Effects of Some Potassium Channel Blockers on the Ionic Currents in Myelinated Nerve

E. W. BETHGE[1], K. H. BOHUSLAVIZKI[2], W. HÄNSEL[3], A. KNEIP[2] and E. KOPPENHÖFER[2]

1 *Department of Physiological Sciences, Valley University, Cali, Columbia*
2 *Institute of Physiology, University of Kiel, Olshausenstr. 40, 2300 Kiel, Germany*
3 *Institute of Pharmacy, University of Kiel, Gutenbergstr. 76—78, 2300 Kiel, Germany*

Abstract. The effects of some potassium channel blockers on the ionic currents and on the so-called K^+-depolarization in intact myelinated nerve fibres were studied. 4-AP, and in particular, Flaxedil, proved to be selective K^+-current blockers. However, TEA, a crown ether (DCH18C6), a longchained triethylammonium compound (C_{10}-TriEA), capsaicin, and the extract from the medicinal herb *Ruta graveolens* proved not to be selective K^+-current blockers; they all block Na^+-currents as well, although to a lesser extent. The sodium inactivation curve did not change under TEA and Flaxedil but was shifted on the potential axis in *negative* direction by DCH18C6, 4-AP, capsaicin and the *Ruta* extract whereas C_{10}-TriEA caused a shift of both sodium inactivation and activation parameters in *positive* direction. Regarding to the kinetics of the persisting K^+-current fraction, two different kinds of blockade were found: 1. Unchanged K^+-kinetic which is typical for the effects of TEA, 4-AP, Flaxedil, and C_{10}-TriEA. 2. Clearly changed K^+-kinetic, characterized by K^+-transients; which is typical for the effects of capsaicin and in particular, for those of DCH18C6 and of the *Ruta* extract. The possibly different modes of action of both groups of blockers are discussed in terms of current models for the action of potassium channel blockers.

Key words: Node of Ranvier — Ionic currents — K^+-channel blockers — K^+-transients — K^+-depolarization

Introduction

Selective pharmacological blockers of either sodium or potassium ionic currents are considered useful tools for a better understanding of the function of the

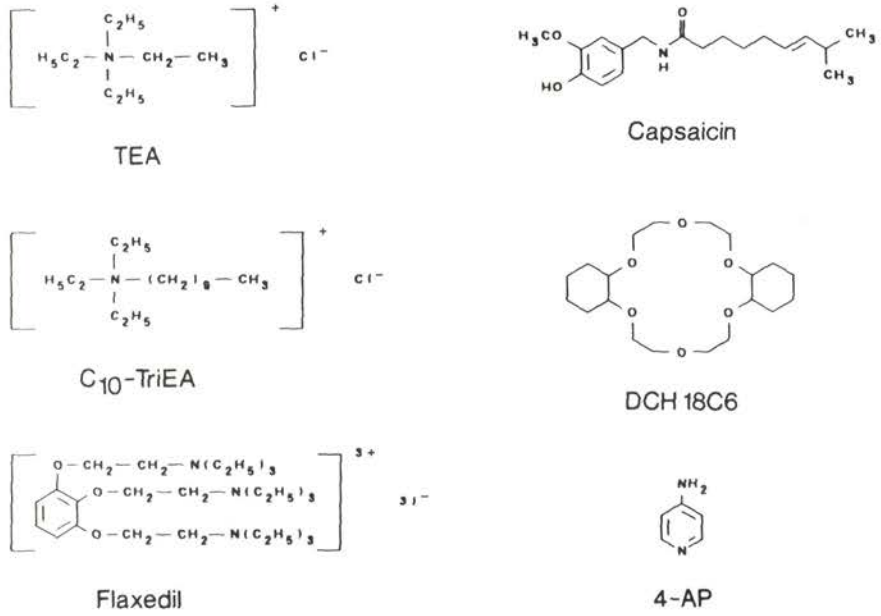

Fig. 1 General molecular structures of the compounds tested.

potential dependent ionic channels in nerve membrane. From a theoretical point of view potassium channel blockers are of great interest because of their dual effects on action potential and resting potential.

Apart from this, recent clinical interest in factors influencing nerve conduction defects in demyelinating diseases, e. g. multiple sclerosis (MS) and a variety of peripheral neuropathies, led to attempts to improve nerve function by the use of the potassium channel blocker 4-aminopyridine (Jones et al. 1983; Davis et al. 1986; Stefoski et al. 1987). The reason is that, although 4-aminopyridine has minimal effects on the action potential waveform of intact mammalian nerve fibres (Schwarz and Eikhof 1987), it has dramatic effects on spike waveform following demyelination (Sherrat et al. 1980; Targ and Kocsis 1985) because plenty of potassium channels are abnormally exposed in demyelinated nerve fibres (Chiu and Ritchie 1982; Grissmer 1986; Chiu and Schwarz 1987; Jonas et al. 1989). They may either "tend to hold the membrane close to the potassium equilibrium potential" (Waxman 1987) or otherwise, they may give rise to a decrease in membrane potential by increasing the external potassium concentration in the direct vicinity of the membrane. At any rate, nerve function would

get rendered difficult (Brismar 1981). Therefore, potassium-channel blocking agents could possibly serve as a symptomatic therapy in MS (Bostock et al. 1981), all the more since, in experimentally demyelinated nerve fibres, the bared axonal membrane remains capable of conducting action potentials (Bostock and Sears 1978).

The aim of the present study was to evaluate the effects of some known potassium channel blockers (see Fig. 1) in the light of current hypotheses on the time course of potassium currents and in connexion with our working hypothesis on malfunction in demyelinated axons (Bautz et al. 1989b).

We carried out *in vitro* experiments on the effects of tetraethylammonium ions (Hille 1967; Koppenhöfer 1967; Schönle and Koppenhöfer 1983), some long-chained triethylammonium compounds (Armstrong 1971; Swenson 1981), Flaxedil (Smith and Schauf 1981), capsaicin (Dubois 1982), the crown ether dicyclohexano-18-crown-6 (Århem et al. 1982; Kristbjarnarson and Århem 1985), 4-aminopyridine (Pelhate and Pichon 1974; Ulbricht et al. 1982) and in addition, of a still unknown potassium current blocker derived from the medicinal herb *Ruta graveolens*. Extracts of *Ruta* are believed to exhibit positive effects on the clinical symptoms in MS (V. Carstens, personal communication).

Parts of the results have been published previously (Bohuslavizki et al. 1988; Bautz et al. 1989a).

Materials and Methods

Chemicals and solutions: Tetraethylammonium chloride (TEA) was purchased from Merck (Darmstadt, Germany), gallamine triethiodide (Flaxedil) from Aldrich-Chemie (Steinheim, Germany), 8-methyl-N-vanillyl-6-nonenamide (capsaicin), dicyclohexano-18-crown-6 (DCH18C6) and 4-aminopyridine (4-AP) were obtained from Sigma Chemie (Deisenhofen, Germany). All drugs including the *Ruta* extract (see below) were added substance to the respective bathing medium except of capsaicin and DCH18C6 of which stock solutions, made by ethanol of 10 mmol/l and 400 mmol/l respectively, were diluted by the normal bathing medium to give the final concentrations. The ethanol effects *per se* were negligibly small (Århem and van Helden 1983). Measurements were not started until the drug effects became stationary. This was regularly the case after 1 to 3 minutes. The duration of subsequent wash-out intervals was chosen to obtain best reversibility. It varied between less than 1 minute (TEA, Flaxedil, *Ruta* extract) and one hour (DCH18C6). All drug effects in this study proved to be fully reversible if not noted otherwise. The effects of 4-AP are known for being not reversible (Århem and Johansson 1989); therefore, wash-out was not tried.

