidylethanolamine | 95.8 ± 5.3 | 97.2 ± 4.7 | 76.2 ± 5.0* | 113.6 ± 10.0 | 114.2 ± 5.6 | 87.5 ± 5.7 |
| Sphyngomyeline | 30.2 ± 2.7 | 34.3 ± 3.4 | 31.7 ± 3.8 | 19.3 ± 2.7 | 20.0 ± 1.3 | 21.3 ± 1.9 |
| Phosphatidylserine | 37.6 ± 3.0 | 41.9 ± 4.5 | 36.4 ± 4.3 | 11.6 ± 1.2 | 12.4 ± 1.2 | 11.2 ± 1.2 |
| Phosphatidylinositol | 41.4 ± 4.7 | 42.0 ± 5.4 | 42.5 ± 5.5 | 43.7 ± 4.8 | 40.7 ± 3.5 | 41.9 ± 2.7 |
| Total phospholipids with high chromatographic mobility including: | 26.1 ± 2.2 | 16.8 ± 3.6 | 38.1 ± 1.5** | 23.1 ± 2.5 | 13.8 ± 2.5* | 40.6 ± 1.2** |
| *N*-acylphosphatidylethanolamine | trace | 1.43 ± 0.16*** | 6.09 ± 0.69*** | trace | 0.69 ± 0.05*** | 3.40 ± 0.48*** |
| Lyso-*N*-acylphosphatidylethanolamine | 3.96 ± 0.16 | 11.13 ± 2.05** | 18.94 ± 3.21** | 8.08 ± 0.79 | 3.78 ± 0.95* | 11.59 ± 2.98* |
| Start zone | 5.6 ± 0.6 | 6.0 ± 0.1 | 6.6 ± 0.8 | 4.2 ± 0.8 | 4.3 ± 0.7 | 5.8 ± 0.7 |
| *N*-acylethanolamine | trace | 0.063 ± 0.004 | 0.021 ± 0.006 | trace | 0.150 ± 0.020 | 0.002 ± 0.0004 |

All lipids—nmol/mg protein; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control.
M ± m; $n = 5$.

phatidylethanolamine. The amount of this phospholipid and its physiologically active metabolite *N*-acylethanolamine increased in plasma membrane and microsomal fraction after cell incubation with both lecithin and with lecithin–cholesterol liposomes, whereas the maximal level of lipids in these cases was different.

The total content of fatty acids loosely bound with cell proteins changed in a different way in cells treated by lecithin and lecithin–cholesterol liposomes. In cholesterol-depleted cells the amount of saturated fatty acids increased while the quantity of unsaturated fatty acids decreased (Table 4). In cholesterol enriched cells we observed an increased level of unsaturated fatty acids, and a reduced amount of saturated ones. It should be stressed that the modification of cholesterol content in undifferentiated and differentiated cells produced different changes of cell lipid composition.

The tightly bound fraction of fatty acids contained less individual fatty acids. While in both fractions the main fatty acids were 16:0, 18:0, 18:1$\omega$9, 20:4$\omega$6, in undifferentiated cells, the double bound index was higher than that in differentiated ones.

The content of fatty acids in the fraction of lipid tightly bound with protein in undifferentiated neuroblastoma cells treated by lecithin and lecithin–cholesterol liposomes changed similarly to changes of fatty acids in lipids loosely bound with protein (Table 5).

Two main phospholipids in the membrane fractions of neuroblastoma cells were phosphatidylcholine and phosphatidylethanolamine (Table 3). It was found that these phospholipids contain different amounts of fatty acyl chains 16:0, 18:1$\omega$9, 18:2$\omega$6, 18:3$\omega$3, 20:4$\omega$6, 22:4$\omega$6, 22:5$\omega$3. In both membrane fractions the amount of saturated fatty acyl chains decreased after incubation with lecithin–cholesterol liposomes and increased after lecithin liposomes treatment in both phosphatidylcholine and in phosphatidylethanolamine. On the contrary, the quantity of unsaturated fatty acyl chains was

