Effects of Three Alkoxypsoralens on Voltage Gated Ion Channels in Ranvier Nodes

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Abstract. The effects of the phototoxic K^+- channel blockers 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP) on Ranvier nodes were compared to those of 5,8-diethoxypsoralen (5,8-EOP) by means of the Hodgkin-Huxley formalism. When these test substances were added individually to the bathing solution (8-MOP: 100 μmol/l; 5-MOP: 50 μmol/l; 5,8-EOP: 10 μmol/l) the following completely reversible effects were observed:

1. 8-MOP, caused a nearly potential-independent decrease of the sodium permeability, P'_{Na}, by ca. 17 %. 5-MOP and 5,8-EOP merely decreased the maximal value of P'_{Na}, by ca. 12 and 8 % respectively, whereas with weak depolarisations P'_{Na} was unchanged.

2. In the tested potential range the potassium permeability, P'_K ,was caused to decrease by ca. 9 % by 8-MOP, ca. 21 % by 5-MOP and ca. 19 % by 5,8-EOP.

3. The potassium currents acquired a phasic time course previously described for 8-MOP and 5-MOP. They reached a relative maximum and approached a lower steady-state value, k_∞, with a time constant τ_k at $V = 120$ mV of about 16 ms (8-MOP), 20 ms (5-MOP) and 94 ms (5,8-EOP).

To obtain dose-response relations the drug-induced effects on peak P'_K and on the steady state value, k_∞, were measured. The corresponding apparent dissociation constants (in μmol/l) were 66.6 and 80.1 (for 8-MOP), 87.6 and 25.8 (for 5-MOP), and 13.5 and 6.5 (for 5,8-EOP). In view of the similarity of the actions of 5-MOP and 5,8-EOP as well as the fact that 5,8-EOP is not phototoxic, in future 5,8-EOP may well prove to be a particularly suitable K^+-channel blocker for the symptomatic therapy of multiple sclerosis and other demyelinating diseases.

Key words: Psoralens — K^+-channel blockers — Node of Ranvier — Demyelinating diseases

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Introduction

Blockers of voltage-gated potassium channels such as 4-aminopyridine (see, e g Sherrat et al 1980) and in particular, some psoralens (Bohuslavizki et al 1994a) have been suggested for specific symptomatic therapy in demyelinating diseases like multiple sclerosis (MS) (Bohuslavizki et al 1993a,b, Koppenhofer et al 1995, Wulff et al 1998a) The reason is that destruction of the myelin sheath uncovers normally "silent" potassium channels which renders nerve function difficult by changing the resting membrane potential (for references, see Bohuslavizki et al 1994a) *In vitro* experiments on intact myelinated axons have shown that, in contrast to 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) is a particularly strong and selective potassium channel blocker (Bohuslavizki et al 1993a) Hence it was not surprising that when 5-MOP was administered as an isolated substance (Koppenhofer et al 1995) or in the form of a 5-MOP-rich diet (Bunger et al 1996), functional deficits due to MS were found to be ameliorated

5-MOP, like 8-MOP, is used in PUVA therapy (psoralen plus ultraviolet-A radiation) of psoriasis, vitiligo and other cutaneous diseases (for references, see Wulff et al 1998b) However, the underlying phototoxic and photomutagenic activity of 5-MOP (Zajdela and Bisagni 1981) might pose serious problems for its use as a potassium channel blocker in the long-term therapy of demyelinating diseases In the search for new psoralens with no phototoxicity but comparable potassium chan nel blocking activity 5,8-diethoxypsoralen (5,8-EOP) has been discovered (Wulff et al 1998b)

The aim of the present study was to compare quantitatively the blocking effects of 5,8-EOP on the ionic currents in intact myelinated nerve fibres to those of 5-MOP and 8-MOP by means of parameters of the Hodgkin-Huxley formalism

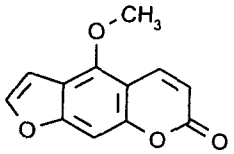
Materials and Methods

Preparation

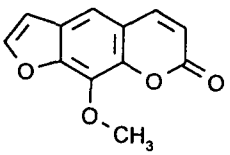
The experiments were carried out on isolated intact myelinated nerve fibres from the sciatic nerve of the toad *Xenopus laevis* The dissection procedure (Koppenhofer et al 1987) delivered nerve fibres 24 μm in diameter (median, range 22–31 μm, $N = 12$)

Chemicals and solutions

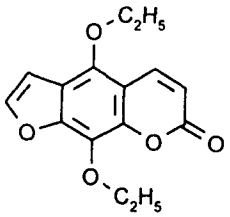
5-Methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP) were purchased from Aldrich Chemie (Steinheim, Germany) 5,8-Diethoxypsoralen (5,8-EOP) was prepared by one of us (H W) as described previously (Wulff et al 1998b) Briefly, 8-MOP was demethylated with magnesium iodide to yield the corresponding alcohol, which was oxidized with chromium trioxide Reduction of the resulting quinone with zinc dust produced 5,8-dihydroxypsoralen, which was reacted with diethylsulfate in the presence of potassium carbonate to give 5,8-diethoxypsoralen Stock



5-MOP



8-MOP



5,8-EOP

Figure 1. Molecular structures of the substances tested. 8-MOP: 8-methoxypsoralen. 5-MOP: 5-methoxypsoralen. 5,8-EOP: 5,8-diethoxypsoralen.

solutions (2 mmol/l) of all three test substances were prepared with dimethyl sulfoxide (DMSO; Fluka AG, Buchs, Switzerland). For the experiments, these were diluted with Ringer solution so that in the final test solutions the concentration of DMSO was 0.7 mol/l throughout. Data collection was begun as soon as the measured effects of the test substance had stabilized at a constant level. In all cases this took less than 1 min. The subsequent washing-out phase was as long as necessary for complete reversibility, 2 min at the most. Note that the current records in each test solution were processed to eliminate the superimposed DMSO effects at the concentration used here before further evaluation (Bohuslavizki et al. 1994a).

The normal bathing medium was Ringer solution (in mmol/l): NaCl 107.0; KCl 2.5; $CaCl_2$ 2.0; N,N-bis (hydroxyethyl)-2-aminoethanesulfonic acid / NaOH buffer (BES) 5.0. The solution for use as artificial intracellular fluid contained (in mmol/l): KCl 108.0; NaCl 5.0; BES 5.0. The pH of all solutions was 7.2 ± 0.1; the temperature during the experiments was 10.0 ± 0.5 °C.

