Degenerative atrophy of central terminals of primary sensory neurons induced by blockade of axoplasmic transport in peripheral nerves*

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Summary. Perineural colchicine- or vinblastine-treatment results in disappearance of non-lysosomal acid phosphatase activity, characterizing axon terminals in the Rolando substance of the normal spinal cord. Ultrastructural alterations are identical with those seen in the course of degenerative atrophy.

Previous investigations in this laboratory revealed that transection of a peripheral nerve induces a degeneration-like process in the central terminals of primary sensory neurons (degenerative atrophy). The question whether this phenomenon is brought about by traumatization of the peripheral axon or rather by cessation of axoplasmic transport, has been studied by topical administration of drugs known to interfere with axonal transport mechanisms.

Material and methods. Investigations were performed on 21 albino rats of both sexes, 200 g average body weight. In Nembutal anesthesia, the left sciatic nerve was exposed and a small (4 mm³) Gelaspon (VEB Jenapharm) fibrin sponge, soaked either in colchicine (0.05%), vinblastine (Gedeon Richter Ltd, Budapest) (0.1%) or saline, was applied around the nerve trunc. The cuffs were removed after 30 min and the skin was sutured. 1–24 days later the animals were subjected to perfusion fixation by Karnovsky's formaldehyde-glutaraldehyde solution. 25 μm frozen cross section of the lumbosacral cord were 'stained' for acid phosphatase, using a slightly modified Gomori incubation solution as described earlier[1]. Small tissue blocks from the Rolando substance were post-fixed in collidine-buffered osmic acid, dehydrated in graded alcohols and embedded in Durcupan (Fluka). Ultrathin sections were obtained on a Reichert Ultratome, stained with uranyl acetate and lead citrate and studied under a Tesla 513 B electron microscope.

Results. In both the colchicine- and vinblastine-treated groups, AP[2] activity of the left Rolando substance in spinal segments L_4–S_1 decreased gradually from the 3rd postoperative day on, resulting in cessation of the enzyme

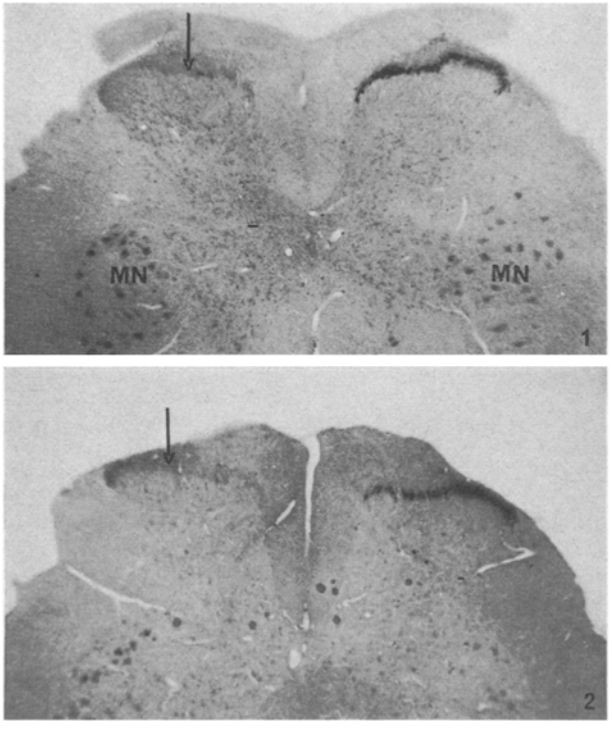

Fig. 1. Effect of local colchicine cuff around the sciatic nerve, upon the acid phosphatase activity of the rat spinal cord. Segment L_6. Note the intense enzyme activity of the right (control) Rolando substance and the virtually abolished reaction on the left side (arrow). Reaction in the motoneurons (MN) is practically unchanged. 16th day after a 30 min local colchicine treatment.

Fig. 2. Effect of a local vinblastine cuff around the sciatic nerve, upon the acid phosphatase activity of the rat spinal cord. Segment L_5. Enzyme activity of the left Rolando substance (arrow) is virtually abolished. 10th day after a 30 min local vinblastine treatment.

