

STRUCTURAL AND FUNCTIONAL CHANGES IN PERIPHERAL NERVES AFTER APPLICATION OF CERTAIN DRUGS

E. I. Chumasov, L. I. Kolosova, A. V. Babin,
K. M. Svetikova, and G. N. Akoev

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The effect of drugs on peripheral nerves is a problem which frequently arises in clinical practice in connection with injection injuries to nerves of the limbs. Several descriptions of clinical observations and studies of the pathogenic features of injuries of this type have been published [2, 3, 6, 8]. Some publications contain experimental data indicating differences in the degree of severity of nerve injury in this type of pathology [5-7].

In the investigation described below, a comprehensive study was undertaken of the morphology and physiological changes taking place as a result of direct application of drugs to peripheral nerves.

EXPERIMENTAL METHOD

The sciatic nerve of male Wistar rats weighing 180-250 g was exposed in the upper third of the thigh under pentobarbital anesthesia (40 mg/kg) under aseptic conditions, and by means of a pipette, 0.1 ml of standard solutions of calcium chloride, pipolphen, and rheopyrine, as used for injection, were applied to its surface drop by drop from a pipette or by application of a gauze pad for 3 min. Physiological saline was used as the control. After application the wound was closed in layers and the site of the wound treated with tincture of iodine and 70° alcohol. The animals were killed with ethyl ether. Material for histologic investigation was taken soon after the operation (1, 3, and 7 days) and fixed in 10% neutral formalin and in Bouin's fluid. Part of the material was cut on a freezing microtome, and part embedded in paraffin wax. Neurohistological methods (impregnation with silver after Bielschowsky and Gros, staining with Sudan Black after Bekker) and general histological (Mayer's hematoxylin and eosin) were used. Parallel with the histologic investigation, a physiological study was carried out. The functional characteristics of the sciatic nerve were studied 3 days after application of the drugs by the method of recording the integral action potential (IAP), derived by means of a special ebonite platform on which silver electrodes were mounted with petrolatum insulation. The platform was fixed proximally to the site of application of the test preparation. Bipolar silver stimulating electrodes were applied distally to the site of application of the pad, at a distance of 20-22 mm from the recording electrodes. The use of a platform to record IAP also enabled the sensitivity of the tissues innervated by the sciatic nerve to be analyzed. For this purpose, spike activity was recorded in microbundles of the sciatic nerve in response to adequate mechanical stimulation of the skin of the foot by means of an electrodynamic stimulator.

EXPERIMENTAL RESULTS

In all cases, 24 h after application of the drugs, inflammatory foci of infiltration of mononuclears and hyperemia of the blood vessels were observed in the surrounding tissues and in the nerve membranes on histological preparations.

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR. A. L. Polenov Leningrad Neurosurgical Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 112, No. 8, pp. 158-160, August, 1991. Original article submitted November 4, 1990.