Table 4. The content of individual fatty acyl chains in lipids loosely bound with proteins of neuroblastoma C1300 N18 cells after 1 h incubation with lecithin and lecithin-cholesterol liposomes (5 $\mu$mol of each lipid per 1 ml)

| Fatty acyl chains | The content of fatty acyl chains (% of total) in: Undifferentiated cells | | | Differentiated cells | | |
|---|---|---|---|---|---|---|
| | Intact cells | Lecithin Liposomes | Lecithin–cholesterol Liposomes | Intact cells | Lecithin Liposomes | Lecithin–cholesterol Liposomes |
| 14:0 | 1.60 ± 0.06 | 1.50 ± 0.02 | 1.22 ± 0.01*** | 0.90 ± 0.22 | 1.33 ± 0.06 | 0.92 ± 0.08 |
| 15:0i | 0.25 ± 0.01 | 0.31 ± 0.1** | 0.22 ± 0.01 | 0.23 ± 0.01 | 0.32 ± 0.02** | 0.19 ± 0.02 |
| 15:0 | 0.60 ± 0.12 | 0.71 ± 0.02 | 0.49 ± 0.01 | 0.50 ± 0.23 | 0.65 ± 0.03 | 0.43 ± 0.02 |
| 15:1$\omega$8 | 0.14 ± 0.02 | 0.10 ± 0.01 | 0.13 ± 0.01 | 0.11 ± 0.01 | 0.08 ± 0.01 | 0.12 ± 0.01 |
| 16:0ai | 0.20 ± 0.03 | 0.96 ± 0.07*** | 0.24 ± 0.02 | 0.20 ± 0.03 | 0.78 ± 0.19* | 0.19 ± 0.02 |
| 16:0i | 0.20 ± 0.02 | 0.82 ± 0.2*** | 0.21 ± 0.01 | 0.13 ± 0.01 | 0.50 ± 0.03*** | 0.10 ± 0.01 |
| 16:0 | 26.20 ± 0.90 | 30.65 ± 0.70** | 18.77 ± 0.58*** | 39.00 ± 2.68 | 41.63 ± 0.99 | 20.81 ± 0.38*** |
| 16:1$\omega$9 | 2.00 ± 0.12 | 1.65 ± 0.02* | 1.94 ± 0.03 | 2.40 ± 0.10 | 1.54 ± 0.04*** | 3.44 ± 0.10*** |
| 16:1$\omega$5 | 0.40 ± 0.09 | 0.14 ± 0.01* | 0.36 ± 0.01 | trace | 0.06 ± 0.01*** | 0.25 ± 0.03*** |
| 17:0ai | 0.27 ± 0.04 | 0.32 ± 0.01 | 0.23 ± 0.01 | 0.22 ± 0.02 | 0.26 ± 0.01 | 0.18 ± 0.01 |
| 17:0i | 0.43 ± 0.03 | 0.51 ± 0.01* | 0.38 ± 0.01 | 0.26 ± 0.03 | 0.42 ± 0.02** | 0.21 ± 0.01 |
| 17:0 | 1.10 ± 0.16 | 1.23 ± 0.02 | 1.02 ± 0.03 | 1.61 ± 0.20 | 1.62 ± 0.10 | 1.08 ± 0.01* |
| 17:1$\omega$10 | 0.44 ± 0.02 | 0.37 ± 0.01* | 0.46 ± 0.01 | 0.47 ± 0.04 | 0.38 ± 0.03 | 1.06 ± 0.05*** |
| 17:1$\omega$8 | 0.40 ± 0.04 | 0.48 ± 0.01 | 0.39 ± 0.01 | 0.11 ± 0.02 | 0.11 ± 0.01 | 0.46 ± 0.04*** |
| 18:0 | 11.00 ± 0.67 | 15.13 ± 0.15*** | 11.14 ± 0.55 | 22.81 ± 0.96 | 22.94 ± 1.20 | 15.17 ± 0.34* |
| 18:1$\omega$9 | 25.30 ± 1.10 | 20.30 ± 0.44** | 29.43 ± 0.56 | 22.40 ± 1.95 | 15.47 ± 0.71* | 32.62 ± 1.09* |
