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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[19,25,38,43]. Multiple sclerosis is another important example of sometimes poor clinicopathological correlation (for review, see Waxman[44]). 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 interneural 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[16]. 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

membrane potential was backed up to −80 mV, this was taken as the resting potential, and the sodium inactivation parameter (h_∞) was then about 0.70. If the intact fiber is found inexcitable and for example h_∞ 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_∞ 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[23]. 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[17]). 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[41]. 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[3]. 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 insulitis 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

found in a fiber from another rat. These changes were similar to those found earlier in alloxan diabetic rats. The equilibrium potential for the initial current was lower in the nodes from the diabetic rats (27 mV ± 13 mV) than in normal rat nodes (39 mV ±10 mV). The specificity of the channels for the initial current was not affected, which indicated that this effect was caused by intracellular Na accumulation. This may be due to hypoinsulinemia, since insulin has a stimulatory effect on the Na pump in muscle[12]. The decreased excitability was, however, essentially due to a decreased Na permeability (decreased maximum P_{Na} and also a negative shift of the inactivation curve along the potential axis). These results are collected in figure 1. The decreased Na permeability in the nodes can reduce the conduction velocity in the diabetic rats - a similar dysfunction may contribute to the human diabetic neuropathy. Speculations about the underlying mechanism can be tested on this experimental model - intracellular changes in ion concentrations as well as toxic metabolites which block Na permeability may be involved. It was noted that the Na permeability changes were similar to those caused by anoxia in normal fibers, and there is possibly a linked pathogenesis.

Uremic neuropathy

Polyneuropathy is a common complication of chronic renal failure. The clinical picture of symmetrical sensorimotor involvement of the extremities is accompanied with distal axonal degeneration with moderate demyelination. The nerve conduction velocity is also decreased proximally and improves rapidly after renal transplantation, which is evidence of a neuronal dysfunction (this of course may be connected to the development of the degenerative changes). As with the diabetic neuropathy it is valuable to find an animal model for the analysis of the nerve fiber function, and these possibilities have recently been tested[40].

Chronic renal failure was induced by surgical removal of the main parts of the kidneys in adult rats, which reduced the glomerular filtration rate to approximately 15%. In spite of this renal failure the motor nerve conduction velocity showed the normal age related increase over the following 4 weeks. A more severe uremia could only be maintained about 48 h with the rats in satisfactory condition (they die after 60–72 h) and was achieved by bilateral ligation of the renal vessels and ureters. These rats exhibited a decreased motor nerve conduction velocity (by about 10–15%) measured 48 h after operation. Measurements in vitro on the de-sheathed sciatic nerve showed the same reduction in nerve conduction velocity. The glomerular filtration rate must probably be decreased below 15% for the development of neuropathy, which is in agreement with the conclusion by Nielsen[27].

A potential clamp analysis was performed with these fibers in order to reveal the mechanism for the conduction defect. The main findings[40] were a decreased maximum Na permeability (after a conditioning negative pulse) and an increased inactivation of the Na permeability (negative shift of the inactivation curve along the potential axis). The K permeability properties were normal. If this effect is caused by a circulating toxic metabolite its binding to the membrane is irreversible (within the hour the nerve fiber is immersed in artificial salt solution), otherwise it may remain intracellularly. It is noteworthy that these changes are similar to those found in anoxia and in diabetic rat fibers, and that both the maximum P_{Na} (related to channel number or the activation system or very slow inactivation changes) and the inactivation (h) system are involved, which seems to be more than a blocking effect.

Demyelination - capacitance and resistance changes

On structural grounds, demyelination can be described as nodal widening-paranodal changes or segmental with long internodal segments devoid of my-

