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Reviews

Electrophysiology and morphology of myelinated nerve fibers

I. Introduction. The Ranvier node, past and future. A personal outlook after forty years of research*

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The modern physiology of single myelinated nerve fibers is based on work done by Tasaki[20] in the late 1930's; this work in turn influenced Professor von Muralt, who decided during World War II to begin research on single nerve fibers of the frog. Soon after becoming a member of his research team I also began to dissect fibers and to try electrophysiological experiments with them. After the International Congress of Physiology in Oxford, 1947, I was invited to join the Rockefeller Unit at Cambridge where Hodgkin and Huxley (HH)[8] were just developing their ionic theory and intended to test it on nerve fibers different from giant axons of the squid. Huxley and I first gave evidence of saltatory conduction[10] and then intended to find a method to measure transmembrane potentials in frog nodes. The circuit of the Poggendorf Null-Method we designed[11] has been, ever since then, the basis of all further work on transmembrane potential measurements and voltage clamp in myelinated nerve fibers. We showed that the resting membrane is not an ideal potassium electrode whereas the enormous increase of sodium conductance during the action potential (AP) temporarily makes it behave like a sodium electrode even if the resting conductance of potassium remains what it was during the resting potential (RP)[12].

Our method which - though unpractical and clumsy - gave excellent values of RP and AP was transformed by means of modern electronics into a fully automatic feedback system by Dodge and Frankenhaeuser[4], preventing flow of longitudinal current in the extracellular current pathway and providing current supply through the other internode. So the potentiostatic method used with the squid membrane, baptized 'voltage clamp' by HH[8], could be applied to single nodes by using one internode as current free voltage controlling pipette measuring the potential inside the nodal membrane, and the other internode as current carrying pipette of a feedback circuit. This method was later simplified and improved by Nonner[15]. A great number of papers have appeared using either one of the methods proving its usefulness and the utility of the node as a test object for comparing myelinated nerve fibers of vertebrates with the squid

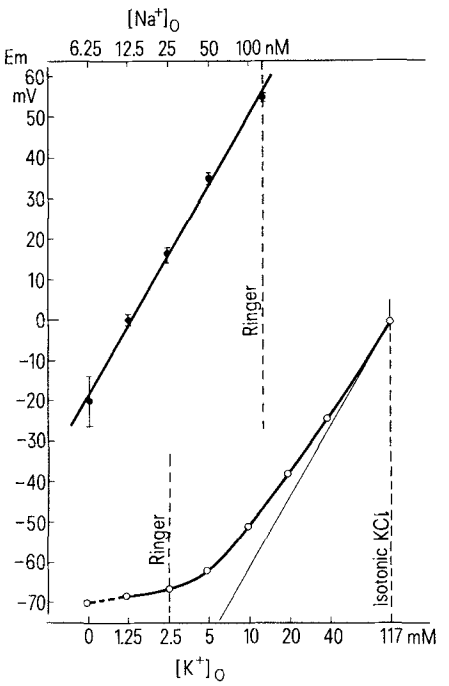

Figure 1. The dependence of the resting potential on $[K^+]_o$ and of the peak of action potential on $[Na^+]_o$ of a frog node. The values are compared with the theoretical linear relation of 58 mV slope for a 10-fold change of ionic activity. Redrawn from Huxley and Stämpfli[11].

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fiber. It is the merit mainly of Frankenhaeuser and his co-workers[4] to have shown to what extent the ionic theory[8] is also applicable to myelinated amphibian fibers. Their work with *Xenopus laevis* showed that permeability coefficients are more adequate than conductances to express their results, but the difference is small and not valid for *Rana pipiens* or possibly for other frogs[19]. As in squid, the main ionic current carrying pathways of the membrane are 1. leak conductance, 2. sodium channels, 3. potassium channels.

As in squid the first conductance is independent of the membrane potential whereas the second and third are nonlinear functions of voltage in which the activation and inactivation parameters obey first-order kinetics in most cases (see the chapter by W. Schwarz). According to Schwarz and Vogel[16] the concept of an inactivation process seemed applicable not only to sodium gating but also to the one of potassium even if it does not affect the action potential in any way comparable to sodium inactivation. Potassium inactivation (k_∞) however, at normal resting potential similar to h_∞ makes only about 80% of the potassium channels activatable. Therefore, hyperpolarization or a reduction of pH (own unpublished results) increase the activatable potassium current owing to the shift of the inactivation curve to the right (fig. 2). This is an early effect, observed in the first 10 sec after changing the pH. Only after longer equilibration in low pH solutions the decline of I_K described by others[2] is observed.

