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INCORPORATION OF LABELLED N-ACYLETHANOLAMINE (NAE) INTO RAT BRAIN REGIONS IN VIVO AND ADAPTIVE PROPERTIES OF SATURATED NAE UNDER X-RAY IRRADIATION

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В роботі розглянуто питання розподілу екзогенного N-пальмітоїлетаноламіну (NPE) в мозку щурів. Крім цього досліджено зміни ліпідного складу мозку щурів під впливом γ-опромінення (2 Гр) та можливість застосування N-стеароїлетаноламіну (NSE) як протектора та адаптогена. Показано, що N-([9,10-^{3}H]-пальмітоїл)етаноламін після перорального введення накопичується в гіпоталамусі, гіпофізі та надниркових залозах, причому кількість мітки в мозку щурів складає 0,95 % від введеної кількості мітки. Через два тижні після опромінення в мозку щурів спостерігаються значні зміни в ліпідному складі. Зокрема, вміст пальмітинової кислоти у фосфоліпідах мозку та вміст плазмалогенної форми фосфатидилхоліну зростає, в той час як кількість вільного холестеролу та діацильної форми фосфатидилхоліну знижується. Рівень 11-ОН-кортикостероїдів (11-ОКС) у крові опромінених щурів порівняно із контрольними тваринами знижено. Введення NSE протягом 10 днів перед опроміненням попереджає перерозподіл діацильної та плазмалогенної форми фосфатидилхоліну та нормалізує рівень 11-ОКС, а введення NSE протягом 14 днів після опромінення зумовлює нормалізацію рівня вільного холестеролу мозку.

Таким чином, накопичення міченого NPE в мозку свідчить про проникнення цієї сполуки через гематоенцефалічний бар'єр, що припускає можливу роль насичених N-ацилетаноламінів у функціонуванні мозкових структур, зокрема в регуляції стресової відповіді організму гіпоталамо-гіпофізарно-адреналовою системою. Введення NSE опроміненим щурам виявляє адаптогенний ефект відносно змін у ліпідному складі мозку та рівні 11-ОКС спричинених впливом γ-опромінення та зумовлює модифікацію жирнокислотного складу фосфоліпідів.

К л ю ч о в і с л о в а: N-ацилетаноламіни, гіпоталамо-гіпофізарно-адреналова система, Х-опромінення, мозок.

Effect of ionizing radiation on the brain is of great interest for many researchers. Mammalian brain is relatively radioresistant but irradiation can lead to progressive mental disturbance, cognitive impairments and neurological abnormalities [1,2]. Low doses of irradiation (1–2 Gy) caused hypothermia and fever as a result of a direct effect on the rat brain [3]. These negative changes are a consequence of alterations which occurred during most biochemical processes in brain tissues. However, changes observed almost depend on radiation dose and time after irradiation. Thus, apoptotic death of different types of brain cells under irradiation [4–6] is a result of elevated level of active oxygen species that causes the stimulation of stress-induced protein kinase [7] and the activation of the whole signal cascade [6], and apoptosis through a p53-dependent pathway [8,9]. On the other hand, X-ray radiation causes a prolonged reduction of cell proliferation [10]. The mutations appeared because of the decreasing of methylation level of nucleotides [11] and arising of double strand break under the impact of free radicals which were actively generated as a consequence of arising/repeated irradiation doses (2–32 Gy) [12,13].

Inflammation of brain tissues is another drastic fact of irradiation. The inflammation occurs as a result of activation of the synthesis of proinflammatory mediators (TNF-α, IL-1 β, IL-6, iNOS) and induction of adhesion molecules (ICAM-1) on the first stages after irradiation [14,15]. Moreover, the cyclooxygenase-2 activity and, respectively, the prostaglandin E_2 and tromboxane level are significantly elevated in the central nervous system that promotes inflammation process too. In addition, the change of the neuromediator and catecholamine content and disturbance of signal transduction took place immediately after irradiation injury of the brain [16–18].

It is necessary to emphasize the role of the activation of oxidative processes, free radical accumulation and disturbance of lipid metabolism among

other fatal consequences of the X-ray exposure in the brain [19]. The activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase were increased and the level of lipid peroxidative products was decreased in the brain cortex after the whole body X-irradiation [20,21]. At the same time S. Ono with co-authors noticed insignificant changes in lipid peroxidation processes in murine brain after whole-body γ-radiation [22].

All the above changes reflect either brain injury or the developing of adaptive reaction in response to the damage effect of irradiation. The system which regulates the mammalian organism adaptation to stress comprises hypothalamus, pituitary and adrenal gland. The multiple pituitary hormone deficiencies depend primarily on the dose received rather than the time interval after irradiation [23]. Corticosteroids produced in the adrenal glands are high-potent metabolic regulators and the development of adaptive reactions significantly depends on them. Adrenal functions are activated and corticosteroid level in serum is markedly increased in short time after exposition of animal to different doses of X-ray irradiation [24–26]. But there is no unequivocal data about adrenal enzymes activity in a rather long term after irradiation [27,28].

N-acylethanolamines (NAE) are cell minor lipids which have high biological activity, possess protective effect under the action of different toxic factors, ischemic damage and/or under stress state or other injuries [29–33]. Among numerous biological characteristics of NAE it is necessary to note their antioxidative effect under the ischemic and neurotoxic brain damage [29,34]. Both saturated and unsaturated NAE affect adrenal cortex function. They modulate steroidogenesis and thus can influence some biochemical processes [35]. One of the NAE namely anandamide [36] and some other congeners [37] are endogenous ligands for cannabinoid receptors (CB-receptors), which are widely distributed in the central nervous system and other mammalian tissues [36]. It was hypothesised the existence either of a new type of CB-receptor – CB-n or a binding site for saturated NAE [38,39]. Recently CB-like receptors has been found in the adrenal gland. Little is known about the involvement of NAE in adaptive responses to irradiation.

Changes induced by X-ray radiation depended on elapsed time after exposure and a dose. A lot of parameters which were found to be changed in a short time after low doses of radiation treatment were restored and the significant adaptive changes appeared as some time passed after the exposure. The alterations of the brain lipid composition and functional state of the adrenal cortex of mammals 2 week after X-ray exposure are poorly studied. A possibility to correct these negative changes with nature membrane protective compounds namely NAE which have adaptogenic properties is not investigated too. Furthermore, the metabolism of exogenous saturated NAE in the rat brain and adrenal gland is insufficiently known. In view of the aforesaid the aim of the present study was to investigate the regional distribution of N-palmitoylethanolamine ($NAE_{16:0}$) in the rat brain tissues and in the adrenal gland; to estimate changes of lipid content which were caused by 2 Gy X-ray exposition and to study the protective effect of N-stearoylethanolamine ($NAE_{18:0}$) under these conditions.