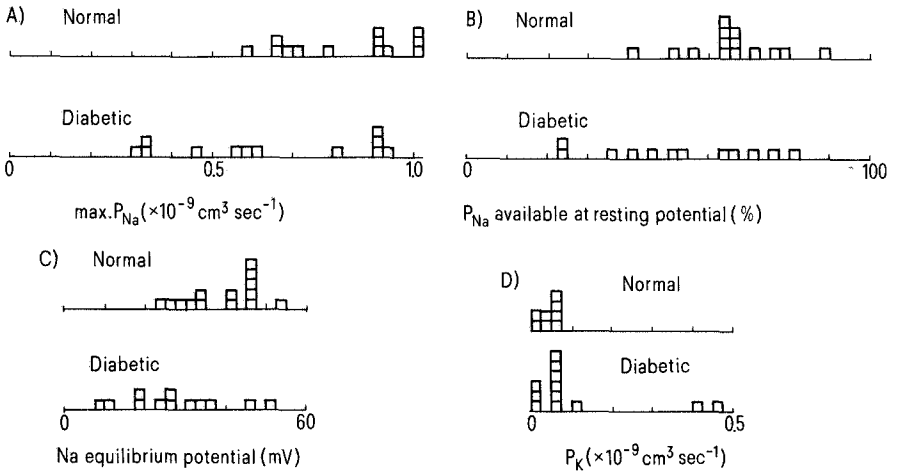

Figure 1. Mechanisms of decreased excitability in spontaneously diabetic rat nerves. *A* The decreased maximum P_{Na} (measured after a conditioning negative pulse) and *B* the increased sodium permeability inactivation (negative shift of the h_∞/potential curve) were the main factors. *C* The Na equilibrium potential was decreased, which by measurement in different external solutions could be attributed to increased axoplasmic [Na]. *D* Marked increase in P_K in 2 fibers. From Brismar[8] and Brismar and Sima[9].

elin. In multiple sclerosis plaques are found in the white matter of the CNS, where the fibers have lost the myelin over long stretches. Diphtheria toxin inoculation (by injection in the nerves or roots or in most species also when given systemically) produces paranodal demyelination, which has become a valuable model to study the physiological consequences of demyelination (see e.g. McDonald[24] for review). Rasminsky and Sears[29], from longitudinal current recordings of the undissected rat ventral roots, identified a decreased internodal conduction time presumably related to increased capacitance and decreased transverse resistance of the internodes.

If the electrical properties of the axolemma in the paranodes is the same as in the node, then already a small paranodal demyelination would short circuit the action current and block the impulse propagation (calculated in computor simulation by Koles and Rasminsky[21]. Rat sciatic nerve fibers with different degrees of demyelination have been isolated and the relation between leak conductance and capacitance of the nodal segment (node plus 100 µm of fiber on each side) was calculated[5]. In fibers with nodal widening or paranodal demyelination (< 90 µm) the increase in conductance was much less then the increase in capacitance (fig. 2). – Are these the electrical properties of remaining myelin or Schwann cell membranes, or do they relate to the axolemma? Since ultramicrographs[1] show that parts of the paranodal axolemma at some stages is completely uncovered, the conclusion that the paranodal axolemma has considerably lower leak conductance than the nodal membrane seems justified. The comparison is made relative to the capacitance changes; it is assumed that these membranes have similar capacitive properties. The nodal capacitance was measured from the time-course of the nodal potential change caused by a pulse of constant current[18]. Strictly, a segment of myelinated internode (about 200 µm) was in parallel with the node, since it was contained in the same pool of the recording chamber, but normally it contributes < 0.3 pF to the measured capacitance of > 1–2 pF. The capacitive artifact, which is due to the vaseline seals becoming tighter during the experiment[13], was avoided by measuring the membrane time constant early. Acute immersion of rabbit myelinated fibers in lysolecithin[11] similarly causes a larger increase in capacitance relative to the increase in leak conductance. Intraneural injection of anti-galactocerebroside serum in rat nerve causes paranodal demyelination within a few hours[33] and from records of external longitudinal currents in undissected roots treated with this serum Lafontaine et al.[22] arrived to the same conclusion that the increase in capacitance was larger than the resistance change.

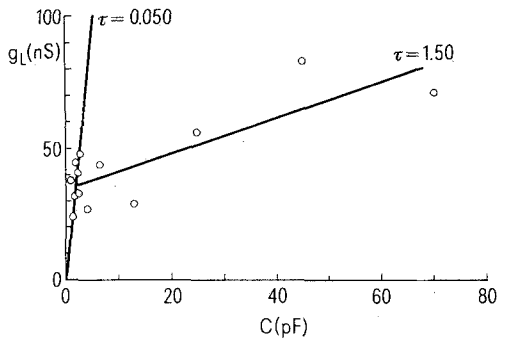

Figure 2. Leak conductance (g_L) versus capacitance (C) of the nodal segment in fibers with various degree of paranodal demyelination. The relation between g_L and C ($\tau 0.050$ msec) is predicted with 'nodal' electrical properties of the demyelinated segment. The measurements indicated a much smaller g_L/C ratio (that is longer membrane time constant, $\tau = 1.50$ msec) of the demyelinated segment. From Brismar[5].

