# Effects of Silperisone on the Excitation Process in Ranvier Nodes

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Abstract. The effect of silperisone on single intact Ranvier nodes of the toad Xenopus was investigated by adding it to the bathing medium. At 100  $\mu$ mol/l the following fully reversible effects were observed:

1. The spike amplitude decreased in a frequency-dependent manner.

2. Both the sodium activation and the inactivation curves as well as the potential dependence of  $\tau_{\rm m}$  were slightly shifted in the negative direction, while  $\tau_{\rm h}$ did not change.

3. The sodium permeability constant  $\overline{P}_{Na}$  decreased by 50%.

4. The potassium currents acquired a phasic time course previously described for certain psoralens. They reached a relative maximum and then approached a lower steady state value,  $k_{\infty}$  with a time constant of about 5 ms.

Concentration-related responses of  $\overline{P}_{Na}$ ,  $\overline{P}_{K}$  and of  $k_{\infty}$ , yielded:

5. The apparent dissociation constant of block of  $\overline{P}_{Na}$  was 110  $\mu$ mol/l.

6.  $\overline{P}_{\rm K}$  proved not to be changed by silperisone in the concentration range tested, while the variable  $k_{\infty}$  yielded a relation similar to that of  $\overline{P}_{\rm Na}$  except that the apparent dissociation constant was 24  $\mu$ mol/l.

The phasic course of the potassium currents in the presence of silperisone may be due to an open channel blockade. In view of the similarities between the actions of silperisone and 5-methoxypsoralen, it is entirely conceivable that silperisone has potential for an antispastic drug, e.g., in demyelinating diseases like multiple sclerosis.

**Key words:** Silperisone — K<sup>+</sup>-channel blocker — Node of Ranvier — Demyelinating diseases — Antispastic drug

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# Introduction

About half a century ago the first reliable, as it seemed at the time, method of measuring ionic currents at individual nodes of Ranvier in myelinated axons under potential-clamp conditions was developed (Dodge and Frankenhaeuser 1958). Within a few years many researchers were trying to use the new technology to learn more about the actions of a vast number of substances that affect the conduction of nervous impulses in the axon. In most cases the real goal was to produce targeted changes in function of the voltage-gated channels involved, so as to gain insight into their structures. In retrospect it is clear that these efforts were largely doomed to fail, not least because of systematic measurement errors and overinterpretations based on technological deficiencies (for references, see Zaciu et al. 1996).

For some years now, since the influences of all the parameters that affect the reliability of such ion-current measurements have been systematically minimized (Koppenhöfer et al. 1987; Bethge et al. 1991; Bohuslavizki et al. 1994b; Zaciu et al. 1996; Düring et al. 2000), data of previously unattainable reliability have been accumulated in large quantities. The isolated myelinated axon has finally become the ideal object with which to undertake dependable, time-saving and inexpensive screening studies to evaluate, for example, substances that are or might prove to be therapeutically useful in neurological disorders (Bethge et al. 1991; Bohuslavizki et al. 1992; 1993a,b; 1994a).

In the search for therapeutically effective antispastic drugs, it was discovered that silperisone (see Fig. 1) acts as a presynaptic inhibitor of spinal reflexes (Bielik et al. 1997; Farkas et al. 1997). The experiments described here were intended to provide an orienting overview, with reference to the parameters of the Hodgkin-Huxley formalism, of such additional actions as silperisone might prove to have on conduction in peripheral axons.



Figure 1. Molecular structure of silperisone hydrochloride.

# **Materials and Methods**

## Preparation

The experiments were carried out on isolated myelinated nerve fibres (median diameter: 25  $\mu$ m; range: 21–27  $\mu$ m; N = 11) from the sciatic nerve of the toad *Xenopus laevis.* 

#### Chemicals and solutions

The normal bathing medium was Ringer solution (in mmol/l) NaCl 107 0, KCl 2 5, CaCl<sub>2</sub> 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, the temperature during the experiments was 10°C Test solutions were produced by adding silperisone (N-[(dimethyl-4-fluorobenzylsilyl)-methyl]-piperidine) as the hydrochloride (kindly supplied by Strathmann AG, Hamburg, Germany) to the Ringer solution in an amount such that the test concentration was 100  $\mu$ mol/l throughout, except for the concentration-response experiments Data collection was started as soon as the measured effects of silperisone had stabilised at a constant level, which took less than 1 min The subsequent washing-out phase took less than 3 min for complete reversibility

