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, $P_{\text{Na}}^\prime$, by ca. 17%. 5-MOP and 5,8-EOP merely decreased the maximal value of $P_{\text{Na}}$, by ca. 12 and 8% respectively, whereas with weak depolarisations $P_{\text{Na}}$ was unchanged.

2. In the tested potential range the potassium permeability, $P_{\text{K}}^\prime$, 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, $k_{\infty}$, with a time constant $\tau_k$ 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 $P_{\text{K}}^\prime$ and on the steady state value, $k_{\infty}$, 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 channel 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
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; CaCl₂ 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 \( (V = -40 \text{ mV}) \) 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 \( \bar{P}_{Na} \) and \( \bar{P}_K \) were found by computer-assisted calculation of \( \bar{P}'_{Na} \) and \( \bar{P}'_K \), 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)

\[
P_{Na} = \bar{P}'_{Na} \cdot [1 - \exp(-t - \delta t)/\tau_m]^{a_1} \cdot \exp(-t - \delta t)/\tau_h
\]

where

\[
\bar{P}'_{Na} = \bar{P}_{Na} \cdot m_\infty
\]
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, $\delta t$.

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

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

where

$$P'_K = P_K \cdot n_\infty^b$$

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)

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

where

$$P'_K = P_K \cdot n_\infty^b \cdot k_0,$$

assuming the starting value of the variable, $k_0$, 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

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

(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 $\overline{P}_K$,

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

where

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

and 2. the drug-induced reduction of $k_\infty$, 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

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

\[ 1 - k_\infty = \frac{(1 - k_\infty)_{\text{max}} \cdot c^x}{c^x + (1 - k_\infty)_{C50}} \]  

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 \( r_n \) (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 (\( \Delta V_h \)) 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 \( \Delta V_h \), leftward shift of the sodium inactivation curve \( k_\infty \), \( \tau_k \) steady-state potassium “inactivation” variable and its time constant at \( V = 120 \) mV Medians, \( N \) number of measurements

<table>
<thead>
<tr>
<th></th>
<th>( c ) [( \mu \text{mol/l} )]</th>
<th>( \Delta V_h ) [mV]</th>
<th>( k_\infty )</th>
<th>( \tau_k ) [ms]</th>
<th>( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-MOP</td>
<td>100</td>
<td>−3.5</td>
<td>0.6</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>5-MOP</td>
<td>50</td>
<td>−2</td>
<td>0.2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>5,8-MOP</td>
<td>10</td>
<td>−3.6</td>
<td>0.2</td>
<td>94</td>
<td>2</td>
</tr>
</tbody>
</table>

**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 quasistationary 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
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 $\tau_m$ and $\tau_h$ of the sodium permeability $P_{Na}$ nor on the delay time of the setup, $\delta t$; $\tau_n$, however, was slightly reduced (not shown). The time constants $\tau_k$ and the steady-state values of the inactivation parameter of $P_K$, $k_\infty$, as measured in the respective test solutions, are given in Table 1. The decrease of $k_\infty$ (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 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)
Permeability-voltage curves

For permeability curves (Figs. 3 and 4) $P'_{\text{Na}}$ and $P_{\text{K}}$ 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 $P'_{\text{Na}}$ values measured in Ringer solution. The extrapolated maximum of the calculated curve gave the permeability constant $\bar{P}_{\text{Na}}$ in Ringer solution in each case, which was then used to normalise the values of $P'_{\text{Na}}$ found for Ringer solution and for the associated test solution. Then Eq. (7) was fitted to the latter values, which gave the permeability constant $\bar{P}_{\text{Na}}$ in the respective test solution. It is evident that all three test substances produce a not inconsiderable decrease of $\bar{P}_{\text{Na}}$. However, with weak depolarisations $P'_{\text{Na}}$ 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

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**Figure 3.** Sodium permeabilities, $P'_{\text{Na}}$, as calculated from sodium currents by the constant-field concept and Eqs (1) and (2). Abscissae test-pulse amplitude, $V$, ordinates $P'_{\text{Na}}$, normalised to the maximum of the continuous curve, $\bar{P}_{\text{Na}}$, 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
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. 1994b).

