Effects of Bencyclane on Normal and Cevadine-modified Na Channels in Frog Skeletal Muscle

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Abstract. The effect of 10^{-5} mol/l bencyclane on the repetitive electrical activity of muscle membrane was studied with the conventional microelectrode technique. Electrical activity was induced by repetitive stimulation in normal Ringer solution (train) or by a single depolarizing current pulse in the presence of 10^{-6} mol/l cevadine (volley). Bencyclane decreased, in a use-dependent manner, the maximum rates of depolarization and repolarization (V^+_{max} and V^-_{max}, resp.) of the action potentials both of the train and the volley. The inhibition of V^+_{max} and V^-_{max} was proportional; however, it was stronger for the volleys than for the trains. The cycle length (mean interspike interval) of the volley was increased by bencyclane; the prolongation was progressive during consecutive cycles. The dissociation of bencyclane from the Na channel was studied by applying trains of different durations with equal pulse numbers. Bencyclane at a higher concentration (5×10^{-5} mol/l) caused a reversible tonic block: the overshoot potentials, V^+_{max} and V^-_{max} were markedly reduced. The reduction of V^-_{max} was slightly stronger than that of V^+_{max}. Slow membrane potential oscillation (SMPO) was evoked by treating the muscle with 10^{-4} mol/l of cevadine. The administration of 5×10^{-6} mol/l bencyclane decreased the frequency of SMPO, while 10^{-5} mol/l bencyclane terminated the slow oscillation activity without changing its baseline potential. The present results indicate that bencyclane induces use-dependent inhibition of Na channels in muscle, similarly as do class 1 antiarrhytmic drugs. Inhibition was observed with both normal and cevadine-modified Na channels.

Key words: Na channel — Skeletal muscle — Antiarrhythmic drugs — Use-dependent action

Introduction

Suppression of enhanced electrical activity of the impaired cardiac tissue, leaving the normal electrogenesis practically unaltered, is a common feature of

action of antiarrhythmic agents. Class 1 drugs of the Vaughan Williams scheme induce this effect through the use-dependent inhibition of the cardiac Na channels (Vaughan Williams 1984). The drugs do not show strict cardioselectivity, since an inhibitory effect on Na channels of skeletal muscle or neural tissues was also demonstrated (Hondeghem and Katzung 1984).

In an earlier paper from this laboratory (Nánási et al. 1987) the inhibitory effect of bencyclane on Na channel was reported in skeletal muscle and canine cardiac Purkinje fiber. Our previous results suggested that bencyclane is capable of displaying class 1 antiarrhythmic action. The drug has already been used in the therapy of different cardiovascular disorders (Braun et al. 1970; Érczy 1970; Széplaki and Széplaki 1976) as a potent smooth muscle relaxant owing to its vagolytic and Ca-antagonistic effects (Széplaki and Széplaki 1976; Fleckenstein 1978).

In the present report the inhibitory effects of bencyclane on Na channels with normal and cevadine-modified gating machinery were compared. Another aim was to study the kinetics of bencyclane — Na channel interaction and to test the antiarrhythmic effect of the molecule in a free-running system, namely on repetitive electrical activity evoked in the presence of 10^{-6} mol/l cevadine.

Materials and Methods

Experiments were performed with superficial fibres of frog sartorius muscle (*Rana esculenta*) with the conventional microelectrode technique (Ling and Gerard 1949). Two glass capillary electrodes, filled with 2.5 mol/l KCl (*DC* resistance 5—10 × 10^6 ohm), were impaled into the same fiber at a distance of approximately 5 mm. One of the intracellular microelectrodes served for electrical stimulation. Repetitive electrical activity of the membrane was evoked by trains of stimuli with appropriate duration and frequency (train), or by a single depolarizing current pulse (0.5 ms—1 μA) applied in the presence of 10^{-6} mol/l cevadine (volley). The action potentials and their first time derivatives, obtained with an analogue differentiator, were fed into a dual beam digital memory oscilloscope (Gould 0S 4000), sampled at 10 kHz and displayed on a chart recorder (Servogor 460).

In tonic block measurements (5×10^{-5} mol/l bencyclane) single action potentials were evoked: first in Ringer solution, then following 60 min treatment with bencyclane, and finally after 90 min washout.