Experimental setup

Measurements of membrane potential changes, V, and membrane currents, I, were carried out by a potential clamp system which minimizes errors in measurement nearly to the limit to which they can be pushed at present (Bethge et al. 1991;

Bohuslavizki et al. 1994b; Zaciu et al. 1996). Nevertheless spontaneous changes, regarding in particular zero drift of the tested axon during the course of the experiment, were still inevitable. Therefore, before the control data in the normal bathing medium were recorded, i.e. before and after each application of test solution, the following were routinely checked and if necessary readjusted: 1. the amount of leakage current compensation, 2. the amount of sodium inactivation (desired value: $h_\infty = 0.8$, to keep the resting potential constant), 3. the amount of positive feedback for compensation of the voltage drop across the nodal series resistance, and 4. the transfer function of the membrane potential (see Bohuslavizki et al. 1994b). As a result, a considerable decrease was achieved in the scatter of the data owing to measurement errors that has traditionally been attributed to "unavoidable biological dispersion". This allowed, together with a consistent minimization of all mechanical stress on the axon throughout the tests, experiments to be continued for several hours with no rundown that measurably affected the results. This in turn brought considerable savings in the number of experiments and thus also in time and funds (cf. also Bethge et al. 1991).

Measuring conditions and calibrations

For current-voltage relations various positive test pulses V were preceded by negative prepulses of amplitude and duration sufficient to make the sodium inactivation variable h unity at the beginning of the test pulses. For sodium inactivation curves the steady-state value of h was measured by the well-known two-pulse protocol (Frankenhaeuser 1959).

Specific currents were calculated from membrane current records and from the fibre dimensions according to Stämpfli and Hille (1976).

Data processing

The procedure for on-line processing of current records comprised the following steps (for details, see Bethge et al. 1991): 1. leakage current compensation by hyperpolarizing test pulses ($V = -40$ mV) assuming a potential-independent leakage conductance, 2. deliberate filtering (for so-called "over-filtering" and aliasing effects, see Albers et al. 1989), 3. appropriate A/D conversion and 4. storage of the data.

To calculate the membrane permeabilities P_{Na} and P_K from the underlying ionic currents I_{Na} and I_K, the constant-field concept was applied (for details, see Bohuslavizki et al. 1994a). The permeability constants $\overline{P}_{Na}$ and $\overline{P}_K$ were found by computer-assisted calculation of P'_{Na} and P'_K, respectively, by means of least-square fitting from the time courses of the currents elicited by test pulses of $V = 40$ to 110 mV and $V = 80$ to 130 mV (50–100 ms in duration), respectively. We applied the simplified equation (Frankenhaeuser 1960)

$$P_{Na} = P'_{Na} \cdot [1 - \exp(-t - \delta t)/\tau_m]^a \cdot \exp(-t - \delta t)/\tau_h \quad (1)$$

where

$$P'_{Na} = \overline{P}_{Na} \cdot m_\infty^a \quad (2)$$

Note that there is considerable evidence for $a = 1$ (Bohuslavizki et al. 1994b). Therefore, the well-known delay of the onset of sodium currents elicited by positive test pulses was accounted for by introducing the delay time of the setup, δt.

For fitting potassium currents the comparably short delay time of the setup was neglected. The corresponding simplified equation for normal potassium currents was

$$P_K = P'_K \cdot [1 - \exp(-t/\tau_n)]^b \tag{3}$$

where

$$P'_K = \overline{P}_K \cdot n_\infty^b \tag{4}$$

For the potassium currents recorded in test solutions, Eq. (3) was expanded by a so-called potassium inactivation variable k (Frankenhaeuser 1963a; Bohuslavizki et al. 1994a)

$$P_K = P'_K \cdot [1 - \exp(-t/\tau_n)]^b \cdot [k_\infty + (1 - k_\infty) \cdot \exp(-t/\tau_k)] \tag{5}$$

where

$$P'_K = \overline{P}_K \cdot n_\infty^b \cdot k_o, \tag{6}$$

assuming the starting value of the variable, k_o, to be close to unity (Frankenhaeuser 1963a).

For permeability curves P'_{Na} and P'_K were plotted *versus* test pulse amplitude V. The P'_{Na} values were fitted by the equation

$$P'_{Na} = \overline{P}_{Na} \frac{1}{1 + \exp[(V_p - V)/k_p]} \tag{7}$$

(Benoit and Dubois 1987; Bohuslavizki et al. 1994b); here, V_p represents the position of the inflection point of the curve on the potential axis, k_p is its maximal slope, and $\overline{P}_{Na}$ is the extrapolated maximum of the calculated curves. It is still questionable whether potassium permeability data should be treated in a similar manner (Frankenhaeuser 1962; Bohuslavizki et al. 1994a). So it seemed a reasonable compromise to take the individual maximum of each data set for $\overline{P}_K$.

To quantify the potassium channel-blocking efficacy of the substances tested, two parameters were employed: 1. the drug-induced reduction of the permeability constant $\overline{P}_K$,

$$B_K = \frac{\Delta \overline{P}_K}{\overline{P}_{K\ \text{control}}}, \tag{8}$$

where

$$\Delta \overline{P}_K = \overline{P}_{K\ \text{control}} - \overline{P}_{K\ \text{test}} \tag{9}$$

and 2. the drug-induced reduction of k_∞, in form of so-called potassium inactivation $(1 - k_\infty)$; both parameters were plotted semilogarithmically *versus* test concentration, c. To the two sets of data the equations

$$B_K = \frac{B_{K\ \max} \cdot c^x}{c^x + B_{K\ C50}} \tag{10}$$

and

$$1 - k_\infty = \frac{(1 - k_\infty)_{max} \cdot c^x}{c^x + (1 - k_\infty)_{C50}} \tag{11}$$

were fitted, respectively (see Jedicke et al. 1988) Both fits yielded values for half-maximum block, $B_{K\ C50}$ and $(1 - k_\infty)_{C50}$, respectively, and for the steepness parameter x.

As a measure of the quality of the curve-fitting we used the nonlinear regression coefficient r_{nl} (Sachs 1984). It was at least 0 986

Results

Sodium inactivation curves

The changes of the potential dependence of sodium inactivation are shown in Table 1 Shifting of the sodium inactivation curve in the negative direction, which is typically induced by most of the neuropharmaceuticals that affect axons, was very slight in the case of these three psoralens. The smallest leftward shift (ΔV_h) was caused by 5-MOP. The slope of the curves (k_h) was not affected by the substances tested here (not shown).