#### Experimental setup

Measurements of membrane currents were carried out by a state-of-the-art potential clamp system (for details, see Bethge et al 1991, Bohuslavizki et al 1994b, Zaciu et al 1996) Nevertheless, some spontaneous changes were still inevitable Therefore, before control data were obtained in the normal bathing medium, i.e. before and after application of a test solution, all the technologically accessible parameters that could influence the scatter of data were checked and, where necessary, readjusted (for details, see During et al 2000) This in turn brought considerable savings in the number of experiments

Action potentials were measured at the output of the potential-recording amplifier in the conventional manner (Frankenhaeuser 1957)

# Pulse protocols and calibrations

For current-voltage relations various positive test pulses V were preceded by negative prepulses of amplitude and duration sufficient that at the beginning of the test pulses the sodium inactivation variable h was unity. For sodium inactivation curves the steady-state value of h was measured by the well-known two-pulse protocol (Frankenhaeuser 1959). In order to minimize the so-called frequency-dependent effects of silperisone (see Fig. 2), in all ion-current measurements the test pulse interval amounted to more than 1s

Specific currents were calculated from the current recordings and from the fibre dimensions according to Stampfli and Hille (1976)

#### Data processing

To calculate the membrane permeabilities  $P_{\text{Na}}$  and  $P_{\text{K}}$  from the underlying ionic currents  $I_{\text{Na}}$  and  $I_{\text{K}}$  the constant field concept was applied  $P'_{\text{Na}}$  and  $P'_{\text{K}}$  were found by means of least-squares fitting from the time courses of the currents records elicited by positive test pulses of V = 40 to 110 mV and V = 80 to 130 mV, respectively. In determining  $P'_{\text{Na}}$  the disturbing influence of the superimposed potassium current was reduced by neglecting the 25 30% close to zero of the peak sodium.

current in each case (for further details, see Düring et al. 2000; Hinck et al. 2001). Hence the simplified equation

$$P_{\mathrm{Na}} = P'_{\mathrm{Na}} \cdot \left[1 - \exp(-t - \delta t)/\tau_{\mathrm{m}}\right] \cdot \exp(-t - \delta t)/\tau_{\mathrm{h}} \tag{1}$$

was used, with

$$P_{\rm Na}' = \overline{P}_{\rm Na} \cdot m_{\infty} \tag{2}$$

In determining  $P'_{\rm K}$  it was only at the beginning of the recording that the 30–35% close to zero of the total amplitude of the steady-state potassium current was neglected because of the superimposed sodium current. The corresponding simplified equation for normal potassium currents was

$$P_{\rm K} = P_{\rm K}' \cdot \left[1 - \exp(-t - \delta t) / \tau_{\rm n}\right]^b \tag{3}$$

where

$$P'_{\rm K} = \overline{P}_{\rm K} \cdot n^b_{\infty} \tag{4}$$

Note that the numerical values used for  $\delta t$  were those previously found with the curve fittings employing Eq. (1). For the potassium currents recorded under silperisone, equation (3) was expanded by the so-called potassium inactivation variable k

$$P_{\mathbf{K}} = P_{\mathbf{K}}' \cdot \left[1 - \exp(-t - \delta t)/\tau_{\mathbf{n}}\right]^{b} \cdot \left[k_{\infty} + (1 - k_{\infty}) \cdot \exp(-t - \delta t)/\tau_{\mathbf{k}}\right]$$
(5)

where

$$P_{\rm K}' = \overline{P}_{\rm K} \cdot n_{\infty}^b \cdot k_{\rm o} \tag{6}$$

assuming the starting value of the variable,  $k_o$ , to be close to unity (Frankenhaeuser 1963). For permeability curves  $P'_{Na}$  and  $P'_{K}$  were plotted *versus* test pulse amplitude V. The  $P'_{Na}$ -values were fitted by the equation

$$P_{\rm Na}' = \overline{P}_{\rm Na} \frac{1}{1 + \exp[(V_{\rm PNa} - V)/k_{\rm PNa}]} eqno(7)$$

(Bohuslavizki et al. 1994b); here  $V_{PNa}$  represents the position of the inflection point of the curve on the potential axis,  $k_{PNa}$  is the maximal slope and the permeability constant  $\overline{P}_{Na}$  is the extrapolated maximum of the calculated curves. It is known that the potential dependence of  $P'_{K}$  does not show a sigmoid course (Bohuslavizki et al. 1994a), so it seemed a reasonable compromise to take the individual maximum of each data set for the permeability constant  $\overline{P}_{K}$ .