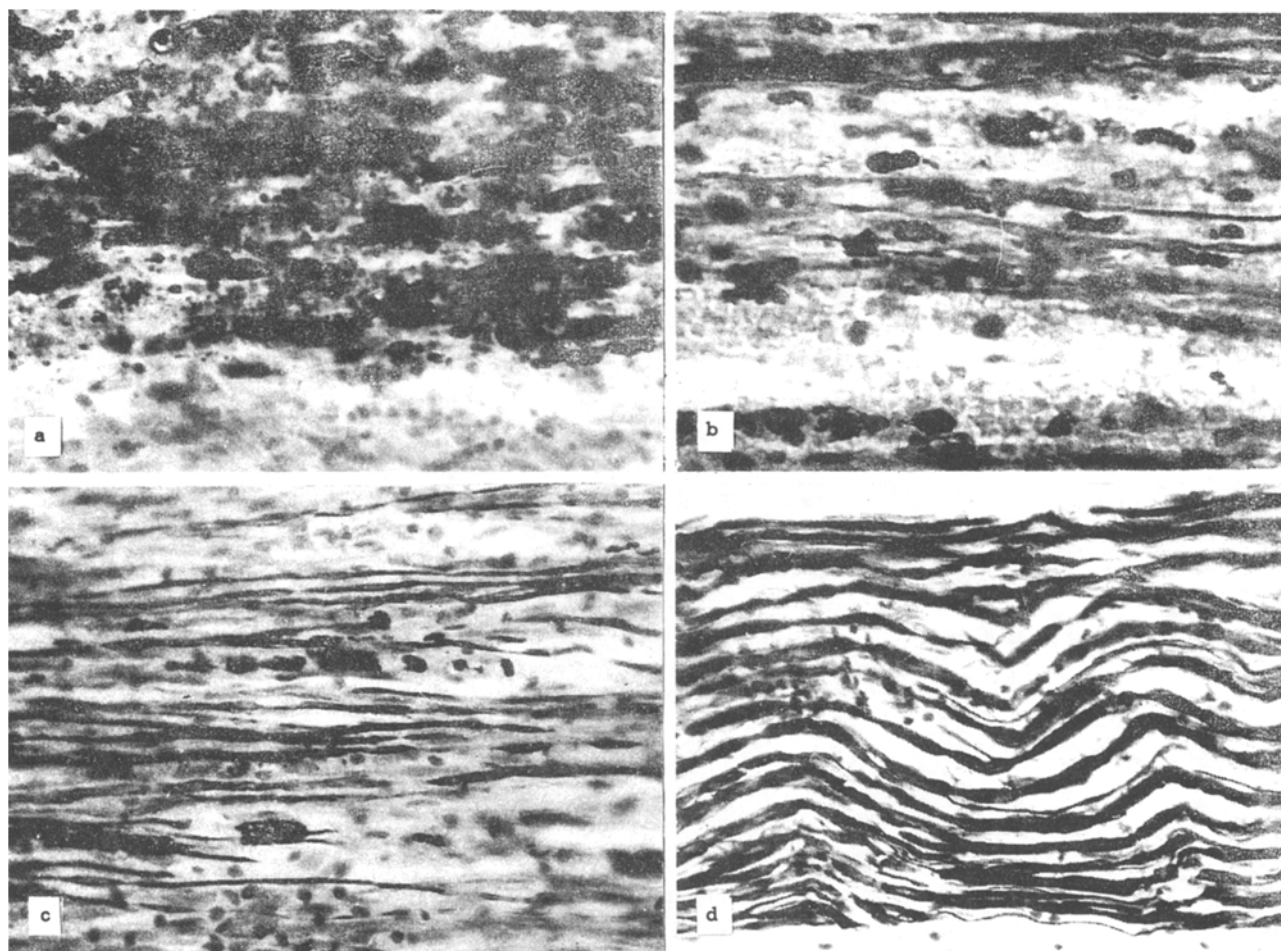


Fig. 1. Structural changes in sciatic nerve after application of drugs: a) marked Wallerian degeneration of nerve fibers after application of calcium chloride (3 days), b) Wallerian degeneration and periaxonal demyelination of nerve fibers after application of rheopyrine (3 days), c) periaxonal demyelination after application of pipolphen (3 days), d) action of physiological saline (control). Magnification 350 \times . a) Bekker's Sudan Black; b, c, d) silver impregnation by Bielschowsky—Gros method.

However, after application of physiological saline to the surface of the nerve, the inflammatory changes which developed were much milder. On the 3rd day after application of calcium chloride, rheopyrine, and pipolphen morphological changes were found not only in the membranes, but in the nerve itself and its branches. The most marked changes were observed after application of calcium chloride and rheopyrine. These substances cause different types of degenerative changes in myelinated nerve fibers: after application of calcium chloride, typical Wallerian demyelination predominated, whereas after application of rheopyrine and pipolphen, a mixed type of degeneration was observed, with predominance of periaxonal demyelination (Fig. 1). Under these circumstances, among the myelin breakdown products, many foci of inflammatory infiltration were observed, consisting of granulocytes, monocytes, and macrophages. On the 7th day, the pattern of breakdown of the nerve fibers and the inflammatory process were even more marked and extended distally from the site of injury as far as the target tissues. Neurohistological analysis of the skin of the foot revealed not only intact, but also destructively changed bundles of nerve fibers and receptor endings beneath the epidermis and in the dermis of the skin. By contrast, application of physiological saline did not give rise to degenerative changes in the skin at any time of observation.