The normal bathing medium was either Ringer solution (containing in mmol/l: NaCl 107.0; KCl 2.5; $CaCl_2$ 2.0; N, N-bis (hydroxyethyl)-2-amino-ethanesulfonic acid/NaOH buffer (BES) 5.0) or a K^+-rich solution (KCl: 80 mmol/l) in which the required amount of Na^+ was replaced by the equivalent amount of K^+. The artificial intracellular fluid contained (in mmol/l): KCl 108.0; NaCl 5.0; BES 5.0. The pH of all solutions was adjusted to 7.2 ± 0.1 at 10.0 ± 0.5 °C.

Synthesis of triethylammonium compounds: One part alkyl bromide and 1.2 parts triethylamine dissolved in nitromethane were refluxed for 1 hour, allowed to cool to room temperature and then

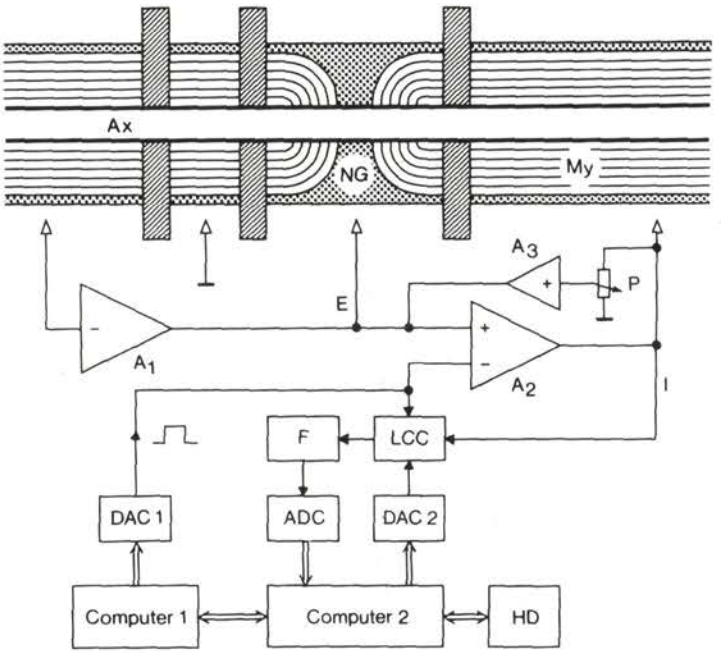

Fig. 2 Schematic diagram of the experimental setup. The nerve fibre (*top*) is laid across four fluid pools of the recording chamber (not shown) which are separated by thin vaseline seals (hatched areas) and connected via conventional electrodes (open triangles) to control circuits formed by operational amplifiers A_1, A_2 and A_3. Ax: axis cylinder bounded by the axonal membrane (heavy border line). My: myelin lamellae enwrapping the axis cylinder except the region of the Ranvier node under investigation (central pool) where the myelin is absent. Stippled areas: simplified Schwann cell structures. NG: nodal gap substance, in the running text termed "series resistance". *P*: adjustment of electronic compensation for the voltage drop across the series resistance. *E*: membrane potential. *I*: membrane current. ⊓ : command pulses. DAC 1 and DAC 2: digital/analog-converters. ADC: analog-digital converter. F: switchable low-pass filter ($f = 80$, 40, 20 kHz, respectively). LCC: leakage current subtractor. HD: hard disk. For further details, see text.

stirred for 24 hours. After evaporating of the solvent under reduced pressure the product was washed with dry ether and recrystallized from a mixture of dry ethanol and ethylic acetate. The dried compounds were tested for purity using thin-layer chromatography.

Treatment of Ruta plant material: For our studies the plant material (stems and leaves) was collected on Ibiza (Spain) and air-dried. The powdered material (a total of 463 g) was macerated with petroleum ether for one day and thereafter, for 5 days with methylene chloride. The solvent was removed in a rotavap. Finally, the obtained paste-like extracts were pooled for drying on a thin layer of kieselgur.

Statistics: The significance of effects was tested on the $\alpha = 0.05$-level by the two sided statistical sign test (Dixon and Mood 1946). Results are given as medians and ranges throughout (Sachs 1984; Koppenhöfer and Bohuslavizki 1988).

Preparation: The experiments were carried out on isolated intact myelinated nerve fibres from the sciatic nerve of the toad *Xenopus laevis*. The dissection procedure (Koppenhöfer et al. 1987) delivered nerve fibres of 24.3 μm in diameter (18.9 to 29.7 μm; $n = 36$) which were mounted across four fluid pools of a recording chamber (see Fig. 2. *above*) in a fashion that the Ranvier node under investigation was placed in the central pool which was continuously perfused by the respective bathing medium. The lateral pools containing the cut ends of the axon were filled with artificial intracellular fluid.

The rationale of mechanical manipulations of the nerve fibres was that any artificial stretch i.e. any loss of reserve length (Clarke and Bearn 1972; Koppenhöfer et al.1987) of the fibres was strictly avoided up to the very end of measurements. Consequently, the preparations survived for 2.5 hours (1.4 to 7.2 h) without clear indications for "rundown" (Fox 1976). The unspecific changes of steady state potassium currents, leakage currents, and of the so-called series resistance (see below) with time amounted to -9 %/hour (-30 to 16 %/h), 14 %/hour (-11 to 142 %/h), and 5 %/hour (-12 to 42 %/h), respectively ($n = 36$). No significant changes of peak sodium currents with time were detectable.

Experimental setup: Measurements of membrane potentials and membrane currents were carried out by means of an improved version of the Frankenhaeuser potential clamp system (Dodge and Frankenhaeuser 1958) which enabled compensation for the ohmic component of the electrical resistance of the Schwann cell structures (see Fig. 2, NG) in series with the nodal membrane, i.e. the so-called series resistance (Wiese and Koppenhöfer 1988). For optimum compensation the potentiometer P was set to give symmetrical peak sodium current-voltage relations in Ringer solution; this corresponded to a median series resistance of 413 kΩ (224 to 916 kΩ; $n = 36$). A_1 and A_2 are operational amplifiers which were connected to the respective fluid pools of the recording chamber *via* Ag/AgCl electrodes (Fig. 2, open triangles), thus forming two feedback loops. E and I represent membrane potentials and membrane currents, respectively. The rectangular symbol ($\sqcap$) stands for command pulses which enable membrane potential steps. Regarding the benefit of long-lasting experiments such axons are obviously superior to those which were available previously (see, e.g. Koppenhöfer and Vogel 1969; Keana and Stämpfli 1974).

Measuring conditions: The holding potential, E_H, was adjusted under the assumption of a steady state value of the sodium inactivation variable $h = 0.8$ (see page 234). For current-voltage relations (see page 230) various positive test pulses were preceded by negative prepulses of sufficient amplitude and duration (see Fig. 5D); thus at the beginning of the test pulses h was unity (Frankenhaeuser 1959). For sodium inactivation curves the potential dependence of the steady state value of the inactivation variable, h, was measured by peak sodium currents, peak I_{Na}, during constant test potentials preceded by conditioning prepotentials E_p of variable amplitude and sufficient duration (see Fig. 7*D*).

Calibrations:Specific currents were calculated from membrane current records and from the fibre dimensions according to the passive standard data of myelinated nerve fibres (Stämpfli and Hille 1976).