| 18:2$\omega$6 | 5.20 ± 0.21 | 3.54 ± 0.7*** | 6.19 ± 0.07** | 2.20 ± 0.21 | 1.95 ± 0.08 | 4.75 ± 0.15*** |
| 18:3$\omega$3 | 0.33 ± 0.01 | 0.15 ± 0.02*** | 0.32 ± 0.01 | 0.12 ± 0.01 | 0.06 ± 0.01** | 0.31 ± 0.01*** |
| 19:1 | 0.51 ± 0.01 | 0.23 ± 0.02** | 0.43 ± 0.02* | 0.13 ± 0.01 | 0.08 ± 0.01* | 0.21 ± 0.01** |
| 20:0 | 0.51 ± 0.01 | 0.60 ± 0.03* | 0.41 ± 0.02** | 0.39 ± 0.01 | 0.48 ± 0.03* | 0.33 ± 0.01** |
| 20:1$\omega$11 | 1.14 ± 0.01 | 0.90 ± 0.07* | 1.21 ± 0.07 | 0.68 ± 0.01 | 0.51 ± 0.04** | 1.08 ± 0.5*** |
| 20:2$\omega$6 | 1.16 ± 0.02 | 1.00 ± 0.04* | 1.17 ± 0.04 | 1.11 ± 0.01 | 0.96 ± 0.05* | 1.14 ± 0.1 |
| 20:3$\omega$9 | 0.17 ± 0.01 | 0.12 ± 0.01* | 0.20 ± 0.01 | 0.14 ± 0.01 | 0.10 ± 0.01* | 0.21 ± 0.01** |
| 20:3$\omega$6 | 0.94 ± 0.01 | 0.94 ± 0.03 | 1.18 ± 0.04** | 1.06 ± 0.01 | 0.82 ± 0.07* | 2.07 ± 0.04*** |
| 20:4$\omega$6 | 11.40 ± 0.03 | 9.70 ± 0.63* | 12.40 ± 0.19** | 2.52 ± 0.49 | 1.76 ± 0.17 | 3.18 ± 0.06 |
| 20:5$\omega$3 | 0.13 ± 0.01 | 0.09 ± 0.01* | 0.15 ± 0.01 | 0.15 ± 0.08 | 0.11 ± 0.01 | 0.29 ± 0.01 |
| 22:0 | 1.26 ± 0.04 | 1.37 ± 0.04 | 1.06 ± 0.02** | 0.23 ± 0.04 | 0.31 ± 0.02 | 0.19 ± 0.01 |
| 22:1$\omega$11 | 1.91 ± 0.17 | 1.67 ± 0.07 | 1.72 ± 0.08 | 0.65 ± 0.17 | 0.49 ± 0.02 | 0.97 ± 0.06 |
| 22:3$\omega$6 | 0.41 ± 0.02 | 0.31 ± 0.01** | 0.72 ± 0.02*** | 0.64 ± 0.02 | 0.57 ± 0.02* | 0.74 ± 0.04* |
| 22:4$\omega$6 | 2.10 ± 0.03 | 1.96 ± 0.06 | 2.66 ± 0.03*** | 2.50 ± 0.03 | 1.71 ± 0.06*** | 2.87 ± 0.07** |
| 22:5$\omega$3 | 3.06 ± 0.04 | 2.58 ± 0.10** | 3.25 ± 0.11 | 3.60 ± 0.07 | 2.16 ± 0.03*** | 4.01 ± 0.09* |
| 22:6$\omega$3 | 0.31 ± 0.01 | 0.24 ± 0.01** | 0.42 ± 0.01*** | 0.50 ± 0.01 | 0.40 ± 0.02** | 0.68 ± 0.03** |
| Saturated | 43.62 ± 1.14 | 54.11 ± 0.72*** | 35.39 ± 0.80*** | 66.48 ± 2.87 | 71.24 ± 1.57 | 39.80 ± 0.52*** |
| Unsaturated | 56.38 ± 1.28 | 45.89 ± 0.54*** | 64.61 ± 0.72*** | 33.52 ± 2.13 | 28.76 ± 0.81 | 60.20 ± 1.18*** |
| Double bound index | 122 | 101 | 138 | 81 | 57 | 112 |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with intact cells.
M ± m; $n = 6$.