Materials and Methods

The studies were carried out on the 3-month aged males of Wistar rats with the weight 150–200 g. All procedures involving animals were performed in accordance with approval of the Institutional Animal Care and Use Committee in our Institute. Animals on arrival in our animal care facility were maintained in colony rooms with average light/dark cycles and constant temperature and humidity. Animals were fed and watered *ad libitum*.

For administration, $NAE_{16:0}$ and $NAE_{18:0}$ were dispersed in distilled water by sonication and given to rats *per os* in all experiments.

Study of the N-([9,10-^{3}H]-palmitoyl)-ethanolamine distribution. The laboratory synthesis of the N-([9,10-^{3}H]-palmitoyl)ethanolamine (^{3}H-$NAE_{16:0}$) was performed as described before [40]. The specific $NAE_{16:0}$ radioactivity was ~5.0 mCi/mg. To study the ^{3}H-$NAE_{16:0}$ distribution over the brain regions it was administered to rats *per os* (~100 mCi, $3.3 \cdot 10^{-5}$ mol/100 g of body weight). Rats were anesthetized by an intraperitoneal injection of sodium ethaminal (50 mg/kg) and sacrificed 20 min later. The hypothalamus, brain cortex, white matter, cerebellum, brain stem, pituitary and adrenal glands were excised and immediately frozen in liquid nitrogen and stored in it until analyzed. Data are means of three different experiments.

Lipid extraction from separated brain regions and adrenal gland was performed by the E. Bligh & W. Dyer method [41] with F. Palmer recommendation for more complete extraction of anionic phospholipids [42]. Tissues frozen in liquid nitrogen were grinded in a pounder before lipid extraction. Lipid extracts obtained were dissolved in benzene and stored at -20 °C. The separation of lipid classes was performed by one-dimensional TLC on plates 6x9 cm with L-5/40 silicagel («Lachema», Czech) using the following systems of solvents: chloroform – methanol – ammonium hydroxide (38%) (80:20:2) (v/v) for NAE identification [43] and hexane – diethyl ether – acetic acid (85:15:1) (v/v) for isolation of phospholipids (PL), free cholesterol (FChol), free

fatty acids (FFA), triglycerides (TG) and cholesterol esters (CE). Aliquots from the lipid extracts and spots of lipid classes scraped from TLC plates were added directly to the scintillation vials with scintillation cocktail for non-aqueous solutions. Radioactivity was measured by the scintillation counter Tracor Analytic («Delta 300», USA).

X-ray treatment of animals. The irradiation treatment was made using the cobalt teletherapy unit (RUM-17) (Russia) at the R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology. Unanaesthetised animals were restrained at the well-ventilated boxes and exposed to deliver the whole body dose 2 Gy (1.33 Gy per min). The animals were divided into five groups: the first "Control" group (7 rats) included intact animals, the second group – "Irradiation" of the animals (10 rats) were treated by single-shot ionizing irradiation with 2 Gy dose. The animals of the third group "$NAE_{18:0}$ pretreatment" (10 rats) received $NAE_{18:0}$ *per os* (50 mg/kg) daily for 10 days and then were irradiated with a dose of 2 Gy two hours after the last $NAE_{18:0}$ administration. The animals of the fourth group "$NAE_{18:0}$ post-treatment" (10 rats) were irradiated with a dose of 2 Gy and then $NAE_{18:0}$ was administered them *per os* (50 mg/kg) daily for 2 weeks. In this group the first $NAE_{18:0}$ administration was made two hours after the irradiation. The irradiated rats were sacrificed on the 14th day after irradiation. The animals of the fifth group "$NAE_{18:0}$ control" (6 rats) were administered $NAE_{18:0}$ *per os* (50 mg/kg) daily for 2 weeks before sacrificing. The rat brain was excised, frozen in liquid nitrogen immediately and stored in it until analyzed.

Lipid extraction and examination procedures. Lipid extraction from the whole brain homogenates was performed as described above. The separation of lipid classes was performed by one-dimensional TLC on the L-5/40 silicagel («Lachema», Czech) with the following system of the solvents: hexane – diethyl ether – acetic acid (85:15:1) (v/v) for isolation of PL, FChol and CE. Phospholipids as well as plasmalogen and diacyl forms of phosphatidylcholine (PC) and phosphatidylethanolamine (PC) were separated by two-dimension TLC on plates 6x6 cm with silicagel KSK-2 (Russia) and measured by the colorimetric method [44]. The following systems of solvents were used:

1) for phospholipids separation: chloroform – methanol – benzene – ammonium hydroxide (38%) (65:30:10:6) (v/v) in the first direction; chloroform – methanol – benzene – acetone – acetic acid – water (70:30:10:5:4:1) in the second one [45];

2) for plasmalogen and diacyl forms of PC and PE separation: chloroform – methanol –ammonium hydroxide (38%) (65:35:5) (v/v) in the first direction; 3 M HCl in methanol in the second one for plasmalogens degradation and chloroform – methanol – benzene – acetone – acetic acid – water (100:40:20:20:10) in the second direction for separation [46].

The methyl esters of fatty acids esterified into the phospholipids were derived by the Christie method [47] and were analyzed by GLC by the chromatograph HRGC 5300 Carlo Erba (Italy).

Protein was mensurated by the Lowry method [48]. The amount of the thiobarbituric acid reactive substances (TBARS) was measured as described [49].

Determination of 11-hydroxycorticosteroids (11-HOCS). Rat blood plasma was obtained by centrifugation of 1000 g of the citrated blood. Adrenal glands were stored at -20 °C until analyzed. The 11-hydroxycorticosteroids (11-HOCS) in the plasma and the adrenal tissue were determined by a spectrofluorimeter HITACHI MPF-4 [50].

Statistical analysis. A comparison of the means ± SEM from different studied groups was done using one-way analysis of variances, and two-tailed Student's *t*-test. An analysis of Contingency Tables was performed by Fisher's Exact Test. Differences with p-value < 0.05 were considered as statistically significant. Programmes GraphPad Prism 4.0 and the Origin 7.0 (OriginLab Corporation) were used for statistical data analysis.

Results and Discussion

The first experiment was aimed to study the biodisposition of ^{3}H-$NAE_{16:0}$ in the rat brain regions 20 min after its *per os* administration. Generally, about 0.95% (0.4 mCi) of ^{3}H-$NAE_{16:0}$ administered to rats was found in the brain. Accumulation of labelled $NAE_{16:0}$ in different rat brain regions and in the adrenal glands is shown in Table 1. The highest accumulation of the label per g of tissue was observed in the hypothalamus and pituitary gland. The considerable relative amount of the label was also found in the adrenal glands. However, the difference between the label accumulation in the pituitary gland vs brain cortex and hypothalamus vs brain cortex reached $1.8 \cdot 10^3$ and $4.1 \cdot 10^3$ times, respectively.

