CYTOCHEMICAL INDICATOR OF THE FUNCTIONAL STATE OF AFFERENT NERVE FIBERS

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To study zones of action potential generation in nerve fibers a morphological method based on the increased affinity of regions of the neuron membrane with a high density of Na channels for Fe ions has been suggested [4]. In particular, the precipitate of the specific cytochemical reaction is deposited in the Ranvier nodes of nerve fibers [3]. The writers observed previously that the nodes far from every afferent fiber give a positive cytochemical reaction, and spontaneous spike activity likewise is not recorded in all fibers [1].

These facts suggested that affinity of the nodes for Fe salts may be connected with the production of bioelectrical activity. The aim of the present investigation was to shed light on this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male and female frogs (*Rana temporaria*). The urinary bladder of the spinal or decapitated frogs was isolated, a two-dimensional preparation was obtained, and neurofilaments in it were isolated by the method described previously [2]. Their spontaneous afferent spike activity was recorded and amplitude discrimination of firing receptor units was carried out. The neurofilaments were then fixed in glutaraldehyde, processed cytochemically with ferrous chloride and potassium ferricyanide by the method in [3], and mounted in glycerol–gelatin for morphological study under the light microscope. There were three series of experiments. In series I (control, 50 filaments) intact fibers were studied, in series II (18 filaments) the animals were kept for 45 h at 10°C after decapitation, and in series III (51 filaments), after spontaneous spike activity had been recorded, 0.5% procaine solution was applied to the preparation for 30 or 60 min (28 and 23 filaments, respectively). Correlation between cytochemical and spike activity of the fibers was determined by calculating the tetrachoric index of correlation.

EXPERIMENTAL RESULTS

In the control series all the filaments studied had spike activity. The calculated value of the tetrachoric index of correlation of spike activity in single fibers and deposition of precipitate in their nodes demonstrate the presence of moderately strong correlation between these features ($r_{++} = 0.51$; $p < 0.05$).

In the experiments of series II spike activity was not recorded in all the neurofilaments. Morphological investigation showed that afferent fibers of neurofilaments without spontaneous or evoked spike activity had degenerative changes. They were not of uniform thickness, their outlines were uneven, and their myelin was fragmented (Fig. 1a). Affinity of the nodes for Fe salts was never observed in the modified fibers, i.e., a negative cytochemical reaction was obtained. Some neurofilaments had spontaneous activity and their fibers contained deposits of precipitate in their Ranvier nodes. The calculated value of the tetrachoric index of correlation for the experiments of this series is evidence of positive correlation between spike activity and deposition of precipitate in the Ranvier nodes ($r_{++} = 0.63$; $p < 0.999$).

In the experiments of series III spike activity was completely blocked by procaine toward the time of fixation in glutaraldehyde, but cytochemical activity of the Ranvier nodes of the

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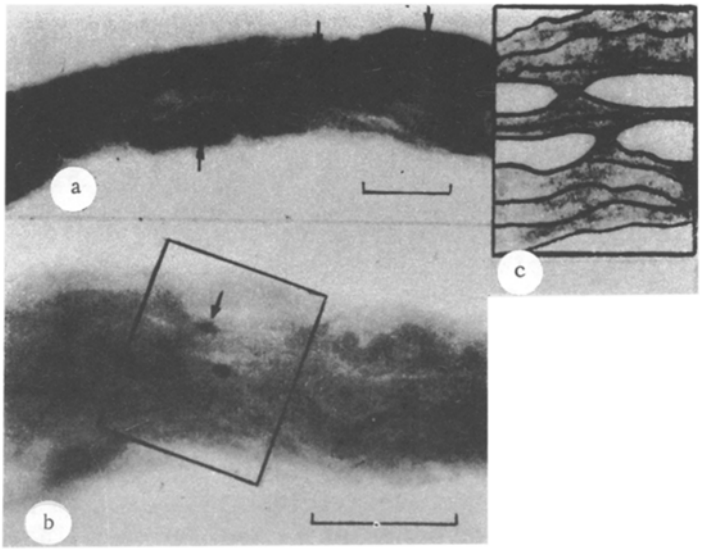

Fig. 1. Nerve filaments treated with ferrous chloride and potassium ferricyanide. a) After keeping at 10°C for 45 h. Arrows indicate degenerating myelinated afferent fibers; b) after treatment with 0.5% procaine solution for 60 min. Arrows indicate deposition of precipitate in Ranvier nodes of afferent fibers; c) explanatory scheme. Objective 40, ocular 10. Scale 60 μ

afferent fibers did not disappear in this case. Just as in the control there were fibers with active nodes (Fig. 1b), and their number was virtually unchanged (22% of active fibers in the control, 14% after treatment with procaine for 30 min, 26% after treatment with procaine for 1 h).

The facts described above are evidence that a positive or negative cytochemical reaction of the nodes is not an indicator of the presence or absence of spontaneous spike activity in afferent fibers at that given moment. However, the tetrachoric index of correlation ($r_{++} > 0.5$) at the same time is a definite indicator of the existence of correlation between these phenomena, as investigations by other workers have confirmed. It has been found, for example, that myelinated fibers of the electric organ of *Sternarchus* contain two types of Ranvier nodes: narrow and wide [5]. Under these circumstances spikes are generated and precipitate deposited only in narrow zones, the wide nodes being cytochemically inactive [3]. It can therefore be concluded that the positive cytochemical reaction is evidence not of the presence of processes of electrogenesis, but rather that the fiber is able to generate action potentials. Complete absence of the precipitate in degenerating fibers confirms this conclusion. Thus, deposition of the precipitate of the specific cytochemical reaction may be an important indicator of the structural and functional integrity of nerve fibers.

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