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Influence of Seizures on Lipids of Homogenate and Neuronal and Glial Nuclei of Rat Neocortex

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Abstract—Lipid composition of homogenate and neuronal and glial nuclei of the brain cortex of Wistar rats was studied under normal conditions and after seizures induced by injection of picrotoxin. Seizures increased contents of lysophosphatidylcholine, sphingomyelin, and total phospholipids in the homogenate. In neuronal nuclei contents of total phospholipids, sphingomyelin, phosphatidylcholine, and phosphatidylserine decreased, and contents of free fatty acids and lysophosphatidylcholine increased. In glial nuclei content of total phospholipids decreased and content of free fatty acids increased. The role of changes in the lipid composition of the neocortex cells during seizures and the involvement of lipid messengers in signal mechanisms are discussed.

Key words: picrotoxin, rat brain cortex, neuronal nuclei, glial nuclei, lipids

Lipids are not only main structural constituents (up to 50% of dry weight) but also the most important regulators of functional activity of nervous system cells. Studies on lipids during seizures are especially interesting because they are likely to stimulate normal intracellular signal mechanisms, which involve lipid messengers [1-3]. Seizures increase contents of diacylglycerides and free fatty acids, which are products of phospholipid degradation [1]. Increase in contents of free fatty acids (in particular, of arachidonic acid) in synaptic terminals correlates with increase in the activity of lipoxygenase that results in products involved in transmission of intracellular signal [1, 4]. Numerous lipid messengers involved in signal mechanisms are found in nuclei of mammalian cells [5]; therefore, studies on the effect of seizures on lipids of cell nuclei from the brain cortex are urgently needed. Literature data on lipid composition of cell nuclei of the brain are scarce [6, 7]. Therefore, it was interesting to study the lipid composition of homogenate and of neuronal and glial nuclei of the neocortex under normal conditions and on injection of picrotoxin, an inhibitor of GABA-A receptors, in the dose inducing clonic seizures in rats.

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MATERIALS AND METHODS

Male Wistar rats with body weight of 180-200 g were used. The experimental animals were injected with picrotoxin (Sigma, USA) in isotonic solution of NaCl in the seizure-inducing dose of 4 mg/kg. Control animal were injected with isotonic solution of NaCl. After termination of seizures (20-30 min after the injection of picrotoxin) the animals were decapitated, and the brain was rapidly taken out, washed in ice-cold isotonic NaCl, and placed in cold 0.32 M sucrose containing 1 mM $MgCl_2$. All procedures were performed at 0-4°C. For each determination, the neocortex of six rats was combined.

Neuronal and glial nuclei were isolated as described in [8] with modifications according to [6]. The cortex was rapidly minced with scissors and homogenized in a glass homogenizer with a Teflon pestle in four volumes (w/v) of 2 M sucrose supplemented with 1 mM $MgCl_2$. The homogenate was diluted with the same volume of the homogenization medium and filtered through kapron tissue. A portion of the homogenate was taken for determination of lipids, protein, and DNA. The remaining portion was used for isolation of nuclei. The homogenate was centrifuged with a Centricon T-2070 centrifuge at 64,000*g* for 30 min. After centrifugation, the supernatant

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fluid was discarded, and walls of the tubes were carefully wiped to prevent contamination of nuclei with cell debris. The precipitated nuclei were resuspended in 4 ml of 2.4 M sucrose supplemented with 1 mM $MgCl_2$, and the suspension of nuclei was placed into centrifuge tubes, with 1.5 ml of 1.8 M sucrose supplemented with 1 mM $MgCl_2$ carefully layered above, and centrifuged at 85,000*g* for 30 min. After the centrifugation, glial nuclei were in the precipitate and the interphase contained the zone corresponding to neuronal nuclei. The upper layer of 1.8 M sucrose was carefully drawn off, the neuronal nuclei in 2.4 M sucrose were poured into another tube, diluted in two volumes of 0.32 M sucrose, and centrifuged at 2000*g* for 3 min.

The neuronal nuclei fraction was additionally washed with 1 M sucrose supplemented with 1 mM $MgCl_2$ and collected by centrifugation at 2000*g* for 3 min. The precipitates of neuronal and glial nuclei were resuspended in 0.32 M sucrose with 1 mM $MgCl_2$, and aliquots were taken for electron microscopy and determination of DNA and protein. The remaining suspension was used to determine lipid contents. Methods used for extraction, chromatography, and determination of lipid contents were described earlier [9]. The protein was determined by the method of Lowry, and the concentration of DNA was determined spectrophotometrically. Purity of the preparations of nuclei was monitored by electron microscopy. Results were processed using Student's *t*-test.