Presence of the labelled NAE in the brain (~98 ng/mg of brain tissue) demonstrated the ability of $NAE_{16:0}$ to penetrate through the blood-brain barrier. The biodisposition of $NAE_{16:0}$ in the mammalian brain has not been studied. K. Willoughby et al. showed that 1–30 min. after the intravenous injection of ^{3}H-anandamide (^{3}H-$NAE_{20:4}\omega6$) to mice (50 mg/kg) the quantity of the label accumulated in the whole brain was about 20 ng/mg of tissue however, the most part of ^{3}H-$NAE_{20:4}\omega6$ was catabolized [51]. In most recent paper F. Oveisi with co-authors denoted that no elevation of N-oleoylethanolamine ($NAE_{18:1}\omega9$) amount was detected in the brain 4 hours

T a b l e 1. Biodisposition of the label and N-([9,10-^{3}H]-palmitoyl)ethanolamine in the rat brain regions and adrenal glands within 20 min after its administration

Label	Organs						
	Hypothalamus	Pituitary gland	Adrenal glands	White matter	Brain stem	Cerebellum	Brain cortex
Label amount, nCi/mg of tissue	16.551	7.121	0.251	0.123	0.026	0.011	0.004
[a] ^{3}H-$NAE_{16:0}$, pmol/mg of tissue	10386.9	2048.3	85.4	64.9	110.2	7.4	2.2

Notes: [a] ^{3}H-$NAE_{16:0}$ – N-([9,10-^{3}H]-palmitoyl)ethanolamine.

after its oral administration [52]. It should be emphasised that this experiment was carried out with 4 hour's interval after $NAE_{18:1}\omega9$ administration and that metabolism of unsaturated NAE is faster than that of saturated ones. The evidence of the latter is our data that 20 min. after $NAE_{16:0}$ administration about 90% of a label remained as NAE (Table 2) whereas 1–30 min. after ^{3}H-$NAE_{20:4}\omega6$ injection 90% of it was metabolized [51]. The analysis of ^{3}H-$NAE_{16:0}$ incorporation into different brain regions and adrenal glands *in vivo* displayed the considerable accumulation of the label in the hypothalamus, pituitary and adrenal glands which have exceptional importance for regulation of homeostasis and organism adaptation. The relative level of the label in the individual brain regions can be arranged as follow: hypothalamus > pituitary gland > brain stem > white matter> cerebellum> brain cortex (Table 1). Hence, a part of the label incorporated into the regulatory brain regions was higher than that accumulated in the other ones. The hypothalamus and pituitary gland are two of the most important regulatory organs of mammalian organism. There are a lot of different receptors in the hypothalamus including CB-receptors. It is well known that the hypothalamus joins and coordinates the regulatory functions of the nervous and endocrine systems, for example, by formation of the hypothalamic-pituitary-adrenal axis etc. In particular, corticotrophin-releasing-factor (CRF) is synthesized in the hypothalamus. The ACTH secretion in the pituitary gland and adrenal gland activity depends on CRF. The huge accumulation of ^{3}H-$NAE_{16:0}$ in the hypothalamus, pituitary and adrenal glands is an important fact which allows us to suppose that $NAE_{16:0}$ can affect the functioning of the hypothalamic-pituitary-adrenal system.

The most quantity of ^{3}H-$NAE_{16:0}$ remained non-metabolized (~90%) in the hypothalamus, brain steam, white matter, brain cortex and adrenal glands 20 min after treatment (Table 2). In contrast, approximately 1/3 of a label was metabolized in the cerebellum and in the pituitary gland. The radioactivities of free fatty acid (FFA), triacylglycerol (TG), cholesterol ester (CE) and phospholipid (PL) fractions were evaluated in different brain regions. The radioactivity was not found at the zone which coin-

T a b l e 2. Label distribution in individual lipid classes in different rat brain regions and adrenal gland within 20 min after N-([9,10-^{3}H]-palmitoyl)ethanolamine administration to rat (% of total label content in each tissue)

Organs	Lipids					
	[a] ^{3}H-$NAE_{16:0}$	[b] NAPE	Phospholipids	Free fatty acids	Triglycerides	Cholesterol esters
Hypothalamus	95.1	1.6	2.1	0.9	0.1	0.1
Pituitary gland	72.2	2.3	3.0	2.6	5.4	14.3
Brain stem	93.6	4.0	2.0	0.4	0.0	0.0
White matter	92.7	2.1	3.4	1.8	0.0	0.0
Cerebellum	66.1	6.6	9.2	4.1	13.5	0.6
Brain cortex	98.0	0.0	2.0	0.0	0.0	0.0
Adrenal glands	85.0	4.2	4.9	2.4	1.9	1.7

Notes: [a] ^{3}H-$NAE_{16:0}$ – N-([9,10-^{3}H]-palmitoyl)ethanolamine; [b] NAPE – N-acylphosphatidylethanolamines.

cides with R_f of free cholesterol. It was surprising to find the significant amount of the label (1.6–6.6%) in a zone with R_f which corresponds to N-acylphosphatidylethanolamine (NAPE) – the endogenous precursor of NAE [53].

Incorporation of the label into different lipid classes of the rat brain regions and adrenal glands (Table 2) allows us to hypothesize the existence of metabolic conversion of exogenous NAE in mammalian tissues. One can suggest that $NAE_{16:0}$ in tissues can be hydrolyzed by amidohydrolases and the formed labelled palmitoyl can reacylate and label other lipid molecules [53]. The label presence in the zone corresponding to R_f of free fatty acids may serve as an evidence in favour of this suggestion (Table 2).

It can be supposed that $NAE_{16:0}$ administered into the organism may be condensed with endogenous phosphatidic acid (PA) with formation of N-palmitoylphosphatidylethanolamine (NPPE). After that the palmitoyl can be involved into the reacylation-deacylation cycle and then may be transferred to more stable phospholipids. It is well known that NAPE hydrolysis by phospholipase of D-type (PLD) with following NAE and PA formation occurs under pathological conditions in the presence of high concentration of Ca^{2+}-ions [54]. It is not excluded that this enzyme under normal physiological conditions can function in reversed direction to condensate exogenous $NAE_{16:0}$ and endogenous PA (Fig. 1) with NPPE formation. We found a label on TLC plates in the zone which corresponded to R_f of NAPE. This fact may serve as an indirect evidence of this hypothesis. The possibility of enzyme functioning in the reverse direction to NAE synthesis was shown by the group of prof. H. H. Shmid and other researchers [55,56]. But another type of PLD – phosphatidylcholine specific phospholipase D cannot function in reverse direction therefore our suggestion requires further experimental investigations. However, the other pathway when $NAE_{16:0}$ is hydrolyzed and palmitoyl is utilized to form other lipid molecules cannot be excluded.

The following part of our work was to evaluate the protective properties of saturated NAE under ionizing irradiation.