Table 1. Effects of the tested psoralens at test concentrations similar to the respective apparent dissociation constants ΔV_h leftward shift of the sodium inactivation curve k_∞, τ_k steady-state potassium "inactivation" variable and its time constant at $V = 120$ mV Medians, N number of measurements

	c [μmol/l]	ΔV_h [mV]	k_∞	τ_k [ms]	N
8-MOP	100	−3 5	0 6	16	10
5-MOP	50	−2	0 2	20	10
5,8-MOP	10	−3 6	0 2	94	2

Time course of current records

The time course of sodium currents elicited by depolarising test pulses was unaffected by all three psoralens (not shown) The potassium currents, however, acquired the phasic time course (Fig 2) previously described for other psoralen derivatives (Bohuslavizki et al. 1994a). Whereas normally, the potassium currents rise monotonically to a *quasi*-stationary value, under the influence of the tested psoralens they first reach a relative maximum within a few milliseconds and then approach a lower steady-state value; as this decline takes tens of milliseconds, it obviously cannot be determined precisely *in praxi* without extrapolation by curve fitting. For all three psoralens the decline of the potassium currents after each

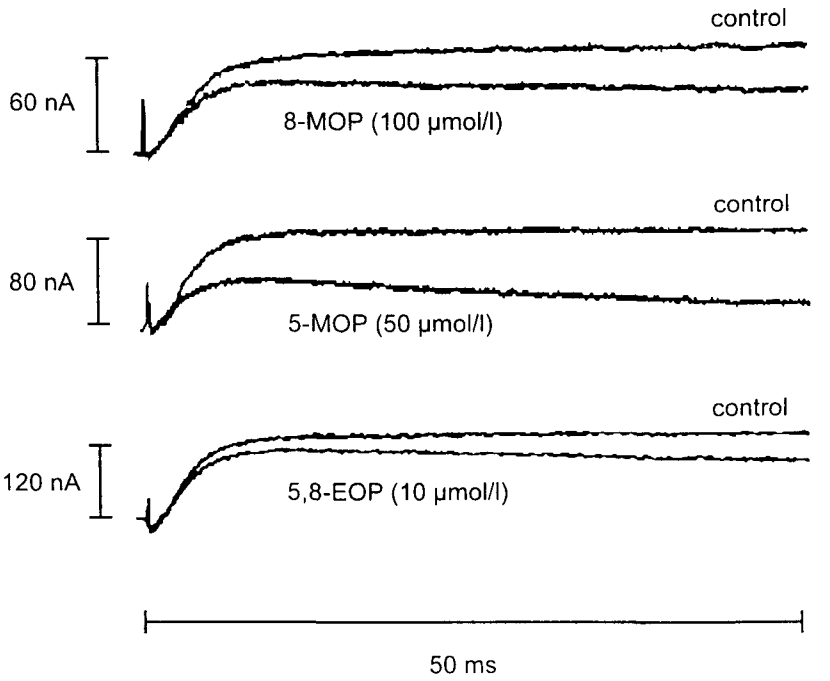


Figure 2. Membrane currents recorded in the presence of psoralens at the indicated test concentrations, in comparison to records in Ringer solution (control) Test-pulse amplitude $V = 130$ mV Note that the chosen test concentrations are close to the respective apparent dissociation constants as measured by the potassium "inactivation" term $(1 - k_\infty)$ (see Table 2)

transient is more rapid at higher than at lower test concentration. Therefore the test concentrations used for Fig. 2 were adjusted to approximate their different efficacies, taking into account the differences in solubility of the substances

Curve fitting

By using Eqs. (1) and (3) to fit curves to the current recordings, no influence of the test solutions was observed, neither on τ_m and τ_h of the sodium permeability P_{Na} nor on the delay time of the setup, δt; τ_n, however, was slightly reduced (not shown). The time constants τ_k and the steady-state values of the inactivation parameter of P_K, k_∞, as measured in the respective test solutions, are given in Table 1. The decrease of k_∞ (normal value = 1.0) was strongest under 5-MOP and 5,8-EOP while the kinetics of k was slowest under 5,8-EOP. Note that the given numerical values, particularly those for 5,8-EOP may not be of usual accuracy because of relatively short test pulses, 100 ms in duration at maximum.

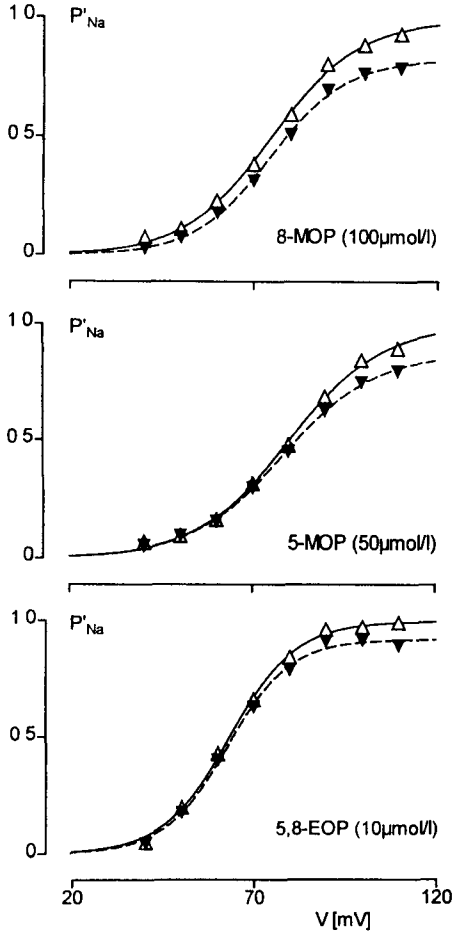


Figure 3. Sodium permeabilities, P'_{Na}, as calculated from sodium currents by the constant-field concept and Eqs (1) and (2) Abscissae test-pulse amplitude, V, ordinates P'_{Na} normalised to the maximum of the continuous curve, $\overline{P}_{Na}$, which was calculated by fitting Eq (7) to the data in Ringer solution before and after application of test solutions (open symbols, continuous curve) Dashed curve corresponding curve fitted to the data in the respective test solution at the given concentrations and normalised to the maximum of the continuous curve (filled symbols) Symbols medians of 10, 10 and 2 measurements, respectively

Permeability-voltage curves

For permeability curves (Figs. 3 and 4) P'_{Na} and P'_K in the various test series were calculated by Eqs. (1), (3), and (5) and plotted *versus* test-pulse amplitude, V First, Eq. (7) was fitted to the P'_{Na} values measured in Ringer solution. The extrapolated maximum of the calculated curve gave the permeability constant $\overline{P}_{Na}$ in Ringer solution in each case, which was then used to normalise the values of P'_{Na} found for Ringer solution and for the associated test solution. Then Eq. (7) was fitted to the latter values, which gave the permeability constant $\overline{P}_{Na}$ in the respective test solution. It is evident that all three test substances produce a not inconsiderable decrease of $\overline{P}_{Na}$. However, with weak depolarisations P'_{Na} is clearly not reduced by either 5-MOP or 5,8-EOP (in contrast to 8-MOP).