K-permeability of demyelinated fibers

The increase in the normally small delayed K-currents in diabetic rat nerve fibers has already been mentioned. The hypothesis that paranodal demyelination had exposed parts of the axolemma with many K-channels[3] gained support from the findings in acute experiments on rabbit fibers that different treatments designed to disrupt the myelin increased the K-currents[10,11]. Also, the effect of K-blocking agents on the longitudinal current of undissected demyelinated nerve roots could be interpreted in this way[36].

In order to test this hypothesis investigations were performed on individual fibers with clearly identified and visible demyelination, which first could be studied microscopically and thereafter with potential

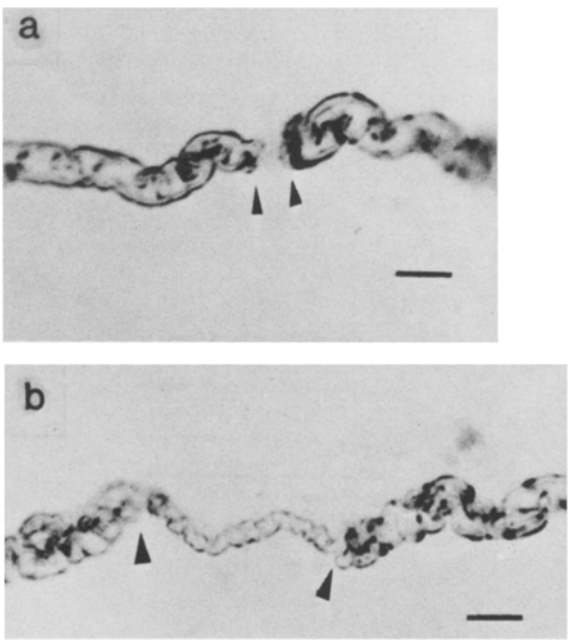

Figure 3. *a* Nodal widening in myelinated fiber 13 days and *b* paranodal demyelination 20 days after diphtheria toxin inoculation. Calibration bars 20 µm. From Brismar[5].

clamp analysis. Diphtheria toxin seemed suitable for these studies since in low doses it produces a demyelination paranodally, leaving the main parts of the internodes intact, which makes it possible to mount the fiber and clamp it in the established way. The analysis of such fibers (fig. 3,a and b) confirmed that they had large delayed currents (fig. 4) and that the K permeability was large in the demyelinated nodal segment[5,6]. The K-permeability was very high also in fibers with only nodal widening and in some light-microscopically normal fibers which were isolated from a demyelinated nerve. It is therefore likely that the early detachment of microvilli and myelin loops had exposed parts of the nodal membrane or the paranodal axolemma which have a high density of K-channels. The K-permeability was increased 10–50-fold in most fibers – 3 fibers with nodal widening (4–6 μm) and 4 with paranodal demyelination (20–90 μm) showed the same order of P_K change. The increased K-permeability had similar features as the small K-permeability found in normal fibers with regard to the potential dependence and the effect of increased external [K][4,8a].

What is the function of these K-channels which normally contribute so little to the membrane current? – In normal or demyelinated fibers there are different indications of a barrier over the K-channels: 1. the blocking effect of tetraethylammonium cations (TEA) is slow and incomplete, 2. the effect of external [K] changes on the large K-currents in demyelinated fibers is slow and 3. regenerative inward K-currents are observed in demyelinated fibers at critical potentials in high external [K] as may be caused by a series resistance over the K-channels. This could be due to the Schwann cell microvilli[22a] covering axolemma patches with high density of K-channels. It could also be that the myelin attachments have loosened from the axolemma (see Berthold and Rydmark, this volume) and opened up a pathway for current to K-channels in the paranodal membrane. These areas of axolemma must then have few or no Na channels, since P_{Na} is not changed in association with the increase in K permeability. There is obviously no contribution to the membrane currents and the nodal potential changes through the covered K-channels in the normal fiber. The findings discussed above emphasize instead the close relation between these channels and the covering structure, the Schwann cell. It may be speculated that the K-ions act as a signal from the axon to the Schwann cell, which may be important for the cooperation of these two cells in nerve growth and regeneration.