New results with sodium and potassium channels are treated in the following contributions by Schwarz, Brismar, Ulbricht and Neumcke. This article, therefore, does not intend to review the modern development of work on saltatory conduction[18] and on the nodal membrane. Having retired from my job and being uncertain as to how much time and opportunity I will have to follow up several questions which I would have liked to solve myself, I submit here a few problems to younger and more efficient specialists. May they take advantage of these recent observations and thoughts. The first one concerns leak currents: The leak conductance is usually associated with a leak electromotive force (e.m.f.) which has been discussed by several authors[5,6,13,19]. The conductance seems related to the density of channels in the membrane because amphibian and mammalian nodes which have very high current densities also have a high leak conductance compared to the squid. The leak pathway might therefore encompass the immediate vicinity of the globular proteins embedded in the membrane leaflets, where the phospholipids of the lipid matrix are likely to be in a fluid state and where the presence of protein molecules disturbs the regular array of the lipid matrix. The leak potential is thought to originate from a preference of the leak pathway for K^+ compared to other cations which are also able to cross it but to a lesser extent[6,19] (but see, also, Stämpfli[17]).

The leak current tends to increase in high external K^+ solutions but remains a linear function of voltage[3]. Most authors therefore use an analogue compensation to subtract the leak current without paying attention to its changed value. As figure 3 shows, however, this increased leak is not due to leak pathways but to selective potassium channels which can be blocked by tetraethylammonium (TEA) ions. If the voltage is clamped to the resting potential, these channels are not closed, probably because they are situated within

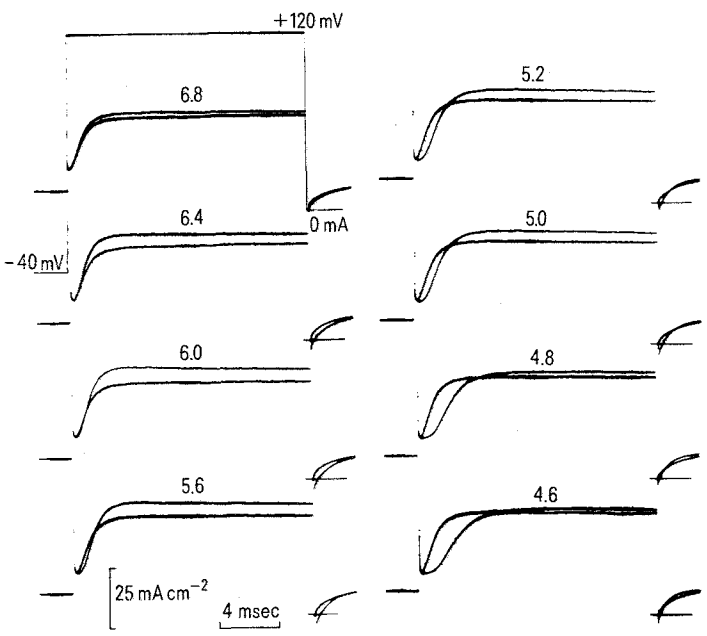

Figure 2. Comparison of potassium current I_K in pH 7 and in reduced pH solutions. The superimposed photographs are taken just before and 6 sec after switching to the pH indicated. Note increase of I_K and delayed turning on of potassium conductance g_K with lowering of pH due to the hyperpolarizing effect of reduced fixed negative charges on the external surface of nodal membrane. Exp. 59/82 motor fiber frog, 22 °C. All solutions contain 300 nM TTX, 1.8 mM $CaCl_2$ and 5 Mm MOPS buffer.