Table 5. The content of individual fatty acyl chains in lipids tightly bound with proteins of neuroblastoma C1300 N18 cells after 1 h incubation with lecithin and lecithin–cholesterol liposomes (5 $\mu$mol of each lipid per 1 ml)

| Fatty acyl chains | The content of fatty acyl chains (% of total) in: Undifferentiated cells Intact cells | Undifferentiated cells Lecithin Liposomes | Undifferentiated cells Lecithin–cholesterol Liposomes | Differentiated cells Intact cells | Differentiated cells Lecithin Liposomes | Differentiated cells Lecithin–cholesterol Liposomes |
|---|---|---|---|---|---|---|
| 14:0 | 1.49 ± 0.01 | 1.37 ± 0.04* | 1.12 ± 0.04*** | 0.51 ± 0.01 | 0.69 ± 0.03*** | 0.52 ± 0.05 |
| 15:0i | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.15 ± 0.01 | 0.13 ± 0.01 | 0.19 ± 0.02* | 0.10 ± 0.01 |
| 15:0 | 1.09 ± 0.02 | 1.13 ± 0.03 | 0.57 ± 0.06*** | 0.22 ± 0.02 | 0.45 ± 0.04** | 0.26 ± 0.03 |
| 15:1ω8 | 0.24 ± 0.01 | 0.14 ± 0.01*** | 0.27 ± 0.03 | 0.17 ± 0.01 | 0.11 ± 0.01** | 0.18 ± 0.01 |
| 16:0ai | 0.42 ± 0.02 | 0.47 ± 0.04 | 0.27 ± 0.01*** | 0.34 ± 0.04 | 0.31 ± 0.02 | 0.25 ± 0.02 |
| 16:0i | 0.39 ± 0.01 | 0.49 ± 0.04* | 0.24 ± 0.03** | 0.18 ± 0.01 | 0.28 ± 0.01*** | 0.16 ± 0.01 |
| 16:0 | 20.50 ± 3.38 | 32.33 ± 0.95* | 12.97 ± 0.75 | 42.20 ± 1.09 | 39.13 ± 3.46 | 34.04 ± 0.84** |
| 16:1ω9 | 4.80 ± 0.76 | 1.79 ± 0.22** | 6.31 ± 0.64 | 2.10 ± 0.28 | 1.38 ± 0.09* | 2.21 ± 0.10 |
| 16:1ω5 | — | — | — | 0.48 ± 0.02 | 0.33 ± 0.02*** | 0.50 ± 0.02 |
| 17:0 | 2.20 ± 0.19 | 2.96 ± 0.12** | 1.53 ± 0.03** | 1.14 ± 0.15 | 2.62 ± 0.10*** | 1.06 ± 0.05 |
| 17:1ω10 | 0.57 ± 0.04 | 0.31 ± 0.02*** | 0.52 ± 0.03 | trace | 0.23 ± 0.01*** | 0.35 ± 0.03*** |
| 17:1ω8 | 0.18 ± 0.01 | 0.13 ± 0.02 | 0.38 ± 0.03*** | 0.23 ± 0.01 | 0.27 ± 0.04 | 0.43 ± 0.1*** |
| 18:0 | 22.60 ± 2.81 | 31.73 ± 1.02* | 13.50 ± 0.45* | 32.60 ± 0.95 | 34.45 ± 0.56 | 28.54 ± 1.11* |
| 18:1ω9 | 13.60 ± 0.14 | 10.77 ± 0.75* | 26.20 ± 1.33*** | 19.30 ± 0.95 | 13.15 ± 0.50** | 22.54 ± 0.60* |
| 18:2ω6 | 2.10 ± 0.27 | 1.62 ± 0.10 | 5.15 ± 0.31*** | 0.19 ± 0.03 | 1.46 ± 0.14*** | 2.12 ± 0.12*** |
| 18:3ω3 | — | — | — | trace | 0.19 ± 0.01*** | 0.46 ± 0.02*** |
| 19:1 | 0.39 ± 0.01 | 0.32 ± 0.02** | 0.53 ± 0.03** | 0.21 ± 0.03 | 0.19 ± 0.03 | 0.82 ± 0.03*** |
| 20:0 | 0.50 ± 0.03 | 0.59 ± 0.03 | 0.30 ± 0.02** | 0.31 ± 0.03 | 0.95 ± 0.06*** | 0.32 ± 0.03 |
| 20:1ω11 | 0.26 ± 0.01 | 0.21 ± 0.01* | 0.30 ± 0.01* | — | — | — |
| 20:2ω6 | 0.12 ± 0.01 | 0.09 ± 0.01 | 0.19 ± 0.01** | 0.13 ± 0.01 | trace | 0.15 ± 0.01 |
| 20:3ω6 | 0.45 ± 0.04 | 0.34 ± 0.01* | 0.82 ± 0.06** | 0.55 ± 0.02 | 0.36 ± 0.02*** | 0.67 ± 0.06 |
| 20:4ω6 | 17.60 ± 1.86 | 9.08 ± 0.73** | 23.02 ± 1.20* | 1.27 ± 0.40 | 0.71 ± 0.03 | 1.83 ± 0.15 |
| 20:5ω3 | 0.15 ± 0.01 | 0.11 ± 0.01 | 0.31 ± 0.03** | 0.12 ± 0.01 | 0.10 ± 0.01 | 0.43 ± 0.5*** |
| 22:0 | 0.46 ± 0.02 | 1.27 ± 0.08*** | 0.31 ± 0.02** | 0.16 ± 0.01 | 1.80 ± 0.10*** | 0.24 ± 0.02* |
| 22:1ω11 | 1.07 ± 0.06 | 0.72 ± 0.04** | 1.02 ± 0.05 | 0.11 ± 0.05 | trace | 0.40 ± 0.03** |
| 22:4ω6 | 2.34 ± 0.72 | 1.89 ± 0.07 | 3.33 ± 0.10 | 2.04 ± 0.51 | 1.25 ± 0.08 | 2.02 ± 0.07 |
| Saturated | 49.82 ± 4.39 | 72.51 ± 0.09*** | 30.96 ± 0.88*** | 77.79 ± 1.45 | 80.87 ± 3.50 | 65.49 ± 1.39*** |
| Unsaturated | 50.18 ± 2.44 | 27.49 ± 1.15*** | 69.04 ± 2.18*** | 22.21 ± 1.22 | 19.13 ± 0.57* | 34.51 ± 0.72*** |
| Double bound index | 107 | 63 | 156 | 39 | 29 | 53 |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with intact cells.
M ± m; $n = 6$.