In conclusion, demyelinating changes in the node and paranode – regardless of its prime cause – is associated with increased K-permeability (with typical characteristics). The mechanism may lie in the uncovering of nodal or possibly paranodal K-channels in the axolemma. Their normal function is unknown and may be unrelated to impulse conduction.

Na-permeability changes

Demyelination can short-circuit the action current and decrease the excitability unless the effect is compensated for by increased Na-current in the nodes. – Are there, in the normal paranodal axolemma, sodium channels which become exposed at demyelination? In fibers which have undergone demyelination the axolemma originally located under the myelin becomes excitatory when new nodes are formed with shorter internodal distance[39]. The 'continuous conduction' in some demyelinated fibers[2] also indicated that the internodal axolemma had become excitable. Are these changes caused by redistribution of nodal Na-channels or by an increased Na-permeability (presumably equivalent to the formation of new Na-channels)? Saxitoxin-binding studies[30] did not provide any evidence for the formation of new Na-channels during the early stage when continuous conduction may develop; the binding capacity had not increased until about 2 months after lysolecithin induction of demyelination (at a time when the number of nodes is higher).

The results of potential clamped diphtheria demyeli-

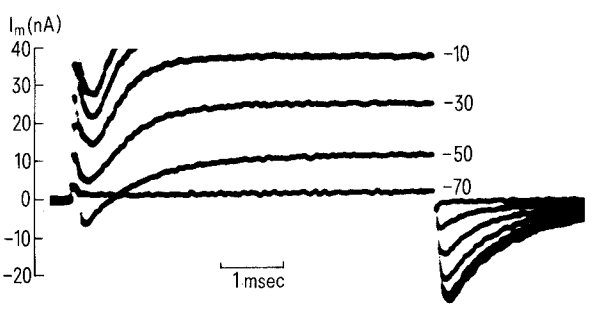

Figure 4. Large delayed outward currents and large inward current tails, corresponding to increased K-permeability in the demyelinated fiber (same as in fig. 3a). The presence of large inward tails at holding potential (−80 mV) suggest external (perinodal) K-accumulation. The Na currents have normal amplitude.

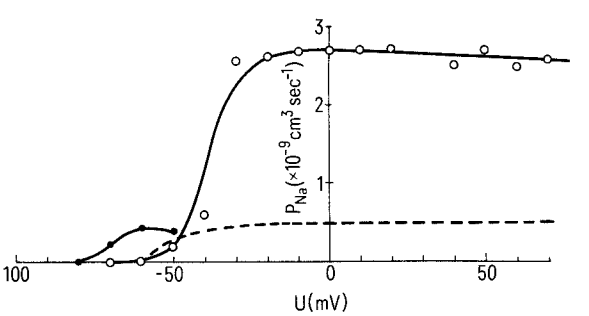

Figure 5. Large increase in P_{Na} in some fibers with paranodal widening. Calculated from initial current peak (open circles) and additional 'hump' of current at small positive steps (filled circles) in records from fiber in fig. 3b. Ordinary sized P_{Na} curve drawn in for comparison (interrupted line). From Brismar[6].

nated fibers lead to a different conclusion[6]. In the early stage of demyelination, where the fiber only had a small widening (4–6 μm) in the node, the Na currents and the Na permeability properties were normal. The reversal potential for the initial current was decreased in some fibers, which decreased the excitability, and this was related to the increase in steady state K-permeability (at resting potential). There was no indication that Na-channels had been exposed by the early widening of the nodes.

Considerably larger Na-currents and P_{Na} -values were found in fibers with pronounced paranodal demyelination (node gap 50–90 μm) as shown in figures 3b and 5. The max P_{Na} was 2–5 times higher than in normal fibers or fibers with only small nodal widening. It shall be noted that the leak conductance and the increase in K-permeability were approximately the same as in fibers with nodal widening (2–6 μm), indicating that the increase in Na-permeability was a specific difference.

