IV. Nodal function of pathological nerve fibers

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The understanding of the disease mechanisms in nerves is linked to the identification of structural changes in human nerve biopsies. Much of the nervous dysfunction that can be identified clinically or as a decrease in nerve conduction velocity has an obvious relation to e.g. axonal degeneration or demyelination. The more detailed quantitative analysis in various clinical studies and animal models has, however, led to the conclusion that in addition there must exist functional changes in the nodes of Ranvier or the myelin sheaths that play an important role in the disease mechanism. For example in both human and animal (spontaneous) diabetes there are changes in nerve conduction velocity that are difficult to relate to morphological changes only. Multiple sclerosis is another important example of sometimes poor clinicopathological correlation (for review, see Waxman). Areas of focal demyelination can be identified postmortally, and the optic nerve conduction velocity may be decreased (increased latency of visually evoked response), without associated clinical symptoms.

The axonal impulse propagation is generally regarded as the strongest link in the chain of nervous signalling. The safety factor for conduction is great, consequently large alterations can appear in nodal function before it causes decreased conduction velocity or propagation block. There is a large uninvestigated field of nerve pathophysiology, where recordings from the single fiber and potential clamp analysis of its membrane properties probably will be necessary to reveal the nervous dysfunction. Squid axons and frog myelinated fibers are invaluable for the exploration of the basic membrane function, but for the understanding of disease mechanisms it seems necessary to turn to mammalian nerve and use the pathological models that have been established. It is, however, more difficult to dissect mammalian nerve fibers than nerve fibers of frog because of the more prominent inter-neural septa and collagen strands. The experiments must be performed at higher temperatures, which puts higher demands on the feed-back circuit since the membrane current changes are then more rapid.

The work with pathological fibers is also coupled to some new methodological considerations. When a demyelinated fiber is selected it is necessary to see that the fiber has the main parts of its internodes intact so it can be mounted in the recording chamber. It can be difficult to find a fiber with the right structure that can be isolated without too much work. The electric feed-back circuit for the potential clamp also sets limits for the changes in leak and capacitive properties that can be tolerated. This is relevant for the studies of demyelinated fibers, which have been restricted to fibers with paranodal demyelination. In the potential clamp work of normal fibers, like in other physiological work, experiments are discarded if they show that the preparation in some way is in poor condition. By experience from the changes during long experiments, an increased sodium inactivation and decreased specific permeabilities can be related to run down of the fiber. Since the threshold for excitation in the intact isolated fiber and in the fiber after it has been cut off were the same when the


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membrane potential was backed up to \(-80\) mV, this was taken as the resting potential, and the sodium inactivation parameter (\(h_\infty\)) was then about 0.70. If the intact fiber is found inexcitable and for example \(h_\infty\) is 0.1 at \(-80\) mV, it is reasonable to exclude this fiber from the material.

A different approach is needed in the work on pathological fibers. All fibers must be included in a series of diseased animals, unless there is some evident technical reason to exclude the results. The incidence of inexcitable fibers or fibers with e.g. low resting value of \(h_\infty\) in a series of normal rats must be known. These considerations are self-evident in clinical or pathological studies, but this is generally not the case in normal physiology. The present review will contain results from potential clamp studies of mammalian fibers in different pathological conditions. The effect of anoxia is a useful subject to start with since it illustrates the pathological changes that can appear in fibers from a normal animal.

**Anoxia**

In single rat nerve fibers anoxia decreases the action potential to only a graded response within about 20 min, which indicated that the sensitivity was much higher than found in other species\(^2\). In these experiments, which were the first that have been described in isolated mammalian nerve fibers, elevation of the external [Ca] improved the survival of the preparation and antagonized the anoxic effect. This was interpreted as an effect related to inactivation through changes in Na and K concentration of the nerve fiber. A distinction between changes in ionic concentration (driving force) and membrane permeability could then not be made but a distinction is readily made with the potential clamp analysis of the nodal membrane.

The effect of anoxia was studied\(^7\) by letting in 100% nitrogen over the surface of the solution pools of the voltage clamp cell. Repeated measurements were first performed during 15 min in oxygenated Ringer to get a reference level. Preliminary work (in 6 fibers) performed in this way showed that the Na permeability decreased within about 15 min of anoxia. This was related both to a decrease of the maximum \(P_Na\) (after a negative conditioning pulse) and a shift of the inactivation curve to more negative potentials, so that only a small part of the Na permeability was available at the resting potential. This rendered the fiber inexcitable and the changes were irreversible. The Na equilibrium potential was not significantly affected.

These results from isolated fibers suggest that the sensitivity to anoxia is much greater than found in in vivo studies (see Fox and Kenmore\(^1\)). In isolated frog nerve fibers, Schoepfle and Bloom\(^34\) found that cyanide and dinitrophenol decreased the action potential spike height, suggesting an inactivation of the Na permeability due to metabolic inhibition and consequently that oxidative metabolism not only is involved in the Na:K pump but also in the mechanism for specific permeability changes. It is necessary to do more extensive in vitro studies of the factors that may influence (temperature, pH and ionic changes) or mimic (metabolic inhibitors) the anoxic effect.

**Diabetic neuropathy**

Symmetric distal neuropathy is one of the late complications to the human diabetes, morphologically it is characterized by segmental demyelination and in severe cases axonal degeneration\(^31\). Generally the nerve conduction velocity is also decreased and it was similarly found that after chemical induction of diabetes in rats the nerve conduction velocity decreases\(^14\). This phenomenon has been extensively studied as a model for diabetic neuropathy but also questioned with regard to the presence of demyelination or other structural changes\(^35\) and the nature of the conduction velocity change\(^20,42\). These rats are usually given a single dose of alloxan or streptozotocin which is toxic to the beta cells and causes a partly transient (dose dependent) reduction in insulin production. They become hyperglycemic but not ketotic and they survive several months without insulin administration before they die after a period of weight loss.

Potential clamp of nerve fibers from diabetic rats showed a large increase in the delayed potassium current, which is very small in the normal rat\(^1\). These findings were explained by the hypothesis that the K-current normally is shunted into the Schwann cell and therefore attenuated, and that the earliest stages of demyelination (with disruption of axolemma-Schwann cell contacts) will result in large K-currents. This presumes that the K-channels were restricted to certain parts of the nodal area\(^3\). A diabetic model that is similar to the human insulin dependent diabetes has been found in the spontaneously diabetic BB-Wistar rat, which gets a severe insulinitis associated with an insulin-dependent diabetes at about the time of sexual maturation\(^26\). The motor nerve conduction velocity decreases after the onset of diabetes but it has not been possible to relate this to structural changes\(^25,37,38\). In search for the mechanism of the conduction velocity decrease, a series of potential clamp experiments were performed in rats with spontaneous diabetes with a duration of up to 6 months\(^9\). A majority of the fibers were inexcitable or had action potentials < 80 mV amplitude, which rarely (in about 1 out of 10 cases) is found in fibers from normal rats. One rat had pronounced demyelination and paranodal swelling with loss of refraction (diabetes duration 6 months) and had very large delayed K-currents; very large K-currents were also