The differences from the permeability curves under normal conditions shown

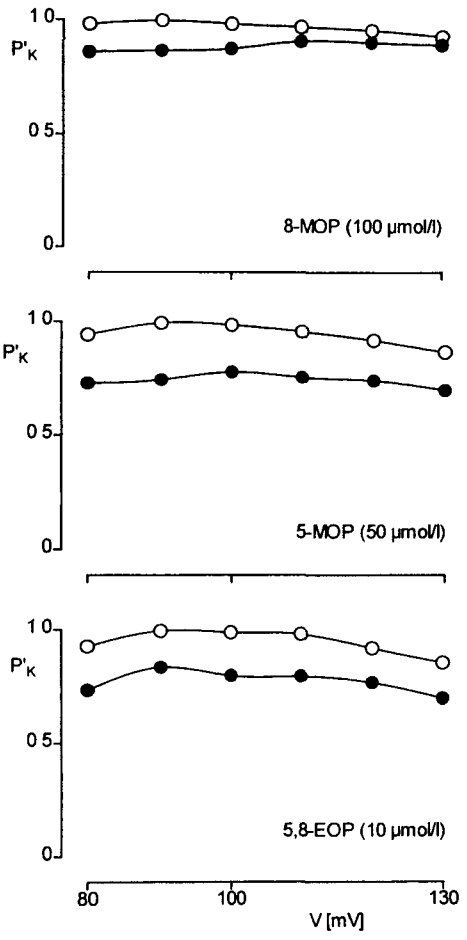


Figure 4. Potassium permeabilities, P'_K, as calculated from potassium currents by the constant-field concept and Eqs (3), (4), (5) and (6) Abscissae test-pulse amplitude, V, ordinates P'_K normalised to maximum of P'_K in Ringer solution, $\overline{P}_K$, before and after application of test solutions (open symbols), filled symbols data from records in the respective test solution at the given concentrations Symbols medians of 10, 10 and 2 measurements, respectively, interconnected by spline interpolation (Wiese und Koppenhöfer 1988)

in Fig. 3 (open symbols) are remarkable: whereas the normal curve in the lower diagram reflects the potential dependence of P'_{Na} usually observed in most axons (cf. Bohuslavizki et al 1994a, Fig. 5), the normal curves in the two upper diagrams rise less steeply and seem to be shifted to the right. This is definitely not a matter of a scatter of data owing to errors in measurement, but rather a peculiarity of axons that are only occasionally observed, among the aberrant characteristics of which are especially small potassium outward currents and unusually large sodium outward currents, although the sodium equilibrium potential has the normal value and the preparation shows excellent long-term stability of all the other quality criteria methodologically accessible to us (Bohuslavizki et al. 1994 b).

The potential dependence of P'_K is shown in Fig. 4. Because it is impossible to determine $\overline{P}_K$ with an equation corresponding to Eq. (7), $\overline{P}_K$ was set equal to the

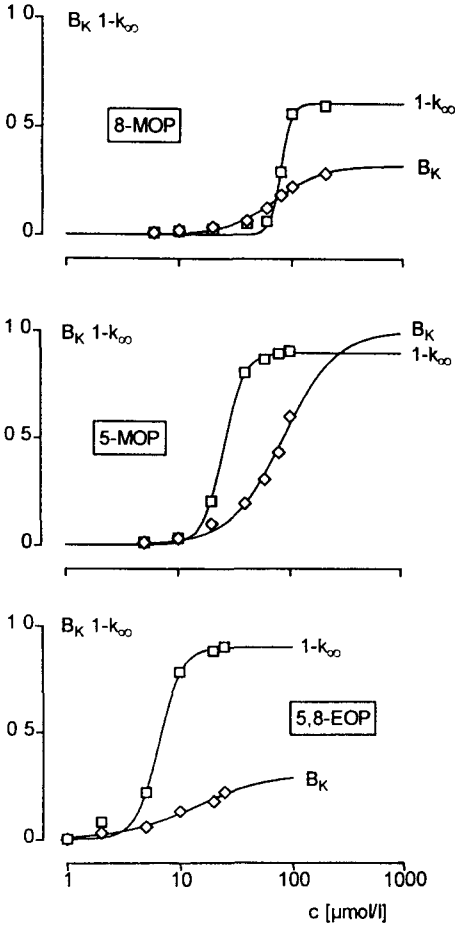


Figure 5. Dose-response relations for the blocking of potassium currents by the tested substances Abscissae test concentrations in µmol/l Ordinates block of the potassium permeability constant, B_K (diamonds), or production of potassium "inactivation", $(1 - k_\infty)$ (squares) at $V = 120$ mV To each set of data Eqs (10) or (11) was fitted, as appropriate The resulting apparent dissociation constants are given in Table 2 Symbols medians and ranges of 4, 5 and 2 measurements, respectively

maximal value of P'_K measured in Ringer solution, and the P'_K values measured in both Ringer solution and the solution being tested were normalised to this value. It is clear that 8-MOP is a considerably less effective potassium channel blocker when compared with 5-MOP and especially with 5,8-EOP. Unfortunately but inevitably, the evaluation procedure used here provides no information about the efficacy of the test substances in the range of weak depolarisations that is particularly relevant functionally.

Dose-response relations

In order to obtain dose-response curves, the blocking $\overline{P}_K$, B_K, and the increase in potassium "inactivation", $(1 - k_\infty)$, were examined at various test concentrations (Fig. 5). An upper limit is placed on the methodologically feasible concentration

range by the different solubilities of the test substances, so that the theoretically achievable maximal effect could only be estimated by fitting Eqs. (10) and (11), respectively, to the measured values and extrapolating the calculated curves. As calculated in this way, B_K approaches the maximal value $B_{K\,max} = 1$ only in the presence of 5-MOP; this was not the case under either 8-MOP or 5,8-EOP (diamonds). When the term $(1 - k_\infty)$ was used as a measure of potassium current blocking (squares), the blockade value $(1 - k_\infty)_{max}$ found by extrapolation to high concentrations was approximately equal to 1 for both 5-MOP and 5,8-EOP, but not for 8-MOP. The apparent dissociation constants $B_{K\,C50}$ and $(1 - k_\infty)_{C50}$ obtained from the fitted curves are shown in Table 2. Here it is evident that 5,8-EOP is the most effective of the three psoralens.

Table 2. Efficacy of the tested psoralens in blocking potassium currents $B_{K\,C50}$ apparent dissociation constant of block of the potassium permeability constant $\overline{P}_K$ $(1 - k_\infty)_{C50}$ apparent dissociation constant of potency to bring about potassium "inactivation", $(1 - k_\infty)$ Medians, N number of measurements

	$B_{K\,C50}$	$(1 - k_\infty)_{C50}$	N
8-MOP	66 6	80 1	4
5-MOP	87 6	25 8	5
5,8-MOP	13 5	6 5	2

Discussion

Mode of action of the tested psoralens

Our results showed that the psoralens tested here act mainly on potassium channels It has been suggested (see Bohuslavizki et al. 1994a) that among the voltage-gated types of potassium channel in the nodal membrane that are experimentally discriminable (Dubois 1981; Safronov et al. 1993; Scholz et al. 1993; Schwarz et al. 1995; Reid et al. 1999), 5-MOP and in particular 5,8-EOP exert the weakest blocking action on those channels that inactivate most rapidly. The present observation that the decline of the potassium transients recorded under the three psoralens tested here is more rapid at higher than at lower test concentrations could support this interpretation If we also take into account that especially 5-MOP and 5,8-EOP accelerate the activation kinetics of the potassium currents to some extent (not shown), the implication could be that the blockers we have tested affect chiefly — in the customary nomenclature for potassium channel types (Chandy and Gutman 1995) — the types Kv1.1 and Kv1.2 (Wang et al. 1993), particularly in view of the finding that the latter is especially readily blocked by 5-MOP (Wulff et al. 1998b).