Data processing: The data processing system shown in Fig. 2 was controlled by two interconnected computers. Command pulses of variable duration and amplitude of either polarity were generated by a 12 bit D/A-converter (DAC 1) under the command of computer 1 at a repetition rate of about 1 Hz if not stated otherwise. Membrane currents, I were corrected automatically for leakage

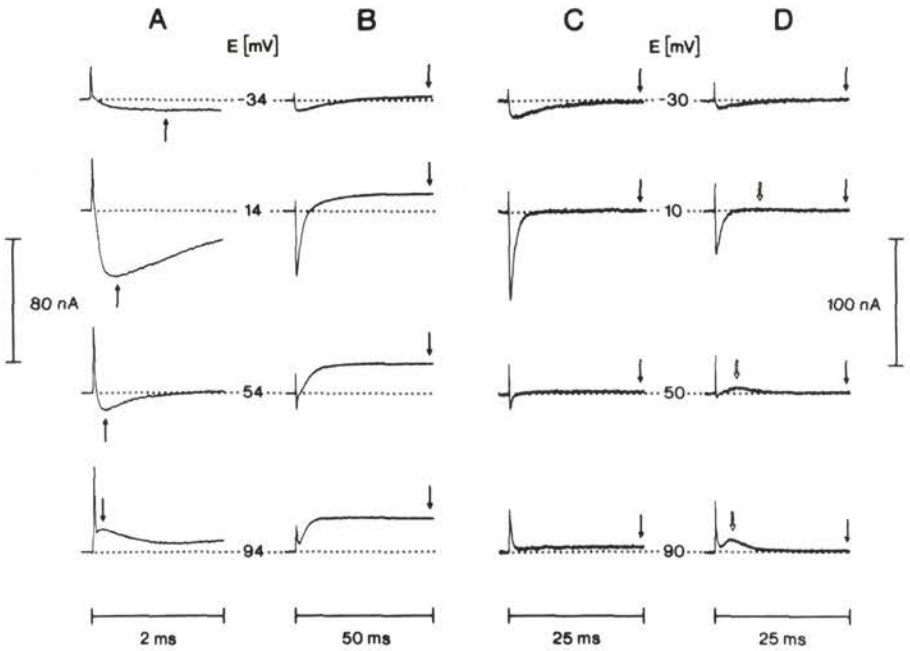

Fig. 3 Time course of membrane current records, elicited by steps to various membrane potentials. *E*. *A* and *B*: records at two different time scales in normal Ringer solution. Arrows: peak sodium currents, peak I_{Na}, (*A*) and steady state potassium currents, $I_{K\,ss}$, (*B*) respectively. *C*: time-idependent depression of $I_{K\,ss}$ (solid arrows) by Flaxedil (1 mmol/l). *D*: time-dependent depression of potassium currents by *Ruta* extract, resulting in barely visible steady state values, $I_{K\,ss}$, (solid arrows) which are preceded by larger potassium transients, peak I_K, (open arrows). Dotted line: zero membrane current.

currents assuming a time and potential independent leakage conductance (Dodge and Frankenhaeuser 1958) by LCC (driven by computer 2 *via* DAC 2) and filtered by a low-pass fourth-order Bessel filter (F) of switchable corner frequency. Deliberate filtering avoided aliasing effects (Azizi 1983) and ensured decent signal to noise ratios without visible indications for the so-called over-filtering. The filtered membrane currents were digitized by a 12 bit A/D converter (ADC) at a minimum of 4 μs intervals and stored on a hard disk (HD) together with the respective pulse parameters delivered by computer 1, both under program control by computer 2.

Results

Current-voltage relations. Membrane currents in Ranvier nodes change with membrane potentials in a characteristic fashion (Dodge and Frankenhaeuser 1958). Fig. 3 shows a set of current records elicited by steps to different

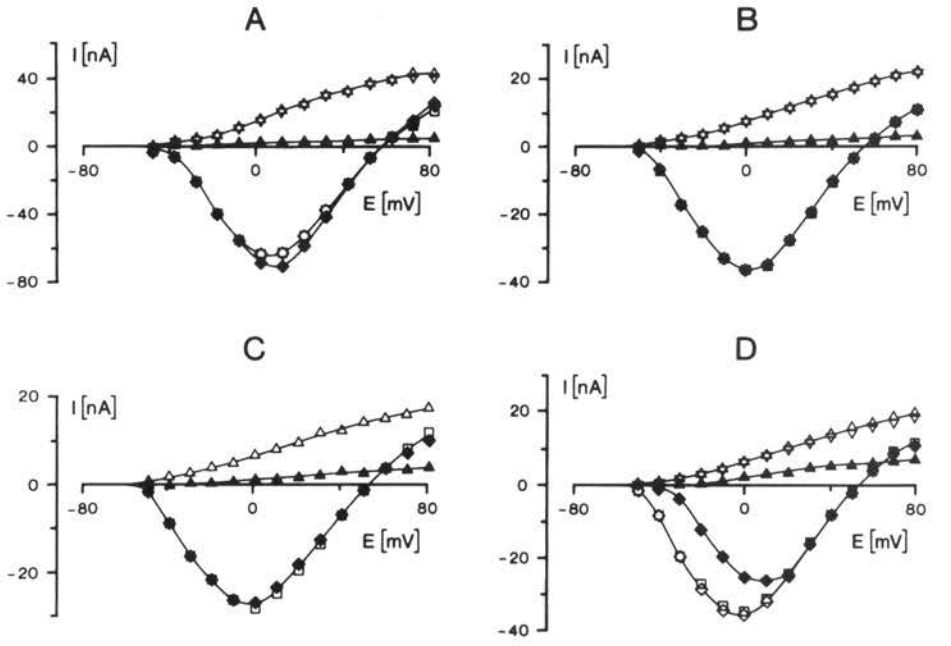

Fig. 4 Current-voltage relations of peak sodium currents (squares and diamonds) and of steady state potassium currents (triangles) before and after application of test solutions (open symbols). Test solutions (filled symbols) in mmol/l: *A*: TEA (10.0); *B*: Flaxedil (1.0); *C*: 4-AP (0.050); *D*: C_{10}-TriEA (1.0). Abscissae: membrane potential during test pulse, *E*. Ordinates: corresponding ionic currents, *I*. Holding potentials (in mV): *A*: −77; *B*: −80; *C*: −79; *D*: −80. The curves were obtained by spline interpolation (Wiese and Koppenhöfer 1988). Note that cumulative effects of 4-AP were minimized by prolonging test pulse intervals to 1 min. Pulse protocol used, see Fig. 5*D*.

membrane potentials at two different time scales in normal Ringer solution (*A*, *B*). The initial transient (*A*) is carried mainly by sodium ions; this follows from the fact that its peak value, peak I_{Na} (arrows), changes its direction as soon as the test pulse potential exceeds the sodium equilibrium potential (56 mV; 39 to 77 mV; $n = 68$). Following the Hodgkin-Huxley-Frankenhaeuser formalism (Hodgkin and Huxley 1952; Frankenhaeuser and Huxley 1964) sodium currents are described by potential- and time-dependent variables, the activation variable m and the inactivation variable h. Both of them change with membrane potential between zero and unity.

The initial sodium transient is accompanied by a delayed potassium current (Fig. 3*B*), with its steady state value, $I_{K\,ss}$ (arrows), increasing monotonously with the increasing test potentials. Thus, under normal conditions or under potassium blockers like Flaxedil (Fig. 3*C*) potassium currents are satisfactorily

described by a single variable, the activation variable n. However, there are potassium blockers like the *Ruta* blocker (Fig. 3*D*) which seem to act in a time dependent manner: the remaining potassium currents exhibit initial transient peak values, peak I_K, (open arrows) followed by comparatively smaller steady state values, $I_{K\,ss}$, (filled arrows).