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
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 $B_K^{\text{max}} = 1$ 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 $(1 - k^\infty)_{\text{max}}$ 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 $P_K$ $(1 - k^\infty)_{C50}$ apparent dissociation constant of potency to bring about potassium "inactivation", $(1 - k^\infty)$ Medians, $N$ number of measurements

<table>
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<tr>
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<th>$B_K^{C50}$</th>
<th>$(1 - k^\infty)_{C50}$</th>
<th>$N$</th>
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<tbody>
<tr>
<td>8-MOP</td>
<td>66.6</td>
<td>80.1</td>
<td>4</td>
</tr>
<tr>
<td>5-MOP</td>
<td>87.6</td>
<td>25.8</td>
<td>5</td>
</tr>
<tr>
<td>5,8-MOP</td>
<td>13.5</td>
<td>6.5</td>
<td>2</td>
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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 discrim- nable (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
During et al.

that, inside the potassium channels, there are binding sites for the tested psoralens to which their access is facilitated if the channels are in the open state. In this case, the time constant \( \tau_k \) given in Table 1 would provide an approximate measure of the binding kinetics of these psoralens, and the final, steady-state level of the potassium currents under these conditions, \( k_{\infty} \), would be a measure of the psoralen-induced blocking of the potassium channels. Evidence for this view is that the dose-response relationships for the term \( 1 - k_{\infty} \), which for simplicity, we have previously called potassium "inactivation", seem to approach 1 at high concentrations of 5-MOP and 5,8-EOP (\( 1 - k_{\infty} \) could thus serve as a suitable measure of the degree of occupation of this reaction site, but the same certainly does not apply to 8-MOP. It is noteworthy that the method of describing the effect of a channel blocker in terms of a decrease in the associated permeability constant (see, e.g., Århem and Frankenhaner 1974) is evidently applicable only to one of the psoralens tested here, namely 5-MOP. In that case, which parameter does best describe the potassium current-blocking effect of these psoralens? It is beyond our present scope to consider the general significance of such a question (Pfaffendorf 1986, Jedicke et al. 1988), however, the most likely inference seems to be that the psoralens we have tested reduce the potassium currents at the nodal membrane by a combination of the mechanisms listed above, and that the quantitative contribution of the individual effects varies from one of these psoralens to another.

Application of our results to human axons

The ensemble of voltage-gated channels in frog myelinated axons is very similar to that in humans (Scholz et al. 1993, Schwarz et al. 1995). In applying neuropharmacological results obtained in amphibians to warm-blooded animals and hence to humans, however, certain differences should be kept in mind. Presumably one such variable is the degree to which suitable potassium channel blockers alter the excitability of the "transition zones" of myelinated axons (Zhou et al. 1999), which are particularly affected in demyelinating diseases, and we can certainly expect the composition of the potassium channel ensemble of the Ranvier node per se to be different in humans (Reid et al. 1999). It is known that the nodal potassium permeability in homiotherms is considerably less than in the poikilotherm (Brismar and Schwarz 1985), thus spikes in intact Ranvier nodes in mammals can hardly be prolonged by the customary potassium channel blockers, unlike those in axons of amphibians. This applies all the more to the tested psoralens, the blocking action of which, according to the open channel blocker hypothesis (see above), begins with some delay after depolarization and therefore does not considerably increase the spike duration even in amphibians (not shown).

Psoralens in demyelinating diseases

In contrast to the longest-known agent for blocking potassium currents in excitable membranes, the tetraethylammonium ion (for references see Štengl and Pučelík 1999), 4-aminopyridine (4-AP) has acquired a certain significance in the symptomatic therapy of demyelinating diseases of various origins. When administered
Effects of Three Alkoxypsoralens in Ranvier Nodes

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, 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 $\delta t$ 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 $\mu s$, with a rising tendency and distinctly increasing scatter of the data owing to measurement errors at weaker depolarizations (not shown). The findings described here with respect to the behavior 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, Ehnder 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 sodium 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, Zacz 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 it 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 \( V > 40 \) 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.

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Effects of Three Alkoxypsoralens in Ranvier Nodes


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