The development of the stress reaction was caused by the ionizing irradiation treatment. One of the important parameters which characterize adaptive response of an organism is 11-HOCS level in the blood. The results of 11-HOCS determination in the blood and adrenal gland tissue are shown in Fig. 1. 11-HOCS level in the adrenal tissue 2 weeks after irradiation was not changed. The amount of 11-HOCS in the adrenal glands was essentially (more than 2 times) increased when $NAE_{18:0}$ was treated for 10 days before irradiation. The 11-HOCS content

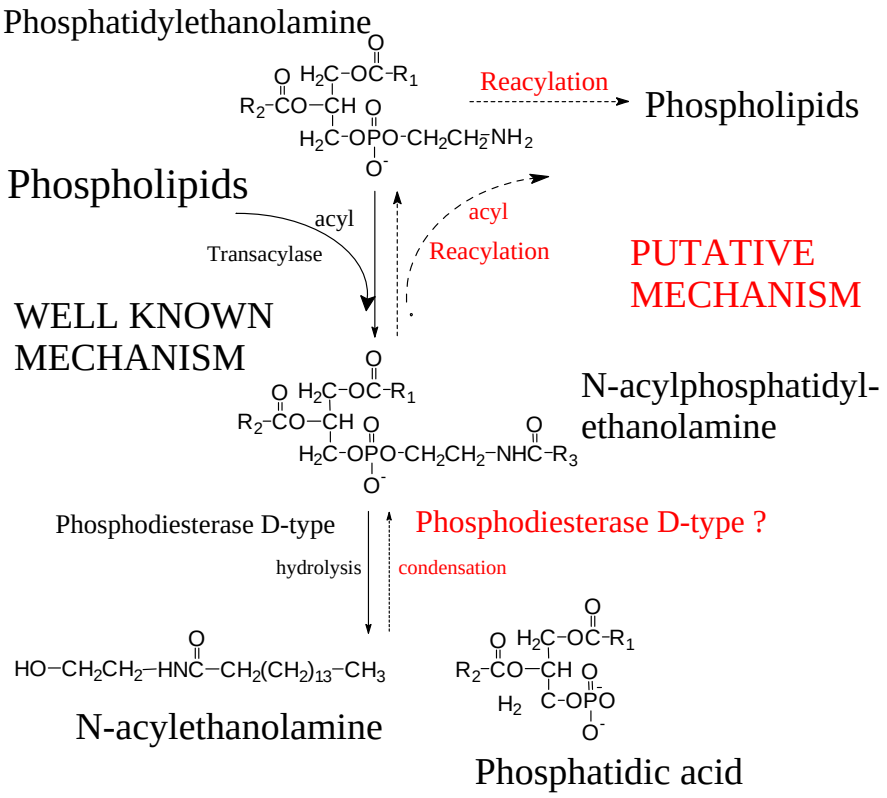

Fig. 1. Possible mechanism of N-palmitoylethanolamine incorporation into the phospholipids.

in the blood was about 2 times decreased after the irradiation (Fig. 2). This alteration was prevented by $NAE_{18:0}$ pre-treatment.

The decrease of 11-HOCS content in the blood two weeks after the irradiation (Fig. 2) can be considered as a sequence of corticosteroid metabolism acceleration because its content in the adrenal tissue did not change under these conditions. Furthermore, such changes may be an after-effect of down-regulating of the hypothalamus-pituitary-adrenocortical axis functions [18] or of decreasing of adrenal 21-hydroxylase activity [27]. On the other hand, the decreasing of the 11-HOCS blood level may be implied with reduction of CRF or ACTH synthesis because of possible damage of the regulatory brain centres by irradiation [57]. $NAE_{18:0}$ pre-treatment of rats restored 11-HOCS level in the blood probably either due to the protective effect of $NAE_{18:0}$ on the adrenal tissue or due to the activation of steroidogenesis by $NAE_{18:0}$ [35]. Also, the pre-treatment with $NAE_{18:0}$ can exert the radioprotective effect on the hypothalamus and pituitary gland. $NAE_{18:0}$ post-treatment was less effective than $NAE_{18:0}$ pre-treatment with respect to 11-HOCS content in the blood plasma and in the adrenal glands. These suggestions are in accordance with well-known data about activation of adrenal cortex function by NAE under the stress conditions [35].

Protective effect of $NAE_{18:0}$ can be also explained by the direct influence of this compound on cell membranes. It is well known, that NAE easily interacts with a cell membrane in a hydrophobic manner. Besides, this effect can be a result of the adrenocorticocyte membrane lipid composition changing that can cause the changes of membrane receptor activity (including cannabinoid receptors). On the other hand, there are some data that $NAE_{18:0}$ possesses signalling activity and may have specific binding sites on the plasma membrane and can affect the activity of some enzymes such as adenylatcyclase and NO-synthase [58].

There were no changes of TBARS content in the adrenal tissue 2 weeks after the irradiation. However, their level in the brain was two-times increased, but not statistically significant (Table 3). The amount of TBARS in the brain tissue was not changed when $NAE_{18:0}$ was post-treated. The effect of NAE treatment before irradiation was more prominent and caused the decrease of the TBARS content in comparison with control and irradiated groups. The catalase activity also was not altered in brain of irradiated and treated with NAE rats (Table 3).

The TBARS level in the brain and adrenal gland tissues was not significantly changed within two weeks after the whole-body irradiation (Table 3). F. S. Erol and co-workers specified the elevation of TBARS content in parietal cortices within ten days after the irradiation [59]. We showed that $NAE_{18:0}$ pre-treatment had an antioxidative effect on brain tissue because in the brain of animals, which received $NAE_{18:0}$ before irradiation, the amount of TBARS was lower (Table 3) as compared to that of