The results suggest that membranes of peripheral nerves (epineurium, perineurium), which are known to be a sufficiently resistant nerve-tissue barrier to diffusion for many exogenous substances [4, 6], are permeable for the drugs tested. In our opinion, these drugs, penetrating into the nerve, exert a cytotoxic effect on myelinated nerve fibers.

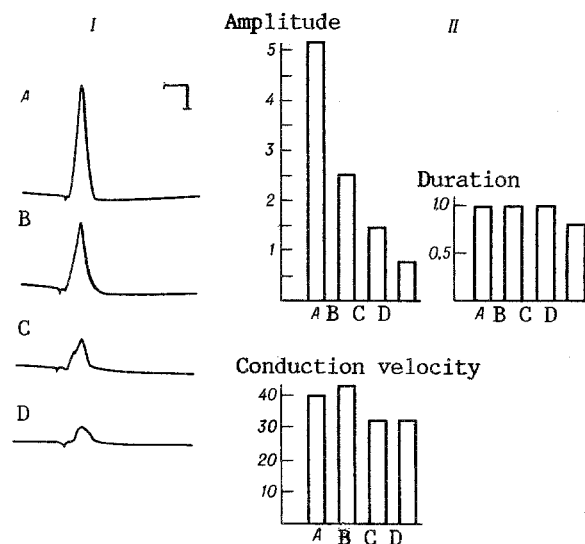


Fig. 2. Integral action potential (IAP) of nerve after application of drugs. I) Traces of responses (calibration 1 mV, 1 msec), II) parameters of IAP. A) Physiological saline, B) calcium chloride, C) pipolphen, D) rheopyrine.

When IAP of the sciatic nerve was recorded, the damaging action of the drugs on the nerve tissue also was revealed (Fig. 2). The most informative parameter of functional changes in the nerve was the amplitude of IAP, which was sharply reduced after application of the drugs, evidence of a decrease in the number of functioning nerve fibers in the nerve trunk. The degree of change of amplitude depended on the preparation used, and amounted after application of physiological saline to $5088 \pm 530 \mu\text{V}$, of calcium chloride to $2534 \pm 146 \mu\text{V}$, of pipolphen to $1449 \pm 334 \mu\text{V}$, and of rheopyrine to $741 \pm 118 \mu\text{V}$. The disparity in the level of severity of the physiological changes observed morphologically in each individual case may in all probability be attributed to differences in the penetrating power of the drugs into the tissues of the nerve trunk.

After exposure of the nerve to pipolphen and rheopyrine, the velocity of impulse conduction showed a significant change: it was 32.1 ± 1.9 and 32.1 ± 2.5 m/sec, respectively, compared with 40.7 ± 1.8 m/sec in the control. The decrease in impulse conduction velocity indicates that the functional properties of the preserved nerve fibers were altered. This conclusion is in agreement with the histological investigations, which showed that after application of these drugs, besides Wallerian degeneration, a process of periaxonal demyelination was also observed in many of the nerve fibers.

It was shown by recording spike activity [1] in response to mechanical stimulation of the skin of the foot that the sensitivity of the skin to the mechanical stimulus was preserved. The reason evidently was that some intact nerve fibers still remain in the injured sciatic nerve.

The drugs which we used for application thus possess a clearly defined pathological action, probably due to their increased permeability through the nerve-tissue barrier and the presence of a cytotoxic effect. The disturbance of nerve function is based on various structural changes in the nerve fibers: the development of Wallerian degeneration and periaxonal demyelination which, in turn, depend on the type of damaging substance.

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