In order to quantify the blocking properties of silperisone, blockage of  $\overline{P}_{Na}$  was defined as

$$B_{\rm Na} = \frac{\Delta \overline{P}_{\rm Na}}{\overline{P}_{\rm Na\,control}} \tag{8}$$

where

$$\Delta P_{\rm Na} = P_{\rm Na\,control} - \overline{P}_{\rm Na\,test} \tag{9}$$

and plotted semilogarithmically against the test concentration. For  $\overline{P}_{K}$  the same procedure was used:

$$B_{\rm K} = \frac{\Delta \overline{P}_{\rm K}}{\overline{P}_{\rm K\,control}}\tag{10}$$

where

$$\Delta \overline{P}_{\rm K} = \overline{P}_{\rm K\,control} - \overline{P}_{\rm K\,test} \tag{11}$$

In addition, the silperisone-induced reduction of  $k_{\infty}$ , in form of the so-called potassium inactivation  $(1-k_{\infty})$ , was plotted in the same way. To the data sets concerning  $B_{\text{Na}}$  the equation

$$B_{\rm Na} = \frac{B_{\rm Na\,max} \cdot c^x}{c^x + B_{\rm Na\,C50}} \tag{12}$$

was fitted (see Jedicke et al. 1988). To the data concerning the so-called potassium inactivation the corresponding equation

$$1 - k_{\infty} = \frac{(1 - k_{\infty})_{\max} \cdot c^{y}}{c^{y} + (1 - k_{\infty})_{C50}}$$
(13)

was fitted. The fits yielded values for the apparent dissociation constants  $B_{\text{Na}C50}$ and  $(1 - k_{\infty})_{C50}$ , respectively, and for the steepness parameters, x and y.

As a measure of quality of the curve fitting we used the nonlinear regression coefficient  $r_{\rm nl}$ . It was at least = 0.988. Numerical values are given as medians and ranges throughout (Sachs 1984).

## Results

#### Resting and action potentials

Addition of silperisone to the normal bath medium affected neither the resting potential nor the time course of the action potentials, but their amplitude was reduced (Fig. 2A). A 12% decrease (8 to 17%; N = 5) in spike amplitude was observed at the end of a volley of 12 identical stimuli and the lowest stimulus frequency tested, 1 Hz. The effect became stronger at solely increasing frequencies, reaching 16% (12 to 23%) at 32 Hz (Fig. 2B). In this experiment the silperisone action reached a stationary plateau at only 16 Hz, but in other experiments the effect approximately leveled off only at 32 Hz. Such a "frequency-dependent" nature of an action is typical of many substances (Courtney 1975). Interestingly, the degree of silperisone-related frequency dependence measured at the stimulus frequency 32 Hz was diminished during hyperpolarization (with direct current) by V = -15 mV; under these conditions the frequency dependence was reduced by about one-third (N = 2).



Figure 2. Effects of silperisone on the action potential A. action potentials elucited by the last stimulus of volleys of 16 pulses of constant stimulus strength (10 mV in amplitude and 2 ms in duration) but of various frequencies of stimuli in Ringer solution (control) and under silperisone B. amplitudes of action potentials, V, at various test frequencies, f, in Ringer solution (open symbols) and under silperisone (filled symbols) Curves were drawn by eye The same experiment as in A

## Sodium inactivation curves

When the equation

$$h_{\infty} = \frac{1}{1 + \exp[(V - V_{\rm h})/k_{\rm h}]} \tag{14}$$

was fitted to the data for sodium inactivation, silperisone could be seen to induce a slight parallel shift of the curve in the negative direction (by  $\Delta V_{\rm h} = 3.0 \text{ mV}$  (1.5 to 4.0 mV; N = 3). Increasing the test concentration to 500  $\mu$ mol/l did not intensify the effect, and at 50  $\mu$ mol/l it was no longer discernible