A second possible way to explain the psoralen-induced slow decline of the potassium transients derives from Armstrong and Hille (1972): the idea here is

that, ınsıde the potassıum channels, there are bındıng sıtes for the tested psoralens to whıch theır access ıs facılıtated ıf the channels are ın the open state In thıs case, the tıme constant τ_k gıven ın Table 1 would provıde an approxımate measure of the bındıng kınetıcs of these psoralens, and the fınal, steady-state level of the potassıum currents under these condıtıons, k_∞, would be a measure of the psoralen-ınduced blockıng of the potassıum channels Evıdence for thıs vıew ıs that the dose-response relatıonshıps for the term $(1 - k_\infty)$, whıch for sımplıcıty, we have previously called potassıum "ınactıvatıon", seem to approach 1 at hıgh concentratıons of 5-MOP and 5,8-EOP $(1 - k_\infty)$ could thus serve as a suıtable measure of the degree of occupatıon of thıs reactıon sıte, but the same certaınly does not apply to 8-MOP It ıs noteworthy that the method of descrıbıng the effect of a channel blocker ın terms of a decrease ın the assocıated permeabılıty constant (see, e g Århem and Frankenhaeuser 1974) ıs evıdently applıcable only to one of the psoralens tested here, namely 5-MOP In that case, whıch parameter does best descrıbe the potassıum current-blockıng effect of these psoralens? It ıs beyond our present scope to consıder the general sıgnıfıcance of such a questıon (Pfaffendorf 1986, Jedıcke et al 1988), however, the most lıkely ınference seems to be that the psoralens we have tested reduce the potassıum currents at the nodal membrane by a combınatıon of the mechanısms lısted above, and that the quantıtatıve contrıbutıon of the ındıvıdual effects varıes from one of these psoralens to another

Applıcatıon of our results to human axons

The ensemble of voltage-gated channels ın frog myelınated axons ıs very sımılar to that ın humans (Scholz et al 1993, Schwarz et al 1995) In applyıng neuropharmacologıcal results obtaıned ın amphıbıans to warm-blooded anımals and hence to humans, however, certaın dıfferences should be kept ın mınd Presumably one such varıable ıs the degree to whıch suıtable potassıum channel blockers alter the excıtabılıty of the "transıtıon zones" of myelınated axons (Zhou et al 1999), whıch are partıcularly affected ın demyelınatıng dıseases, and we can certaınly expect the compositıon of the potassıum channel ensemble of the Ranvıer node *per se* to be dıfferent ın humans (Reıd et al 1999) It ıs known that the nodal potassıum permeabılıty ın homoıotherms ıs consıderably less than ın the poıkılotherm (Brısmar and Schwarz 1985), thus spıkes ın ıntact Ranvıer nodes ın mammals can hardly be prolonged by the customary potassıum channel blockers, unlıke those ın axons of amphıbıans Thıs applıes all the more to the tested psoralens, the blockıng actıon of whıch, accordıng to the open channel blocker hypothesıs (see above), begıns wıth some delay after depolarızatıon and therefore does not consıderably ıncrease the spıke duratıon even ın amphıbıans (not shown)

Psoralens ın demyelınatıng dıseases

In contrast to the longest-known agent for blockıng potassıum currents ın excıtable membranes, the tetraethylammonıum ıon (for references see Štengl and Pučelík 1999), 4-amınopyrıdıne (4-AP) has acquıred a certaın sıgnıfıcance ın the symptomatıc therapy of demyelınatıng dıseases of varıous orıgıns When admınıstered

to MS patients, 4-AP was found to cause significant improvements (Stefoski et al. 1987; van Diemen et al. 1992; Bever et al. 1994; Schwid et al. 1997) as long as the plaques involved contained a high enough proportion of borderline axons, i.e. axons in which continuity is still preserved. The mechanism of action of 4-AP in demyelinating diseases presumably is to block internodal potassium channels that have become exposed as a result of the disease, with the result that the deterioration of excitability is counteracted (Waxman 1987; see, however, Smith et al. 2000); 5-MOP administered to MS patients is thought to act by a similar mechanism (for details, see Bohuslavizki et al. 1992, 1994a). Although in *in vitro* experiments on myelinated axons 5-MOP and 5,8-EOP resemble 4-AP in that they are highly selective, blocking only potassium currents, there are clear functional differences between the psoralens and 4-AP. In *in vitro* experiments the block produced by the psoralens tested here, unlike that by 4-AP (Dubois 1982; Ulbricht et al. 1982), is not use-dependent, but the potassium currents have a phasic time course. In particular, however, administration of 5-MOP to MS patients does not produce the side effects often described for 4-AP (Jones et al. 1983; Stefoski et al. 1991; Bertelsmann et al. 1992; van Diemen et al. 1992, 1993a,b; Bever et al. 1994; Polman et al. 1994). This is not surprising, in view of the fact that 5-MOP and 8-MOP at the normal dosage are known to have a relatively negligible potential for side effects (Wulf 1982). However, in this regard it should be kept in mind that in the case of MS and comparable demyelinating diseases psoralen therapy must be prolonged, and evidently no long-term systematic toxicological studies are available (see Herold et al. 1981).

Under UVA irradiation 5-MOP and 8-MOP form 2 + 2 cycloadducts with pyrimidine bases of DNA (Musajo et al. 1965), an effect that has been utilised clinically for some time in the PUVA therapy of psoriasis (Mortazawi 1972). On the other hand, 5-MOP and 8-MOP in combination with UVA irradiation are probably to be classified as carcinogenic if the period of treatment is very long (> 15 years) or a large number of treatments (> 250) are performed (Stern et al. 1997). Hence, caution dictates that when 5-MOP is used in neurology, the patients should avoid UVA radiation. Another difference between 4-AP and 5-MOP is that, so far, the neurological applications of 4-AP have been tested to a far greater extent: whereas 4-AP is already in Phase III of clinical testing (Schwid et al. 1997), for 5-MOP only individual case studies are available so far (Bohuslavizki et al. 1993b; Koppenhöfer et al. 1995; Gerst 1997).