The potential dependences of peak I_{Na} and $I_{K\,ss}$ are documented in the shapes of the current-voltage relations (Fig. 4). In normal Ringer solution (open symbols) and under proper measuring conditions the absolute slope of the two branches of the peak I_{Na}-voltage relation (squares and diamonds) was basically the same as intentionally adjusted by proper setting of potentiometer P (see page 229). Under 10 mmol/l TEA (Fig. 4*A*) the potassium currents at $E = 80$ mV were reduced by 86 % (75 to 91 %; $n = 9$). A similar reduction was achieved by Flaxedil (Fig. 4*B*) even at 1 mmol/l (85 %; 73 to 89 %; $n = 11$). Moreover, Flaxedil offers a real opportunity to selectively block potassium currents in Ranvier nodes without any visible changes of the peak sodium current-voltage relation. This does not hold for TEA because the minimum of the corresponding peak I_{Na}-voltage relation increased by 3 % (−3 to 12 %; $n = 9$). These findings are in accordance with observations of previous investigations (Hille 1967; Koppenhöfer 1967; Smith and Schauf 1981; Schönle and Koppenhöfer 1983). The block of potassium currents by 4-aminopyridine is mostly irreversible and clearly voltage dependent (Meves and Pichon 1977; Ulbricht et al. 1982; Århem and Johansson 1989). As it can be seen from Fig. 4*C*, 4-AP blocks potassium currents as selectively as does Flaxedil (Fig. 4*B*) at lower concentrations (0.05 mmol/l) but in an irreversible manner (not shown). In 7 experiments the reduction of $I_{K\,ss}$ at $E = 80$ mV amounted to 70 % (63 to 76 %).

It has been reported that a variety of triethylammonium compounds produce a marked time-dependent block of potassium currents in the unmyelinated axon, decyl-triethylammonium being already efficacious at a few micromolar (Swenson 1981). Hexyl-triethylammonium to undecyl-triethylammonium, phenylpropyl-triethylammonium, and benzyl-triethylammonium were tested and found to be several hundred times less efficacious in the nodal membrane than it has been reported for the unmyelinated giant axon (Armstrong 1971; Swenson 1981). In 8 experiments 1 mmol/l C_{10}-TriEA (Fig. 4*D*) reduced $I_{K\,ss}$ at $E = 80$ mV largely time-independent (also see Armstrong and Hille 1972) by 62 % (44 to 73 %). Moreover, chains longer than C_8 clearly shifted the negative branch of the peak sodium current-voltage relation in positive direction. For C_{10}-TriEA the shift amounted to 8 mV (6 to 12 mV; $n = 8$), the minimum of the curve being diminished by 31 % (24 to 39 %).

The observed shift of the experimental values in *positive* direction initiated calculations on the underlying potential dependence of the steady state value of the activation variable, m. We used the constant field equation, introduced by

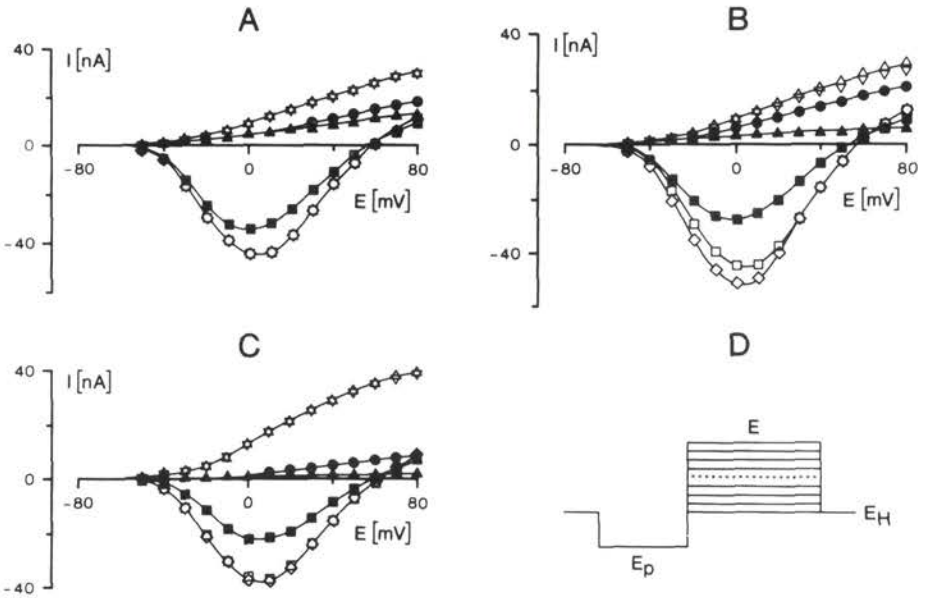

Fig. 5 Current-voltage relations of peak sodium currents (squares and diamonds) and of steady state potassium currents (triangles) before and after application of test solutions (open symbols). Test solutions (filled symbols): *A*: capsaicin (50 μmol/l); *B*: DCH18C6 (50 μmol/l), test pulse intervals: 2 s; *C*: *Ruta* extract (corresponding to 2 g plant material/100 ml); Abscissae: membrane potential during test pulse, *E*. Ordinates: corresponding ionic currents, *I*. *D*: pulse protocol used (not drawn to scale). *E*: test potentials of various sizes preceded by constant prepotentials E_p. Dotted line: zero membrane potential. Holding potential E_H: -80 mV throughout. The curves were obtained by spline interpolation (Wiese and Koppenhöfer 1988).

Dodge and Frankenhaeuser (1959), in the potential range of biological relevance (Albers et al. 1989) for the calculation of peak P_{Na} from peak I_{Na}. The equation

$$\text{peak } P_{Na} = \text{peak } P_{Na\,max} \left[\frac{1}{1 + \exp[(E_m - E)/k_m]}\right]^a \qquad (1)$$

(Benoit and Dubois 1987; Albers et al. 1989) was fitted to the data. Assuming both the ratio of the time constants of sodium currents and the power of the activation term, $a = 2$, to be potential independent (Frankenhaeuser 1960), and after normalizing the data by the theoretical maximum of peak P_{Na}, peak $P_{Na\,max}$, we obtained the so-called activation curve. In normal Ringer solution the potential at $m = 0.5$, E_m, and the maximum steepness of the curve, k_m, corres-

ponded to data presented previously (Albers et al. 1989), $E_m = -23$ mV (-26 to -19 mV; $n = 8$) and $k_m = 14$ mV (13 to 16 mV). Under C_{10}-TriEA, however, the curve was shifted in *positive* direction by 10 mV (7 to 11 mV; $n = 8$) as it was expected from the underlying current-voltage relation shown in Fig. 4*D*.

Capsaicin, the pungent principle in certain species of red pepper (*Capsicum sp.*), has already been used as a tool to separate different types of potassium channels (Dubois 1982; Röper and Schwarz 1989). In 16 experiments nominal 50 μmol/l capsaicin reduced $I_{K\,ss}$ (at $E = 80$ mV) by 51 % (36 to 67 %) and, to a lesser extent, the minimum of the peak I_{Na}-voltage curve by 22 % (15 to 36 %). Both effects are shown in Fig. 5*A*. The block of potassium currents occurred in a time-dependent manner similar to the records shown in Fig. 3*D*; thus, the steady-state values at the end of the pulses, $I_{K\,ss}$, (Fig. 5*A*, filled triangles) were systematically smaller than the preceding potassium transients, peak I_K, (filled dots) which as compared to $I_{K\,ss}$ in the untreated node were reduced by 36 % (21 to 47 %), respectively. In this respect the capsaicin block obviously resembles the effects of crown ether and of the *Ruta* potassium current blocker (see below). However, under capsaicin the difference between $I_{K\,ss}$ and peak I_K varied from experiment to experiment between nil and a number considerably larger than that shown in Fig. 5*A*; what this was due to, remained unclear.

From several crown ethers DCH18C6 has been found to be the only substance to have measurable effects (Århem at al. 1982). The potassium currents were reduced by DCH18C6 (50 μmol/l) in a clearly time-dependent manner (Fig. 5B); the blocks of $I_{K\,ss}$ and peak I_K (at $E = 80$ mV) as compared to $I_{K\,ss}$ in the untreated node were 83 % (65 to 87 %; $n = 8$) and 33 % (22 to 64 %), respectively. The concomitant reduction of the minimum of the peak I_{Na}-voltage curve amounted to 45 % (34 to 67 %) and was characterized by a remarkable fact, namely that during wash-out the peak I_{Na}-values (diamonds) systematically exceeded the corresponding initial values (squares) by 15 % (3 to 32 %).