The use-dependent effect of bencyclane during the train or volley was evaluated as the decrease in the maximum rate of depolarization (V^+_{max}) normalized to the V^+_{max} value of the first action potential. For the Xth action potential:

$$\text{inhibition (\%)} = \left(1 - \frac{V^+_{maxXB}/V^+_{max1B}}{V^+_{maxXC}/V^+_{max1C}}\right) \times 100,$$

where B refers to the bencyclane-treated and C to control fibres.

Slow membrane potential oscillation (SMPO) was recorded from muscles incubated with 10^{-4} mol/l cevadine. Impalements were made in the 60th—90th min of cevadine treatment and the microelectrode was kept in the same fiber throughout the experiment including the application of bencyclane. In the presence of 10^{-4} mol/l cevadine SMPO developed spontaneously within 30—

40 minutes without any other stimulation.

The experiments were performed at room temperature in Ringer solution (in mmol/l: Na^+ 120.2, K^+ 2.5, Cl^- 121.1, Ca^{2+} 1.8, HPO_4^{2-} 2.15, $H_2PO_4^-$ 0.85) buffered to pH 7.0 ± 0.05.

Cevadine was purchased from ICN Pharmaceuticals, Inc. (N.Y., USA) and other chemicals were from REANAL (Hungary). Bencyclane (Halidor® ampoules, EGIS, Hungary) was freshly diluted to the final concentration from a stock solution (10^{-3} mol/l).

Mean values ± SE are given. Student's *t*-test for paired or unpaired data was used to determine statistical significance. The results were considered significant for $p < 0.05$.

Results

Effect of bencyclane on the action potential in the initial phase of repetitive activity

The effects of bencyclane on two types of repetitive electrical activity is illustrated in Fig. 1. Trains with 10 ms cycle length were evoked by repetitive stimulation in Riger solution (*A*) and after 60 min incubation with 10^{-5} mol/l bencyclane (*B*). A gradual decrease in overshoot potential for consecutive spikes was characteristic for bencyclane, in contrast to negligible changes observed in Ringer solution. The overshoot of the first action potential, (OSP_1) decreased from 28.9 ± 1.9 mV to 25.5 ± 1.9 mV (insignificant); while OSP_3 dropped from 26.4 ± 1.9 mV to 19.9 ± 1.7 mV ($p < 0.05$, $n = 6$) after bencyclane treatment. Similar results were obtained with the volley (Figs. 1*C* and *D*): 60 min incubation with 10^{-5} mol/l bencyclane reduced OSP_3 from 20.5 ± 1.9 mV to 9.8 ± 3.7 mV ($p < 0.02$, $n = 10$), while the decrease of OSP_1 was statistically insignificant. The resting potential was never changed by the bencyclane concentration used.

The maximum and minimum values of the first time derivatives (V^+_{max}, V^-_{max}) referring to the consecutive action potentials of the train (*A*) and volley (*B*) are presented in Fig. 2 for control conditions (empty columns) and in the presence of bencyclane (hatched columns). The values of V^+_{max} and V^-_{max} of the Xth action potential were normalized to those of the first one to reduce error due to non-uniform drug-sensitivities of the individual specimens. Each column in the graph represents data for 6 (train) or 10 (volley) muscles, and 6—8 recordings were averaged for each muscle. The stepwise reduction of V^+_{max} and V^-_{max} after bencyclane treatment clearly indicates use-dependent inhibition of Na channels by the drug. The results shown in Figs. 2*A* and *B* are summarized in Fig. 2*C*. In relation to Fig. 2*C* three points should be stressed.