RESULTS

Seizures induced by injection of picrotoxin significantly increased contents of lysophosphatidylcholine and sphingomyelin in the rat brain cortex homogenate. Increase in the content of total phospholipids was significant but less (Table 1), whereas the content of free fatty acids did not change.

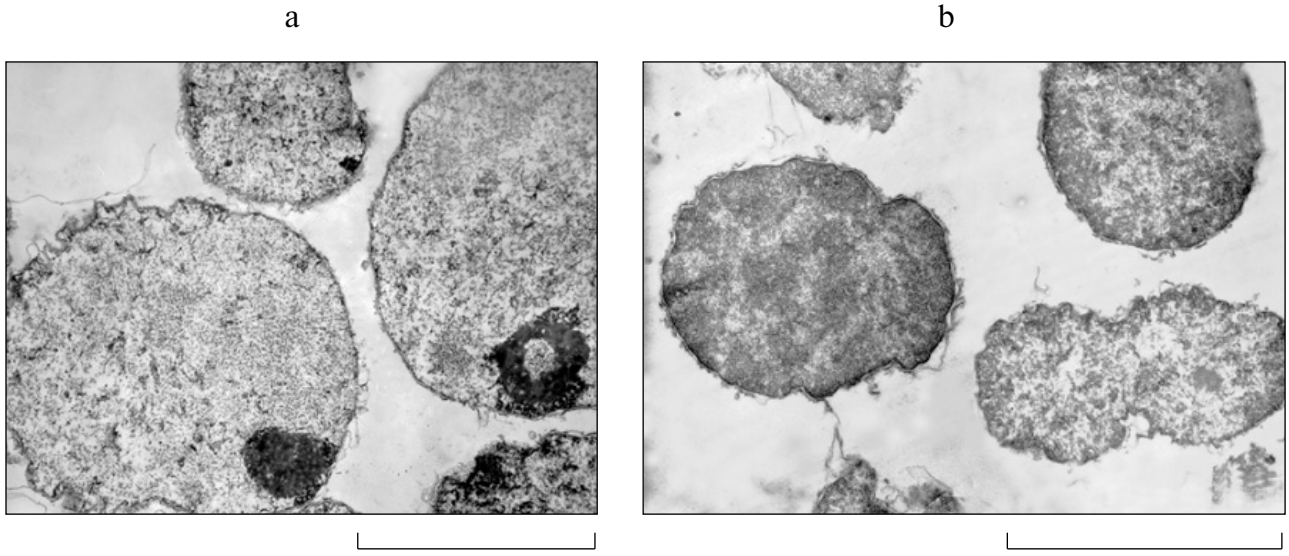
The above-described procedure used for isolation of neuronal and glial nuclei resulted in 45-50% yield of total nuclei (by DNA) from DNA of the homogenate. The ratio of DNA/protein in the neuronal nuclei was 1 : 4.6, and in the glial nuclei it was 1 : 5.5. Electron microscopy indicated a high purification degree of the nuclear preparations (figure, a and b). The high degree of purification of our preparations was additionally characterized by the ratio of phosphatidylserine and phosphatidylinositol in them: the amount of phosphatidylinositol was twofold higher than the amount of phosphatidylserine (Table 2). These findings are consistent with data on the ratio of phosphatidylinositol and phosphatidylserine in neuronal and glial nuclei of rabbit brain cortex, whereas the ratio of these phospholipids in the microsomal fraction is approximately the same [6, 7]. The content of phosphatidylserine is significantly higher than the content of phosphatidylinositol in the synaptosomal fraction [10], presynaptic membranes [11], in myelin [12], and in homogenate of the rat brain cortex (Table 1). In neurons and glia of the rat brain the content of phosphatidylserine is higher (although not so significantly) than the content of phosphatidylinositol [13]. Thus, the twofold excess phosphatidylinositol compared to phosphatidylserine in the neuronal nuclei proves that the preparations of nuclei are not contaminated with pieces of membranes of other organelles and cells.