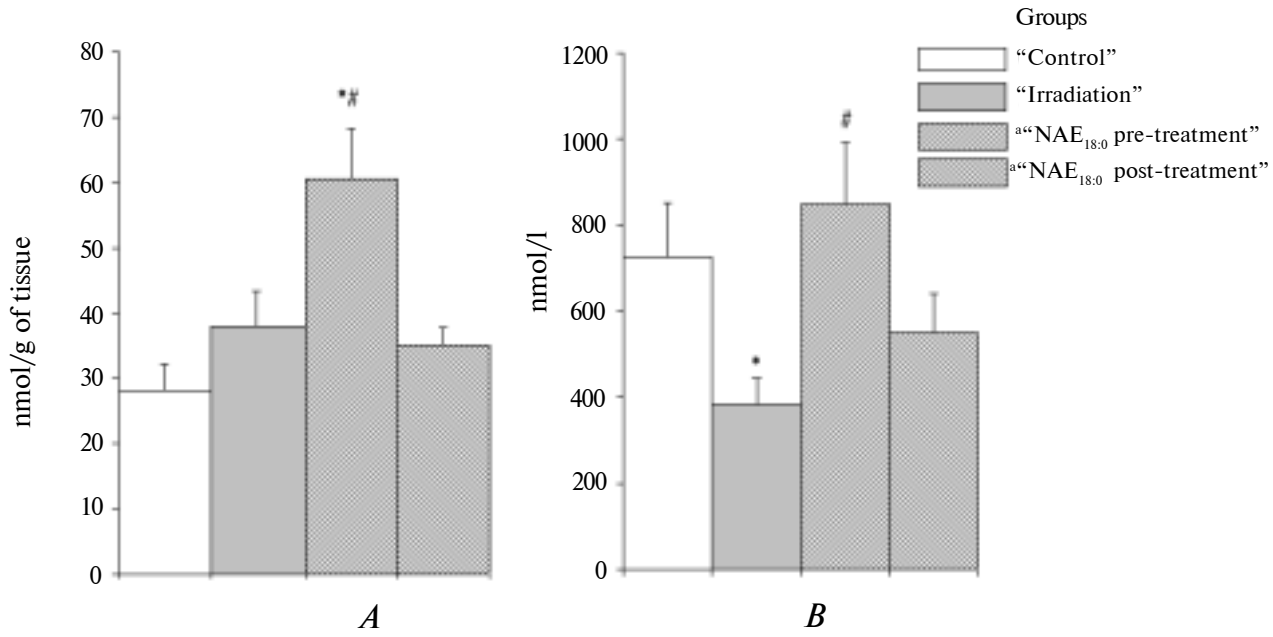

*Fig. 2. The content of 11-OH-corticosteroids in the adrenal tissue (nmol/g of tissue), A; in blood of irradiated rats (nmol/l), B. Notes: * – $p < 0.05$ compared with "Control" group; # – $p < 0.05$ compared with "Irradiation" group; [a] $NAE_{18:0}$ – N-stearoylethanolamine.*

T a b l e 3. Content of the thiobarbituric acid-reactive substances (TBARS) in the brain (μmol/g of tissue) and adrenal gland homogenates (nmol/g of tissue), catalase activity in the brain (ncat/g of tissue)

Tissue		Groups			
		"Control"	"Irradiation"	[a] "$NAE_{18:0}$ pre-treatment"	[a] "$NAE_{18:0}$ post-treatment"
TBARS content	Brain, μmol/g tissue	21.4 ± 3.0	44.2 ± 15.7	10.4 ± 0.9 *#	18.0 ± 2.2
	Adrenal glands, nmol/g tissue	0.18 ± 0.02	0.19 ± 0.03	0.21 ± 0.02	0.19 ± 0.01
Brain catalase activity, ncat/g of tissue		3.6 ± 0.5	4.8 ± 0.7	3.5 ± 0.4	3.5 ± 0.3

*Notes: * − $p < 0.05$ compared with "Control" group; # − $p < 0.05$ compared with "Irradiation" group; [a] $NAE_{18:0}$ −N-stearoylethanolamine.*

irradiated rats. These data confirm well-known membrane protective and antioxidative effects of NAE. As it was previously shown in our laboratory and by other researchers, NAE inhibited Fe^{2+}-induced oxidative damage of heart mitochondria [60] prevented damage action of veratridine on neubroblastoma cell membrane [61], decreased the level of POL products under metastatic Lewis carcinoma and under hypoxia injury [62,63].

Ionizing radiation did not change the total protein and phospholipid content in the brain (these results are not shown). The analysis of the brain individual phospholipids did not reveal any essential alterations after the irradiation (Table 4). The lysophosphatidylcholine (LPC) level was diminished in case when $NAE_{18:0}$ was pre-treated. $NAE_{18:0}$ treatment after the irradiation did not affect the phospholipid composition of the brain.

In addition, the content of minor phospholipids such as N-acylated phospholipids namely N-acylphosphatidylserine (NAPS) and NAPE, as well as diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG), was measured in the rat brain but these phospholipids were defined only in a part of rats. For example, NAPS and DPG were detected in 14.3% of intact animals, in 50% of animals from the group "Irradiation", in 30% of animals from the group "$NAE_{18:0}$ pre-treatment", in 70% of animals from the group "$NAE_{18:0}$ post-treatment" and in 20% of animals from the group of "NAE control". NAPE was measured in 14%, 30%, 20%, 40% and 20% of animals of the corresponding groups and PG was determined in 14.3%, 60%, 30%, 60% and 20% of evaluated animals of respective groups. Analysis Contingency Tables using Fisher's Exact Test (programme GraphPad Prism 4.0) indicated that the occurrence of NAPS as well as that of DPG were statistically significant only in the group "$NAE_{18:0}$ post-treatment".

The balance between plasmalogen and diacyl forms of PC was shifted towards more of the plasmalogen form in the brain of irradiated rats (Table 5). These changes were prevented as a result of the $NAE_{18:0}$ pre-treatment. PE was found at the equal quantities in its diacyl and plasmalogen forms and this proportion was not altered under the irradiation or $NAE_{18:0}$ treatment.

The decreasing of the PC diacyl form level

T a b l e 4. Brain phospholipid composition of control and irradiated rats (10^{-8} mol/mg of protein)

Phospholipids	Groups			
	"Control"	"Irradiation"	[a] "$NAE_{18:0}$ pre-treatment"	[a] "$NAE_{18:0}$ post-treatment"
Phosphatidylcholine	6.21 ± 1.39	4.86 ± 0.48	4.68 ± 0.35	6.46 ± 1.19
Phosphatidylethanolamine	6.70 ± 1.29	5.99 ± 1.04	3.69 ± 0.71	5.19 ± 0.76
Phosphatidylserine	2.04 ± 0.45	1.81 ± 0.38	1.38 ± 0.18	2.03 ± 0.30
Sphingomyelin	1.21 ± 0.34	0.66 ± 0.09	0.78 ± 0.29	0.63 ± 0.16
Phosphatidylinositol	0.69 ± 0.26	0.19 ± 0.05	0.34 ± 0.07	0.31 ± 0.06
Lysophosphatidylcholine	0.39 ± 0.16	0.20 ± 0.09	0.02 ± 0.02*	0.35 ± 0.14
Not identified P_i	1.07 ± 0.50	2.10 ± 0.67	0.83 ± 0.13	1.30 ± 0.29

*Notes: * − $p < 0.05$ compared with "Control" group; [a] − $NAE_{18:0}$ − N-stearoylethanolamine.*

T a b l e 5. Content of phosphatidylcholine and phosphatidylethanolamine plasmalogen and diacyl forms in the brain of control and irradiated rats (% of sum of diacyl and plasmalogen forms)

