Effect of Xymedone on Posttraumatic Survival of Sensory Neurons

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The effect of pyrimidine derivative xymedone promoting survival of sensory neurons and stimulating Schwann cell proliferation on regeneration of myelinated nerve fibers was studied. In rats treated with xymedone, the number of neurons in ipsilateral L4-L5 spinal ganglia on days 60 and 90 after transection of the sciatic nerve increased by 22 and 26%, myelinated fibers by 21.3 and 14.7%, and Schwann cells by 35.7 and 44.1%, respectively.

Key Words: *peripheral nerve; sensory neuron; regeneration; xymedone*

The search for new drugs stimulating regeneration of peripheral nerve is a key problem of neuropharmacology. Recently considerable attention was focused on neural effects of pyrimidine derivatives. For instance, MS-818 stimulates regeneration of unmyelinated fibers in the early period after nerve trauma in mice, but does not affect the number of myelinated fibers, Schwann cells, and macrophages in injured nerve [10]. It increases thickness of myelin sheath in regenerating sciatic nerve [12] and stimulates regeneration of peripheral nerve and migration of Schwann cells [14]. MS-430 promotes recovery of motor function of compressed sciatic nerve in rats [11]. Neurotropic activity of pyrimidine derivatives MS-818 and MS-430 was observed *in vitro* on cultured human and mouse neuroblastoma cells [4,9]. Simultaneous injection of pyrimidine derivatives uridine monophosphate and cytidine monophosphate after compression of the sciatic nerve improved conduction in sensory fibers and promoted myelination [16]. However, another pyrimidine derivative, isoxanine (N-isopropyl-amino-2-pyrimidine orthophosphate) does not affectsensory recovery after experimental sciatic nerve compression [15].

Efficiency of regeneration depends on a number of factors. The most important of them is the ability of damaged neurons to survive in the posttraumatic period, which depends on the entry of the neurotrophic factors released by the non-neural cells and, in particular Schwann cells, into axon and their retrograde transport to the perikaryon [3]. The objective of the present study was to assess the efficiency of regeneration of myelinated fibers under the effect of pyrimidine derivative xymedone (1,2-dihydro-4,6-dimethyl-N-(β-oxyethyl) pyrimidone-2), a preparation with low toxicity sensory neurons and stimulating of Schwann cell proliferation maintaining [1]

MATERIALS AND METHODS

The study was carried out on 40 male albino rats (body weight 120-200 g). The rats were anesthetized with intraperitoneal ketamine (Calipsol, Gedeon Richter, 150 mg/kg) and the sciatic nerve was cut at the middle part of the thigh under aseptic conditions. Experimental rats (n=20) were daily received xymedone (30 mg/kg, Institute of Organic Chemistry, Kazan Division of Academy of Science) starting from postoperation day 1 to the day when nerve samples were taken. Dosage, volume, and the mode of administration in control and experimental groups were described previously [2].

On postoperation days 60 and 90, 6-mm distal fragments of the sciatic nerve were isolated in 10 rats of each group under ketamine anesthesia. The specimens were fixed in glutaraldehyde and osmium tetroxide and after dehydration embedded in Epon-Araldite. Regenerating myelinated fibers were counted on semithin cross-sections of the sciatic nerve stained with methylene blue [2].

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In addition, 5-mm proximal fragments of the same nerve were isolated, fixed in 10% neutral formalin, dehydrated, and embedded in paraffin. Paraffin sections (4 μ) were used for immunohistochemical detection of protein S100 (Schwann cell marker) by indirect immunoperoxidase method (polyclonal antibodies against S100, Dako). Myelinated fibers and $S100^+$-cells in the sciatic nerve were counted under a Jenaval light microscope (×600) with incorporated ocular grid.

After laminectomy, the spinal ganglia were isolated bilaterally at the L4-L5 level, fixed in 10% neural formalin, and embedded in paraffin. Serial sections (7 μ) were stained with methylene blue. Sensory neurons with visible nucleoli [8] were counted on every fifth section. The ratio of perikaryon number on operated/contralateral side was calculated. The results were statistically analyzed using Student's *t* test.

RESULTS

On postoperation days 60 and 90, the number of sensory neurons in spinal ganglia of the operated side in the experimental group surpassed the control values by 22 and 26%, respectively ($p<0.05$, Fig. 1, *a*). Correspondingly, the numbers of myelinated fibers in the experimental group were higher than in the control by 21.3 and 14.7% ($p<0.05$, Fig. 2), while the number of Schwann cells surpassed the control value by 35.7 and 44.1% ($p<0.05$, Fig. 3). Thus, xymedone applied after nerve transection prevented posttraumatic death of sensory neurons, stimulated regeneration of myelinated fibers, and increased the number of Schwann cells.