The results presented here show that 8-MOP is a distinctly less effective potassium channel blocker than 5-MOP and, furthermore, it also blocks sodium channels to a considerable extent. For both these reasons 8-MOP has not yet been tested therapeutically by us. The potential neurological benefits of 5,8-EOP seem much greater to us; it is even more effective than 5-MOP in blocking potassium channels. Furthermore, with the method we used there was nearly no detectable block of sodium channels, which of course would be undesirable, by either 5,8-EOP or 5-MOP, particularly in the functionally important threshold region. But what may be the crucial therapeutic advantage of 5,8-EOP as opposed to 5-MOP is that

5,8-EOP is not phototoxic (Wulff et al. 1998b). However, no studies of the toxicology of 5,8-MOP have yet been carried out, so that at present a clinical trial of 5,8-EOP would be problematic.

Therapeutic aspects

Evidently, the action of potassium channel blockers in demyelinating diseases, as mentioned above, consists chiefly in blocking internodal potassium channels exposed by the disease but normally silent, which are active in the region of the resting potential (Koh et al. 1992). Several authors have already pointed out that in order for potassium channel blockers to exert a direct action in demyelinating diseases, axonal continuity must still be maintained. However, according to recent studies transected axons are common in MS lesions (Trapp et al. 1998) and, conversely, the portion of preserved but demyelinated axons, so-called borderline axons, is really smaller than had been presumed earlier. This finding is consistent with the observation that the proportion of responders to treatment of MS with potassium channel blockers is quite limited. The success rate appears to be lower, the longer the disease has lasted, a clinical observation (unpublished) presumably based on axonal dystrophy as a consequence of long-term demyelination (Raine and Cross 1989).

Borderline axons are also found in quite different pathophysiological contexts, for instance in the most frequent nerve-entrapment syndrome — the carpal tunnel syndrome — and also in patients with traumatic, nonpenetrating spinal cord injury (Waxman 1998). In both cases the borderline axons are probably produced by pressure-induced atrophy of the associated Schwann cell and the myelin sheath. For this reason, treatment of some incomplete tetraplegics with 4-AP resulted in clear improvement (Potter et al. 1998). Regarding treatment of carpal tunnel syndrome with 5-MOP, we have observed some impressive, but unfortunately not quantified improvement of the clinical findings; furthermore, the same applies to certain forms of leukodystrophy and occasional cases of therapeutic success following unsuccessful discotomy (unpublished).

Regardless of the etiology underlying the destruction of the myelin sheath, in lesions with persistent, demyelination-induced conduction block axons may degenerate as a direct consequence of their enforced prolonged silence (Scolding and Franklin 1998), which contributes to the progressive deterioration of the clinical condition. In this respect the observed symptomatic action of 5-MOP, and perhaps also of 5,8-EOP, would be expected to help to slow the progress of functional deficits resulting from, for example, MS.

Furthermore, it may well be that the therapeutic usefulness of 5-MOP, in MS for instance, not only resides in a symptomatic action on the impaired nerve conduction (Bohuslavizki et al. 1993b; Koppenhöfer et al. 1995), but also involves a kind of causal therapy in the sense of an attenuation of the inflammatory demyelinating process, given that 5-MOP and, in particular also 5,8-EOP, are capable of effectively blocking the potassium channel Kv1.3 of the T lymphocytes (Wulff et al. 1998b). This channel plays a crucial role in control of membrane potential,

production of lymphokines and proliferation of human T-cells (Lewis and Cahalan 1995)

Observations on the reliability of our results

As a result of recent progress in technology and experimental design (Bethge et al 1991, Bohuslavizki et al 1994b, Zaciu et al 1996, p 351, this paper), measurement errors of whatever origin have been minimized, with a corresponding reduction in the scatter of data in experiments directed to identical questions For example, the precision of the findings obtained after application of neuropharmaceuticals with completely reversible actions is now all that could be desired (see, e g , Bethge et al 1991, Figs 4–7, Bohuslavizki et al 1994a, Fig 4, Fig 5, this paper) Therefore, no differing results are to be expected in replicates of a given experiment — as long as the sets of data to be compared differ systematically from one another Of course, this applies only to the extent that individual experimental objects are not detectably different from one another in any of the parameters that could affect the outcome of the test The 12 axons included in this report, out of about 60 to 80 axons tested, met this criterion without exception

Regarding the reliability of individual ion-current recordings from isolated myelinated axons, many reports have been published and need not be discussed again here (for references, see Zaciu et al 1996), nor need we consider the problems associated with data acquisition and processing (see Albers et al 1989, Bethge et al 1991) The delay time δt of the measurement system we used, measured as the time elapsed from the pulse onset to the detectable onset of the sodium current, in the potential range from $V = 50$ to 110 mV was between 120 and 40 μs, with a rising tendency and distinctly increasing scatter of the data owing to measurement errors at weaker depolarisations (not shown) The findings described here with respect to the behaviour of the potassium currents at the nodal membrane remain a valid approximation when such system-related delay times are neglected (see page 351) Their possible significance in relation to data in the literature on the kinetics of sodium activation and, above all, of capacitive currents and the so-called gating currents (see, e g Neumcke et al 1976) is beyond our present scope It should be mentioned, however, that all older technologies are likely to suffer from considerably longer system-related delay times due to their particularly low bandwidths (Zaciu et al 1996)

The computer-assisted curve fittings used previously (Bohuslavizki et al 1994a) and also in the present study to quantify the results have now become so technically simple and can be carried out so rapidly that so-called tail current measurements became avoidable in most cases — which is particularly desirable since the latter were only too likely to lead to absurd overinterpretations (cf Albers et al 1989, Fig 9 and Fig 14, Elinder and Århem 1997, Fig 2) Furthermore, there is surely no doubt as to the fundamental suitability of the Hodgkin-Huxley formalism for quantifying pharmacological actions on voltage-gated sodium or potassium channels, especially since from the parameters of the formalism it is possible to calculate action potentials and their alterations (see, e g Frankenhaeuser and

Huxley 1964, Schwarz et al 1995) — including changes in the propagation of excitation Here, though, two aspects should be kept in mind First, the agreement between the action potentials calculated from so-called standard data for the node of Ranvier (Frankenhaeuser 1960, 1963a) and the directly recorded action potentials (Frankenhaeuser and Huxley 1964), considered so remarkable at the time, was by no means such a convincing proof of either the validity of the Hodgkin-Huxley formalism or, in particular, the accuracy of the numerical values of its parameters It turned out later that at least some of the numerical parameter values obtained with old technologies and involved in the especially rapid process of sodium activation (Frankenhaeuser 1960) and even so-called peculiarities of the more than one decade slower inactivation process (Frankenhaeuser 1963b, Koppenhofer 1967, Kniffki et al 1981, Schonle and Koppenhofer 1983) were systematically erroneous (Albers et al 1989, Bohuslavizki 1989, Bohuslavizki et al 1994b, Zaciu et al 1996) or based on overinterpretations (Bohuslavizki et al 1994b) The second consideration is that the Hodgkin-Huxley formalism in the original form, as used in this paper, is a macroscopic theory, a typical example of a so-called black-box method Hence, as is well known, it provides no information about the nature of the molecular events underlying the permeability changes However, this by no means limits the usefulness of the formalism in quantifying neuropharmacological effects at the axons of poikilotherms