This increase in Na-permeability was interpreted as due to increased number of Na-channels, but it is evident that theoretically the permeability of already existing channels may have increased. In line with the hypothesis for the increase in K-permeability, it is possible that the demyelination had exposed Na-channels in the nodal/paranodal membrane. In this case they must have another location relative to the K-channels (since there was no increase in P_{Na} in fibers with only nodal widening). In freeze-fractured axolemma of myelinated nerve from frog brain, particles similar to those in the node have been found in paranodal 'lakes', which could be aggregates of Na-channels[31,32] (see also Rosenbluth, this volume). The parallel is remarkable, but it should be noted – apart from the difference in fiber type and species – that the increase in Na permeability in 1 fiber was 5 times the normal, inferring a correspondingly large amount of particles in the axolemma outside the node.

The other alternative is that new Na channels had been formed, which implies a mechanism by which the channel molecules (likely to be proteins) are activated or built into the axolemma. The demyelinated region was not completely bare in these fibers but had a thin covering structure (fig. 3b) as in the early stage of remyelination, indicating that these fibers were at a later stage when new Na channels could have been formed. This could be the counterpart to the increased ferric ion-ferrocyanide staining in axolemma regions outside the node after demyelination[15]. This capacity to increase the Na permeability must be crucial for the appearence of continuous conduction after demyelination as well as for the formation of Na permeability sites in new areas of the axolemma in the remyelinated fiber with its closely spaced nodes – similarly these changes are probably not due to redistribution but to the appearence of new Na-channels.

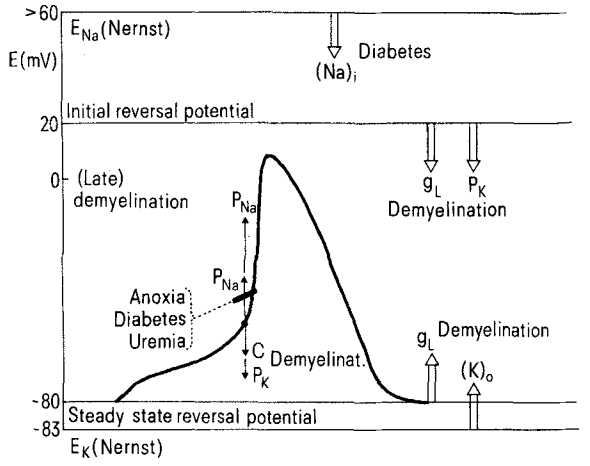

Figure 6. Diagram illustrating how the membrane action potential is influenced by pathological changes in the membrane properties. The ionic batteries (E_{Na} and E_K) set the theoretical limits for action potential size which are more narrow in nodes with axoplasmic Na accumulation (diabetes) or perinodal K-accumulation (demyelination). In practice the reversal potentials for the total initial current (after a positive potential step) and at steady state (= resting potential) are closer to zero potential because of leak of other ions. In demyelinated fibers the steady state P_K and g_L are especially high. The Na-battery is efficient only when the P_{Na} is large, excitation is prohibited by P_{Na} block and inactivation in conditions such as anoxia, diabetes and uremia. In some demyelinated fibers a large increase in P_{Na} compensates for the high P_K and large membrane capacitance (C).

Conclusion

Two major types of functional changes have been identified in pathological states of the node of Ranvier. One is the decrease in Na permeability (anoxia, diabetes mellitus and uremia), the other is the increase in capacitance, leak conductance, K-permeability and – most important – in Na-permeability associated with demyelination. A further analysis may show whether the physiological consequences of demyelination differ with respect to their cause (diabetes mellitus, inoculation of diphtheria toxin, anti galactocerebroside serum or lysolecithin) – that is, what factors can influence the 'compensatory' increase in the Na permeability or other phenomena which may be related to regeneration?

A schematic picture which summarizes the various pathological effects on the node and their immediate influences on the excitability is attempted in figure 6. The action potential is drawn in as a fluctuation of the membrane potential between the 2 theoretical limiting values (E_{Na} and E_K) defined by the Nernst equation and the respective ionic concentrations. Intracellular Na-accumulation (in e.g. spontaneously diabetic rat fibers) or K-accumulation in the nodal space (in some demyelinated fibers) will affect these levels. The total membrane current *reversal* potentials are, however, closer to zero potential due to the leak of ions (g_L) and – as for the initial current after a positive potential step – because of the steady state P_K, which

will keep down the maximum amplitude of the action potential. Therefore the pathological increase in g_L and P_K (in demyelinated fibers) will decrease excitability. Within the range set by the reversal potentials, the positive potential change depends on the size of P_{Na} (related to the product of the *maximum* P_{Na} and the resting value of h_∞) which was decreased in several conditions (anoxia, uremia, diabetes). In some instancies (with demyelination) the factors that hold back the positive potential change – high P_K and high nodal capacitance (C) – are unusually strong. In some of these fibers excitation can still be upheld by the large increase in P_{Na}. This is also the only efficient way to compensate for the increased current load in these conditions.

