Lipid Composition of Rat Somatic Nerves under the Effect of Damaging Factors

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Ligation of the sciatic nerve in rats led to reversible decrease in excitability and changes in lipid composition of myelinated nerve fiber: increase in the content of lysophosphatidylcholine, lysophosphatidylethanolamine, and free fatty acids and phospholipase A_2 activity. Xymedon and laminin accelerate nerve regeneration and restore its lipid composition.

Key Words: *myelinated nerve fiber, phospholipids; free fatty acids; xymedon; laminin*

Transmission of rhythmic excitation is the main function of peripheral nerves. This function depends on transmembrane potential and peculiarities of plasma membrane proteins and lipids. Products of lipid metabolism, such as 1,2-diacylglycerol, lysolipids, free fatty acids (FA), regulate activities of phospholipase A_2, protein kinase C, function of ionic channels, and transmembrane potential during rhythmic excitation [1]. Degeneration of the crossed nerve is accompanied by changes in its phospholipid and FA composition and synthesis of phospholipases [5,7]. However, qualitative and quantitative changes in lipid composition of the nerve during reparation and recovery of rhythmic excitation are little studied. Reparative processes in damaged nerves usually take from few weeks to several months. These processes can be accelerated by some stimulators, but the mechanism of their action remains poorly understood.

Here we studied changes in lipid composition of somatic myelin nerves after mechanical trauma and during stimulated reparative process.

MATERIALS AND METHODS

Experiments were carried out on 123 Wistar rats weighing 200-250 g maintained on a standard ration. Mechanical trauma was modeled by ligation of the sciatic nerve. After surgery the animals were divided into 3 groups: group 1 rats (n=58) daily received ximedon (30 mg/kg intraperitoneally), group 2 rats (n=7) received 0.25 mg/kg laminin on days 3 and 6 after surgery, and group 3 rats were controls (n=58). The animals of groups 1 and 3 were sacrificed on days 1 (n=14), 5 (n=28), 10 (n=32), 15 (n=28), and 20 (n=14) after nerve ligation; group 2 rats were sacrificed on day 10 (n=7). Functional activity of the nerve was evaluated by its ability to transmit rhythmic excitation. Action potential (AP) of the nerve was recorded in a temperature-controlled chamber using a S1-68 oscillograph; the nerve was stimulated with rectangular electrical pulses generated by an ESL-1 generator.

Lipid fractions were separated by microthin layer chromatography, the number of phospholipids fractions was determined. FA composition was analyzed on a Kristall 5000.1 gas chromatograph with a HP-FFAP 50m capillary column (0.32 mm, 0.5 μ). Phospholipase A_2 activity in nerve homogenate was measured by accumulation of free FA after phosphatidylcholine cleavage by the method of gas chromatography; protein content was measured by the method of Lowry.

The experimental data were processed statistically.

RESULTS

Mechanical trauma led to blockade of rhythmic excitation. On day 20 after damage we observed

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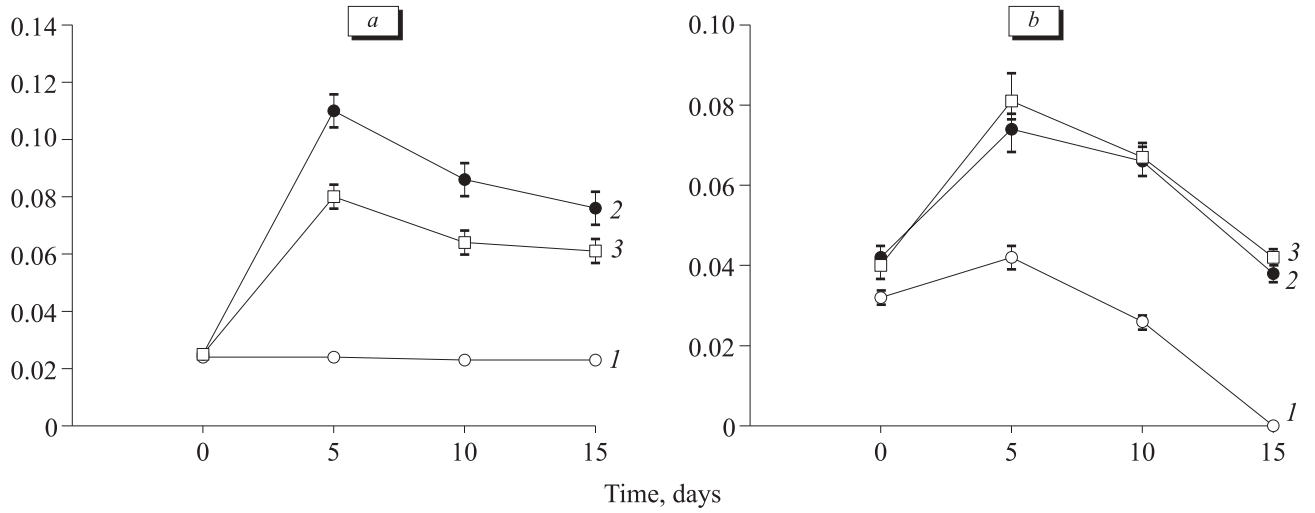

Fig. 1. Dynamics of LPC (*a*) and LPE (*b*) content (μg) in nerve after mechanical trauma. *1*) control, *2*) trauma, *3*) trauma+xymedon. Here and on Fig. 2: ordinate: LPC phosphorus to lipid phosphorus ratio.

considerable changes in lipid composition of the nerve, which depended to the amplitude of damage and duration of the posttraumatic period.