Phospholipids	Groups			
	"Control"	"Irradiation"	[a] "$NAE_{18:0}$ pre-treatment"	[a] "$NAE_{18:0}$ post-treatment"
PC – diacyl	97.47 ± 0.77	95.19 ± 0.48 *	97.32 ± 0.52 #	95.24 ± 0.77
PC – plasmalogen	2.53 ± 0.77	4.81 ± 0.48 *	2.68 ± 0.52 #	4.76 ± 0.77
PE – diacyl	50.74 ± 2.60	45.45 ± 1.44	45.23 ± 2.09	46.09 ± 1.20
PE – plasmalogen	49.26 ± 2.60	54.55 ± 1.44	54.77 ± 2.09	53.91 ± 1.20

*Notes: * – $p < 0.05$ compared with "Control" group; # – $p<0.05$ compared with "Irradiation" group; [a] – $NAE_{18:0}$ – N-stearoylethanolamine.*

under irradiation may indicate the disturbance of phospholipid synthesis and of reacylation-deacylation processes in the brain tissue (Table 5). On the other hand, ionizing radiation changed membrane physicochemical properties [64]. $NAE_{18:0}$ prevented these changes and thus revealed the membrane-stabilizing and antioxidative properties. As it is shown in tables 1 and 2 the long-chain saturated NAE can penetrate through blood-brain barrier and act directly on the brain cell membrane. A. Ambrosini and D. Epps with co-authors described the fact of lipid membrane stabilisation by NAE [65,66].

The analysis of fatty acids esterified into the phospholipids showed that the amount of the palmitic acid ($C_{16:0}$) increased after the irradiation, while $NAE_{18:0}$ treatment did not affect its level (Table 6, the fatty acids which amounts did not exceed 1% of total quantity are not presented). The amount of the oleic acid ($C_{18:1}\omega9$) increased and the level of the isopalmitic one ($iC_{16:0}$) was decreased in the group "$NAE_{18:0}$ post-treatment". The amount of some minor fatty acids such as isostearic ($iC_{18:0}$), eicosanoic ($C_{20:0}$), docosamonoenoic ($C_{22:1}\omega11$) and docosatrienoic ($C_{22:3}\omega9$) esterified into the phospholipids were decreased in the brain of animals treated with $NAE_{18:0}$ both before and after irradiation in comparison with non-irradiated rats.

The fatty acid range of total brain phospholi-

T a b l e 6. The content of fatty acid esterified into brain phospholipids of control and irradiated rats (% of total fatty acid amount)

Fatty acids	Groups			
	"Control"	"Irradiation"	[a] "$NAE_{18:0}$ pre-treatment"	[a] "$NAE_{18:0}$ post-treatment"
$iC_{16:0}$	0.82 ± 0.20	0.56 ± 0.16	0.66 ± 0.23	0.14 ± 0.05 *#
$C_{16:0}$	12.39 ± 0.62	14.81 ± 0.61*	14.52 ± 0.31 *	15.64 ± 0.50 *
$iC_{18:0}$	3.04 ± 0.53	2.25 ± 0.83	1.38 ± 0.59 *	0.41 ± 0.14 *
$C_{18:0}$	28.51 ± 2.61	25.94 ± 1.14	23.95 ± 0.62	25.79 ± 0.53
$C_{18:1}\omega9$	21.31 ± 1.74	22.12 ± 0.69	23.83 ± 0.82	24.36 ± 0.56 #
$C_{18:2}\omega6$	0.66 ± 0.08	0.72 ± 0.03	0.70 ± 0.08	0.78 ± 0.05
$C_{20:0}$	1.42 ± 0.09	1.73 ± 0.44	1.08 ± 0.12 *	1.06 ± 0.08 *
$C_{20:1}\omega11$	4.29 ± 0.49	3.03 ± 0.49	4.36 ± 0.32 #	4.11 ± 0.23
$C_{20:4}\omega6$	8.30 ± 1.01	9.20 ± 0.73	8.46 ± 1.06	9.41 ± 0.81
$C_{22:0}$	1.15 ± 0.12	0.92 ± 0.13	1.12 ± 0.12	1.18 ± 0.12
$C_{22:1}\omega11$	0.96 ± 0.08	0.77 ± 0.15	0.41 ± 0.06 *	0.41 ± 0.02 *#
$C_{22:3}\omega9$	1.93 ± 0.58	1.29 ± 0.50	0.43 ± 0.06 *	0.41 ± 0.04 *
$C_{22:4}\omega6$	1.53 ± 0.22	2.16 ± 0.24	1.97 ± 0.34	2.51 ± 0.19
$C_{22:5}\omega6$	1.93 ± 0.26	1.87 ± 0.19	1.36 ± 0.14	1.59 ± 0.07
$C_{22:6}\omega3$	7.58 ± 1.24	7.98 ± 1.18	5.46 ± 0.58	6.35 ± 0.55

*Notes: * – $p<0.05$ compared with "Control" group; # – $p<0.05$ compared with "Irradiation" group; [a] – $NAE_{18:0}$ – N-stearoylethanolamine.*

*Fig. 3. Effect of NAE on the content of cholesterol in the brain of irradiated rats (μmol/ g of tissue). Notes: * – $p < 0.05$ compared with "Control" group; # – $p < 0.05$ compared with "Irradiation" group; [a] $NAE_{18:0}$ – N-stearoylethanolamine.*

pids (Table 6) was almost not changed after irradiation. The percentage of the palmitic acid was slightly increased. That could be a result of the decrease of unsaturated acids percentage in phospholipids that can cause the abnormality of saturated/unsaturated fatty acids ratio. The $NAE_{18:0}$ administration modified minor fatty acid composition of brain phospholipids. The amount of $C_{22:1}\omega11$ and $C_{22:3}\omega9$ was decreased and the level of $C_{18:1}\omega9$ and $C_{20:1}\omega11$ was increased under $NAE_{18:0}$ treatment. That may indicate inhibition of elongation and desaturation processes of unsaturated fatty acids ω9-, ω11-series. The diminishing of $iC_{16:0}$, $iC_{18:0}$ and $C_{20:0}$ levels can be a result of $C_{16:0}$ accumulation and probably of depression of saturated fatty acids elongation. Such NAE effect can be explained by direct $NAE_{18:0}$ influence on the brain cell membrane, modification of their physicochemical properties [65,66] and as a result of activity of membrane-associated proteins, in particular, functioning of lipid metabolism enzymes (fatty acid elongases and desaturases, different types of phospholipases, plasmalogenase etc.).

The contents of free cholesterol and its esters were also studied for more complete characteristic of changes which occurred in lipid components of the brain cell membranes of the irradiated animals (Fig. 2). The amount of free cholesterol in the brain of the irradiated rats was significantly diminished. This alteration did not appear when animals were treated with $NAE_{18:0}$ after the irradiation. However, the cholesterol ester level was drastically increased in this group.

The decreasing of free cholesterol amount also can reflect the damage effect of X-ray radiation on brain cell membranes (Fig. 2). $NAE_{18:0}$ post-treatment eliminated this change. These data confirm the reparative and protective characteristics of this compound.