1. In the initial phase of the train or volley, the increase in inhibition strength was nearly linear.

2. The inhibition of V^+_{max} was proportional to that of V^-_{max}.

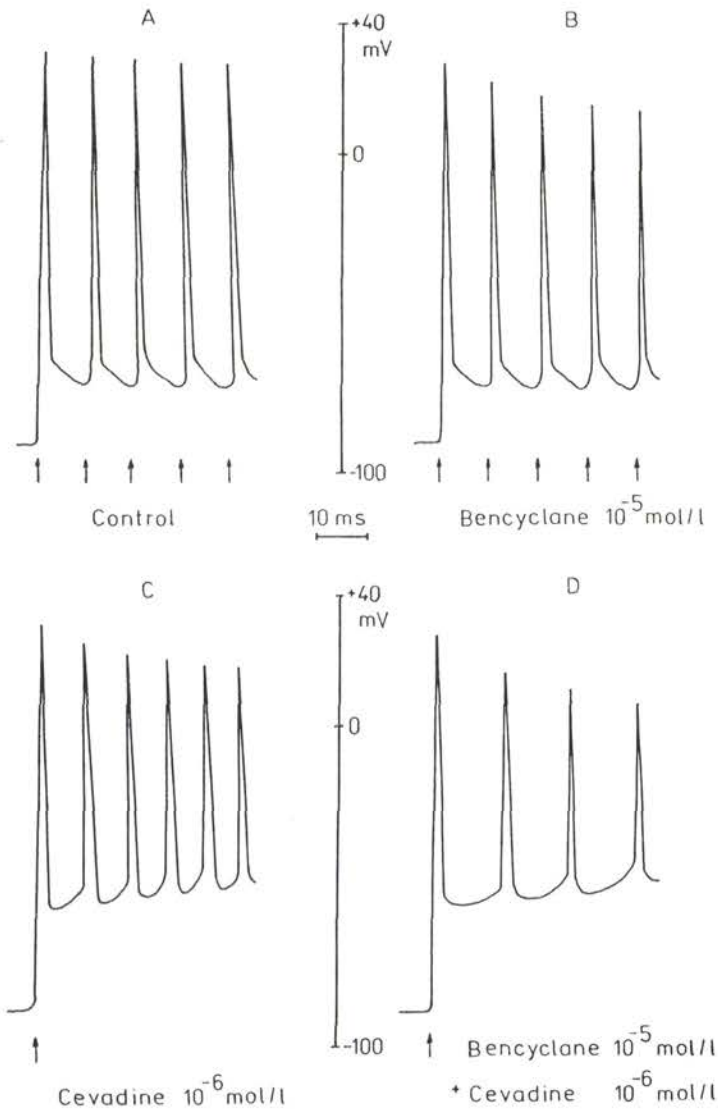

Fig. 1. Effect of bencyclane on the initial 50 ms of the train (*A*, *B*) and volley (*C*, *D*). *A*: train in Ringer solution, *B*: train recorded in the same muscle after 60 min bencyclane-treatment, *C*: volley induced by a single stimulus in the presence of 10^{-6} mol/l cevadine, *D*: volley from the same muscle incubated with bencyclane + cevadine. Note that overshoot potential decreased gradually during the volley in both cases, whereas the rate of the decrease was significantly larger in the bencyclane treated that in the control muscle. Stimulation is indicated by arrows.