The potassium current blocking principle from the *Ruta* plant could not be identified so far. Hence *Ruta* extract (see page 228) was added to the bathing medium, corresponding to 2 g plant material/100 ml. In 9 experiments peak I_{Na} was reduced by 44 % (23 to 55 %). The block of potassium currents exceeding clearly the block of peak I_{Na} (Fig. 5*C*, filled symbols) resembled that seen with capsaicin, and with DCH18C6, i.e. the potassium currents remaining at the end of the pulses $I_{K\,ss}$ (filled triangles) were obviously smaller than the peak values of the preceding potassium transients, peak I_K (filled dots). The decrease of $I_{K\,ss}$ and peak I_K (at $E = 80$ mV) as compared to $I_{K\,ss}$ in the untreated node amounted to 92 % (66 to 100 %) and 67 % (44 to 82 %), respectively, indicating that for the concentrations chosen the potassium block by the *Ruta* extract is more efficient than the other drugs tested.

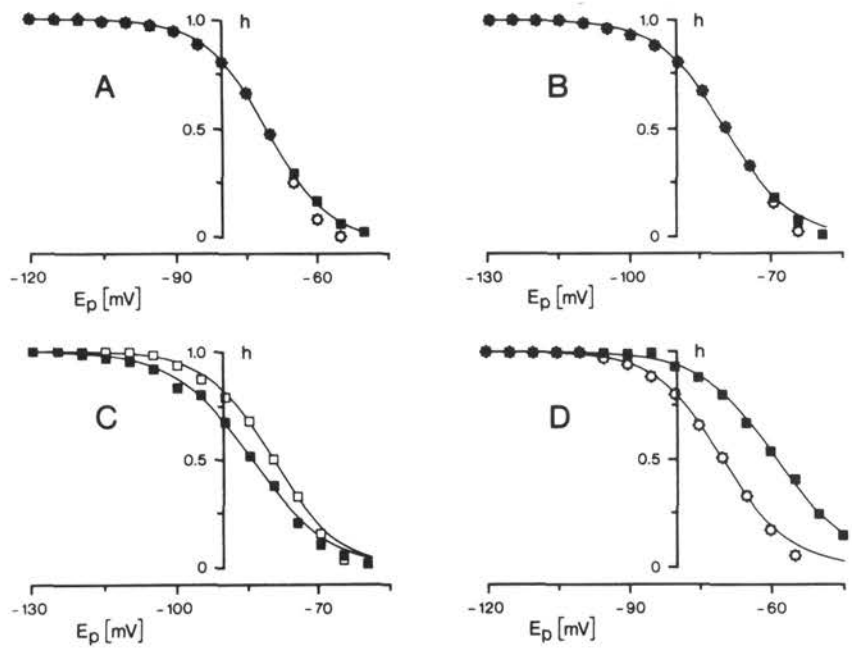

Fig. 6 Sodium inactivation curves in Ringer solution (open symbols) before (squares) and after (diamonds) application of test solutions (filled symbols). Test solutions in mmol/l: *A*: TEA (10.0); *B*: Flaxedil (1.0); *C*: 4-AP (0.050); *D*: C_{10}-TriEA (1.0). Abscissae: membrane potential during prepotentials, E_p. Ordinates: inactivation variable, h. The respective holding potentials are indicated by the position of the ordinates on the abscissae. The curves were calculated by equation (2). Note that cumulative effects of 4-AP were minimized by prolonging test pulse intervals to 1 min. Pulse protocol used, see Fig. 7*D*.

Sodium inactivation. The potential depedence of the sodium inactivation variable, h, was measured by peak sodium currents, peak I_{Na}, elicited by the two-pulse protocol shown in Fig. 7*D*. After converting peak I_{Na} in peak P_{Na} (Dodge and Frankenhaeuser 1959) the equation

$$\text{peak } P_{Na} = \text{peak } P_{Na\,max} \frac{1}{1 + \exp\left[(E\text{-}E_h)/k_h\right]} \tag{2}$$

(Frankenhaeuser 1959) was applied to each set of data measured. The fit yielded a theoretical maximum of peak P_{Na}, peak $P_{Na\,max}$, E_h, i.e. the potential where peak P_{Na} is 50 % of peak $P_{Na\,max}$, and of the steepness factor k_h. Normalizing the experimental values by peak $P_{Na\,max}$ delivered the so-called inactivation curve. In

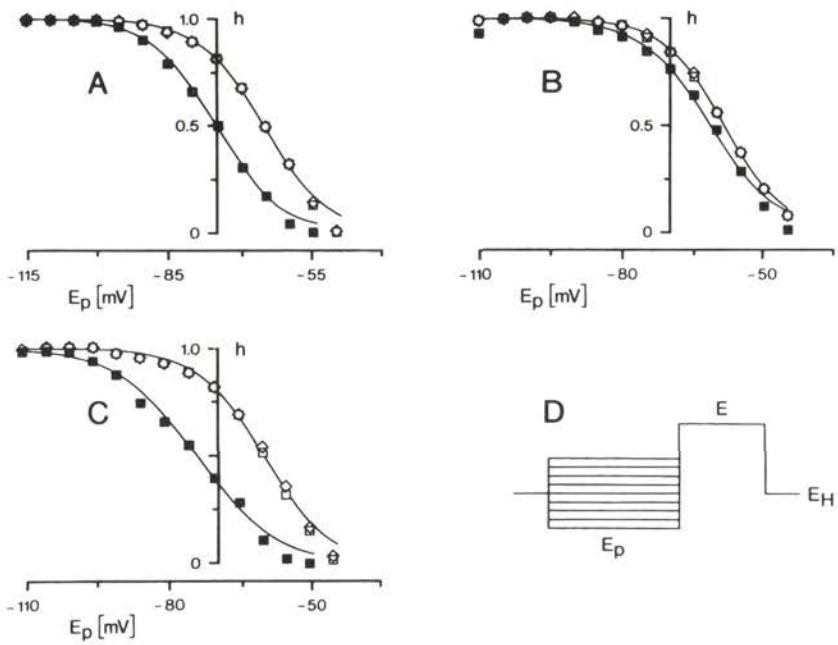

Fig. 7 Sodium inactivation curves in Ringer solution (open symbols) before (squares) and after (diamonds) application of test solutions (filled symbols). *A*: capsaicin (50 μmol/l); *B*: DCH18C6 (50 μmol/l) pulse intervals: 2 s; *C*: *Ruta* extract (corresponding to 2 g plant material/100 ml); Abscissae: membrane potential during prepotentials, E_p. Ordinates: inactivation variable, *h*. *D*: pulse protocol used (not drawn to scale). E_p: prepotentials of various sizes followed by constant test potentials *E*. E_H: holding potentials as given by the position of the ordinates on the belonging abscissae in *A*—*C*. The curves were calculated by equation (2).

normal Ringer solution (Figs. 6 and 7, open symbols) the medians of E_h and k_h were -68 mV (-79 to -48 mV) and 7 mV (6 to 9 mV) respectively ($n = 67$); these results agree reasonably well with corresponding data reported by Frankenhaeuser (1959). The small but systematic deviations of the measuring points from the calculated curves were obviously due to superimposed potassium currents and disappeared upon application of potassium blockers such as TEA (Fig. 6*A*) or Flaxedil (Fig. 6*B*) which definitely did not change the inactivation curve.

The effect of the *Ruta* extract on the inactivation curve is illustrated in Fig. 7*C*. The experimental values (filled symbols) were shifted to more *negative* potentials by 15 mV (8 to 18 mV; n = 8). The slight decrease in steepness of the curve by about 1 mV was in the range seen with 4-AP and C_{10}-TriEA. Under capsaicin

(Fig. 7*A*) and, to a somewhat lesser extent under 4-AP (Fig. 6*C*) and DCH18C6 (Fig. 7*B*), the inactivation curves were also shifted in *negative* direction by 11, 5, and 3 mV (7 to 15 mV; $n = 16$; 1 to 7 mV; $n = 7$; 2 to 7 mV; $n = 8$, respectively). C_{10}-TRiEA (Fig. 6*D*), however, shifted the sodium inactivation curve in *positive* direction by 11 mV (9 to 12 mV; $n = 8$), agreeing qualitatively with the C_{10}-TriEA effects as mentioned above.