The increasing of cholesterol ester level in the brain of animals post-treated with NAE can be explained by the increasing of free cholesterol. The amount of the brain cholesterol esters consisted of less than 1% of free cholesterol amount, that is why negligible variations of nonesterified cholesterol quantity could cause significant changes in cholesterol esters level. Besides, this fact can be elucidated by membranotrophic properties of NAE. $NAE_{18:0}$ could affect membrane bounded enzymes of cholesterol esters metabolism such as acyl-CoA:cholesterol acyltransferase and cholesteryl ester hydrolase.

Thus, the results of this study showed that different brain regions and adrenal glands may be a target for exogenous saturated NAE. The observed decrease of 11-HOCS content in the rat blood and changes of brain lipid composition such as, shifting of the balance between plasmalogen and diacyl forms of PC, increasing of the palmitic acid amount, decreasing of free cholesterol amount might be conside-

red as compensatory post-radiation perturbations of brain metabolism within two week after the irradiation. It was established that $NAE_{18:0}$ possesses adaptive properties under X-ray irradiation. NAE treatment caused the reduction of irradiation-induced changes in phospholipid composition, redistribution of fatty acids and free cholesterol content. It was shown that the animals treated with $NAE_{18:0}$ were significantly affected adrenal function that was altered after the irradiation. The considerable accumulation of labelled $NAE_{16:0}$ in different brain regions gives the opportunity to speculate about possible role of saturated long-chain NAE in the brain functioning and in the regulation of response of the hypothalamus-pituitary-adrenal axis to the stress. Furthermore, the protective effect of $NAE_{18:0}$ under X-ray irradiation and under other neurodegenerative states allows us to suppose that this compound may be useful in pharmacotherapy of neurodegenerative changes.

INCORPORATION OF LABELLED N-ACYLETHANOLAMINE (NAE) INTO RAT BRAIN REGIONS IN VIVO AND ADAPTIVE PROPERTIES OF SATURATED NAE UNDER X-RAY IRRADIATION

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S u m m a r y

Regional distribution of exogenous N-palmitoylethanolamine in the rat brain was investigated in the study. Possible protective and adaptive effect of N-stearoylethanolamine under 2 Gy whole-body X-irradiation and changes of brain lipid composition were also studied. It was found that after per os administration to rats N-([9,10-^{3}H]-palmitoyl)-ethanolamine was primarily accumulated in hypothalamus, pituitary and adrenal glands and the label amount in brain was 0.95% of the oral dose. Quantities of palmitic acid in total brain phospholipids and plasmalogen form of phosphatidylcholine were increased; free cholesterol and diacyl form of phosphatidylcholine were decreased in 2 weeks after irradiation. 11-OH-corticosteroid level in the blood of exposed rats was decreased in comparison with control animals. N-stearoylethanolamine pre-treatment prevented from increasing the plasmalogen form of phosphatidylcholine and decreasing its diacyl form and restored 11-OH-corticosteroid level in the blood of irradiated rats. Recovering of brain free cholesterol level was observed when N-stearoylethanolamine was post-treated. So, the accumulation of N-([9,10-^{3}H]-palmitoyl)ethanolamine in brain indicates its penetration through blood-brain barrier and suggests the possible role of saturated N-acylethanolamines in brain functioning, particularly, in stress response regulation of the organism by hypothalamus-pituitary-adrenal system. N-stearoylethanolamine treatment of irradiated rats causes protective effect concerning the of irradiation induced changes in the brain lipid composition and in 11-OH-corticosteroid level and modifies phospholipid fatty acid composition.

K e y w o r d s: N-acylethanolamines, hypothalamus-pituitary-adrenal glands axis, X-ray irradiation, brain.

1. *Mizumatsu S., Monje M. L., Morhardt D. R. et al.* // Cancer Res. – 2003. – **63**. – P. 4021–4027.
2. *Raber J., Rola R., LeFevour A. et al.* // Radiat. Res. – 2004. – **162**. – P. 39–47.
3. *Kandasamy S. B., Hunt W. A., Mickley G. A.* // Ibid. – 1988. – **114**. – P. 42–53.
4. *Lu F. G., Wong C. S.* // Int. J. Radiat. Biol. – 2004. – **80**. – P. 39–51.
5. *Guelman L. R., Zieher L. M., Fiszman M. L.* // Neurochem. Int. – 1996. – **29**. – P. 521–527.
6. *Nagai R., Tsunoda S., Hori Y., Asada H.* // Surg. Neurol. – 2000. – **53**. – P. 503–507.
7. *Chen Y. R., Meyer C. F., Tan T. H.* // J. Biol. Chem. – 1996. – **271**. – P. 631–634.
8. *Toda H., Hasegawa M., Hayakawa K. et al.* // Anticancer Res. – 2000. – **20**. – P. 165–170.
9. *Wang X., Matsumoto H., Okaichi K., Ohnishi T.* // Ibid. – 1996. – **16**. – P. 1671–1674.
10. *Tada E., Parent J. M., Lowenstein D. H., Fike J. R.* // Neuroscience. – 2000. – **99**. – P.33–41.
11. *Tawa R., Kimura Y., Komura J. et al.* // J. Radiat. Res. (Tokyo). – 1998. – **39**. – P.271–278.
12. *Ono T., Ikehata H., Vishnu Priya P., Uehara Y.* // Int. J. Radiat. Biol. – 2003. – **79**. – P. 635–41.
13. *Gobbel G. T., Bellinzona M., Vogt A. R. et al.* // J. Neurosci. – 1998. – **18.** – P. 147–155.
14. *Lynch A. M., Moore M., Craig S. et al.* // J. Biol. Chem. – 2003. – **278**. – P. 51075–51084.
15. *Kyrkanides S., Olschowka J. A., Williams J. P. et al.* // J. Neuroimmunol. – 1999. – **95**. – P. 95–106.
16. *Foulon O., Lalouette F., Lambert F. et al.* // J. Neurosci. Res. – 1999. – **55**. – P. 770–775.
17. *Miyachi Y., Kasai H., Ohyama H., Yamada T.* // Neurosci. Lett. – 1994. – **175**.– P. 92–94.