On postoperation day 60, the number of myelinated fibers on the operated side increased by 92.2% in comparison with the contralateral side, while the cor-

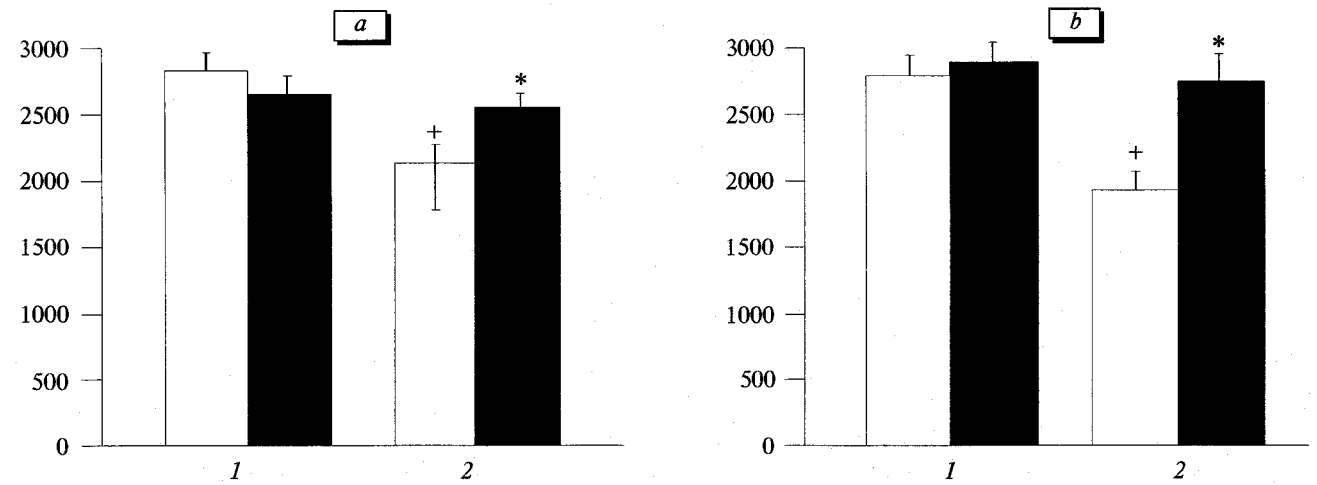

Fig. 1. Effect of xymedone on the number of sensory neurons in L4-L5 spinal ganglia on postoperation days 60 (*a*) and 90 (*b*). Ordinate: total number of sensory neurons in L4-L5 spinal ganglia. Here and in Figs. 2 and 3: *1*) on ipsilateral (operated) side, *2*) on the contralateral side. Light bars: control; dark bars: experiment. $p<0.05$: *compared to the control, **compared to the corresponding indices on the contralateral side.