Potassium-depolarization. In accordance with common belief the decrease of the absolute value of resting potential in potassium-rich extracellular solution, i.e. potassium-depolarization, is due to a diffusion potential across the nodal membrane predominantly permeable to potassium ions and to a smaller degree, to sodium ions (Huxley and Stämpfli 1951) (see, however, Århem et al. 1973). Therefore, potassium channel blockers should be expected to *increase* the potassium-depolarization; however, as it has already been shown for TEA (Schmidt 1965), just the opposite is true.

We tested the effects of various compounds on the resting potential in potassium-rich solution (80 mmol/l). Under TEA (10 mmol/l) and under *Ruta* extract the potassium-depolarization *decreased* to 46 % (32 to 81 %; $n = 9$) and 56 % (25 to 66 %; $n = 7$) of their normal values. Flaxedil and C_{10}-TriEA (1 mmol/l each) proved less efficacious (73 %; 42 to 83 %; $n = 10$ and 75 %; 68 to 79 %; $n = 7$) while 4-AP (0.5 mmol/l), capsaicin (0.05 mmol/l), and DCH18C6 (0.05 mmol/l) were almost inert (92 %, 80 to 100 %, $n = 8$; 100 %, 73 to 100 %, $n = 9$; 95 %, 91 to 100 %, $n = 7$, respectively).

Discussion

Selectivity. Among the drugs tested in this study 4-AP, and in particular, Flaxedil, are the only ones which act selectively on potassium channels as shown by peak sodium current voltage relations. Nevertheless, the well-known voltage dependence of 4-AP block (Meves and Pichon 1977; Ulbricht et al. 1982; Århem and Johansson 1989), the slight shift of the inactivation curve by 4-AP, and the concomitant lack of effects on the potassium-depolarization under 4-AP suggest remarkable functional dissimilarities between these drugs. The other drugs tested cannot be called selective potassium channel blockers, as documented by their mostly strong effects on sodium currents.

The observed shifts of both sodium activation and inactivation curves on the potential axis in *positive* direction under C_{10}-TriEA are in clear contrast to the effects of most of the drugs acting on potential-controlled ion channels in nerve membrane. They usually shift the inactivation curve in *negative* direction while the corresponding activation curve generally proves less susceptible to such

substances. Increased cation concentrations are known to cause similar shifts of membrane parameters in *positive* direction (Frankenhaeuser and Hodgkin 1957; Chandler et al. 1965; Vogel 1974; Brismar and Frankenhaeuser 1975; Hille et al. 1975); this has been referred to a so-called electrostatical screening of negative fixed charges at the external membrane surface. Thus, it seems likely that quarternary ammonium compounds like C_{10}-TriEA might interact with fixed charges at the external surface membrane in a way similar to the hypothesis suggested by Jedicke and collaborators (1988) concerning the action of some amino benzoic derivatives at the internal surface membrane. The observation that under C_{10}-TriEA no shift of the curve of the activation potassium variable n (Frankenhaeuser 1963) was detectable supports the assumption that the charge density near the potassium channel is obviously lower than that near the sodium channel (Vogel 1974).

The blocking effects of capsaicin, DCH18C6 and of the *Ruta* extract reveal two common features: All these drugs unequivocally block both types of channels and the remaining potassium currents are clearly time-dependent. In this connexion, it should be kept in mind that *Ruta* extract is a largely unknown mixture of numerous compounds (Bautz et al. 1989b).

The time course of potassium currents. The mechanisms responsible for the time-dependence of potassium currents as seen with capsaicin, DCH18C6 and in particular with *Ruta*, are still open to question. Similarly shaped potassium currents seen under strychnine led to the conclusion that only open channels could be blocked (Shapiro 1977). However, starting from the Hodgkin-Huxley-Frankenhaeuser formalism (Frankenhaeuser 1963; Frankenhaeuser and Huxley 1964) the observed decay of potassium currents under sustained positive pulses could obviously reflect a markedly enhanced potassium inactivation which, under normal conditions is so slow (Schwarz and Vogel 1971) that potassium currents seem to reach stationary values, $I_{K\,ss}$, (see Fig. 3) for a pulse length of 50 ms as used in this study. This concept has already proved useful for the description of potassium transients seen both under DCH18C6 (Århem et al. 1982; Kristbjarnarson and Århem 1985) and under the antiarrhythmic drug quinidine (Wong 1981). Screening experiments revealed that in Ranvier nodes quinidine resembles local anesthetics rather than blockers which act predominantly on potassium channels.

For an alternative interpretation of eventually time-dependent potassium currents in unmyelinated giant axons, one of the landmarks of the ionic theory of excitation (Hodgkin and Huxley 1952) has been questioned (French and Shoukimas 1981), i.e. the belief that the ionic concentrations on both sides of the axonal membrane do not change during single pulses. In face of the already well-established perinodal diffusion barrier in the myelinated axon (Dubois and

Bergman 1975; Katalymov 1978; Attwell et al. 1980; Moran et al. 1980; Berthold and Rydmark 1983a, 1983b) relatively long-lasting potassium currents could give rise to potassium-accumulation and/or potassium-depletion with time on either side of the axonal membrane; this in turn could result in a decrease of the electromotive driving force of potassium ions and thereby in time-dependent potassium current records. However, since in unaltered Ranvier nodes potassium currents usually saturate monotonously it seems likely that an effective potassium-uptake by the Schwann cell microvilli in the nodal gap, similar to glial cells in other preparations (Orkand et al. 1966; Orkand 1977) and known as spatial buffering of potassium (Gardner-Medwin 1983a, 1983b), potassium- siphoning (Newman 1985), potassium-homoeostasis (Gardner-Medwin 1986) or simply, control of the extracellular potassium-concentration (Henn et al. 1972), might compensate effectively for such changes in the electromotive driving force of potassium ions.

Taking this model into account, the observed time dependence of potassium-currents under some drugs like the *Ruta* extract could be explained by a time-dependent potassium-accumulation caused by a pharmacological block of potassium-uptake through the Schwann cell membrane. In that case the driving force of potassium ions would become time-dependent in a way which could qualitatively explain the observed potassium transients. This could mean that such drugs predominantly block potassium channels of the Schwann cell membrane in the nodal gap while blockers which act TEA-like predominantly reduce the permeability constant of the axonal membrane *per se*, $\bar{P}_K$, (Frankenhaeuser 1963). This model seems to conflict with some observations on cultured Schwann cells (Ritchie 1987) and in particular with our previous model (Bautz et al. 1989b) explaining the beneficial effects of the *Ruta* potassium channel blocker on demyelinated nerve fibres in MS. Nevertheless, the possibility that potassium channels of the axonal membrane could be pharmacologically discriminated from those of the Schwann cell membrane is worthy of being thoroughly investigated.

Potassium-depolarization. The amplitude of potassium-depolarization in potassium-rich solution was reduced by the tested drugs to various degrees. Unfortunately, we have not been able to read the underlying informations of these findings so far. Undoubtedly, the observed *decrease* of potassium-depolarization by potassium channel blockers favours the hypothesis that the resting potential of Ranvier nodes is controlled by a co-operation of the unquestioned potassium permeability with a relatively high leakage conductance rather than with an appreciable "sodium error" (Schmidt 1965).