Seizures significantly decreased contents of sphingomyelin, phosphatidylcholine, phosphatidylserine, and total phospholipids in lipids of neuronal nuclei. But the

Table 1. Contents of lipids (μg lipid per mg protein) in rat brain cortex homogenate under the influence of picrotoxin ($n = 4$)

Fraction studied	Control	Experiment	Experiment (% of control on pair comparison)
Free fatty acids	169.6 ± 14.2	156.4 ± 17.5	92 ± 10
Cholesterol	137.0 ± 10.7	149.1 ± 6.0	109 ± 4
Total phospholipids	350.9 ± 20.0	394.6 ± 23.0	112 ± 3*
Lysophosphatidylcholine	14.5 ± 2.5	21.8 ± 2.2	156 ± 16*
Sphingomyelin	19.0 ± 2.1	34.7 ± 4.9	183 ± 18*
Phosphatidylcholine	132.5 ± 11.2	128.7 ± 25.3	98 ± 6
Phosphatidylserine	40.7 ± 1.8	46.8 ± 2.9	115 ± 6
Phosphatidylinositol	15.1 ± 2.5	14.1 ± 2.5	96 ± 7
Cardiolipin	17.3 ± 1.0	16.5 ± 1.6	95 ± 6
Phosphatidylethanolamine	111.8 ± 7.3	132.0 ± 13.6	118 ± 7

* The difference between the control and experiment is significant, $p < 0.05$.



Electron microscopy of nuclei isolated from neurons (a) and glial cells (b) of rat neocortex. The scale is 5 μm

contents of free fatty acids and lysophosphatidylcholine increased under the influence of the convulsant (Table 2).

The number of glial nuclei isolated by the procedure used was significantly lower than the number of neuronal nuclei (the protein ratio in the fractions of neuronal and glial nuclei was (1 : 4)-(1 : 5)). Under normal conditions, in the fraction of glial nuclei the levels of cholesterol and total phospholipids calculated per mg protein of the fraction were virtually the same as in the neuronal nuclei. The content of free fatty acids in the glial nuclei was nearly twofold higher than in the neuronal nuclei (Tables 2 and 3). Picrotoxin decreased the content of total phospholipids and increased the content of free fatty acids in the glial nuclei (Table 3).

DISCUSSION

Supposing that seizures should stimulate normal signal mechanisms including lipid messengers [1], a single seizure incident can be used as a model system to study changes in lipid metabolism caused by hyperactivation of cells. Changes in lipid metabolism initiated by a convulsant may be also considered as the brain response to dam-

Table 2. Contents of lipids (μg lipid per mg protein) in neuronal nuclei of rat brain cortex under the influence of picrotoxin ($n = 4$)

Fraction studied	Control	Experiment	Experiment (% of control on pair comparison)
Free fatty acids	163.0 ± 21.0	216.4 ± 9.2	130.0 ± 7.0*
Cholesterol	63.3 ± 19.4	59.7 ± 15.3	102.9 ± 14.3
Total phospholipids	71.3 ± 2.5	52.9 ± 33.0	73.9 ± 8.0*
Lysophosphatidylcholine	1.8 ± 0.4	2.3 ± 0.33	127 ± 8.4*
Sphingomyelin	3.7 ± 0.35	2.9 ± 0.35	71.1 ± 8.1*
Phosphatidylcholine	33.0 ± 3.1	26.6 ± 3.0	79.1 ± 4.3**
Phosphatidylserine	3.5 ± 0.5	2.5 ± 0.3	64.1 ± 7.7**
Phosphatidylinositol	6.7 ± 0.5	5.8 ± 0.4	86.2 ± 4.9
Cardiolipin	2.8 ± 0.2	2.8 ± 0.6	99.0 ± 16.5
Phosphatidylethanolamine	11.2 ± 0.8	9.3 ± 1.5	82.5 ± 10.0

* The difference between the control and experiment is significant, $p < 0.05$.
** The difference between the control and experiment is significant, $p < 0.02$.

Table 3. Contents of lipids (μg lipid per mg protein) in glial nuclei of rat brain cortex under the influence of picrotoxin ($n = 3$)

Fraction studied	Control	Experiment	Experiment (% of control on pair comparison)
Total phospholipids	67.8 ± 5.0	47.2 ± 2.4	69.8 ± 0.8**
Free fatty acids	310.5 ± 21.0	364.6 ± 43.3	118.6 ± 3.8*
Cholesterol	58.9 ± 14.7	56.8 ± 12.0	88.9 ± 6.0

* The difference between the control and experiment is significant, $p < 0.05$.
** The difference between the control and experiment is significant, $p < 0.001$.

age, which also includes lipid-involving compensatory processes.