The validity of Eqs (1), (3) and (5), which we used for curve fitting, is limited by the known potential dependence of the variables m and h as well as n and k We assumed that the resulting prerequisites $m_0 = 0$, $h_0 = 1$, $h_\infty = 0$, $n_0 = 0$ and $k_0 = 1$ were met to a sufficient degree of accuracy by test pulses with $V \geq 40$ mV and prepulses with $V = -40$ mV and a duration of 50 ms The necessary separation of sodium and potassium currents was undertaken by suitably restricting the amplitude and potential ranges of the current record (Bohuslavizki et al 1994a), the errors that resulted were clearly not appreciable

References

Albers M , Bohuslavizki K H , Koppenhofer E (1989) High standard one-loop potential clamp device for Ranvier nodes Gen Physiol Biophys **8**, 409—433

Århem P , Frankenhaeuser B (1974) Local anesthetics effects on permeability properties of nodal membrane in myelinated nerve fibres from Xenopus Potential clamp experiments Acta physiol scand **91**, 11—21

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

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

Bertelsmann F W , Polman C H , van Diemen H A , de Waal R , Koetsier J C (1992) A comparison between 4-aminopyridine and 3,4-diaminopyridine in the treatment of multiple sclerosis Ann Neurol **32**, P100

Bethge E W , Bohuslavizki K H , Hansel W , Kneip A , Koppenhofer E (1991) Effects of some potassium channel blockers on the ionic currents in myelinated nerve Gen Physiol Biophys **10**, 225—244

Bever, Jr C T , Young D , Anderson P A , Krumholz A , Conway K , Leslie J , Eddington N , Plaisance K I , Panitch H S , Dhib-Jalbut S , Fossler M J , Devane J , Johnson K P (1994) The effects of 4-aminopyridine in multiple sclerosis patients Results of a randomized, placebo-controlled, double-blind, concentration-controlled, crossover trial Neurology **44**, 1054—1059

Bohuslavizki K H (1989) Zur Kinetik der Natriumionenstrome des Ranvierschen Schnurrings unter optimierten Messbedingungen Thesis University of Kiel, Medical Faculty

Bohuslavizki K H , Hansel W , Kneip A , Koppenhofer E , Reimers A (1992) Potassium channel blockers from *Ruta* - a new approach for the treatment of multiple sclerosis Gen Physiol Biophys **11**, 507—512

Bohuslavizki K H , Hansel W , Kneip A , Koppenhofer E , Reimers A , Sanmann K (1993a) Blocking of potassium channels in Ranvier nodes by 4,5,6,7-substituted benzofurans and its significance on demyelinating diseases Gen Physiol Biophys **12**, 293—301

Bohuslavizki K H , Hinck-Kneip Ch , Kneip A , Koppenhofer E , Reimers, A (1993b) Reduction of MS-related scotomata by a new class of potassium channel blockers from *Ruta graveolens* Neuro-ophthalmol **13**, 191—198

Bohuslavizki K H , Hansel W , Kneip A , Koppenhofer E , Niemoller E , Sanmann K (1994a) Mode of action of psoralens, benzofurans, acridinons, and coumarins on the ionic currents in intact myelinated nerve fibres and its significance in demyelinating diseases Gen Physiol Biophys **13**, 309—328

Bohuslavizki K H , Kneip A , Koppenhofer E (1994b) State-of-the-art potential clamp device for myelinated nerve fibres using a new versatile input probe Gen Physiol Biophys **13**, 357—376

Brismar T , Schwarz J R (1985) Potassium permeability in rat myelinated nerve fibres Acta physiol scand **124**, 141—148

Bunger A , Erbersdobler H F , Gerst F , Grunwald G , Koppenhofer E (1996) Erfahrungen mit einer psoralenreichen Diat bei multipler Sklerose (1996) Akt Ernahr -Med **21**, 229—234

Chandy K G , Gutman G A (1995) Voltage-gated potassium channel genes In Handbook of Receptors and Channels (Ed R A North) pp 1—71, CRC press, Boca Raton, Florida

van Diemen H A , Polman C H , van Dongen T M , van Loenen A C , Nauta J J , Taphoorn M J , van Walbeek H K , Koetsier J C (1992) The effect of 4-aminopyridine on clinical signs in multiple sclerosis A randomized, placebo-controlled, double-blind, cross-over study Ann Neurol **32**, 123—130

van Diemen H A , Polman C H , Koetsier J C , van Loenen A C , Nauta J J , Bertelsmann F W (1993a) 4-Aminopyridine in patients with multiple sclerosis dosage and serum level related to efficacy and safety Clin Neuropharmacol **16**, 195—204

van Diemen H A M , Polman C H , van Dongen M M , Nauta J J , Strijers R L , van Loenen A C , Bertelsmann F W , Koetsier J C (1993b) 4-aminopyridine induces functional improvement in multiple sclerosis patients a neurophysiological study J Neurol Sci **116**, 220—226

Dubois J M (1981) Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane J Physiol (London) **318**, 297—316

Dubois J M (1982) Properties and physiological roles of K^+ currents in frog myelinated nerve fibres as revealed by 4-aminopyridine In Aminopyridines and Similarly Act-

ing Drugs (Eds P Lechat, S Thesleff and W C Bowman), pp 43—51, Pergamon Press, Oxford

Elinder F , Århem P (1997) Tail currents in the myelinated axon of *Xenopus laevis* suggests a two-open-state Na channel Biophys J **73**, 179—185

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 (1962) Potassium permeability in myelinated nerve fibres of *Xenopus laevis* J Physiol (London) **160**, 54—61

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

Frankenhaeuser B (1963b) Inactivation of the sodium-carrying mechanism in myelinated nerve fibres of *Xenopus laevis* J Physiol (London) **169**, 445—451

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

Gerst F (1997) Psoralene zur symptomatischen Therapie MS-bedingter Funktionsausfalle - Vorarbeiten fur kunftige klinische Studien Thesis University of Kiel, Medical Faculty

Herold M , Berney B , Angigbard D , le Duc R (1981) Toxicological study of the compound 5-methoxy-psoralen (5-MOP) In Psoralens in Cosmetics and Dermatology (Eds J Cahn, P Forlot, C Grupper, A Maybeck and F Urbach) pp 303—309, Pergamon Press, New York

Jedicke U , Haller R , Koppenhofer E (1988) Wirkung von Aminobenzoesaure-Derivaten auf die Ionenstrome myelinisierter Nervenfasern 1 Mitteilung Dosis-Wirkungs-Beziehungen Arzneim -Forsch /Drug Res **38** (I), 801—805

