A METHOD OF APPLYING COLCHICINE TO THE RAT VAGUS NERVE TO BLOCK AXON TRANSPORT

T. A. Uzbekova, I. A. Chernova, V. I. Savchuk,
G. F. Dobrovol'skii and T. M. Semushkina

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Blocking fast axon transport by colchicine is one of the techniques used to study neurotropic influences, the mechanism of which has been associated, in the context of intercellular relations, with axoplasmic transport of substrates of the nerve cell and their secretion through synaptic contacts [1, 5, 6]. The use of small and medium doses of colchicine over a long period of time is considered to be optimal [7].

In experiments *in vivo* great importance is attached to the method of local application of colchicine. Injection of a solution of colchicine beneath the perineurium has been criticized [4] for the risk of trauma to the nerve could not be ruled out, and a systemic perineural effect was observed. Irrigating the nerve or applying a sponge soaked in colchicine solution had a short and reversible action, the brevity of which had to be compensated by increasing the concentration of the compound.

Albuquerque et al. [4] developed a method of applying colchicine to the rat sciatic nerve in a silicone cuff. Our own attempts to use this technique for applying colchicine to the rat vagus nerve was unsuccessful. Application of a cuff made from the silicone Elastosil 173-180 to the cervical part of the nerve invariably led to disturbance of the circulation of perineural fluid, edema of the tissues, and swelling of the cuff, with compression of the perineural vessels and infiltration of the perineurium by polymorphs. On the 3rd-4th day degenerative changes were found in the neurofibrils. Cangiano and Fried [5] also observed edema and compression of the rat sciatic nerve beneath a silicone cuff. The method of applying colchicine polymerized in polyvinyl alcohol [3] is evidently traumatic also, for the authors cited observed complete destruction of the nerve after 72 h.

The traumatic nature of the methods of long-term local action of colchicine on nerve fibers described above, preventing any unambiguous assessment of the results, necessitated a search for a method of application that would be adequate for the study of neurotrophic influences, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 16 noninbred rats weighing 180-250 g. The depot material was a mixture of wax (m.p. 38-40°C) and paraffin (m.p. 58-60°). Crystalline colchicine DAB-7 was crushed and mixed with the base in the proportions of 1:100 and 1:50 by weight. By heating the mixture in a simple mold made from foil, blocks were prepared, cut into sections, and weighed on torsion scales. The dose of colchicine was estimated relative to the weight of filler. Application of the filler without colchicine was carried out on four rats. Doses of 25, 50, 75, and 100 μg were tested on 12 animals. Three intact rats also were studied.

A midline skin incision was made in the neck under intraperitoneal pentobarbital anesthesia (50 mg/kg). The muscles above the neurovascular bundle on the right side were restricted and the layers of fascia separated. The colchicine in its filler was applied directly to the vagus nerve, without mobilizing the nerve, and was fixed by the fascias and muscles. The wound was sutured.

The filler was removed from the anesthetized animals four to six days later, when it consisted of a finely granular mass, in contact with the perineurium and separated from the muscles by a thin connective-tissue capsule. The vagus nerve was isolated and its total electrical activity (the electroneurogram – ENG) was recorded by bipolar platinum electrodes and a system of biopotentials amplifiers on a Mingograph-82 instrument.

After investigation of the ENG the cervical part of the nerve was excised and fixed in 10% neutral formalin or 2.5% glutaraldehyde solution. Longitudinal sections 15 μ thick were cut in a cryostat from fragments of the nerve fixed in formalin, and impregnated with silver by the Bielschowsky–Gros method. The nerves fixed in glutaraldehyde were rinsed

Department of Morphology and Cytology, Medicobiological Faculty, N. I. Pirogov Second Moscow Medical Institute.
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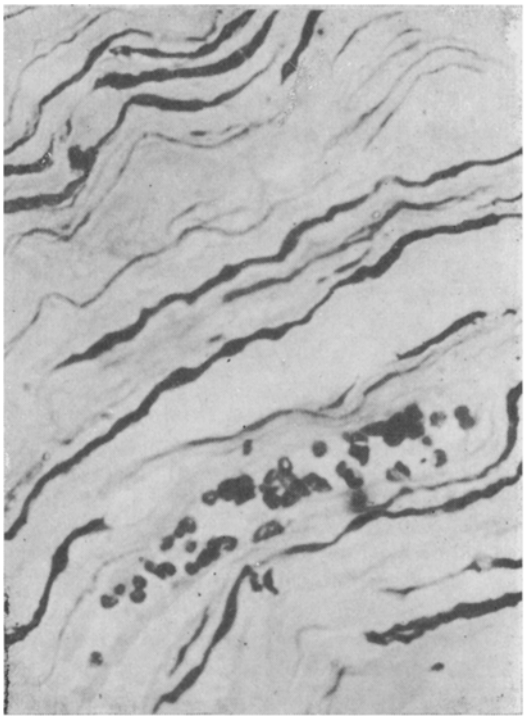