increased in sterol enriched cells and decreased in the sterol-depleted ones (Tables 6 and 7).

## DISCUSSION

The main purpose of the present study was to investigate the changes of lipid constituents in neuroblastoma C1300 N18 cells with modified level of cholesterol.

Cholesterol is one of the main lipids in many types of cells. In neuroblastoma C1300 N18 cells the cholesterol/phospholipid ratio is 1:2.[12] However, it is well known that the content of cholesterol in cells is not constant and depends on many effectors.

Our data show that the cholesterol amount in differentiated neuroblastoma cells was higher than in undifferentiated ones. Cholesterol incorporation into undifferentiated cells after incubation with lecithin–cholesterol liposomes was higher than that into differentiated ones. This effect is likely to depend on different physical and physiological properties of plasma membrane of mature and immature cells.

It has been established that cholesterol changes are accompanied by changes of membrane protein function.[1,31]

Membrane proteins interact with membrane lipids through several types of forces. In this work we have established that in neuroblastoma cells nearly 5% of total cholesterol was tightly bound with protein and could not easily be extracted by organic solvents. A tightly bound lipid fraction was obtained only after mild alkaline or acidic hydrolysis of the protein pellet, delipidated by the method of Bligh and Dyer, and additionally by ethanol at 70°C. This fraction of sterol did not change after cell incubation with liposomes. Significant changes were found only in the loosely bound cholesterol fraction. These investigations tend to suggest that loosely, but not tightly bound, cholesterol affected protein function after liposome treatment.

The changes in cholesterol content in plasma membrane and microsomal fraction were accompanied by similar changes of cholesterol esters and lysolecithin. Cholesterol is known to reduce membrane fluidity and permeability, while cholesterol esters and lysophospholipids lead to their increase.[6,8,18] Possibly, the opposite effects of cell lipid constituents serve to maintain constant membrane fluidity and other physical properties of these membranes.

The diminished cholesterol level was observed in

Table 6. The content of individual fatty acyl chains in the phosphatidylethanolamine of plasma membrane and microsomal fraction of neuroblastoma C1300 N18 cells after 1 h incubation with lecithin and lecithin–cholesterol liposomes (5 μmol of each lipid per 1 ml)