## Time course and voltage dependence of the current records

The actions of silperisone on the sodium and potassium currents are shown in Fig. 3. The peak sodium current is distinctly reduced and the time to peak is



Figure 3. Effects of superisone on membrane currents A. peak sodium currents (arrows) in Ringer solution (control) and under superisone Test pulse amplitude V = 70 mV B. steady-state potassium currents (arrows) in Ringer solution (control) and under superisone Note that potassium currents under superisone acquire a phasic time course Test pulse amplitude V = 130 mV

slightly shorter (A, arrows), indicating that silperisone evidently has only a weak effect on the sodium-current kinetics. However, the potassium current (B), which normally rises monotonically, is massively altered by silperisone: it becomes phasic, although the final steady-state value is reached only slightly later than under normal conditions (arrows).

The potential dependence of the peak sodium currents and the steady-state potassium currents is illustrated in the form of current-voltage relations (Fig. 4). The action of silperisone consisted in a largely potential-independent reduction of both parameters



Figure 4. Current-voltage relations of peak sodium currents (triangles) and of steadystate potassium currents (squares) in Ringer solution before and after silperisone treatment (open symbols) and under silperisone (filled symbols). Abscissa: test pulse amplitude V; ordinate: corresponding ionic currents, I. Curves were drawn by spline interpolation. Data from one individual experiment.



Figure 5. Potential dependence of the time constant of sodium activation in Ringer solution before and after silperisone treatment (open symbols) and under silperisone (filled symbols). Abscissa: test pulse amplitude, V; ordinate: time constant,  $\tau_{\rm m}$ . Curves were drawn by eye. Data from one individual experiment.

## Curve fitting

By using equations (1) and (3) to fit curves to the current records, median values were obtained for the time constants of activation of the sodium and potassium permeabilities,  $\tau_{\rm m}$  and  $\tau_{\rm n}$ , respectively, as well as the delay parameter  $\delta t$  and the

V	$k_\infty$	$ au_{ m k}$
[mV]		[ms]
100	0.25	6.7
	0 22 to 0 28	63 to 7
110	0.22	5.7
	0 19 to 0 24	53 to 6
120	0.20	5.4
	0 18 to 0 22	49 to 58
130	0.19	4.8
	0 18 to 0 21	39 to 66

Table 1. Influence of superisone  $(100 \ \mu \text{mol/l})$  on the parameter  $k_{\infty}$  and on its time constant  $\tau_k$  Medians and ranges of 4 measurements

exponent b, the time constant of sodium inactivation  $\tau_{\rm h}$  and the permeabilities  $P'_{\rm Na}$ and  $P'_{\rm K}$  In addition, under the formal assumption that silperisone brings about potassium inactivation, curve fitting with equation (5) provided median values for the time constant of potassium inactivation,  $\tau_{\rm k}$ , as well as for the variable  $k_{\infty}$  The fits showed a small decrease of  $\tau_{\rm m}$  (see Fig 5), in accordance with the observed decrease of the time to peak in Figure 3A, but no significant changes of  $\tau_{\rm h}$ ,  $\tau_{\rm n}$  and the exponent b under the influence of silperisone, note that the delay parameter also did not change The parameters of silperisone-induced potassium inactivation,  $\tau_{\rm k}$  and  $k_{\infty}$ , are given in Table 1, they seem to change with membrane potential in direction of the Hodgkin-Huxley formalism

# Permeability-voltage relations

Sodium permeability-voltage curves, i.e. the potential dependence of  $P'_{\rm Na}$  in Ringer solution and under silperisone are shown in Fig. 6A. First, equation (7) was fitted to the  $P'_{\rm Na}$  values measured in Ringer solution. The extrapolated maximum of the calculated curve gave the permeability constant  $\overline{P}_{\rm Na}$  in Ringer solution which was then used to normalise the values of  $P'_{\rm Na}$  found for both Ringer solution and silperisone. Applying equation (7) to the latter the silperisone-related decrease in  $\overline{P}_{\rm Na}$  amounted to 50% (45 to 53%, N = 3). When the  $P'_{\rm Na}$  curve (Fig. 6A, dashed curve) found for silperisone is normalized to 1 it shows the potential dependence of the activation variable  $m_{\infty}$  (B, dashed curve). The main difference from the activation curve obtained under normal conditions (B, continuous curve), apart from a slight increase in slope, is that it is shifted to the left by 5 mV (3 to 6 mV, N = 3).