This picture is simplified: it is, for example, very likely that the resting potential of the node changes in pathological conditions which – mediated by changes in Na inactivation – will have a strong influence on membrane excitability. In practice this may take place in demyelinated fibers after a train of impulses, when the high P_K causes extracellular K-accumulation[3]. More elaborate models involving resting potential changes must be discussed with great caution, since already the resting potential of the normal (frog) fiber is more complex than an ionic battery and possibly dependent on active, electrogenic transport systems[1a].

And the perspectives? The present potential clamp studies seem like a few steps in a vast field of nerve fiber pathophysiology. But rather than mapping the features of various animal models of neuropathy, it ought to be important to carry on the studies on the 2 main pathophysiological mechanisms (decreased P_{Na} and changes related to demyelination), that now have emerged. More detailed and quantitative studies on the connection of function and structure will certainly be useful in studies of demyelination. Another line of future work may involve the attempt to integrate the knowledge of the individual node dysfunction into models of propagation and nervous signalling, thereby increasing our understanding of the symptomatology and the diagnostic possibilities in the neuropathies.

Acknowledgment. This work was supported by grants from the Foundations of the Karolinska Institute, the Swedish Diabetic Association and the Swedish Medical Research Council (project No. 14X-4255).

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0014-4754/83/090946-08$1.50 + 0.20/0

V. Intramembranous particle distribution in nerve fiber membranes

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What are intramembranous particles?

When tissue specimens, either fixed or fresh, are frozen into a vitreous state and then mechanically cracked open, the fracture tends to cleave the lipid bilayer of membranes into outer and inner leaflets[16]. The interior of the membranes thus exposed can be replicated with evaporated platinum, and the replicas of the two 'fracture faces', designated 'E' (external) and 'P' (protoplasmic) respectively, can then be studied by electron microscopy (fig. 1).

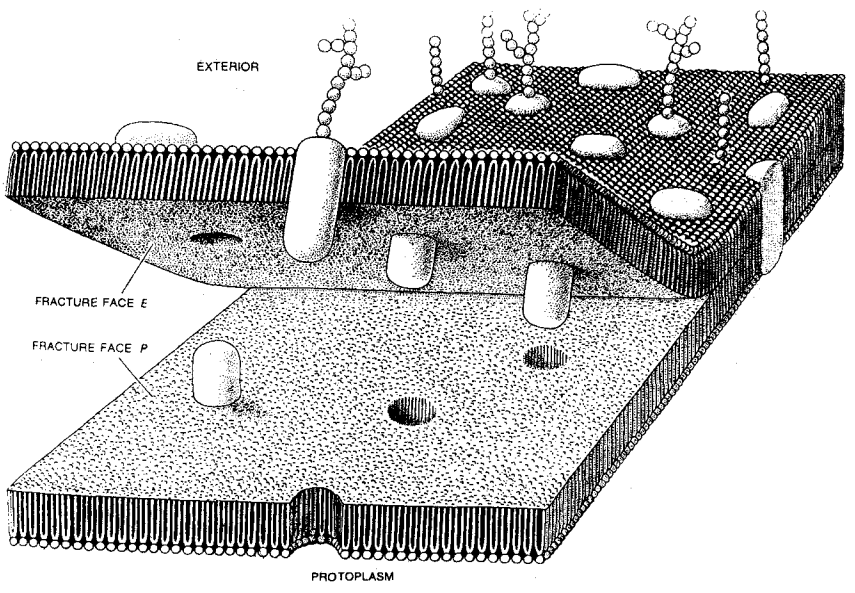

Figure 1. Diagram showing cleavage of a membrane bilayer into inner (protoplasmic) and outer (exterior) leaflets. Integral membrane proteins remain intact attached to one or the other leaflet and appear as 'particles' projecting from that fracture face with 'pits' in the complementary face. (From: 'The first steps in secretion' by B. Satir. Copyright 1975 by Scientific American, Inc. All rights reserved.)