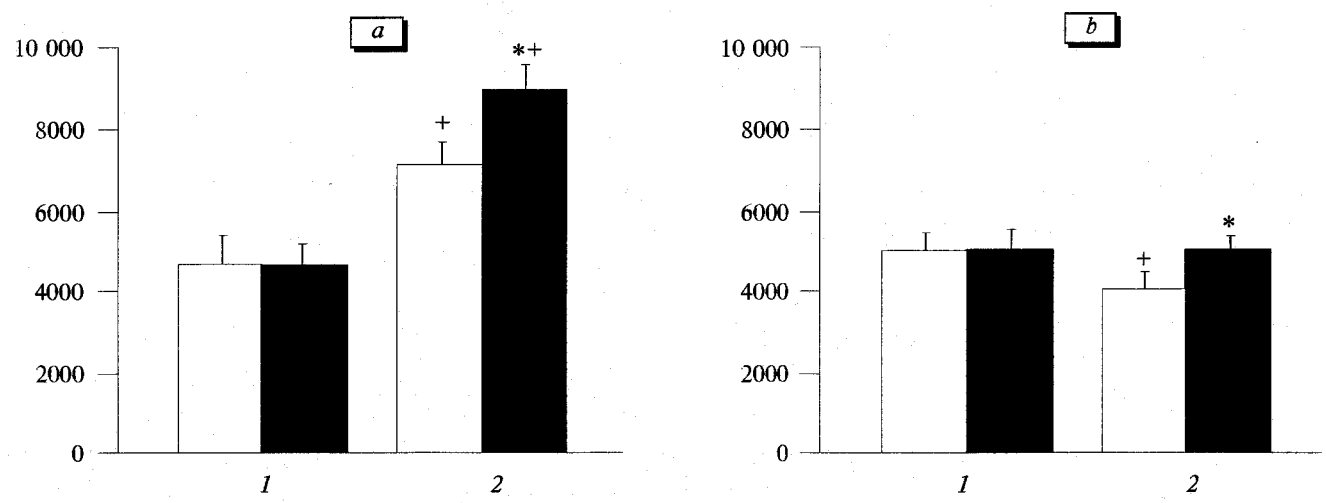

Fig. 2. Effect of xymedone on the number of myelinated fibers in the distal part of sciatic nerve on postoperation days 60 (*a*) and 90 (*b*). Ordinate: number of myelinated fibers in the total cross-section area.

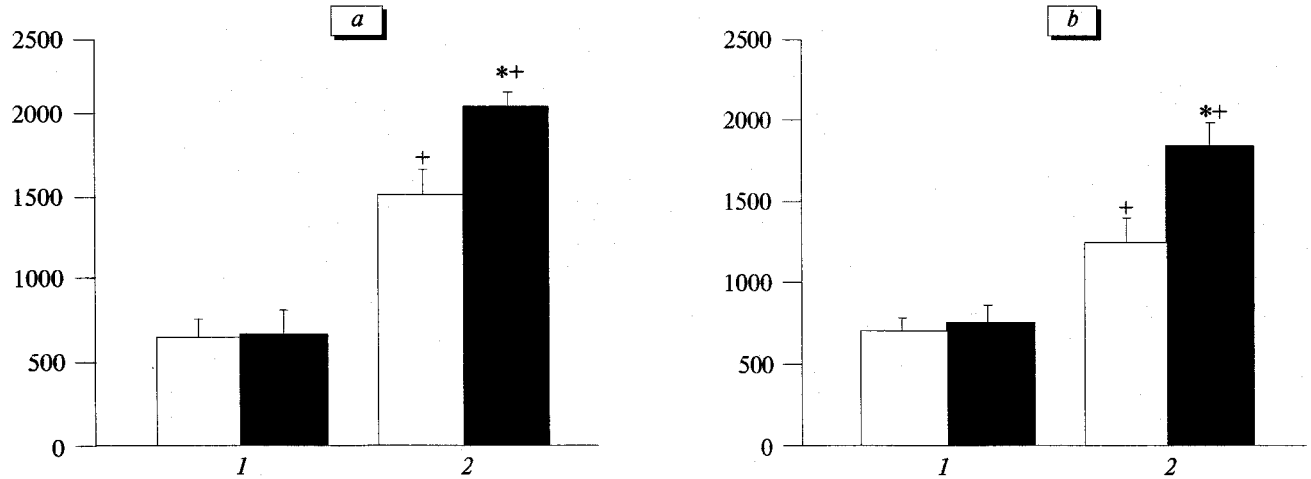

Fig. 3. Effect of xymedone on the number of Schwann cells in the distal part of sciatic nerve on postoperation days 60 (*a*) and 90 (*b*). Ordinate: number of Schwann cells in the total cross-section area.

responding increase in the control group was 53.7%. On postoperation day 90, the corresponding changes were +0.4% (insignificant, $p>0.05$) and -14.3% ($p<0.05$).

The efficiency of regeneration of nerve fibers depends on neuron survival, microenvironment in the potential axonal elongation space, and recovery of specific neural connections with the innervated target organ.

It was found that the axotomized sensory neurons underwent apoptosis [7]. In our experiments, xymedone acted directly on sensory neurons (inhibited apoptosis) or indirectly (via other cell types, for example, Schwann cells). It is suggested that neuronal death depends on insufficient retrograde transport of the neurotrophic factors. After axotomy, the non-neural cells and, in particularly, Schwann cells synthesize several neurotrophic factors [5,13]. We observed an increase in the number of Schwann cells in traumatized nerve under the effect of xymedone. In our experiments, the stimulating effect of xymedone on regeneration of nerve fibers can be explained by enhanced proliferative activity of Schwann cells. This increase in the number of Schwann cells in the traumatic region in the experimental group is probably related to stimulating effect of xymedone on activity of macrophages, which release cytokines and mitogenes for Schwann cells [6]. Therefore, xymedone maintains regeneration of myelinated fibers by stimulating neuron survival and increasing the number of Schwann cells in the potential axonal elongation space.

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