For potassium permeability-voltage curves (not shown) the maximum of the  $P'_{\rm K}$  values in Ringer solution was taken as representing the potassium permeability constant  $\overline{P}_{\rm K}$  (see page 160) and all the  $P'_{\rm K}$  values to be compared were normal-



Figure 6. Sodium permeabilities,  $P'_{\rm Na}$ , and sodium activation variable,  $m_{\infty}$ , as calculated by equations 1 and 2, respectively Abscissa test pulse amplitude, VOrdinates A.  $P'_{Na}$  normalized to the maximum of the continuous curve which was fitted to the data in Ringer solution (open triangles), dashed line corresponding curve fitted to the data under silperisone (filled triangels) B. continuous curve sodium activation curve in Ringer solution, identical to the corresponding  $P'_{\rm Na}$ -V curve (A, continuous curve) Dashed curve sodium activation curve under silperisone normalized to its own extrapolated max- $\operatorname{imum} (= \overline{P}_{Na} \text{ under silperisone})$ All data from one individual experiment

ized to this value. Surprisingly,  $\overline{P}_{\rm K}$  proved not to be influenced by superisone (cf. Fig. 7B).

#### Concentration-response relations

The blocking actions of silperisone at various concentrations on the sodium permeability constant,  $\overline{P}_{Na}$ , is shown in Fig. 7A When equation (8) was fitted to the measured data (filled symbols) it became evident that they conform closely to that equation; the apparent dissociation constant  $B_{Na C50}$  thus calculated (see eq. (12)) was 110  $\mu$ mol/l at x = 1.7 (99 to 118  $\mu$ mol/l; N = 3). The potassium permeability constant  $\overline{P}_{K}$ , on the other hand, was not affected at all by silperisone in the concentration range investigated (Fig. 7B, open symbols).

However, when the parameter for potassium inactivation  $(1 - k_{\infty})$  obtained from the same experimental data is plotted as a function of test concentration (Fig. 7B, filled symbols), the measured data can be seen to follow an S-curve that





can be reproduced very well with equation (13). The apparent dissociation constant  $(1 - k_{\infty})_{C50}$  thus determined was 24  $\mu$ mol/l at y = 1.2 (20 to 26  $\mu$ mol/l; N = 4).

# Discussion

#### Action potentials

At the node of Ranvier silperisone decreases the amplitude of action potentials to a degree that depends on stimulus frequency, just as a lidocaine derivative has been found to do (Courtney 1975), although such an effect was not found for silperisone in patch-clamp experiments on the dorsal root ganglion of the rat (Bielik et al 1997). Frequency dependence of block is typical of many local anaesthetics (see, e.g., Bokesch et al. 1986). This phenomenon associated with the action, for example,

of lidocaine derivatives, procaine, trimecaine, benzocaine and tetracaine is weakened by hyperpolarization (Strichartz 1973, Khodorov et al 1976, Hille 1977) In the present experiments, too, the frequency dependence of the silperisone-induced decrease in spike amplitude became less pronounced during hyperpolarization

The decrease in spike amplitude under silperisone at very low stimulus frequencies, often termed "tonic block", is thought to lower conduction velocity in the nerve *in vivo* and thereby to limit the discharge rate – an action more significant in sensory axons, with their generally higher discharge rates, than in the relatively lowfrequency motor axons (Franz and Perry 1974) The frequency-dependent "phasic block" observed in the present experiments would enhance this effect, as is well known, this interpretation is confirmed by relevant clinical observations during the administration of local anaesthetics

The fact that the "phasic block" of the actions of certain local anaesthetics is affected by membrane potential has been associated (Khodorov et al 1976, Koppenhofei and Sommer 1988) with an increase in the so-called slow sodium inactivation (Brismar 1977) However, it is more likely to reflect another possibility, namely that some local anaesthetics have better access to their sites of action when the sodium channel is open (Strichartz 1973, Hille 1977) The consequences of the potential dependence of the silperisone-related frequency dependence *in vivo* are thought to be most significant where excitatory and inhibitory synapses modulate the passage of a sensory input From what has been said above, one can postulate that as a signal is being sent along a chain of neurons, for example, neuronal facilitation would be attenuated