| Fatty acyl chains | The content of fatty acyl chains (% of total) in: Plasma membrane | | | Microsomal fractions | | |
|---|---|---|---|---|---|---|
| | Intact cells | Lecithin–cholesterol Liposomes | Lecithin | Intact cells | Lecithin–cholesterol Liposomes | Lecithin |
| 14:0 | — | — | — | 6.43 ± 0.57 | 1.27 ± 0.11*** | 8.69 ± 0.66* |
| 15:0(i + n) | — | — | — | 0.12 ± 0.01 | trace*** | 0.37 ± 0.04*** |
| 15:1ω8 | — | — | — | 0.09 ± 0.01 | 0.38 ± 0.04*** | trace*** |
| 16:0(ai + i + n) | 6.49 ± 0.36 | 4.19 ± 0.32** | 16.62 ± 0.89*** | 11.07 ± 0.62 | 7.03 ± 0.47*** | 19.62 ± 1.13*** |
| 16:1(ω9 + ω5) | 2.14 ± 0.12 | 3.17 ± 0.27** | 0.47 ± 0.06*** | 6.15 ± 0.47 | 8.50 ± 0.63* | 3.18 ± 0.26*** |
| 17:0(ai + i + n) | 0.63 ± 0.03 | 0.40 ± 0.04** | 1.05 ± 0.08** | 0.11 ± 0.01 | 0.12 ± 0.01 | 0.09 ± 0.01 |
| 17:1(ω10 + ω8) | 2.09 ± 0.15 | 2.19 ± 0.26 | 1.58 ± 0.12* | 0.12 ± 0.01 | 0.11 ± 0.01 | 0.09 ± 0.01 |
| 18:0 | 15.61 ± 0.93 | 10.61 ± 0.65** | 32.78 ± 1.83*** | 17.94 ± 0.88 | 9.06 ± 0.68*** | 24.46 ± 1.27*** |
| 18:1ω9 | 11.52 ± 0.72 | 16.64 ± 0.64*** | 7.47 ± 0.61** | 16.56 ± 0.63 | 21.13 ± 1.13** | 12.35 ± 0.60** |
| 18:2ω6 | 2.78 ± 0.35 | 3.65 ± 0.21 | 2.06 ± 0.22 | 4.37 ± 0.45 | 7.45 ± 0.62** | 2.01 ± 0.17** |
| 18:3ω6 | 0.12 ± 0.01 | 0.26 ± 0.02*** | trace*** | 0.44 ± 0.06 | 0.79 ± 0.07** | 0.10 ± 0.01*** |
| 19:1 | 0.21 ± 0.01 | 0.10 ± 0.01*** | 0.36 ± 0.02*** | — | — | — |
| 20:0 | 0.40 ± 0.05 | 0.18 ± 0.01** | 3.28 ± 0.41*** | 0.82 ± 0.05 | 0.37 ± 0.02*** | 2.67 ± 0.23*** |
| 20:1ω11 | 2.30 ± 0.17 | 3.11 ± 0.34 | 1.31 ± 0.08*** | 1.67 ± 0.11 | 2.08 ± 0.21 | 0.44 ± 0.06*** |
| 20:2ω6 | 0.21 ± 0.03 | 0.28 ± 0.02 | 0.17 ± 0.01 | 0.84 ± 0.06 | 1.23 ± 0.14* | 0.38 ± 0.05*** |
| 20:3(ω9 + ω6) | 2.23 ± 0.21 | 3.16 ± 0.25* | 1.33 ± 0.12** | 1.79 ± 0.10 | 2.41 ± 0.20* | 0.85 ± 0.07*** |
| 20:4ω6 | 17.20 ± 0.86 | 22.41 ± 1.35* | 10.16 ± 0.67*** | 12.38 ± 0.58 | 15.16 ± 0.64* | 8.93 ± 0.49** |
| 20:5ω3 | 0.07 ± 0.01 | 0.06 ± 0.01 | trace*** | — | — | — |
| 22:0 | 0.24 ± 0.02 | trace*** | 4.49 ± 0.52*** | 0.75 ± 0.07 | 0.21 ± 0.02*** | 5.64 ± 0.51*** |
| 22:1ω11 | 1.11 ± 0.12 | 1.48 ± 0.11 | 0.93 ± 0.05 | 1.49 ± 0.11 | 2.64 ± 0.23** | 0.51 ± 0.04*** |
| 22:3ω6 | 2.98 ± 0.33 | 3.66 ± 0.27 | 1.41 ± 0.14** | 4.81 ± 0.34 | 6.06 ± 0.41* | 2.27 ± 0.22*** |
| 22:4ω6 | 16.54 ± 0.74 | 19.29 ± 0.89* | 10.83 ± 0.53*** | 12.05 ± 0.52 | 14.00 ± 0.69 | 7.35 ± 0.48*** |
| 22:5ω3 | 2.17 ± 0.30 | 2.86 ± 0.23 | 1.62 ± 0.14 | — | — | — |
| 22:6ω3 | 2.96 ± 0.34 | 3.41 ± 0.31 | 2.08 ± 0.12* | — | — | — |
| Saturated | 23.37 ± 1.00 | 15.38 ± 0.73*** | 58.22 ± 2.14*** | 37.24 ± 1.22 | 18.06 ± 0.83*** | 61.54 ± 1.91*** |
| Unsaturated | 76.63 ± 1.64 | 84.62 ± 2.00 | 41.78 ± 1.92*** | 62.76 ± 1.26 | 81.94 ± 1.81*** | 38.46 ± 0.99*** |
| Double bound index | 205 | 258 | 129 | 155 | 197 | 96 |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with intact cells.
M ± m; $n = 5$.

cells after incubation with lecithin liposomes. Simultaneously the amount of cholesterol esters in plasma membrane and microsomal fraction decreased too. These data also support the idea that changes of cholesterol content in cells caused the response directed to compensate the alteration of membrane fluidity and to maintain constant properties in the cells. The level of lysophosphatidylcholine was lowered only in the plasma membrane, but not in the microsomal fraction.

