

Short communication

Blocking of Potassium Channels in Ranvier Nodes by 4,5,6,7-substituted Benzofurans and Its Significance on Demyelinating Diseases

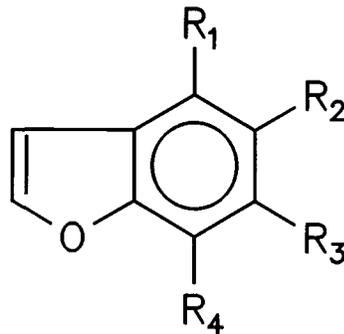
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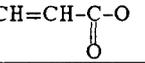
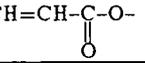
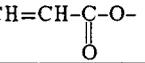
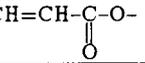
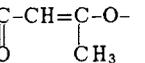
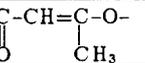
Demyelinating diseases like multiple sclerosis (MS) uncover internodal potassium channels in myelinated axons (Chiu and Ritchie 1982) which tend to clamp the axonal membrane close to the equilibrium potential, thus rendering impulse propagation difficult (Waxman 1987; Bautz et al. 1990). Therefore, the role of potassium channel blockers in the symptomatic treatment of MS has received much attention (Jones et al. 1983; Davis et al. 1986; Stefoski et al. 1987, 1991; Bohuslavizki et al. 1988; 1992; van Diemen et al. 1991; Aronson 1992).

Figure 1. General structure of the tested compounds.



In the search for further potassium channel blockers of therapeutical use in demyelinating diseases we discovered 4,5,6,7-substituted benzofurans of the general structure outlined in Fig. 1. Various compounds (see Table 1) were tested on isolated intact myelinated nerve fibres of the toad *Xenopus laevis* using an improved version of the potential clamp technique described elsewhere (Bethge et al. 1991).

Table 1. Benzofurans used with their groups R_1 to R_4 and ranked in decreasing order of efficacy for blocking steady state potassium currents, $B_{K_{ss}}$, $B_{K_{tr}}$, efficacy of blocking potassium transients. Efficacies are given in percentages of the normal steady state value (maximum blockade = 100%) Note that corrections were made for the DMSO effect S selectivity of blockade as defined by $B_{K_{ss}}/B_{Na}$, where B_{Na} denotes the corresponding block of peak sodium currents at $h = 1$. $B_{K_{ss}}$, $B_{K_{tr}}$, and S are given as medians and ranges ($n = 10$). Potassium currents and sodium currents were measured at $E = 80$ mV and at $E = -10$ mV, respectively. $-\Delta E_h$ shift of the midpoint of the sodium inactivation curve in negative direction given in mV. Drug concentrations: 100 μ mol/l throughout. * commercially available, content > 95%. ** synthesized by one of us (K S), tested for purity by conventional techniques.

Compounds	R_1	R_2	R_3	R_4	$B_{K_{ss}}$	$B_{K_{tr}}$	S	$-\Delta E_h$
5 Acetyl-4-methoxy-6-hydroxybenzofuran**	OCH ₃		OH	H	94 (81-96)	62 (43-72)	5.0 (4.0-16.2)	5.9 (3.2-7.5)
5 Acetyl-6-benzyloxy-4,7-dimethoxybenzofuran**	OCH ₃		O-CH ₂ -C ₆ H ₅	OCH ₃	90 (84-92)	59 (54-62)	1.7 (1.3-2.1)	13.9 (12.4-23.3)
5-Methoxy psoralen*	OCH ₃			H	84 (72-89)	61 (47-73)	14.1 (6.8-72.2)	3.3 (2.0-6.6)
5,8-Dimethoxy psoralen*	OCH ₃			OCH ₃	78 (61-82)	28 (13-34)	8.7 (8.2-61.1)	3.0 (2.1-3.5)
5-Acetyl-4,7-dimethoxy-6-hydroxybenzofuran*	OCH ₃		OH	OCH ₃	77 (68-83)	32 (24-36)	9.6 (7.6-33.9)	2.9 (2.3-4.2)
5-Acetyl-6-allyloxy-4,7-dimethoxybenzofuran**	OCH ₃		O-CH ₂ -CH=CH ₂	OCH ₃	50 (37-62)	37 (24-43)	5.3 (5.2-5.6)	4.1 (3.6-4.7)
8-Methoxy psoralen*	H			OCH ₃	34 (26-41)	20 (11-21)	3.8 (3.4-6.4)	3.6 (2.0-5.2)
Psoralen*	H			H	26 (19-50)	17 (10-27)	4.4 (4.2-6.2)	3.1 (1.9-3.9)
5,8-Dimethoxy-2-methylfurochromone*	OCH ₃			OCH ₃	22 (9-33)	10 (1-21)	5.6 (3.7-8.9)	2.9 (1.7-3.7)
5-Methoxy-2-methylfurochromone*	OCH ₃			H	20 (7-33)	14 (1-17)	5.0 (3.7-6.7)	2.2 (0.1-2.6)