18. *Wan H., Gong S. L., Liu S. Z.* // Biomed. Environ. Sci. – 2001. – **14**. – P. 248–255.
19. *Riley P. A.* // Int. J. Radiat. Biol. – 1994. – **65**. – P. 27–33.
20. *Yamaoka K., Edamatsu R., Itoh T., Mori A.* // Free Radic. Biol. Med. – 1994. – **16**. – P. 529–534.
21. *Yamaoka K., Nomura T., Wang D. H. et al.* // Physiol. Chem. Phys. Med. NMR. – 2002. – **34**. – P. 133–144.
22. *Ono S., Cai L., Cherian M. G.* // Radiat. Res. – 1998.– **150**. – P. 52–57.
23. *Shalet S. M., Beardwell C. G., MacFarlane I. A. et al.* // Acta Endocrinol. (Copenh.). – 1977. – **84**. – P. 673–680.
24. *Groza P., Vladescu C.* // Physiologie. – 1975. – **12**. – P. 53–55.
25. *Flemming K., Hellwig J.* // Acta Radiol. Suppl. – 1971. – **310**. – P. 124–136.
26. *Geierhaas B., Flemming K.* // Radiobiol. Radiother. (Berl.). – 1970. – **11**. – P. 215–219.
27. *Inano H., Suzuki K., Ishii-Ohba H. et. al.* // Radiat. Res. – 1989. – **117**. – P. 293–303.
28. *Suzuki K., Takahashi M., Ishii-Ohba H. et al.* // J. Steroid. Biochem. – 1990. – **35**. – P. 301–305.
29. *Hansen H.S., Lauritzen L., Moesgaard B. et al.* // Biochem. Pharmacol. – 1998. – **55**. – P. 719–725.
30. *Kondo S., Sugiura T., Kodaka T. et al.* // Arch. Biochem. Biophys. – 1998. – **354**. – P. 303–310.
31. *Epps D. E., Schmid P. C., Natarajan V., Schmid H. H.* // Biochem. Biophys. Res. Commun. – 1979. – **90**. – P. 628–633.
32. *Berdyshev E. V., Schmid P. C., Dong Z., Schmid H. H.* // Biochem. J. – 2000. – **346**. – P. 369–374.
33. *Hansen H. S., Lauritzen L., Strand A. M. et al.* // Biochim. Biophys. Acta. – 1995. – **1258**. – P. 303–308.
34. *Schmid P. C., Krebsbach R. J., Perry S. R. et al.* // FEBS Lett. – 1995. – **375**. – P. 117–120.
35. *Mikosha A., Kovzun O., Zhukov A., Gulaya N.* // Med. Sci. Res. – 1998. – **26**. – P. 85–88.
36. *Berdyshev E. V., Boichot E., Lagente V.* // J. Lipid. Mediat. Cell. Signal. – 1996. – **15**. – P. 49–67.
37. *Skaper S. D., Buriani A., Dal Toso R. et al.* // Proc. Natl. Acad. Sci. USA. – 1996. – **93**. – P. 3984–3989.
38. *Lambert D. M., Di Marzo V.* // Curr. Med. Chem. – 1999. – **6**. – P. 757–773.
39. *Maccarrone M., Cartoni A., Parolaro D. et al.* // Mol. Cell. Neurosci. – 2002. – **21**. – P. 126–140.
40. *Маргітич В. М., Жуков О. Д., Артамонов М. В., Гула Н. М.* // Укр. біохім. журн. – 1999. – **71**, № 6. – С. 108–110.
41. *Bligh E. G., Dyer W. I.* // Can. J. Biochem. Physiol. – 1959. – **37**. – P. 911–917.
42. *Palmer F. B.* // Biochim. Biophys. Acta. – 1971. – **231**.– P. 134–144.
43. *Schmid H. H., Schmid P. C., Natarajan V.* // Prog. Lipid. Res. – 1990. – **29**. – P. 1–43.
44. *Vaskovsky V. E., Kostetsky E. Y., Vasendin I. M.* // J. Chromatogr. – 1975. – **114**. – P. 129–141.
45. *Vaskovsky V. E., Terekhova T. A.* // J. High Resol. Chromatogr. & C.C. – 1979. – **2**. – P. 671–672.
46. *Vaskovsky V. E., Dembitzky V. M.* // J. Chromatogr. – 1975. – **115**. – P. 645–647.
47. *Christie W. W.* / Lipid analysis. – Pergamon Press, Oxford, 1979. – P. 338.
48. *Lowry O. H., Rosenbrough N. J., Farr A. L., Randall R. J.* // J. Biol. Chem. – 1951. – **193**. – P. 265–275.
49. *Владимиров Ю. А., Арчаков А. И.* / Перекисное окисление липидов в биологических мембранах. – Наука, Москва, 1972. – С. 252.
50. *de Moor P., Steeno O., Raskin M., Hendrix A.* // Acta Endocrinol. – 1960. – **33**. – P. 297–307.
51. *Willoughby K. A., Moore S. F., Martin B. R., Ellis E. F.* // J. Pharmacol. Exp. Ther. – 1997. – **282**. – P. 243–247.
52. *Oveisi F., Gaetani S., Eng K. T., Piomelli D.* // Pharmacol. Res. – 2004. – **49**. – P. 461–466.
53. *Natarajan V., Schmid P. C., Reddy P. V., Schmid H. H.* // J. Neurochem. – 1984. – **42**. – P. 1613–1619.
54. *Reddy P. V., Schmid P. C., Natarajan V. et al.* // Biochim. Biophys. Acta. – 1984. – **795**. – P. 130–136.
55. *Schmid P. C., Schwindenhammer D., Krebsbach R. J., Schmid H. H.* // Chem. Phys. Lipids. – 1998. – **92**. – P. 27–35.
56. *Kurahashi Y., Ueda N., Suzuki H. et al.* // Biochem. Biophys. Res. Commun. – 1997. – **237**.– P. 512–515.
57. *Schmiegelow M., Feldt-Rasmussen U., Rasmussen A. K. et al.* // J. Clin. Endocrinol. Metab. – 2003. – **88**. – P. 3149–3154.
58. *Maccarrone M., Pauselli R., Di Rienzo M., Finazzi-Agro A.* // Biochem. J. – 2002. – **366**. – P. 137–144.
59. *Erol F. S., Topsakal C., Ozveren M. F. et al.* // Neurosurg Rev. – 2004. – **27**. – P. 65–69.
60. *Parinandi N. L., Schmid H. H.* // FEBS Lett. – 1988. – **237**. – P. 49–52.
61. *Gulaya N. M., Melnik A. A., Balkov D. I. et al.* // Biochim. Biophys. Acta. – 1993. – **1152**. – P. 280–288.
62. *Гулая Н. М., Смирнов И. М., Шмалько Ю. П. и др.* // Укр. биохим. журн. – 1993. – **65**, № 6. – С. 96–101.

63. *Gulaya N. M., Kuzmenko A. I., Margitich V. M. et al.* // Chem. Phys. Lipids. – 1998. – **97**. – P. 49–54.
64. *Benderitter M., Vincent-Genod L., Pouget J. P., Voisin P.* // Radiat. Res. – 2003. – **159**. – P. 471–483.
65. *Epps D. E., Cardin A. D.* // Biochim. Biophys. Acta. – 1987. – **903**. – P. 533–541.
66. *Ambrosini A., Bertoli E., Tanfani F. et al.* // Chem. Phys. Lipids. – 1994. – **72**. – P. 127–134.

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