The total amount of phospholipids did not change under the action of liposomes, though the amount of some individual phospholipids and their acyl chains changed.

These results are in agreement with previously published data about some phospholipid reorganization in human erythrocyte membrane upon cholesterol depletion.[17] It has also been established that membrane phospholipid metabolism alters in response to cholesterol changes. Sterol depletion correlates with an increase in ratio of unsaturated and saturated fatty acyl chains in membrane phospholipids.[2] In our experiments we found significant changes in the content of lysophosphatidylcholine, phosphatidylethanolamine and in the zone of phospholipids with high chromatographic mobility. The most dramatic changes were observed in the content of *N*-acylphosphatidylethanolamine and its metabolite *N*-acylethanolamine. The amount of *N*-acylethanolamine increased in plasma membrane and microsomal fraction as a result of the cholesterol depletion after incubation with lecithin liposomes. This fact may be of physiological significance because *N*-acylethanolamine reduced plasma membrane permeability.[10] The increased amount of *N*-acylethanolamine in this case can also be attributed to the adaptive cell response to reduction of cholesterol.

The investigation of fatty acyl chains in some individual phospholipids and in the content of lipid fractions loosely and tightly bound with proteins showed that enhanced levels of cholesterol in cells always caused the increase of double bound index of fatty acids. In contrast, the reduced level of cholesterol in cells resulted in the decline of fatty acid unsaturation. These phenomena correlate with changes of many lipid constituents in cell. Variations in individual phospholipid content and in the fatty acid profile of membrane phospholipids are involved in the modulation of membrane properties. The activities of membrane-associated enzymes and transport across membranes are affected by their lipid environment.[9,13,17,19] Probably, the alteration of these

Table 7. The content of individual fatty acid chains in the phosphatidylcholine of plasma membrane and microsomal fraction of neuroblastoma C1300 N18 cells after 1 h incubation with lecithin and lecithin–cholesterol liposomes (5 $\mu$mol of each lipid per 1 ml)