The normal bathing medium was Ringer solution (in mmol/l): NaCl 107.0, KCl 2.5, CaCl₂ 2.0, N,N-bis(2-hydroxyethyl)-2-amino-ethanesulfonic acid/NaOH (BES) buffer 5.0 (pH 7.2; T = 10°C). For experiments with high external potassium concentration the bathing medium contained (in mmol/l): KCl 108.0, NaCl 5.0, CaCl₂ 2.0, BES 5.0. Test solutions were prepared immediately before use by dissolving the compound to be tested in dimethyl sulfoxide (DMSO; 99.96%); dilution by the corresponding bathing medium gave final concentrations of the drug and the solvent of 50 to 100 μmol/l and 0.7 mol/l, respectively. All drug effects were proved to be fully reversible within 5 min, including the minor DMSO-induced decrease in potassium permeability. Test pulses were preceded by constant hyperpolarizing prepulses, thus the sodium inactivation variable, h , was unity (Frankenhaeuser 1959) except for experiments concerning the potential dependence of h_{∞} , i. e. the inactivation curve, where prepulses were varied instead of test pulses.

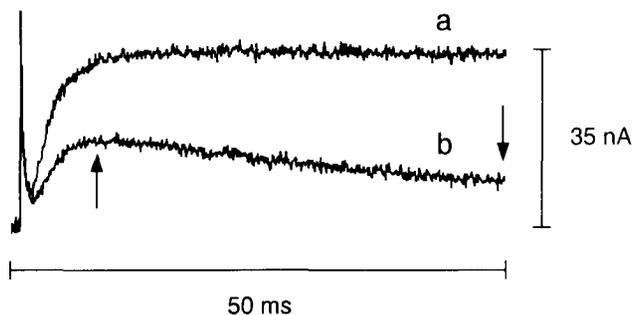


Figure 2. Membrane currents of an intact Ranvier node elicited by rectangular test pulses to $E = 80$ mV. a: Ringer solution; b: addition of 5-methoxypsoralen (100 μmol/l). ↑: transient peak potassium current; ↓: steady state potassium current. Dotted line: zero membrane current.

It is a peculiarity of the tested benzofurans that potassium currents elicited by positive test pulses were depressed in a time dependent manner as shown in Fig. 2: the remaining transient peak value (↑) is followed by a comparatively smaller steady state value (↓). Undoubtedly, the effect of benzofurans resembles the effect of capsaicin and dicyclohexano-18-crown-6 in Ranvier nodes (Bethge et al. 1991) and that of some substituted ammonium compounds in unmyelinated giant axons (Armstrong 1971; French and Shoukimas 1981; Swenson 1981). The potency of blocking steady state potassium currents, B_{Kss} , varies with the substituents chosen, as shown in Table 1; the same holds for the potency of blocking transient peak values, B_{Ktr} , the selectivity of block, S , and for the undesired shift of the midpoint of the sodium inactivation curve (Frankenhaeuser 1959) in negative direction, $-\Delta E_h$.

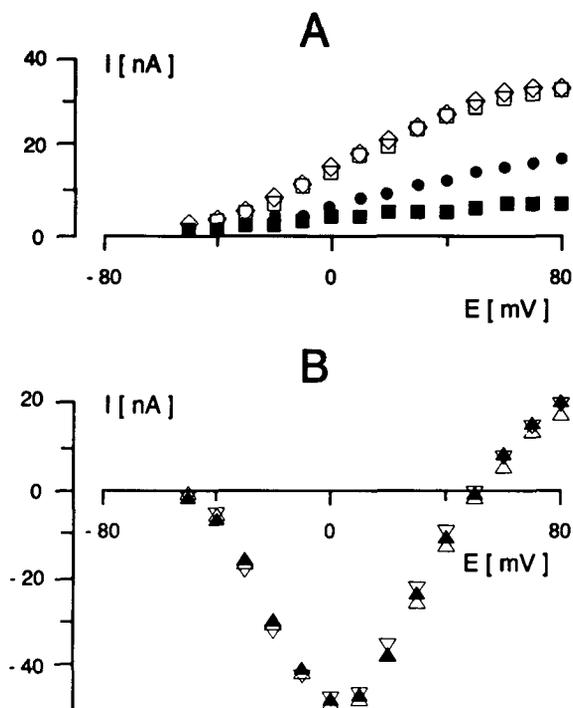


Figure 3. Current-voltage relations of potassium currents (A) and of peak sodium currents (B) before and after application of the test solution (open symbols) and during application of 100 $\mu\text{mol/l}$ 5-methoxypsoralen (filled symbols). Squares and diamonds: steady state potassium currents. Filled circles: potassium transients. Abscissa: membrane potential, E , in mV. Ordinate: membrane current, I , in nA.

