Effect of Stimulation of Nerve Regeneration on Posttraumatic Neuronal Survival in Dorsal Root Ganglia

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The survival of neurons is a key condition for complete posttraumatic regeneration of the peripheral nerve. In experiments on rats we studied survival capacity of different neuronal subpopulations in L_{IV}-L_V dorsal root ganglia after ligation or transection and suturing of the sciatic nerve. Experiments with nerve ligation showed that IB_4^+ neurons are more sensitive to the injury than $NF200^+$ neurons. By day 90 after ligation of the sciatic nerve IB_4^+ neurons were virtually not detected in the dorsal root ganglia. By day 90 after nerve transection the number of surviving $NF200^+$ and IB_4^+ neurons decreased by 26.1 and 21.4%, respectively, in comparison with intact animals. Treatment with xymedon, a regeneration stimulator, led to a 48.5% increase in the number of surviving $NF200^+$ neurons by day 30 after ligation of the nerve and a 50.7% increase by day 90. The number of surviving IB_4^+ neurons increased more than 8-fold by this term after ligation of the nerve and drug stimulation. Xymedon had a neuroprotective effect towards both neuron subpopulations, more intensely preventing apoptosis of IB_4^+ neurons.

Key Words: *sensory neuron; apoptosis; IB_4^+; NF200*

Three populations of neurons are distinguished in the dorsal root ganglia by the morphofunctional criteria: small, medium, and large, in which different subpopulations are distinguished on the basis of their phenotypical characteristics. Small neurons are mainly nociceptive neurons and include 2 subpopulations: neurons binding *Bandeiraea simplicifolia* B_4 isolectine (IB_4) [12], expressing glial neurotrophic factor (GDNF) receptors [9] and primarily resistant to neurotrophins [7]; and neurons not interacting with IB_4 and sensitive to nerve growth factor [9]. High-molecular component of neurofilament triplet NF200 serves as the marker of neurons forming A-fibers (populations of large proprioceptive and medium tactile neurons) [8].

Signals regulating, for example, the production of neurofilament triplet proteins and tubulines enter the dorsal root ganglion neuron perikaryon retrogradely along the growing axon [6]. The impact of the axon elongation factor on the survival of its own neurons was not investigated. We evaluated the survival capacity of neurons of some populations using two experimental models: under conditions precluding axon regeneration and restoration of specific nerve bonds (nerve ligation) and under conditions allowing axon elongation (transection and suturing of the nerve).

We previously showed that pyrimidine derivative xymedon maintains posttraumatic survival of rat sensory neurons [2]. However it remained unclear, whether neurons of different subpopulations differ by sensitivity to neuroprotectors and whether this sensitivity depends on the neuronal process elongation factor. We compared the effects of pharmacological stimulator of regeneration on the number of surviving $NF200^+$ and IB_4^+ neuron subpopulations under conditions of ligation or transection/suturing of nerves.

MATERIALS AND METHODS

Experiments were carried out on 60 male albino rats (120-200 g). The left sciatic nerve was ligated (n=32) or cut and sutured (n=16) at the level of the middle of the thigh under aseptic conditions under ketamine nar-

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cosis (150 mg/kg intraperitoneally) [1,2]. Starting from the next day after surgery and to sacrifice 16 rats with ligated nerve and 8 animals subjected to the nerve transection and suturing received regeneration stimulator xymedon (Institute of Organic Chemistry, Kazan Branch of the Russian Academy of Sciences) in a single daily dose of 30 mg/kg [1]. Other operated animals of both series did not treated with xymedon served as controls.

Dorsal root ganglia at the L_{IV}-L_v level on the left side were collected after laminectomy under ketamine narcosis on days 30 and 90 after the nerve ligation (16 animals per term), on day 90 after the nerve transection and suturing (16 rats), and in 12 intact animals. The material was fixed in 10% neutral formalin, dehydrated, and embedded in paraffin. The subpopulation of IB_4^+ neurons was isolated by immunocytochemical method in every 5th serial section (7 μ) of dorsal root ganglia. The sections were incubated in IB_4 solution (2 μg/ml) conjugated with horseradish peroxidase (Sigma). $NF200^+$ neurons were detected in the same material in serial sections using monoclonal antibodies to neurofilament triplet protein with a molecular weight of 200 kDa (Sigma, 1:100 dilution). NF200- and IB_4-positive neurons with visible nucleoli [5] were counted in L_{IV}-L_V dorsal root ganglia on the left side in intact animals and after ligation and transection of the nerve in control and experimental animals.

The results were statistically processed using Student's *t* test.

RESULTS

The number of IB_4^+ neurons in L_{IV}-L_V dorsal root ganglia of intact animals was 23.6%, that of $NF200^+$ neurons 15%. IB_4^+ neurons belong to the population of small neurons. Some neurons with large and medium-sized perikaryons express NF200.