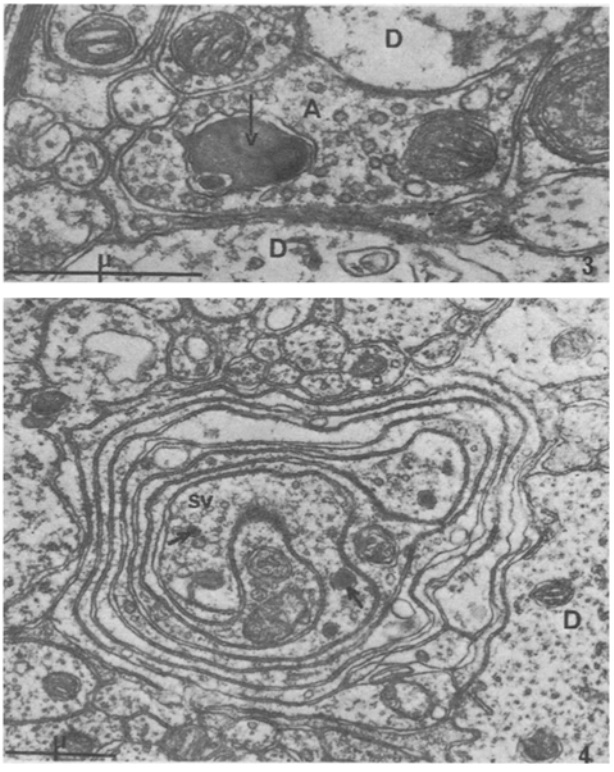

Fig. 3. Degenerative atrophy of a primary sensory nerve terminal in the Rolando substance, 10 days after a 30 min application of a local vinblastine cuff around the ipsilateral sciatic nerve. Note the large, homogeneous osmiophilic degenerative body (arrow) within the terminal axoplasm (A). D: dendrites.

Fig. 4. Degenerative atrophy in the Rolando substance, 10 days after a 30 min local vinblastine cuff around the ipsilateral sciatic nerve. Note the spirally wound labyrinth formed by the terminal axoplasm, suggesting futile regenerative efforts. sv: synaptic vesicles in the terminal; arrows point at dense-core vesicles. D: dendrite.

activity on the 10th day. No enzyme reactivation could be seen throughout the period investigated. At the same time, both the electron dense sinusoid terminals in the Rolando substance, exerting AP activity under normal conditions, and other primary axon terminals in this area, exhibited signs of ultrastructural desorganization essentially identical with those occurring in the course of degenerative atrophy that ensues after surgical transection or traumatization of the respective peripheral spinal nerve (figures 1–4). No alterations could be seen in the control (saline-treated) group.

Discussion. It has been shown that local colchicine and vinblastine treatment induces blockade of axoplasmic transport mechanisms in peripheral nerves[3–8]. In pioneering studies performed by injecting small amounts of these drugs, nerve truncs may have suffered microinjuries resulting in Wallerian degeneration of peripheral axons[9]. The use of perineural cuffs, however, excludes the possibility of a mechanical injury; therefore, the effects of colchicine and vinblastine observed by us should be ascribed to arrested axoplasmic transport.

Both colchicine and the Vinca alcaloids are known to induce a metaphase mitotic arrest[10]. By virtue of the analogy between mitotic spindle filaments and neurotubuli, the theory was forwarded that fast axoplasmic transport is related to mechanochemical activity of neurotubuli. Our observations indicate that, in addition to arresting axoplasmic transport, mitotic metaphase inhibitors induce histochemical and ultrastructural alterations in the central terminals of primary sensory neurons identical with degenerative atrophy caused by transection, crush or ligature of peripheral axons[11]. Such 'transcellular' or 'transganglionic' alterations were sporadically reported since 1880 (Stiénon, cit. Scharf[12], Grant[13], etc.[14]); however, only recent studies in this laboratory revealed the fine structural and histochemical aspects of this process[15]. Degenerative atrophy, obviously necessitating a new formulation of the doctrine of 'neuronal trophical entity', is characterized by the disappearance of AP activity from the central terminals of sensory neurons in the Rolando substance, and by a series of ultrastructural alterations resembling or identical with, those of a secondary Wallerian degeneration.

The present investigations support the idea that the very reason of degenerative atrophy is the blockade of microtubule-dependent fast transport mechanisms in the peripheral axon. It can be assumed that axoplasmic arrest elicits a signal for the perikaryon, initiating a 'state of emergency' throughout the domain of the primary sensory neuron. Degenerative atrophy of the central terminal appears to be one of the symptoms of this emergency.

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Effect of the pentosanpolysulfate SP 54 on the collagen of embryonic limb buds cultured in vitro

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Summary. After addition of SP 54 to limb buds from 11-day-old mouse embryos in tissue culture, collagen with an altered structure is produced.

Proteoglycans (PG) and glycosaminoglycans (GAG) are bound to collagen by salt-like bindings. These substances influence the aggregation of collagen to filaments and fibrils in vitro, the strongest effects being produced by highly sulphated PG and GAG respectively[1–8]. They also seem to play an important part in the development of some properties of the fibrils in vivo, such as thickness, length, cross-striation pattern, and packing density[9–11]. However, rather contradictory results on the influence of GAG on collagen have been obtained from in vitro experiments. These are probably due to the different properties of the various collagen and GAG preparations used. For studying the influence of GAG on collagen fibril formation with natural procollagen and natural collagen respectively, limb bud cultures from 11-day-old mouse embryos are especially suitable as they produce large amounts of type II collagen[12–14]. We therefore studied in this system the influence of GAG with different degrees of sulfatation on fibrillogenesis.

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