the spiral of paranodal myelin loops and are connected to the nodal gap through a high series resistance. The leak conductance in isotonic KCl can be reduced to approximately the same value as in Ringer's by shifting the holding potential V_H by -20 to -40 mV. Such channels could also be responsible for the very big K-current fluctuations observed in isotonic KCl solutions at $V_H=0$. As the paranodal channels are not clamped to the resting potential but through the series resistance to a more positive value, they may escape the clamp, suddenly depolarize to zero, inactivate, recover to an activatable state by repolarization and continue to show in a random fashion regenerative depolarization[17]. The resulting K-current fluctuations can be reduced either by more negative holding potentials or by TEA, which corroborates this explanation. It would be worthwhile to find ways of uncovering the myelin loops and to prove that in this case the leak conductance does not change in isotonic KCl in frog nodes. K-current fluctuations should then be reduced as well. Such proof would, in my opinion, point to drawbacks in the study of potassium channels by fluctuation analysis in nodes of Ranvier. There might be results indicating another population of potassium channels which, however, could be ordinary channels with a high series resistance.

The reversal potential of the leak current, if only arising by a preference of the leak pathway for K^+ ions should be zero in isotonic KCl. But under these circumstances the leak I(V)-curve is not crossing the V-axis at zero reversal potential but at considerably more negative values (fig. 3). Furthermore, there is in current clamp experiments a marked hyperpolarization if the nodal potassium channels are blocked by 12 mM external TEA (fig 4). In voltage clamp at $V_H=0$ the inward current necessary to maintain the resting potential is strongly reduced by TEA. If the membrane is clamped to $V_H=+70$ mV, where in isotonic KCl no current is needed to keep the potential constant, addition of TEA to isotonic KCl produces outward currents. This could be explained by assuming an e.m.f. in parallel with the membrane which is not influenced by external K^+ and which tends to repolarize the membrane as soon as the membrane impedance rises by blocking potassium channels. This effect is not specific for TEA and thus cannot be attributed to a TEA permeability of the depolarized membrane. Ordinary inactivation of potassium channels which occurs after a step change to isotonic KCl (fig. 4) produces an increase of mem-

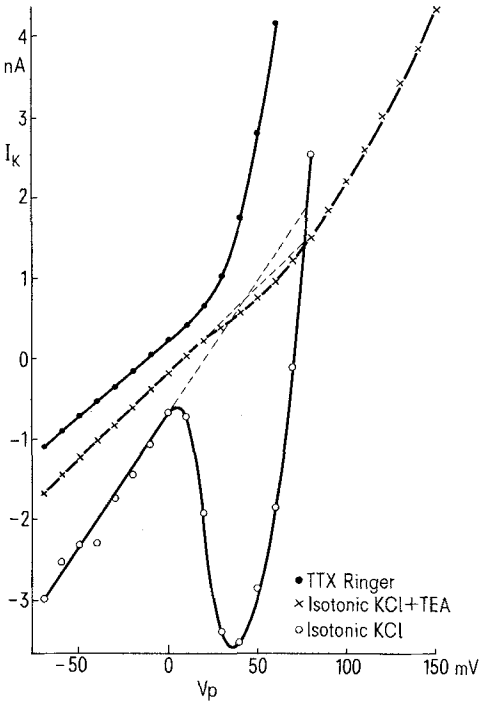

Figure 3. Lower part of uncorrected current-voltage curves in Ringer and isotonic KCl without and with 12 mM TEA, all solutions with 300 nM TTX. Note irregular leak values (due to increased current fluctuations) and steeper slope in normal isotonic KCl and disappearance of both if TEA is added. The leak conductance g_L is then almost the same as in Ringer but 12 mM TEA does not block all channels as revealed by inward and outward current deviation from linear relationship. g_L is 37 $mScm^{-2}$ in Ringer, 66 $mScm^{-2}$ in KCl and 42.4 $mScm^{-2}$ if TEA is added. The corresponding maximum K conductances $g_{K_{max}}$ are 370 $mScm^{-2}$ in Ringer and 620 $mScm^{-2}$ in KCl. Exp. 6/83 sens. fiber, 19 °C.