Fig. 1

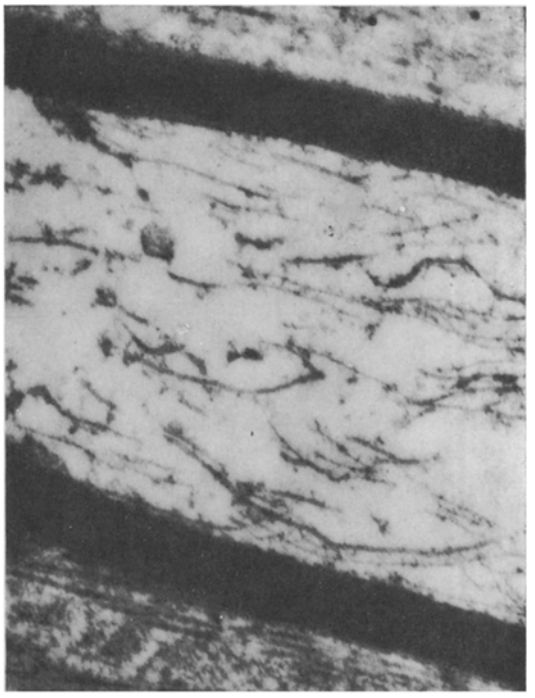

Fig. 2

Fig. 1. Right vagus nerve of rat 4 days after application of 100 μg colchicine. Impregnation with silver by Bielschosky–Gros method, 200 X. Irregular thickening of neurofibrils of axis cylinders. Blood vessel of nerve trunk on right.

Fig. 2. Myelinated axon of right vagus nerve of rats 4 days after application of 75 μg colchicine. Single microtubules scattered in axoplasm, many neurofilaments present, 44,000 X.

with phosphate buffer, postfixed with 1% OsO_4, and dehydrated in acetones of increasing concentration. The material was embedded in Araldite. Longitudinal and transverse sections 30-50 nm thick were cut on an ultramicrotome. Electron micrographs were obtained in the Hitachi II-E electron microscope under magnifications of between 2500 and 44,000 X.

EXPERIMENTAL RESULTS

The right vagus nerve, after application of filler without colchicine and with colchicine in doses of 25 and 50 μg, did not differ significantly from the nerves of intact animals. After application of colchicine in doses of 75 and 100 μg, changes were discovered which increased with an increase in the dose: irregular thickening of the neurofibrils and more intensive impregnation with silver (Fig. 1). No degenerative changes in the axis cylinders or proliferative reaction on the part of the Schwann cells were observed. Investigation of the left vagus nerve in order to exclude any systemic effect revealed no abnormality. No ultrastructural changes were found in the right vagus nerve as a result of application of filler without colchicine. When colchicine was applied in doses of 75 and 100 μg, however, changes in the ultrastructure of the neurofibrillary apparatus of some axons of myelinated nerve fibers were observed after 4 days. Under these circumstances a disturbance of the parallel arrangement of the neurofilaments and a reduction in the number of microtubules were found (Fig. 2). The axoplasm appeared translucent and clusters of neurofilaments were seen in the subneurolemmal zones. Aggregation of vescicular structures and edema of the mitochondria were observed. The cytolemma was not damaged.

The character of electrical activity in the vagus nerve of the experimental rats did not differ from that of the control (Fig. 3). No difference likewise was found in spike activity when the nerve was tested at different levels: at the site of application of the filler with colchicine and above and below it.

The technique of colchicine application to nerve fibers in order to study the cytological mechanisms of neurotropic influences must satisfy two demands: It must block axon transport, but at the same time, it must not disturb conduction of spikes along the nerve fiber.

The effectiveness of the method was evaluated by electron microscopy. A reduction in the number of microtubules, an increase in the number of neurofilaments, and aggregation of organelles are known to be dependent on the dose of colchicine [7]. Similar changes were found in the ultrastructure of the axis cylinders. No degenerative changes such as are

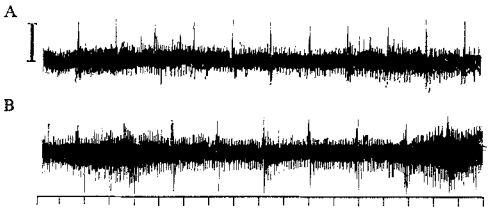

Fig. 3. Total ENG of right vagus nerve of rat. A) Control; spike discharge of respiratory fibers of vagus nerve of intact animal; B) no change in electrical activity 4 days after application of 100 μg colchicine. Mingograph-82, calibration 20 μV.

observed as early as 24 h after sectioning or ischemization of a nerve were found. Impregnation with silver revealed no fragmentation of the axis cylinders over a long length of the nerve.

The fact that spike conduction along nerve fibers can be preserved during blockage of axoplasmic transport is supported on theoretical grounds by the structural independence of these two processes [8] . Total activity in the nerve recorded on the ENG reflected its functional integrity [2].

We were looking for the least traumatic method of applying colchicine, without disturbing the blood supply to the curve or the integrity of the perineurium. We found that there is no need to isolate the nerve or to surround it by a sponge cuff, as was done by Albuquerque et al. in their experiments [4] . Application of colchicine in a filler and contact with the nerve trunk over a distance of 5 mm ensured an adequate pharmacological action of the substance. The principle of contact action of colchicine is familiar in oncologic practice.

The use of a mixture of wax and paraffin as depot base has advantages over the silicone sponge for the filler does not swell as a result of an inflow of tissue fluid, and it compresses the nerve trunk less severely. The closeness of the melting point of the wax to the rat's body temperature facilitates the liberation of colchicine. The addition of paraffin with a higher melting point, however, makes the filler more convenient for fixation in the tissues.

The suggested method thus permits the prolonged action of colchicine without compression of or injury to the nerve, in consequence of which the after-effect of the blocking of transport of neurotropic factors on metabolism of the innervated structures can be unambiguously evaluated. The method can be used for the experimental study of axon transport and of neurotrophic influences, and it is particularly relevant with regard to the study of the trophic influence of the vagus nerve on the heart and other organs.

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