The potential dependences of steady state potassium currents, initial transient potassium currents and of peak sodium currents are presented in form of current-voltage relations (Fig. 3). Under normal conditions (open symbols) the measuring points exhibited their well-known potential dependence. In the case of 5-methoxypsoralen (5-MOP) the steady state potassium currents (filled squares) were reduced in a potential-independent manner; naturally, the potassium transients (filled circles) remained higher. As expected from Table 1 the sodium currents stayed largely unaffected.

Experiments with high potassium concentration exhibited the well-known normal steady state potassium current-voltage relation (Frankenhaeuser 1962), as shown in Fig. 4 (open symbols). Addition of 5-MOP (filled symbols) led to the rectifier behavior: outward (= upward) directed currents were only diminished while inward (= downward) directed currents were suppressed to almost zero.

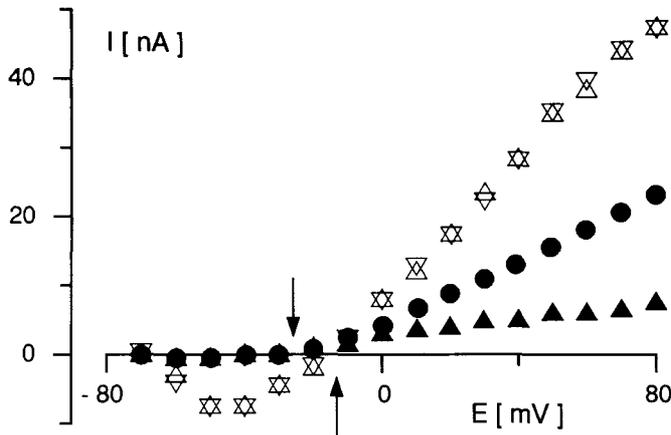


Figure 4. Current-voltage relations of potassium currents with high potassium concentration, before and after application of the test solution (open symbols) and during application of $50 \mu\text{mol/l}$ 5-methoxypsoralen (filled symbols). Triangles: steady state potassium currents. Filled circles: potassium transients. Abscissa: membrane potential, E , in mV. Ordinate: membrane current, I , in nA. Reversal potentials without application (\uparrow) and during application (\downarrow) of the drug.

The anomalous rectification, i. e. selective block of potassium outward currents, occurring in the squid axon on internal application of tetraethylammonium ions (TEA), has been referred to the axonal membrane being swept clear of TEA by potassium inward currents (Armstrong and Binstock 1965). Therefore, the observed block of potassium inward currents in Ranvier nodes caused by 5-MOP (applied externally) may be due to the blockade of potassium channels of the axolemma *per se* which are washed by potassium outward currents. In that case the observed time course of the remaining potassium outward currents (Fig. 2b) may reflect a dramatic enhancement of pharmacologically induced inactivation of axolemmal potassium channels which under normal conditions is negligibly slow (Fig. 2a). At any rate, the observed shift of the reversal potential with high potassium concentration by 5-MOP in *negative* direction (see Fig. 4, arrows) is inconsistent with the idea of pharmacological block of potassium homeostasis produced by block of potassium channels of the Schwann cell membrane (Gardner-Medwin 1986; Bethge et al. 1991).

At present, the effects of benzofurans tested on membrane currents in Ranvier nodes can be summarized as follows:

1. Potassium currents are blocked in a time dependent manner: the remaining potassium currents exhibit initial transient peak values followed by comparatively smaller steady state values.

2. The onset of potassium blockade starts with a decrease of the steady state value followed by formation of an initial potassium transient; subsequent wash-out occurs in reverse order (both not shown).

3. With high potassium concentration the block of potassium inward currents exceeds considerably the decrease of corresponding outward currents. Moreover, the reversal potential is shifted in negative direction.

4. The associated disturbance of sodium channels, as given by block of peak sodium currents and by the shift of the inactivation curve, remains comparatively small.