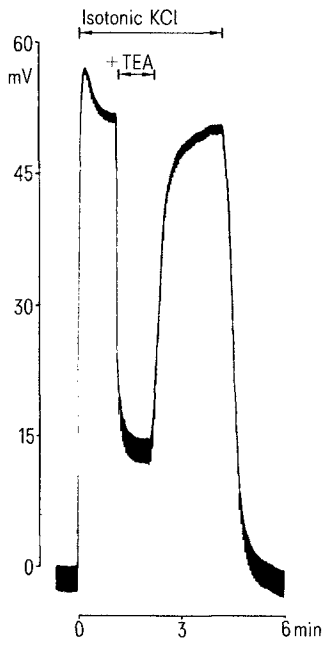

Figure 4. Current clamp record of nodal potential change in isotonic KCl solution (with 1.8 mM $CaCl_2$). The first depolarization is reduced by inactivation which produces a repolarization of 5 mV and an increase of membrane resistance. Addition of 12 mM TEA to the isotonic KCl yields a further rapid repolarization by 37 mV. Returning to TTX Ringer brings the membrane to 66 mV instead of 65 mV. Note resistance changes indicated by hyperpolarizing pulses of 10 mV and 16 msec duration (gives not full deflection owing to inertia of ink writer). All solutions contain 300 nM TTX, isotonic KCl: 117 mM KCl, Ringer: 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$ and 5 mM MOPS buffer at pH 7.2, temperature 19 °C, motor fiber 88/82.

brane resistance and accordingly a hyperpolarization. 4-Aminopyridine (4-AP) has a similar effect – it is less efficient than TEA owing to its potential dependent action.

My conclusion is that the nodal membrane is under the influence of an e.m.f., which so far was called leak potential but which may have a different origin. My guess is that the myelin by its structure and by continuous action of the resting potential has reached an ionic distribution producing an e.m.f. which, owing to the high resistance of the myelin sheath, is not immediately affected by either action potential or changes of external K^+. The paranode where the great number of myelin layers reach the neurilemma and undergo junctions of several types with it[14] may be the region where the myelin potential can influence the nodal membrane potential if the membrane resistance reaches values similar to or higher than the internal resistance of the paranodal myelin battery.

Such a mechanism may be much more important for the repolarization of action potentials than the delayed rectification of potassium channels in the nodal membrane. It must be particularly efficient in mammalian fibers where the nodal membrane is practically devoid of potassium channels[9]. Repetitive activity in sensory fibers might be induced by a shortening of the AP by fast repolarization due to this mechanism and faster availability of a sufficient number of activatable sodium channels to overcome leak and potassium outward currents in long lasting depolarization. A comparative study of the leak reversal potential in motor and sensory fibers should be made. Comparison of the paranodal morphology of sensory and motor fibers is necessary because of the still uncertain origin of their different electrophysiological behavior[19]. One working hypothesis might be that sensory fibers have less potassium channels in the nodal neurilemma but more within the paranodal loops. This would explain why TEA does not appreciably depolarize sensory nodes and why the increase of delayed rectification begins only at stronger depolarizations (when the paranodal potassium channels can contribute to outward K^+ current by becoming activated despite of the high series resistance)[19].