The unduly high scatter of data as documented by the observed wide ranges of drug effects under high potassium concentrations (see page 237) suggests that

membrane potential measurements in Ranvier nodes are masked by some unkown interferences between different sites of action in the axonal membrane and in the Schwann cell membrane. In this connexion we are aware that the commonly accepted belief of membrane potential measurements in isolated Ranvier nodes not being influenced by the attached Schwann cell has never been verified. Therefore, it is likely that scaling procedures depending on membrane potential measurements may give rise to an increase in scatter of experimental data. In case of compensation of the influence of the series resistance uncertainties of that kind were avoided by replacing the "$E = 0$"-criterion (Koppenhöfer et al. 1984; Wiese and Koppenhöfer 1988; Albers et al. 1989) by the "symmetry"-criterion which is obviously less susceptible to uncertainties in membrane potential measurements. Thus, in this study experiments of several hours in duration could easily be utilized (see page 229) whereby the number of animals to be sacrificed was considerably reduced.

Acknowledgments. This study was supported by the Karl und Veronica Carstens-Stiftung im Stifterverband für die Deutsche Wissenschaft and the Alfried Krupp von Bohlen und Halbach-Stiftung. The authors wish to thank Chr. Bautz for treatment of the *Ruta* plant material, E. Niemöller for synthesis of the triethylammonium compounds, and A. Koppenhöfer for help with the English text.

References

Albers M., Bohuslavizki K. H., Koppenhöfer E. (1989): High standard one-loop potential clamp device for sodium current measurements in Ranvier nodes. Gen. Physiol. Biophys. **8,** 409—433

Armstrong C. M. (1971): Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. J. Gen. Physiol. **58,** 413—437

Armstrong C. M., Hille B. (1972): The inner quarternary ammonium ion receptor in potassium channels of the node of Ranvier. J. Gen. Physiol. **59,** 388—400

Århem P., Frankenhaeuser B., Kristbjarnarson H. (1982): Inactivation of potassium transport system of myelinated nerve in the presence of a cyclic ionophore. Acta Physiol. Scand. **114,** 593—600

Århem P., Frankenhaeuser B., Moore L. E. (1973): Ionic currents at resting potential in nerve fibres from *Xenopus laevis*. Potential clamp experiments. Acta Physiol. Scand. **88,** 446—454

Århem P., van Helden D. (1983): Effects of aliphatic alcohols on myelinated nerve membrane. Acta Physiol. Scand. **119,** 105—197

Århem P., Johansson S. (1989): A model for the fast 4-aminopyridine effects on amphibian myelinated nerve fibres. A study based on voltage-clamp experiments. Acta Physiol. Scand. **137,** 53—61

Attwell D., Dubois J. - M., Ojeda C. (1980): Fully activated potassium current-voltage relationship in the Ranvier node. Pflügers Arch. **384,** 49—56

Azizi S. A. (1983): Entwurf und Realisierung digitaler Filter. Oldenbourg, München

Bautz Ch., Bohuslavizki K. H., Hänsel W., Koppenhöfer E. (1989a): Effects of *Ruta* on the excitation process in myelinated nerves. Planta Medica, 37th Ann. Congr. Med.Plant Res., P2—14

Bautz Ch., Bohuslavizki K. H., Hänsel W., Kneip A., Koppenhöfer E., Möller W.-D. (1989b): Über neurogene Wirkungen von *Ruta graveolens*. In: Naturheilverfahren — Zum Stand der Forschung, (Eds. H. Albrecht, E. Franz) pp. 87—114, Springer, Berlin

Benoit E., Dubois J.-M. (1987): Properties of maintained sodium current induced by a toxin from *Androctonus* scorpion in frog node of Ranvier. J. Physiol. (London) **383,** 93—114

Berthold C.-H., Rydmark M. (1983a): VI. Anatomy of the paranode-node-paranode region in the cat. Experientia **39,** 954—976

Berthold C.-H., Rydmark M. (1983b): Electron microscopic serial section analysis of nodes of Ranvier in lumbosacral spinal roots of the cat: Ultrastructural organization of nodal compartments in fibres of different sizes. J. Neurocytol. **12,** 475—505

Bohuslavizki K. H., Koppenhöfer E., Hänsel W., Möller W.-D. (1988): A new approach in treating demyelinating diseases? J. Neuroimmunol. **20,** 251—252

Bostock H., Sears T. A. (1978): The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. J. Physiol. (London) **280,** 273—301

Bostock H., Sears T. A., Sherrat R. M. (1981): The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. J. Physiol. (London) **313,** 301—315

Brismar T. (1981): Specific permeability properties of demyelinated rat nerve fibres. Acta Physiol. Scand. **113,** 167—176

Brismar, T., Frankenhaeuser B. (1975): Effects of ionic concentration on sodium permeability properties of myelinated nerve fibres of *Xenopus laevis*. J. Physiol. (London) **249,** 549—559

Chandler W. K., Hodgkin A. L., Meves H. (1965): The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. J. Physiol. (London) **180,** 821—836

Chiu S. Y., Ritchie J. M. (1982); Evidence for the presence of potassium channels in the internode of frog myelinated nerve fibres. J. Physiol. (London) **322,** 485—501

Chiu S. Y., Schwarz W. (1987): Sodium and potassium currents in acutely demyelinated internodes of rabbit sciatic nerves. J. Physiol. (London) **391,** 631—649

Clarke E., Bearn J. G. (1972): The spiral nerve bands of Fontana. Brain **95,** 1—20

Davis F. A., Stefoski D., Bindokas J., Schauf C. L. (1986): 4-aminopyridine administered orally improves clinical sings in multiple sclerosis. Ann. Neurol. **20,** 152

Dixon W. J., Mood A. M. (1946): The statistical sign test. J. Amer. Statist. Assoc. **41,** 557—566

Dodge F. A., Frankenhaeuser B. (1958): Membrane currents in isolated frog nerve fibre under voltage clamp conditions. J. Physiol. (London) **143,** 76—90

Dodge F. A., Frankenhaeuser B. (1959): Sodium currents in the myelinated nerve fibre of *Xenopus laevis* investigated with the voltage clamp technique. J. Physiol. (London) **148,** 188—200

Dubois J. M. (1982): Capsaicin blocks one class of K^+ channels in the frog node of Ranvier. Brain Res. **245,** 372—375

Dubois J. M., Bergman C. (1975): Potassium accumulation in the perinodal space of frog myelinated axons. Pflügers Arch. **358,** 111—124

Fox J. M. (1976): Ultra-slow inactivation of the ionic currents through the membrane of myelinated nerve. Biochim. Biophys. Acta **426,** 232—244

Frankenhaeuser B. (1959): Steady state inactivation of sodium permeability in myelinated nerve fibres of *Xenopus laevis*. J. Physiol. (London) **148,** 671—676

Frankenhaeuser B. (1960): Quantitative description of sodium currents in myelinated nerve fibres of *Xenopus laevis*. J. Physiol. (London) **151,** 491—501

Frankenhaeuser B. (1963): A quantitative description of potassium currents in myelinated nerve fibres of *Xenopus laevis*. J. Physiol. (London) **169,** 424—430

Frankenhaeuser B., Hodgkin A. L. (1957): The action of calcium on the electrical properties of squid axons. J. Physiol. (London) **137,** 218—244

Frankenhaeuser B., Huxley A. F. (1964): The action potential in the myelinated nerve fibre of *Xenopus laevis* as computed on the basis of voltage clamp data. J. Physiol. (London) **171,** 302—315

French R. J., Shoukimas J. J. (1981): Blockage of squid axon potassium conductance by internal tetra-n-alkylammonium ions of various sizes. Biophys. J. **34,** 271—291

Gardner-Medwin A. R. (1983a): A study of the mechanisms by which potassium moves through brain tissue in the rat. J. Physiol. (London) **335,** 353—374

Gardner-Medwin A. R. (1983b): Analysis of potassium dynamics in mammalian brain tissue. J. Physiol. (London) **335,** 393—426

Gardner-Medwin A. R. (1986): A new framework for assessment of potassium-buffering mechanisms. Ann. N. Y. Acad. Sci. **481,** 287—302