5. Both the selectivity and the potency of potassium blockade vary considerably with the R groups introduced.

However, further investigations are necessary for a closer understanding of the action of benzofurans in myelinated nerve.

Comparing the results of the tested benzofurans leads to the conclusion that 5-MOP offers the best compromise between potency in blocking potassium currents and selectivity of block in the intact axon. Thus, at present 5-MOP fits best the requirements of our current working hypothesis on beneficial effects of potassium channel blockers on pulse propagation in demyelinated axons (Bautz et al. 1990; Bohuslavizki et al. 1992). Corresponding investigations in MS-patients were reasonable because 5-MOP and in particular, 8-methoxypsoralen (8-MOP) are already well-established commercially available drugs for the treatment of leucoderma (El Mofty 1948) and psoriasis (Mortazawi and Oberste-Lehn 1973).

Lesions in the optic pathway (Gartner 1953; Lumsden 1970) and abnormal visual fields (Patterson and Heron 1980; D'Cruz and Ellenberger 1983) are a paradigm for patients with definite MS. Therefore, it seemed promising to test the efficacy of 5-MOP in MS-patients by a computer-controlled version of profile perimetry (Harms 1960; Bohuslavizki et al. 1993). Threshold measurements were carried out by variation of the luminance of a white target according to the so-called up and down method (see, e.g. Bebie et al. 1976) using standard techniques for static quantitative perimetry. The profile of a patient shown in Fig. 5 exhibits an extensive depression in sensitivity as compared to a corresponding normal profile of healthy subjects (dotted line). After dispensing 5-MOP a marked increase in sensitivity occurred (black areas) which lasted for at least one day. Undoubtedly, there is a striking similarity between the results shown in Fig. 5 as compared to the effects of administering a single dose of tea made from the medicinal herb *Ruta graveolens* (Bohuslavizki et al. 1992; 1993). In fact, extracts made from *Ruta* block potassium currents in isolated myelinated axons in a similar fashion (Bethge et al. 1991) as do benzofurans (see Fig. 1). Since effective potassium channel blockers like 5-MOP have been detected in *Ruta* (Gray and Waterman 1978) we conclude that the beneficial effects of *Ruta* tea against the functional deficits in MS (Bohuslavizki et al. 1992; 1993) are mainly due to the benzofurans mentioned.

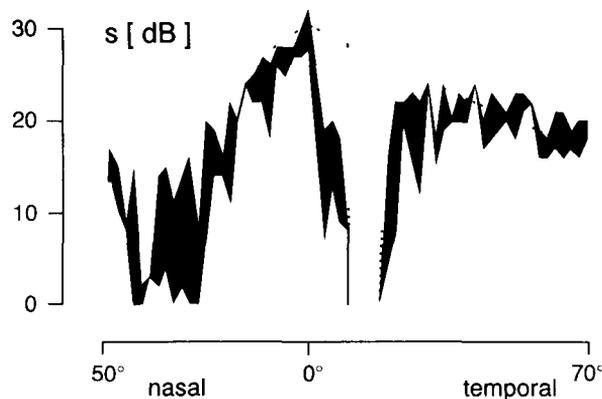


Figure 5. Profile perimetry in an MS-patient with an extensive visual field defect. Abscissa: meridian which hits the blind spot; 0° : fovea centralis. Ordinate: contrast sensitivity, s , in dB; $0 \text{ dB} = 318.3 \text{ cd/m}^2$. Black areas: improvement of sensitivity, 7 hours after oral administration of 20 mg 5-methoxypsoralen. Dotted line: corresponding normal profile for healthy subjects. Background luminance: 0.32 cd/m^2 . Distance of test points: 2° .

The well-known photosensitization by *Ruta* may be due to the fact that *Ruta* contains psoralen derivatives which are used for the so-called PUVA-therapy (see, e.g. Tronnier and Loehning 1974). It should be noted that the improvement shown in Fig. 5 was achieved by a dose which was much lower than necessary for dermatological purposes. No irritations of the skin or other side effects were noticed.

The blocking ability of various types of potassium channels in the heart by so-called class III-antiarrhythmic agents (see, e.g. Philipson and Miller 1992) is a much discussed mechanism for the suppression of serious cardiac arrhythmias following coronary artery occlusion or digitalis overdosage (see, e.g. Steinberg et al. 1984). In view of the relatively low potency and poor pharmacological selectivity of some of these agents (Gwilt et al. 1991) it also seems reasonable to test benzofurans for therapeutic use in disorders of cardiac rhythm.

In a subsequent communication we will report on the effects of further potassium channel blockers in the nerve.

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