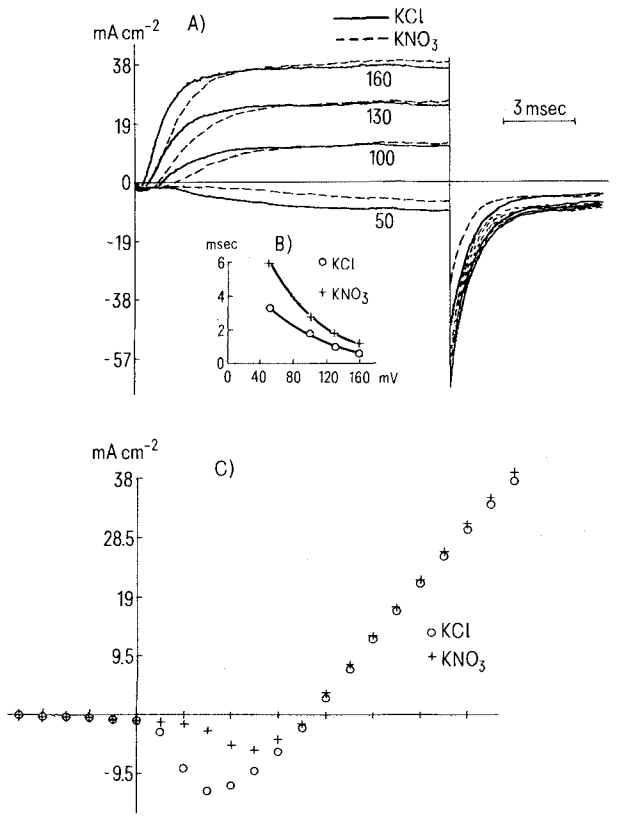

Figure 5. Effect of substitution of NO_3^- for Cl^- in isotonic K solution. *A* Potassium currents I for potential clamps V to 160, 130, 100 and 50 mV, full lines Cl^-, hatched lines NO_3^-. *B* Time of current rise to half maximum value for these 4 pulse potentials after subtraction of initial delay of 0.295 msec for KCl and 0.579 for KNO_3. *C* IV-curve. Note: Shift of inward current maximum to the right in *C*, changed kinetics in *A* and *B*. Both solutions contain 1.8 mM $CaCl_2$, 5 mM MOPS buffer at pH 7.2., 300 nM TTX and 117 mM KCl or KNO_3. Exp. 11/83, motor fiber at 20 °C. Holding potential $V_H = -30$ mV.

A last observation deals with recent experiments on substitution of non-diffusable anions for chloride: In isotonic potassium solutions with NO_3^- or $CH_3SO_3^-$ the 'on' kinetics of potassium currents is slowed by a factor of more than 2 while the inward tails are not influenced. The maximum potassium current I_K is equal to or bigger than in Cl^--Ringer, the K conductance-voltage $g_K(V)$ curve is shifted to the right by 30–40 mV and the initial delay increases (fig. 5). This effect of external anions is completely different from the one described by Dani et al.[1] (1983) and Koppenhöfer[13] on sodium currents. It is also observed, but to a lesser extent, in Ringer's solution with Na-methylsulphate or $NaNO_3$ instead of NaCl. To my surprise nobody seems to have observed this striking difference between sodium and potassium channels which indicates that besides the shift of activation and inactivation curves for potassium channels to the right by reducing the number of negative fixed charges by protons (fig. 2) another similar effect is obtained by substituting non-diffusible anions for Cl^-. There is no doubt that the phenomenon is reproduceable and needs an explanation. Frankenhaeuser[3] was unable to detect a change of the chloride permeability in *Xenopus* fibers, although he substituted Na-methylsulphate for NaCl. My own measurements with *Rana esculenta* showed in methylsulphate Ringers a clear slowing of the 'on' kinetics of currents but this was much less pronounced than in isotonic potasium methylsulphate. There might be a pathway for Cl^- ions coupled to the opening of potassium channels permitting a short and fast inflow of Cl^- which speeds up the current rise so far believed to be potassium current only. Or the absence of Cl^- permits stronger screening

of fixed negative charges by Ca^{++} or protons. As the shift of g_K curve vs V is 20–40 mV such an effect should shed light on some particular chemical structure in the immediate vicinity of the potassium channel. These few observations may serve others in the attempt to fill the gap which still exists in the full understanding of the functions of the complicated nodal structure. In my opinion, the simple views of an easily accessible bit of excitable membrane with clear delimitations by a myelin-covered inexcitable membrane in the paranodal region does need some amendments. The role of the paranodal loops of myelin as well as the one of the microvilli of Schwann cell cytoplasm is not yet understood and needs elucidation. It is probable, however, that most of the work done on sodium channels is valid. Potassium channels in warmblooded and poikilothermic animals are more difficult to approach and further experimental work is needed into, among other things, the leak problem mentioned in this paper.

* Dedicated to Professor Alexander von Muralt on the occassion of his 80th birthday.

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II. Sodium and potassium channels in myelinated nerve fibers

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The role of sodium and potassium channels in the excitability of the node of Ranvier

The time course of an action potential is determined by time- and voltage-dependent charge movements within and across the cytoplasmic membrane. By means of the voltage-clamp technique it was demonstrated that membrane currents in the node of Ranvier of *Xenopus laevis* can be described by equations (see e.g. Stämpfli and Hille[33]) very similar to the description of membrane current in squid giant axons developed by Hodgkin and Huxley[17]. Also for myelinated nerve fibers of other vertebrates the same kinetic description with only slight modifications can be applied to the membrane currents[7,12]. In general, the total membrane current in the node of Ranvier consists of 4 components:

$$I_{tot} = I_{Na} + I_K + I_C + I_L;$$

in addition to sodium- and potassium-specific currents (I_{Na} and I_K, respectively), capacity current I_C and unspecified leak currents I_L are involved. A further component I_P has been introduced by Fran-