Grissmer S. (1986): Properties of potassium and sodium channels in frog internode. J. Physiol. (London) **381,** 119—134

Henn F. A., Haljamäe H., Hamberger A. (1972): Glial cell function: active control of extracellular K^+-concentration. Brain Res. **43,** 437—443

Hille B. (1967): The selective inhibition of delayed potassium currents in the nerve by tetraethylammonium ion. J. Gen. Physiol. **50,** 1287—1302

Hille B., Woodhull A. M., Shapiro B. I. (1975): Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. Phil. Trans. Roy. Soc. London B. **270,** 301—318

Hodgkin A. L., Huxley A. F. (1952): A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (London) **117,** 500—544

Huxley A. L., Stämpfli R. (1951): Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. J. Physiol. (London) **112,** 496—508

Jedicke U., Haller R., Koppenhöfer E. (1988): Wirkung von Aminobenzoesäure-Derivaten auf die Ionenströme myelinisierter Nervenfasern. 2. Mitteilung: Inaktivierung der Natrium-Permeabilität. Arzneim.-Forsch.-Drug Res. **38,** 873—876

Jonas P., Bräu M. E., Hermsteiner M., Vogel W. (1989): Single-channel recording in myelinated nerve fibres revealed one type of Na channel but different K channels. Proc. Nat. Acad. Sci. USA **86,** 7238—7242

Jones R. E., Heron J. R., Foster D. H., Snelgar R. S., Mason R. J. (1983): Effects of 4-aminopyridine in patients with multiple sclerosis. J. Neurol. Sci. **60,** 353—362

Katalymov L. L. (1978): Confirmation of the hypothetical diffusion barrier in the region of the Ranvier node of myelinated nerve fibers. Bull. Eksp. Biol. Med. **86,** 517—519

Keana J. F. W., Stämpfli R. (1974): Effects of several "specific" chemical reagents on the Na^+, K^+ and leakage currents in voltage-clamped single nodes of Ranvier. Biochim. Biophys. Acta **373,** 18—33

Koppenhöfer E. (1967): Die Wirkung von Tetraäthylammoniumchlorid auf die Membranströme Ranvierscher Schnürringe von *Xenopus laevis*. Pflügers Arch. **293,** 34—55

Koppenhöfer E., Bohuslavizki K. H. (1988): Inconsistent sodium current records derived on Ranvier nodes with a commercially available potential clamp device according to Nonner. Gen. Physiol. Biophys. **7,** 557—567

Koppenhöfer E., Vogel W. (1969): Wirkung von Tetrodotoxin und Tetraäthylammoniumchlorid and der Innenseite der Schnürringsmembran von *Xenopus laevis*. Pflügers Arch. **313,** 361—380

Koppenhöfer E., Wiese H., Schumann H., Wittig J. (1984): Experimente zum Einfluß des Serienwiderstandes auf die Potentialabhängigkeit der Natriumspitzenströme des Ranvierschen Schnürrings. Funkt. Biol.Med. **3,** 61—64

Koppenhöfer E., Sommer R.-G., Froese U. (1987): Effects of benzocaine and its isomers on sodium permeability and steady state inactivation in the myelinated nerve, obtained by an improved dissection technique. Gen. Physiol. Biophys. **6**, 209—222

Kristbjarnarson H., Århem P. (1985): The efect of crown ethers on ionic currents in myelinated nerve fibres from *Xenopus laevis*. Acta Physiol. Scand. **123**, 261—268

Meves H., Pichon Y. (1977): The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. J. Physiol. (London) **268**, 511—532

Moran N., Palti Y., Levitan E., Stämpfli R. (1980): Potassium ion accumulation at the external surface of the nodal membrane in frog myelinated fibres. Biophys. J. **32**, 939—954

Newman E. A. (1985): Voltage-dependent calcium and potassium channels in retinal glial cells. Nature (London) **317**, 809—811

Orkand R. K. (1977): Glial cells. In: Handbook of Physiology, Vol. 1, Part 2, The Nervous System (Eds. J. M. Brookhart, V. B. Mountcastle, E. R. Kandel, St. R. Geiger), pp. 855—875, American Physiological Society, Bethesda

Orkand R. K., Nicholls J. G., Kuffler S. W. (1966): Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J. Neurol. **29**, 788—806

Pelhate M., Pichon Y. (1974): Selective inhibition of potassium current in the giant axon of the cockroach. J. Physiol. (London) **242**, 90P—91P

Ritchie J. M. (1987): Voltage-gated cation and anion channels in mammalian Schwann cells and astrocytes. J. Physiol. (Paris) **82**, 248—257

Röper J., Schwarz J. R. (1989): Heterogeneous distribution of fast and slow potassium channels in myelinated rat nerve fibres. J. Physiol. (London) **416**, 93—110

Sachs L. (1984): Applied Statistics. A Handbook of Techniques. Springer, New York

Schmidt H. (1965): Die Wirkung von Tetraäthylammoniumchlorid auf das Membranpotential und den Membranwiderstand von Bündeln markhaltiger Nervenfasern. Pflügers Arch. **282**, 351—361

Schönle Ch., Koppenhöfer E. (1983): Zur Selektivität der Wirkung gereinigten Tetraethylammoniumchlorids am Ranvierschen Schnürring. Funkt. Biol. Med. **2**, 49—52

Schwarz J. R., Eikhof G. (1987): Na currents and action potentials in rat myelinated nerve fibres at 20 and 37 °C. Pflügers Arch. **409**, 569—577

Schwarz J. R., Vogel W. (1971): Potassium inactivation in single myelinated nerve fibres of *Xenopus laevis*. Pflügers Arch. **330**, 61—73

Shapiro B. I. (1977): Effects of strychnine on the potassium conductance of the frog node of Ranvier. J. Gen. Physiol. **69**, 897—914

Sherrat R. M., Bostock H., Sears T. A. (1980): Effects of 4-aminopyridine on normal and demyelinated mammalian nerve fibers. Nature (London) **283**, 570—572

Smith K. J., Schauf C. L. (1981): Gallamine triethiodine (Flaxedil): tetraethylammonium-like effects in myelinated nerve fibres. Science **212**, 1170—1172

Stämpfli R., Hille B. (1976): 1. Electrophysiology of the peripheral myelinated nerve. In: Frog Neurobiology (Eds. R. Llinás, W. Precht), pp. 3—32, Springer, Berlin

Stefoski D., Davis F. A., Faut M., Schauf C. L. (1987): 4-aminopyridine improves clinical signs in multiple sclerosis. Ann. Neurol. **21**, 71—77

Swenson Jr. R. P. (1981): Inactivation of potassium current in squid axon by a variety of quaternary ammonium ions. J. Gen. Physiol. **77**, 255—271

Targ E., Kocsis J. D. (1985): 4-aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. Brain Res. **328**, 358—361

Ulbricht W., Wagner H.-H., Schmidtmayer J. (1982): Effects of aminopyridines on potassium current of the nodal membrane. In: Advances in the Biosciences, Vol. 35, Aminopyridines and Similar Acting Drugs (Eds. P. Lechat, S. Thesleff, W. Bauman), pp. 29—41, Pergamon Press, Oxford

Vogel W. (1974): Calcium and lanthanum effects at the nodal membrane. Pflügers Arch. **350,** 25—39

Waxman St. G. (1987): Conduction properties and pathophysiology of myelinated and non-myelinated nerve fibres. In: Handbook of Clinical Neurology, Vol. 7 (51), Neuropathies, (Ed. W. B. Matthews), pp. 41—62, Elsevier, Amsterdam

Wiese H., Koppenhöfer E. (1988): Minimizing the influence of the series resistance in potential clamped Ranvier nodes. Gen. Physiol. Biophys. **7,** 143—156

Wong B. S. (1981): Quinidine interaction with *Myxicola* giant axons. Mol. Pharmacol. **20,** 98—106

Final version accepted January 4, 1991