After nerve ligation the number of survived IB_4^+ neurons in L_{IV}-L_v dorsal root ganglia markedly decreased, and by day 90 the neurons with this phenotype were virtually not detected (Fig. 1, *a*). The number of IB_4^+ neurons survived by day 90 after nerve transection decreased by 21.4% and that of $NF200^+$ neurons by 26.1% in comparison with intact animals ($p<0.05$). Hence, experiments with nerve ligation showed that IB_4^+ neurons were more sensitive to injury than $NF200^+$ neurons. These results are in line with previous data on low resistance of small neurons to traumatic damage [13] of their processes and neurotoxins [11] compared to other populations of sensory neurons. The decrease in the number of surviving IB_4^+ neurons after nerve transection was less pronounced than after ligation, which can be explained by more effective delivery of GDNF produced by Schwann cells into the perikaryon with retrograde axon transport [4]. The axolemma surface area does not constantly increase and hence, the number of receptors for neurotrophic factors produced by Schwann cells in the potential space of axon growth did not increase under conditions of nerve ligation, when elongation of the processes was precluded (in contrast to nerve transection allowing axonal regeneration).

By day 30 after nerve ligation the number of surviving IB_4^+ neurons in the group treated with xymedon more than 8-fold surpassed their level in the control and approached the level in intact animals (Fig. 1, *a*). A similar picture was observed on day 90 after nerve ligation, while after nerve transection there were no significant differences in the number of surviving IB_4^+

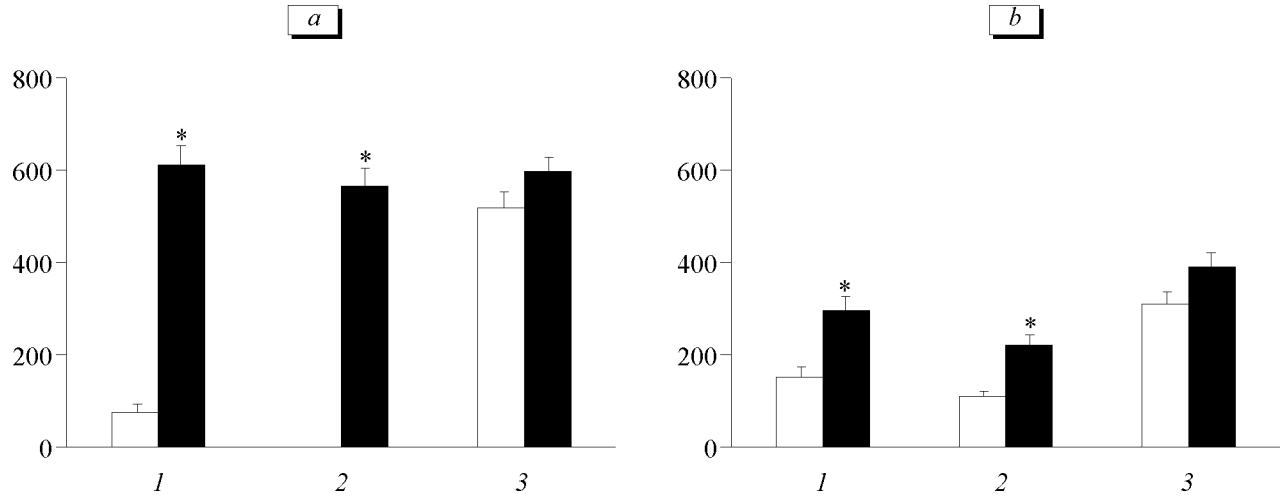

Fig. 1. Posttraumatic survival of IB_4^+ (*a*) and $NF200^+$ (*b*) neurons in L_{IV}-L_V dorsal root ganglia under conditions of drug stimulation of nerve regeneration. *1*, *2*) ligature, days 30 and 90, respectively; *3*) nerve transection, day 90. Ordinate: total number of neurons in L_{IV}-L_V dorsal root ganglia on one side. Dashed line: number of neurons in L_{IV}-L_V dorsal root ganglia in intact animals. Light bars: without xymedon; dark bars: xymedon treatment. *$p<0.05$ compared to the group without xymedon treatment.

neurons in the control and experimental groups by this term (Fig. 1, *a*).

After ligation of the nerve the count of $NF200^+$ neurons decreased in experimental and control groups. By day 30 after nerve ligation the number of surviving $NF200^+$ neurons in experimental group increased by 48.5% ($p<0.05$) in comparison with the control and by day 90 it increased by 50.7% ($p<0.05$; Fig. 1, *b*). By day 90 after nerve transection there were no appreciable differences in the numbers of surviving $NF200^+$ neurons between the control and experimental groups (Fig. 1, *b*).

Hence, treatment with nerve regeneration stimulator xymedon decreases the probability of posttraumatic apoptosis for neurons of both studied populations only after the nerve ligation. Since ligation of the nerve can involve a decrease of neurotrophic factors transport into the perikaryon, the neuroprotective effect of xymedon can be realized due to stimulation of neurotrophic factors production by cells surrounding the neuronal perikaryons. This hypothesis is in line with the data on the possibility of GDNF synthesis in satellite cells [4].

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