Short communication

Blocking of Potassium Channels in Ranvier Nodes by 1,2,3,4,10-substituted Acridin-9-ons and its Possible 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), thus rendering impulse propagation difficult (Waxman 1987). Therefore, the role of potassium channel blockers like 4-aminopyridine (Stefoski et al. 1991; van Diemen et al. 1991) and substituted benzofurans (Bohuslavizki et al. 1993) in the symptomatic treatment of MS recently received much attention.

In the search for further potassium channel blockers of therapeutical use in demyelinating diseases we discovered 1,2,3,4,10-substituted acridin-9-ons (=acridons) of the general structure outlined in Figure 1. Various compounds (see Table 1) were tested on isolated intact myelinated nerve fibres of the toad *Xenopus laevis* using the potential clamp technique (Bethge et al. 1991). The experiments were carried out both under normal external potassium concentration (Ringer solution) and under high potassium concentration (for the respective ionic concentrations see Bohuslavizki et al. 1993). 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 the final concentrations of the drug and the solvent of 50 to 100 μmol/l and 700 mmol/l, respectively. Both onset and in particular washout of the effects of the tested acridons took decisively more time than the corresponding effects of benzofurans (Bohuslavizki et al. 1993). In general, after about 10 min the observed changes were fully reversible. 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 so-called inactivation curve, where prepulses were varied instead of test pulses.

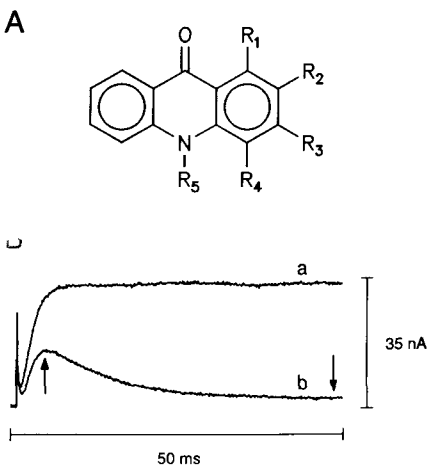

Figure 1. *A* General structure of the tested compounds *B* Membrane currents of an intact Ranvier node elicited by rectangular test pulses to $E = 80$ mV *a* Ringer solution *b* addition of 4-MOMA (100 μmol/l), ↑ transient peak potassium current, ↓ steady state potassium current Dotted line zero membrane current

The effect of the tested acridons on the time course of potassium currents is close to the effect of benzofurans (Bohuslavizki et al 1993) as shown in Figure 1 the remaining transient peak value (↑) is followed by a comparatively smaller steady value (↓) The potency of blocking steady state potassium currents, $B_{K\,ss}$, varies with the substituents, R_n, chosen as shown in Table 1, the same holds for the potency of blocking transient peak values, $B_{K\,tr}$, the selectivity of blockade, S, and for the undesired shift of the midpoint of the sodium inactivation curve (Frankenhaeuser 1959) in negative direction, $-\Delta E_h$

Methylation of a weak blocker (4-MOA) on the nitrogen (4-MOMA) results in an increase of blocking potency at constant selectivity Methylation of a strong blocker (4-EOA) shows similar effects, however a decrease of selectivity (4-EOMA) Of the methoxy-acridon-isomers (1-MOA, 2-MOA, 3-MOA, 4-MOA) 3-MOA shows strongest blocking potency at relatively good selectivity On the other hand, 2-MOA can be considered as a sodium channel blocker ($S < 1$) while 1-MOA (not shown) is almost inert After N-methylation of these substances (2-MOMA 3-MOMA 4-MOMA) no differences in their effects are observed 2-MOMA becomes in contrast to 2-MOA a potassium channel blocker It was further observed that N-ethylated derivatives (2-MOEA 4-MOEA) show very similar effects to the corresponding N-methylated derivatives (2-MOMA 4-MOMA) In the light of a desired combination of efficient blockade of potassium currents, B and high selectivity of blockade S, the acridons listed in Table 1 are less convincing than the earlier tested benzofurans (Bohuslavizki et al 1993 Table 1)

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 In Ringer solution (Fig 2*A* open symbols) the mea-

Table 1 Acridons used with their groups R_1 to R_5 and ranked in decreasing order of efficacy for blocking steady state potassium currents $B_{K\,ss}$ as given percentage of the corresponding normal value (maximum blockade = 100%) Note that corrections were made for the DMSO effect $B_{K\,tr}$ efficacy of blocking potassium transients S selectivity of blockade as defined by $B_{K\,ss}/B_{Na}$, where B_{Na} denotes the concomitant blockade 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 μmol/l throughout * commercially available, content > 95 % ** synthesized by one of us (E N), tested for purity by conventional techniques