| Fatty acid chains | The content of fatty acid chains (% of total) in: Plasma membrane | | | Microsomal fraction | | |
|---|---|---|---|---|---|---|
| | Intact cells | After incubation with: Lecithin–cholesterol Liposomes | Lecithin Liposomes | Intact cells | After incubation with: Lecithin–cholesterol Liposomes | Lecithin Liposomes |
| 14:0 | 0.23 ± 0.01 | 0.20 ± 0.02 | 0.87 ± 0.04*** | 0.78 ± 0.04 | 0.43 ± 0.04*** | 1.09 ± 0.11* |
| 15:0(i + n) | 0.39 ± 0.03 | 0.37 ± 0.04 | 0.74 ± 0.05*** | 0.83 ± 0.06 | 0.88 ± 0.11 | 1.24 ± 0.08** |
| 15:1$\omega$8 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.27 ± 0.01 | 0.40 ± 0.03** | 0.26 ± 0.04 |
| 16:0(ai + i + n) | 19.74 ± 0.66 | 9.74 ± 0.73*** | 29.06 ± 1.19*** | 28.42 ± 1.08 | 11.76 ± 0.97*** | 36.95 ± 2.17*** |
| 16:1($\omega$9 + $\omega$5) | 2.13 ± 0.25 | 3.81 ± 0.43** | 1.31 ± 0.17* | 1.23 ± 0.08 | 4.33 ± 0.58*** | 0.71 ± 0.05*** |
| 17:0(ai + i + n) | 0.63 ± 0.04 | 0.21 ± 0.05*** | 2.67 ± 0.36*** | 0.14 ± 0.01 | 0.12 ± 0.01 | 0.16 ± 0.01 |
| 17:1($\omega$10 + $\omega$8) | 2.11 ± 0.12 | 4.19 ± 0.43** | 1.86 ± 0.09 | 0.27 ± 0.02 | 0.48 ± 0.06* | 0.22 ± 0.01 |
| 18:0 | 19.03 ± 1.17 | 11.81 ± 0.79*** | 33.94 ± 1.02*** | 32.84 ± 1.63 | 21.95 ± 1.15*** | 38.79 ± 1.74* |
| 18:1$\omega$9 | 31.18 ± 2.14 | 38.15 ± 1.83* | 17.47 ± 0.71*** | 19.36 ± 0.84 | 27.58 ± 1.30*** | 12.03 ± 0.83*** |
| 18:2$\omega$6 | 7.01 ± 0.32 | 10.44 ± 0.68** | 2.89 ± 0.24*** | 4.11 ± 0.31 | 8.74 ± 0.92** | 2.26 ± 0.31** |
| 18:3$\omega$6 | 0.74 ± 0.02 | 0.88 ± 0.08 | 0.06 ± 0.01*** | 0.10 ± 0.01 | 0.39 ± 0.05*** | trace*** |
| 19:1 | 0.12 ± 0.01 | 0.08 ± 0.02 | 0.13 ± 0.03 | — | — | — |
| 20:0 | 0.24 ± 0.01 | 0.06 ± 0.01*** | 0.77 ± 0.03*** | 0.32 ± 0.02 | 0.14 ± 0.01*** | 1.79 ± 0.20*** |
| 20:1$\omega$11 | 2.93 ± 0.37 | 3.36 ± 0.39 | 2.08 ± 0.18 | 1.64 ± 0.14 | 2.87 ± 0.29** | 0.61 ± 0.04*** |
| 20:2$\omega$6 | 0.34 ± 0.04 | 0.38 ± 0.03 | 0.27 ± 0.02 | 1.89 ± 0.17 | 2.43 ± 0.18 | 0.77 ± 0.08*** |
| 20:3($\omega$9 + $\omega$6) | 0.97 ± 0.04 | 1.23 ± 0.06** | 0.61 ± 0.03*** | 0.83 ± 0.04 | 1.59 ± 0.20** | 0.35 ± 0.02*** |
| 20:4$\omega$6 | 4.72 ± 0.21 | 6.17 ± 0.36** | 1.76 ± 0.17*** | 3.08 ± 0.26 | 8.62 ± 0.64*** | 1.16 ± 0.10*** |
| 20:5$\omega$3 | 0.07 ± 0.01 | 0.14 ± 0.02** | trace*** | — | — | — |
| 22:0 | trace | trace | 0.31 ± 0.01 | — | — | — |
| 22:1$\omega$11 | 2.85 ± 0.18 | 3.22 ± 0.23 | 1.94 ± 0.12** | 1.43 ± 0.11 | 2.71 ± 0.34** | 0.26 ± 0.02*** |
| 22:3$\omega$6 | 1.01 ± 0.09 | 1.18 ± 0.08 | 0.62 ± 0.04** | 0.62 ± 0.05 | 1.68 ± 0.21** | 0.31 ± 0.04** |
| 22:4$\omega$6 | 3.48 ± 0.26 | 4.09 ± 0.21 | 0.26 ± 0.02** | 1.84 ± 0.22 | 2.90 ± 0.25* | 1.05 ± 0.08* |
| 22:5$\omega$3 | 0.14 ± 0.01 | 0.18 ± 0.01* | 0.05 ± 0.01*** | — | — | — |
| 22:6$\omega$3 | 0.06 ± 0.01 | 0.14 ± 0.01*** | 0.04 ± 0.01 | — | — | — |
| Saturated | 40.26 ± 1.34 | 22.39 ± 1.08*** | 68.36 ± 1.57*** | 63.33 ± 1.96 | 35.28 ± 1.51*** | 80.01 ± 2.79** |
| Unsaturated | 59.74 ± 2.22 | 77.61 ± 2.15*** | 31.64 ± 0.82*** | 36.67 ± 0.99 | 64.72 ± 1.92*** | 19.99 ± 0.90*** |
| Double bound index | 102 | 128 | 44 | 61 | 118 | 31 |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with intact cells.
M ± m; $n = 5$.

processes is the reason why neuroblastoma cells are very susceptible to the described changes in lipid composition. Seventy per cent of the cells survive after 60 min of incubation with liposomes and only 30% after 90 min.[30]

## CONCLUSION

It can be concluded from the data of the present study that cells possess a programme to prevent changes in plasma membrane fluidity caused by altered cholesterol content. In sterol-depleted cells the amount of lipids increased, membrane microviscosity. In sterol enriched cells the level of some lipids increased which is known to reduce membrane microviscosity. The analysis of our data showed that this mechanism is mainly realized by controlling the process of fatty acid saturation and desaturation. While other compensatory processes can also play a special role, these results regarded from the viewpoint of regulation of cell survival under the changed cholesterol content, suggest that the observed changes in fatty acyl chains and other lipid content may be a vital process for maintaining plasma membrane fluidity and other properties under steady-state conditions. The exact mechanism by which such regulation is accomplished is still open to investigation.

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