Increase in the level of sphingomyelin in the homogenate (Table 1) under the influence of picrotoxin seems to be specific for seizures. This is also confirmed by data on increase in the level of sphingomyelin at the peak of seizures [14], whereas pentobarbital, which has an anticonvulsant activity, decreased the content of sphingomyelin in homogenate of neocortex [15]. Unlike single seizures, repeated seizures are known to produce apoptotic-like death of neurons [16, 17]. Moreover, products of sphingomyelin hydrolysis are shown to induce apoptosis [18]. Increase in the level of sphingomyelin in the homogenate in our experiments seems to be due to inhibition of sphingomyelinases and is likely to be a compensatory mechanism directed to prevent cell death. It seems also that increase in the level of sphingomyelin in the cortex homogenate changes the sphingomyelin/cholesterol ratio in the cells and influences functioning of special microdomains (rafts) on the surface of cell membranes involved in signal transduction [19]. Increase in the level of lysophosphatidylcholine in the homogenate, similarly to that observed at the peak of seizures [14], may be explained by activation of phospholipase A_2 in the course of depolarization of neurons [20, 21]. However, the increase in the lysophosphatidylcholine content in the homogenate was not accompanied by decrease in the content of phosphatidylcholine and increase in the level of free fatty acids. Moreover, the content of total phospholipids increased. Therefore, the increase in the content of lysophosphatidylcholine was due to either activation of its *de novo* synthesis in certain cellular compartments or its increased utilization by the cortex cells from the blood. Such changes can occur because this lipid is a precursor of the platelet activation factor, which is one of main transmitters of the signal transduction in the nervous system [3].

During seizures, in the neuronal nuclei the level of total phospholipids, in particular, of phosphatidylcholine, decreased, and this was associated with increase in the content of free fatty acids (Table 2). Concurrently, the content of lysophosphatidylcholine, a precursor of the platelet activation factor, increased. The platelet activation factor can induce expression of genes of the early response [2, 3], similarly to that observed during seizures [22]. Receptors of the platelet activation factor have been found on the nuclear membrane [23], and its possible synthesis from lysophosphatidylcholine in neuronal nuclei has been also shown [24]. But, considering the increase in lysophosphatidylcholine content in the neocortex homogenate during seizures, its transport from the homogenate into the nucleus after hyperactivation of the cells seems also possible. In this case, the decrease in the phosphatidylcholine level in the nuclei can be caused not by phospholipase A_2 but its hydrolysis by phospholipase C resulting in diacylglycerides which act as lipid messengers. Hydrolysis of phosphatidylcholine and phosphatidylinositol results in diacylglycerides with different composition of fatty acids involved in signal mechanisms responsible for various cell responses [25, 26]. In response to different stimuli, activation of the polyphosphoinositol cycle in nuclei of mammalian cells is recorded for 2-4 min. And this is associated with noticeable changes in incorporation of the labeled precursor into phosphatidylinositolmono- and phosphatidylinositoldiphosphate, whereas its incorporation into phosphatidylinositol changes insignificantly [27]. This is likely to be associated with a significant decrease in the content of phosphatidylinositol within the period of our study. The sphingomyelin cycle is activated later [28, 29]. It has been shown in model experiments that modification of the lipid bilayer due to changes in the ratio of phosphatidylcholine, phosphatidylserine, and diacylglycerides regulates the activity of protein kinase C [30-32]. Such a regulation seems also to occur in the neocortex under the influence of picrotoxin, because it is associated with phosphorylation of synaptic proteins [33]. It is not improbable, that the modification of membranes of neuronal nuclei in our experiments caused by decrease in the content of phosphatidylcholine is also associated with activation of nuclear protein kinases.

Similar changes in neuronal and glial nuclei, such as decrease in the content of total phospholipids and

increase in the content of free fatty acids, suggest the presence of similar processes in response to convulsant. The observed changes are caused by involvement of lipid transmitters in signal mechanisms and seem to be associated with universal responses of mammalian cells to various damaging agents, because similar changes in lipids, such as increase in content of free fatty acids and decrease in content of phospholipids, have been found in nuclei of rat thymocytes under the influence of ionizing radiation [34].

Our findings suggest complicated rearrangements in lipids of the neocortex after transient seizures. Changes in lipids of neuronal and glial nuclei of the brain cortex cells are especially important, because they suggest that phospholipids of nuclear membranes, along with other cellular membranes [3], should be a depot of signal molecules for transmission of information into the genetic apparatus of the cells.

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