## Sodium currents

The silperisone-induced decrease in the peak sodium currents proved to be largely independent of the amplitude of the test pulses Almost all substances that block axonal sodium channels produce a typical leftward shift of the inactivation curve, but in the case of silperisone this shift is relatively slight in view of silperisone's strong blocking action  $(B_{\text{Na}C50} = 110 \ \mu \text{mol/l})$  The potential dependence of the time constant of sodium activation was also shifted slightly to the left Calculation of the variable  $P'_{Na}$  likewise revealed a silperisone-related leftward shift of the activation curve However, because the slope of the curve was simultaneously increased, in the region of the threshold potential the increase in  $m_{\infty}$  is less than would be expected from the amount of leftward shift On the other hand, the  $P'_{Na}$ -V curve (which for technical reasons was not measured in the region of the threshold potential) makes it appear certain that there is a decrease of  $P'_{Na}$  in that region under silperisone In tests of the concentration-dependence of the block of the sodium permeability constant  $\overline{P}_{Na}$  superisone was found to be about ten times as effective as, for example, lidocaine (Arhem and Frankenhaeuser 1974), and four times as effective as benzocaine (Jedicke et al 1988) In this respect, then, it surpasses most local anaesthetics in its *in vitro* efficacy on axonal sodium channels. We may well wonder how the interactions of the various effects – the leftward shifts of the potential dependencies of the parameters  $h, m, \tau_{\rm m}$ , the decrease in  $P_{\rm Na}$  and the above-mentioned frequency dependence of the spikes – affect the processes of spike generation and conduction in the intact organism. But this is a very complex matter, dependent on a number of other mechanisms (see Strichartz 1987) with respect to which silperisone has evidently not yet been investigated and it is beyond the scope of this discussion.

# Potassium currents

A phasic time course of the potassium currents at the Ranvier node like that found in the presence of silperisone has previously been observed during the intracellular action of certain triethylammonium ions (Armstrong and Hille 1972) and as a result of adding various other substances to the bathing medium: strychnine (Shapiro 1977), certain crown ethers (Århem et al. 1982), capsaicin (Dubois 1982), flurazepam (Schwarz and Spielmann 1983) and certain psoralens (Bohuslavizki et al. 1994a). From the viewpoint of the clinician, the similarity of the potassium currents recorded under silperisone to those obtained under 5-methoxypsoralen (5-MOP) (Bohuslavizki et al. 1993b) deserves special attention because 5-MOP has been used in the symptomatic therapy of multiple sclerosis (Bohuslavizki et al. 1993c; Koppenhöfer et al. 1995). It is not only the similarity between the time courses of the potassium currents observed under silperisone and under 5-MOP that suggests a potential clinical use for silperisone in future. The apparent dissociation constants of the two substances, calculated under the assumption of potassium inactivation, are also nearly the same, about 20  $\mu$ mol/l (Düring et al. 2000). Of course, the analogy with the axonal action of 5-MOP should not be taken too far. For one thing, the permeability constant  $P_{\rm K}$  is not affected at all by silperisone in the concentration range tested, whereas it is by 5-MOP; the implication is that the two act on the nodal potassium channel each in a different way. For another, silperisone is by no means as selective a potassium channel blocker as, for example, 5-MOP is (Bohuslavizki et al. 1994a): the apparent dissociation constant for blockade of the sodium channel by silperisone is hardly one order of magnitude greater than that for potassium channels.

# Mode of action on potassium channels

The conversion of the potassium currents from monotonic to phasic under silperisone could be based on various mechanisms. A first plausible explanation is that the constancy of the potassium ion concentration under the multiply documented perinodal diffusion barrier (for references, see Bethge et al. 1991), cannot be maintained during prolonged depolarizing pulses in the presence of silperisone and similarly acting substances because of their blocking activity on potassium channels in the part of the Schwann cell membrane that faces the axon (Bohuslavizki et al. 1994a). In this case the time course of the potassium currents under silperisone would reflect the collapse of the electromotive driving force for potassium ions in the region of the nodal membrane.