In intact nerves the content of lysophosphatidylcholine (LPC) did not exceed 1% of total phospholipids, and only trace amounts of lysophosphatidylethanolamine (LPE) were detected. The content of LPC and LPE in the nerve increased after trauma (Fig. 1), but decreased by the 15th day. Increasing the duration of nerve ligation promoted accumulation of both LPC and LPE. The increase in the percentage of LPC and LPE in the nerve after damage attests to the involvement of these lysolipids in the regeneration process.

FA composition of lipids in the nerve changed after trauma. The percent of long-chain FA increased in all analyzed fractions. The content of free FA gradually increased and peaked on day 10, but then decreased. The percent of unsaturated FA first increased, but on day 15 practically did not differ from the control.

Thus, the content of free FA correlated with changes in LPC and LPE content in the nerve after trauma. Accumulation of these lipids in damaged nerve probably resulted from activation of phospholipase A_2, because this enzyme catalyses hydrolysis of phospholipids primarily in sn-2 position typical of unsaturated FA [9].

Thus, recovery of conduction of rhythmic excitation is preceded by normalization of the lipid composition of the nerve.

In our experiments pyrimidine derivatives xymedon and laminin were used for acceleration of reparation processes.

Administration of xymedon to experimental animals promoted recovery of rhythmic excitation by the 15th day after trauma. The content of LPC in the damaged nerve was lower throughout the experiment (Fig. 1, *a*), the content of LPE remained unchanged (Fig. 1, *b*), while the content of unsaturated free FA decreased. Thus, xymedon promoted reparative processes, thus reducing the duration of degenerative processes in the damaged nerve.

Xymedon modulates the cell system of active transport regulation, LPO intensity, and activity of the antioxidant system [2]. The effect of this drug on the damaged nerve can be explained by inhibition of lipolytic enzymes, in particular, phosphatidyl-specific phpspholipase A_2, which is accompanied by a decrease in the content of LPC and unsaturated FA in the damaged nerve.

Another promising drug stimulating tissue reparation laminin belongs to the family of highly active extracellular matrix glycoproteins triggering the mechanisms of cell growth and differentiation,

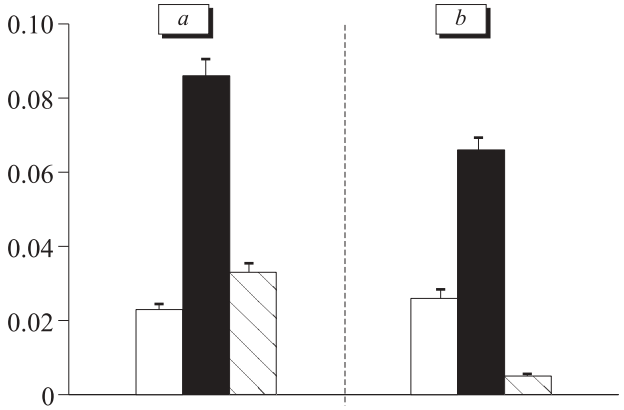

Fig. 2. Effect of laminin on LPC (*a*) and LPE (*b*) content in damaged nerve. Open bars: control; dark bars: trauma; hatched bars: trauma+laminin.

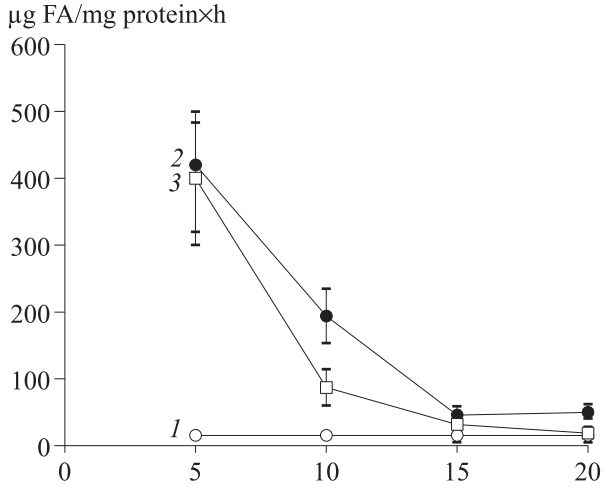

Fig. 3. Effect of xymedon on phospholipase A_2 activity in damaged nerve. *1*) control, *2*) trauma, *3*) trauma+xymedon.

neuronal growth, and nerve regeneration [8]. Laminin treatment decreased the content of LPC and LPE in the damaged nerve by 62 and 92%, respectively (Fig. 2).

Phospholipase activity of nerve homogenate was evaluated in the dynamics of degeneration and regeneration processes in the damaged nerve. This parameter peaked on day 5 after damage. On day 10 phospholipase activity sharply decreased and on days 15-20 approached the control level (Fig. 3). In rats treated with xymedon phospholipase activity was lower. On day 10 after trauma this parameter was 2-fold lower than in damaged nerve of control animals. On day 20 phospholipase activity returned to the control level.

Thus, mechanical trauma blocked rhythmic excitation, which was accompanied by activation of phospholipase A_2, accumulation of lysophospholipids and free FA, and increase in the relative content of unsaturated FA. Treatment with regeneration stimulators xymedon and laminin accelerated recovery of nerve excitability and lipid composition.

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