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

Kniffki K D , Siemen D , Vogel W (1981) Development of sodium permeability inactivation in nodal membranes J Physiol (London) **313**, 37—48

Koh D S , Jonas P , Brau M , Vogel W (1992) A TEA-insensitive flickering potassium channel active around the the resting potential in myelinated nerve J Membrane Biol **130**, 149—162

Koppenhofer E (1967) Die Wirkung von Tetraathylammoniumchlorid auf die Membranstrome Ranvierscher Schnurringe von *Xenopus laevis* Pflugers Arch **293**, 34—55

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

Koppenhofer E , Ditzen G , Gerst F , Reimers A (1995) Kaliumkanalblocker in der Neurologie TW Neurologie Psychiatrie **9**, 585—591

Lewis R S , Cahalan M D (1995) Potassium and calcium channels in lymphocytes Annu Rev Immunol. **13**, 623—653

Mortazawi S M A (1972) Meladinine und U V A bei Vitiligo, Psoriasis, Parapsoriasis und Akne vulgaris Derm Mschr **158**, 908—909

Musajo L , Rodighiero G , Dall'Acqua F (1965) Evidence of photoreaction of the photosensitizer furocoumarin with DNA and with pyrimidine nucleosides and nucleotides Experientia **21**, 24—25

Neumcke B , Nonner W , Stampfli R (1976) Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve Pflugers Arch **363**, 193—203

Pfaffendorf M (1986) Observations on the coupling between receptor occupation and different consecutive effects Naunyn-Schmiedeberg's Arch Pharmacol **332** (suppl), R2

Polman C H , Bertelsmann F W , de Waal R , van Diemen H A , Uitdehaag B M , van Loenen A C , Koetsier J C (1994) 4-Aminopyridine is superior to 3,4-diaminopyridine in the treatment of patients with multiple sclerosis Arch Neurol **51**, 1136—1139

Potter P J , Hayes K C , Hsieh J T , Delaney G A , Segal J L (1998) Sustained improvements in neurological function in spinal cord injured patients treated with oral 4-aminopyridine three cases Spinal-Cord **36**, 147—155

Raine C S , Cross A H (1989) Axonal dystrophy as a consequence of long-term demyelination Lab Invest **60**, 714—725

Reid G , Scholz A , Bostock H , Vogel W (1999) Human axons contain at least five types of voltage dependent potassium channel J Physiol (London) **518**, 681—696

Sachs L (1984) Applied statistics A Handbook of Techniques Springer, New York

Safronov B V , Kampe K , Vogel W (1993) Single voltage dependent potassium channels in rat peripheral nerve membrane J Physiol (London) **460**, 675—691

Scholz A , Reid G , Vogel W , Bostock H (1993) Ion channels in human axons J Neurophysiol **70**, 1274—1279

Schonle Ch , Koppenhofer E (1983) Zur Selektivitat der Wirkung gereinigten Tetraethylammoniumchlorids am Ranvierschen Schnurrings Funkt Biol Med **2**, 49—52

Schwarz J R , Reid G , Bostock H (1995) Action potentials and membrane currents in the human node of Ranvier Pflugers Arch **430**, 283—292

Schwid S R , Petrie M D , McDermott M P , Tierney D S , Mason D H , Goodman A D (1997) Quantitative assessment of sustained-release 4-aminopyridine for symptomatic treatment of multiple sclerosis Neurology **48**, 817—821

Scolding N , Franklin R (1998) Axon loss in multiple sclerosis The Lancet **352**, 340—341

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

Smith K J Felts P A , John G R (2000) Effects of 4-aminopyridine Brain **123**, 171—184

Stampfli R , Hille B (1976) Electrophysiology of the peripheral myelinated nerve In Frog Neurobiology (Eds R Llinas and 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

Stefoski D , Davis F A , Fitzsimmons W E , Luskin S S , Rush J , Parkhurst G W (1991) 4-Aminopyridine in multiple sclerosis prolonged administration Neurology **41**, 1344—1348

Štengl M , Pučelík P (1999) Effects of strontium ions on contraction and action potential in rabbit papillary muscles A comparison with effects of tetraethylammonium ions Gen Physiol Biophys **18**, 19—33

Stern R S , Khanh T N , Vakeva L H (1997) Malignant melanoma in patients treated for psoriasis with methoxsalen (psoralen) and ultraviolet A radiation (PUVA) N Engl J Med **336**, 1041—1045

Trapp B D , Peterson J , Ransohoff R M , Rudick R , Mork S , Bo L (1998) Axonal transection in the lesions of multiple sclerosis N Engl J Med **338**, 278—285

Ulbricht W , Wagner H H , Schmidtmayer J (1982) Effects of aminopyridines on potassium currents of the nodal membrane In Aminopyridines and Similarly Acting

Drugs (Eds P Lechat, S Thesleff and W C Bowman), pp 29—41, Pergamon Press, Oxford

Wang H , Kunkel D D , Martin T M , Schwartzkroin P A , Tempel B L (1993) Heteromultimeric K^+ channels in terminal and juxtaparanodal regions of neurons Nature **365**, 75—79

Waxman S G (1987) Conduction properties and pathophysiology of myelinated and nonmyelinated nerve fibers In Handbook of Clinical Neurology, Vol **7** (51), Neuropathies (Ed W B Matthews), pp 41—62, Elsevier, Amsterdam

Waxman S G (1998) Demyelinating diseases – new pathological insights, new therapeutic targets N Engl J Med **338**, 323—324

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

Wulf H Ch (1982) Aspects of the toxicology of 8-methoxypsoralen and longwave ultraviolet light Acta Dermato-Venereologica, suppl **106**, The Finsen Institute, Copenhagen

Wulff H , Koppenhofer E , Hansel W (1998a) Potassium channel blocking psoralens, benzofurans, acridones and coumarins for the treatment of multiple sclerosis and related diseases Curr Res Ion Channel Modulators **3**, 207—212

Wulff H , Rauer H , During T , Hanselmann Ch , Ruff K , Wrisch A , Grissmer St , Hansel W (1998b) Alkoxypsoralens, novel nonpeptide blockers of *Shaker*-type K^+ channels synthesis and photoreactivity J Med Chem **41**, 4542—4549

Zaciu C , Koppenhofer E , Maskaliunas R (1996) Reliability of ionic current measurements in Ranvier nodes Gen Physiol Biophys **15**, 89—107

Zajdela F , Bisagni E (1981) 5-Methoxypsoralen, the melanogenic additive in sun-tan preparations is tumorigenic in mice exposed to 365 nm u v radiation Carcinogenesis **2**, 121—127

Zhou L , Messing A , Chiu S Y (1999) Determinants of excitability at transition zones in Kv 1 1-deficient myelinated nerves J Neurosci **19**, 5768—5781

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