A second possible way to explain the silperisone-induced slow decline of the potassium currents after a peak has been passed is related to the fact that in both amphibians (Dubois 1981) and mammals (Safronov et al 1993) the nodal membrane comprises several different types of voltage-gated potassium channels. Therefore the time course of the potassium currents, which can be observed up to relatively high test concentrations, could imply the existence of a particularly fast inactivated channel type that silperisone blocks only at much higher test concentrations than those needed to block the other, slower types. This would be consistent with the finding that the phasic nature of the potassium-current recordings is distinctly less obvious at lower test concentrations, perhaps because in this case a larger proportion of the other channel types is still contributing to the overall current than at high test concentrations. Note, however, that the observed  $\tau_k$ -values under silperisone (see Table 1) are more than two decades smaller than those one should expect (Dubois 1981)

A third, more plausible way to explain the silperisone-induced slow decline of the potassium currents derives from Armstrong and Hille (1972) the idea here is that in the potassium channel (as it was mentioned above with reference to the sodium channel) there are binding sites for silperisone to which the access of the agent is facilitated if the channels are in the open state. In this case the time constants given in Table 1 would provide an approximate measure of the binding kinetics of silperisone, and the final, steady-state level of the potassium currents under these conditions would be a reliable measure of the silperisone-induced blocking of the potassium channels. Evidence favouring this view is that the concentrationresponse relationship for the term  $(1 - k_{\infty})$ , which for simplicity we have previously called "potassium inactivation", approaches 1 at high concentrations of the agent It could thus serve as a suitable measure of the degree of occupation of this reaction site (Pfaffendorf 1986a,b), but the same certainly does not apply to the permeability constant  $\overline{P}_{\rm K}$ 

#### Concluding remarks

It has been difficult to make useful comparisons between the silperisone findings described here and older potential-clamp studies on the neuropharmacology of the node of Ranvier The main problem laid in the widespread technological deficiencies that formerly prevailed (Zaciu et al 1996) and the resulting systematic measurement errors, which in turn not uncommonly led to unfounded interpretations. The only exceptions here are some of the much more recent publications on the axonal actions of psoralens

Nowadays there is no longer any doubt that ion-current measurements at a structure as complex as a whole Ranvier node, with its two neighbouring internodes, are not a suitable means of investigating molecular mechanisms. On the other hand, the technique of the "whole node clamp" provides, in a unique way, readily quantified basic information about the action of potentially therapeutically useful substances on the myelinated axon as a whole. Therefore computer simulations of the propagation of the nervous impulse, by incorporating data from such experiments, should in future substantially facilitate the targeted development of neuropharmaceuticals with specific actions on nervous dysfunction, for "there is a large uninvestigated field of nerve pathophysiology, where recordings from the single fibre and potential clamp analysis of its membrane properties probably will be necessary to treat nervous dysfunctions in an optimized manner" (Brismar 1983).

Studies of this kind have so far mostly employed amphibian axons, because it is obviously much harder to isolate the axons of mammals (Brismar 1983) and to perform experiments that yield data of comparable reliability. In the context of application-oriented research, then, it is fortunate that the composition of the ensemble of voltage gated channels in frogs 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 (Brismar and Schwarz 1985) and hence to humans (Schwarz et al. 1995), however, certain differences should be kept in mind (Reid et al. 1999). Moreover, the potassium permeability of the Ranvier node is considerably less in the latter than in the cold-blooded forms; the spikes in homoiotherm axons, for example, can hardly be prolonged at all by the customary potassium channel blockers, unlike those in the axons of poikilotherms. This applies all the more to silperisone, the blocking action of which, according to the open channel blocker hypothesis, begins with some delay after depolarization and therefore does not measurably increase the spike duration even in amphibians (cf. Fig. 2). Therefore, if superisone does eventually prove therapeutically useful, for instance in demyelinating diseases, it will presumably act, like 5-MOP, mainly by blocking internodal "flickering" potassium channels (Koh et al 1992), which evidently have a crucial influence on axonal excitability by regulating the resting potential. Definitive clarification here will require experiments on the action of silperisone on demyelinated axons. There is no need to detail here the other kinds of investigation that will be required, on the pharmacokinetics, toxicity, systemic actions, undesirable side effects etc. of silperisone in warm-blooded animals and ultimately in humans.

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