Effect of Picrotoxin-Induced Seizures on Lipid Composition of Cortical Tissue Homogenate and Its Nuclear Fraction in Rats

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The content of lipids in rat cortical tissue homogenate and fraction of neuronal nuclei was determined under normal conditions and after picrotoxin-induced seizures. Changes in lipid composition of homogenate and nuclear fraction differed considerably. In homogenate, the content of sphingomyelin, lysophosphatidylcholine, and total phospholipids increased, while the content of free fatty acid remained unchanged; in the nuclear fraction the total phospholipid content decreased, while the concentration of free fatty acids increased.

Key Words: *picrotoxin; neocortex; neuronal nuclei; phospholipids; free fatty acids*

Changes in brain lipid metabolism during seizures are of special interest because lipids play an important role in the mechanisms of intracellular signaling. It is known that epileptiform discharges can stimulate the expression of immediate early genes [14], which correlates with activation of polyphosphoinositol and sphingomyelin (SPM) cycles [8]. The content of diacylglycerol and free fatty acids (FFA), products of phospholipids (PL) degradation, also increased during seizure activity [10]. Accumulation of FFA (in particular, arachidonic acid) in synaptic terminals during seizures correlates with increased lipoxygenase activity involved in intracellular signaling [10].

Despite considerable interest to this problem, there are only limited data on changes in brain lipids caused by convulsants and practically no relevant data for neuronal nuclei. In this study we investigated the effects of seizure activity on lipid composition of cortical tissue homogenate and its nuclear fraction in rats.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 180-200 g. For each measurement cortex samples from 6 animals were pooled. The rats received intraperitoneal picrotoxin (Sigma) in a convulsant dose of 4 mg/kg or isotonic NaCl (controls). The experimental rats were decapitated 20-30 min after convulsions, the brain was rapidly removed, washed with cold isotonic NaCl, and the neocortex was homogenized in a cold medium. The homogenate was used for isolation of the nuclear fraction [15] and for lipid assay. Extraction, chromatography, and identification of neutral lipids and PL were performed as described elsewhere [4]. A calibration curve for measuring the FFA content was constructed using arachidonic acid as a standard. The content of protein in homogenates determined by the method of Lowry was 86.1±5.5 and 89.3±2.6 mg/g tissue in the control and experimental groups, respectively. The data were analyzed statistically using Student's paired *t* test.

RESULTS

Picrotoxin considerably changed the lipid composition of cortical homogenates (Table 1). The content of SPM in the neocortex dramatically increased, probably due to inhibition of sphingomyelinase. It can be assumed, that seizure activity is accompanied by activation of the SPM cycle. This assumption is supported by the high correlation between the level of SPM and sphin-

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gomyelinase activity in rat hippocampus under the influence of tumor necrosis factor-α [6]. The decrease in neocortical SPM caused by anticonvulsant pentobarbital [1] suggests that accumulation of SPM is specific for seizure activity. It was also shown that picrotoxin-induced cortical seizures were accompanied by the increase in the total SPM+phosphatidylinositol fraction [5]. Metabolic pathways of SPM and cholesterol are known to be interconnected [12]. In our experiments, picrotoxin induced no changes in the neocortical content of cholesterol, which agree with previous findings in rabbit neocortex [7]. It was hypothesized that there are several cholesterol pools with different rates of turnover in the brain, which ensures constant level of cholesterol [13]. Oscillations in the cerebral content of cholesterol were observed in rats receiving tumor necrosis factor-α [6]. These findings suggest that picrotoxin exerts selective effects on some lipids in rat neocortex.

Apart from elevation of SPM content, picrotoxin increased the level of lysophosphatidylcholine in cortical homogenates, which agrees with published data [5]. This increase can be explained by activation of phospholipase A_2 caused by neuronal depolarization [1]. PL hydrolysis by this phospholipase leads to accumulation of fatty acids and lysoPL (mainly, lysophosphatidylcholine) and a decrease of PL content. However, our study revealed no FFA accumulation (PL degradation products), therefore, it is most likely that the increased content of lysophosphatidylcholine results from its *de novo* synthesis as PL intermediate. Activation of *de novo* synthesis is confirmed by elevation of the total PL concentration during seizure activity. On the other hand, the accumulation of PL and lysophosphatidylcholine could be explained by inhibition of phospholipase C hydrolyzing these lipids. However, phospholipase C was shown to be activated rather than inhibited during seizure activity [11]. Constant content of phosphatidylcholine also attests to *de novo* synthesis of lypophosphatidylcholine. Thus, changes in lipid components of cortical homogenates reflect both hyperactivation-induced damage and compensatory processes preserving structural integrity of the neocortex. They should be considered as an integral index of alterations of the lipid composition of different cellular and subcellular structures.

In the nuclear fraction, changes in total PL content were opposite to those in cortical homogenates. In the control group, the total PL content was 71.3±2.5 μg/mg protein, which is comparable with values reported for neuronal nuclei from rabbit neocortex [9]. Seizure activity decreased the content of PL to 52.9±3.3 μg/mg protein (73.9±8.0% of the control, $p<0.05$) and increased the content of FFA to 216.4±9.2 protein *vs.* 163±21 μg/mg protein in the control (130±7%, $p<0.05$). The observed changes in the content of nuclear lipids can be explained by activation of phospholipase A_2. These findings are in line with the concept of "lipid effect" on neuronal membranes caused by seizure activity. It is suggested that seizure activity stimulates intracellular signal transduction systems via lipid messengers, and this stimulation is responsible for "lipid effect" [10]. Our previous experiments demonstrated the effects of fractionated γ-irradiation on lipids in thymocyte nuclei [3,4]. One hour after irradiation we observed a sharp increase in the content of FFA and a decrease in the level of phosphatidylcholine and phosphatidylethanolamines, the most abundant lipids of nuclear membranes. These effects were not observed in thymus homogenates. Thus, radiation stimulating lymphocyte apoptosis and convulsant picrotoxin cause similar changes in the nuclear lipids in different cells (thymocytes and neurons), which suggests that intracellular signaling systems in various cells have some common mechanisms.

TABLE 1. Effect of Seizure Activity on Lipid Composition of Homogenates of Rat Neocortex ($M\pm m$, n=4-5)

Components	Control	Experiment	Percent of control
Lysophosphatidylcholine	14.5±2.5	21.8±2.2**	156±16
Phosphatidylcholine	132.5±11.2	128.7±25.3	98±6
SPM	19.0±2.1	34.7±4.9*	183±18
Phosphatidylserine	40.7±1.8	46.8±2.9	115±6
Phosphatidylinositol	15.1±2.5	14.1±2.5	96±17
Phosphatidylethanolamine	111.8±7.3	132.0±13.6	118±7
Cardiolipin	17.3±1.0	16.5±1.6	95±6
Total phospholipids	348±20	388±23**	112±3
Cholesterol	137.0±10.7	149.1±6.0	109±4
Free fatty acids	169.6±14.2	156.4±17.5	92±10

Note. *$p<0.02$, **$p<0.05$ in comparison with the control (paired t test).

Specific changes in the lipid composition of neuronal nuclei revealed in our study indicate that even single seizure episode can activate the lipid-mediated mechanisms of intracellular signaling.

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