Compounds	R_1	R_2	R_3	R_4	R_5	$B_{K\,ss}$	$B_{K\,tr}$	S	$-\Delta E_h$
4-Methoxy-10-methylacridin-9-on** (4-MOMA)	H	H	H	OCH_3	CH_3	93 (91 97)	52 (48–54)	3 3 (3 1–4 0)	9 9 (8 4–10 6)
4-Ethoxy-10-methylacridin-9-on** (4-EOMA)	H	H	H	OC_2H_5	CH_3	89 (86–92)	52 (46–65)	2 3 (1 4–3 1)	16 6 (14 5–19 5)
3-Methoxy-10-methylacridin-9-on** (3 MOMA)	H	H	OCH_3	H	CH_3	87 (81–92)	60 (55–63)	4 0 (3 4–4 3)	11 4 (8 4–17 2)
2 Methoxy-10-methylacridin-9-on** (2-MOMA)	H	OCH_3	H	H	CH_3	87 (80–91)	78 (68–84)	3 8 (2 8–4 7)	6 9 (5 8–9 5)
10-Ethyl-4-methoxyacridin-9-on** (4-MOEA)	H	H	H	OCH_3	C_2H_5	82 (68 88)	37 (20–49)	3 4 (1 8–4 1)	8 4 (4 6–13 4)
10-Ethyl-2-methoxyacridin-9-on** (2-MOEA)	H	OCH_3	H	H	C_2H_5	72 (59 80)	41 (24–49)	3 1 (2 8 3 9)	6 0 (4 5 6 7)
4-Ethoxyacridin-9-on** (4-EOA)	H	H	H	OC_2H_5	H	70 (51–85)	33 (23–42)	7 8 (3 1–8 5)	6 8 (5 4–8 9)
3-Methoxyacridin-9-on** (3-MOA)	H	H	OCH_3	H	H	67 (57 71)	32 (27–41)	4 5 (3 6–5 7)	6 7 (6 0–8 2)
2-Methoxyacridin-9-on** (2-MOA)	H	OCH_3	H	H	H	56 (36–58)	19 (12 24)	0 7 (0 6-0 8)	9 1 (8 2 15 0)
4-Methoxyacridin-9-on* (4-MOA)	H	H	H	OCH_3	H	37 (33 50)	19 (8–23)	2 7 (2 6–8 3)	6 1 (5 4–7 4)
Acridin-9-on*	H	H	H	H	H	26 (10–56)	17 (7 28)	2 6 (2 0–5 0)	6 3 (3 3 12 5)

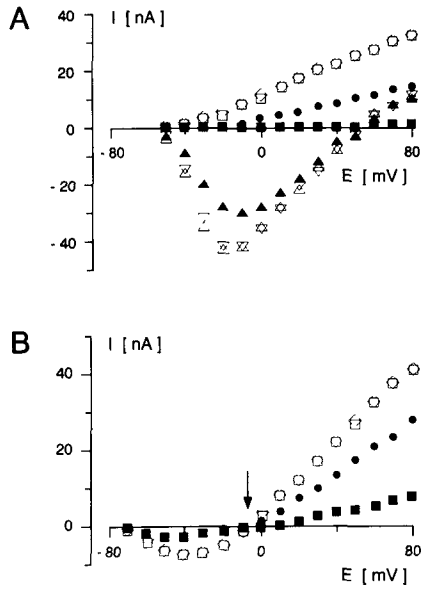

Figure 2. Current-voltage relations under normal (2.5 mmol/l) potassium concentration (A) and under high (120 mmol/l) potassium concentration (B). Open symbols: before and after application of the test solution; filled symbols: during application of 4-MOMA (A: 100 μmol/l, B: 50 μmol/l); triangels: peak sodium currents; squares and diamonds: steady state potassium currents; filled circles: potassium transients. Abscissa: membrane potential, E, in mV. Ordinate: membrane current, I, in nA. Arrow: potassium equilibrium potential.

suring points exhibit their well-known potential dependence. Upon addition of 4-methoxy-10-methylacridon (4-MOMA) the steady state potassium currents (filled squares) were reduced in a mainly potential-independent manner; the potassium transients (filled circles) remained naturally higher. As expected from Table 1 the sodium currents were much less diminished. Experiments with high potassium concentration (Fig. 2B) exhibited the well-known normal steady state potassium current-voltage relation (open symbols) (Frankenhaeuser 1962). Addition of 4-MOMA (filled symbols) led surprisingly to a uniform depression of potassium currents of either direction. Moreover, no change of the potassium equilibrium potential (arrow) was detectable. Both observations differ clearly from the effects which are typical for benzofurans (Bohuslavizki et al. 1993).

Nevertheless, apart from considerably slower onset and washout the effects of acridons tested on membrane currents in Ranvier nodes are similar to those of benzofurans (Bohuslavizki et al. 1993). They 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. With high potassium concentration an inconspicious blockade of potassium currents occurs. 3. The associated disturbance of sodium channels remains comparatively small, as given by blockade of peak sodium cur-

rents and by the shift of the inactivation curve 4 Both selectivity and potency of potassium blockade vary considerably with the *R* groups Like with benzofurans, further investigation, e g dose-response curves, are necessary for a better understanding of the action of acridons on potassium channels in the intact nerve

Comparing the results of the tested acridons 4-MOMA seems to be the best available compromise between potency in blocking potassium currents and selectivity of blockade Thus 4-MOMA meets best the prerequisites of our working hypothesis on beneficial effects of potassium channel blockers on pulse propagation in demyelinated axons (Bautz et al 1990, Bohuslavizki et al 1992) As far as benzofurans are concerned this holds unequivocally for 5-methoxypsoralen which has already been tested successfully in MS-patients by profile perimetry (Bohuslavizki et al 1993) In contrast to psoralens, there is still a great lack of knowledge about possible toxic side effects of the acridons listed in Table 1 (see, e g Reisch et al 1972) Therefore, these substances could not yet be tested in MS-patients

The blocking ability of potassium channels in the heart by so-called class III-antiarrhytmic agents (see, e g Philipson and Miller 1992) is a much discussed mechanism for the suppression of cardiac arrhythmias It might be that acridons could be also of some therapeutic use in this field

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