3. When expressed in terms of dV/dt; the inhibition was considerably stronger with the volley than with the train.

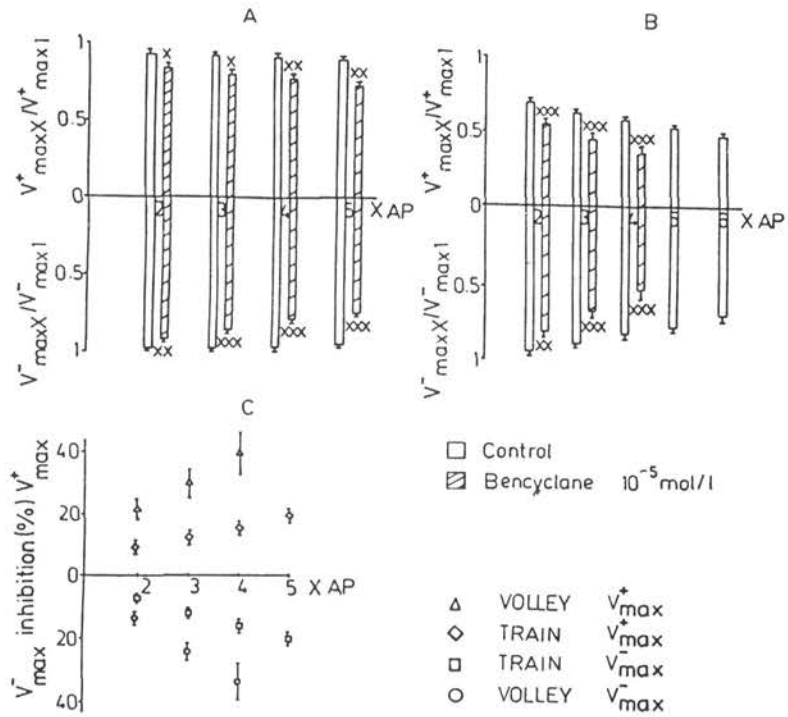

Fig. 2. Effects of bencyclane on the V^{+}_{max} and V^{-}_{max} values during train (*A*) and volley (*B*). The data were normalized to the corresponding parameters of the first action potential. In *C* inhibition is plotted on the ordinate as percentages of the control values. The columns and bars represent mean ± SE; $n = 6$ (train); $n = 10$ (volley). × — $p < 0.05$, × × — $p < 0.01$, × × × — $p < 0.001$.

Effect of bencyclane on single action potentials

Since bencyclane at 10^{-5} mol/l induced only small alterations in the parameters of the first action potential (V^{+}_{max1} of the train was reduced by 13.9 ± 2.5 %, $p < 0.02$; and the 3.5 mV decrease of OSP_1 was statistically insignificant), the steady-state effects of the drug on single action potentials were studied at 5×10^{-5} mol/l bencyclane. As obvious from Table I, 5×10^{-5} mol/l bencyclane produced a marked, though almost fully reversible, suppression of the action potential. The reduction of V^{-}_{max} was stronger than that of V^{+}_{max}, suggesting some inhibition of K channels at this concentration.

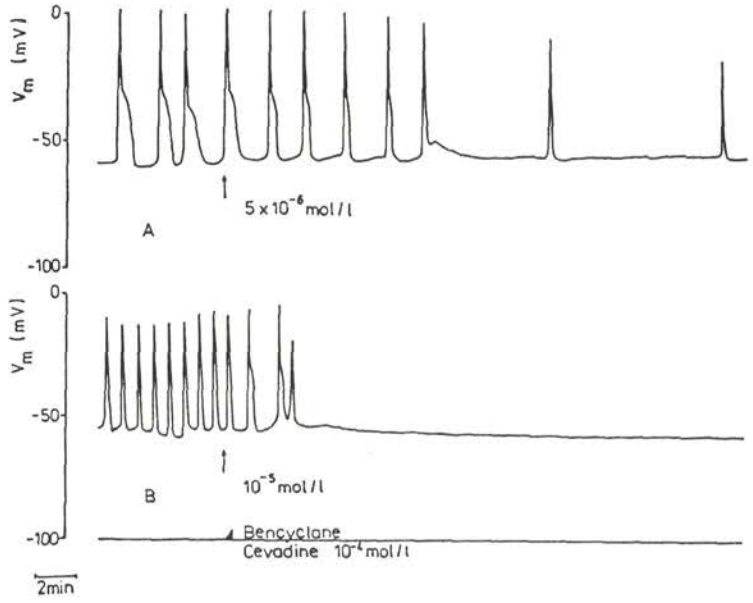

Fig. 5. Effect of bencylane on slow membrane potential oscillation (SMPO). The membrane potential changes were recorded continuously after 60 min incubation with 10^{-4} mol/l cevadine. The arrows indicate the addition of bencyclane (5×10^{-6} mol/l in *A* and 10^{-5} mol/l in *B*).

train. This is in good agreement with our previous assumption that during the initial 50 ms of a train or volley dissociation can be neglected. With longer cycles, the block weakened with the increasing cycle length, presumably due to the greater degree of dissociation of bencyclane from Na channels.

Effect of bencyclane on volley cycle length

A characteristic feature of the volley is that the duration of each consecutive cycle shows a decreasing tendency (Fig. 1*C*). Bencyclane prolonged the cycle length of the volley (Fig. 1*D*). The same is true for the experiment illustrated in Fig. 2*B*: 6 spikes were observed during the initial 50 ms of recording, whereas there were but 4 in the presence of bencyclane. Results of a quantitative analysis of the bencyclane-induced prolongation of cycle length are shown in Fig. 4. The value of T_1 (cycle length following the first spike) increased from 11.7 ± 1.1 ms to 16.9 ± 1.2 ms, that of T_n (cycle length of the last but one spike) from 8.9 ± 0.63 ms to 16.0 ± 0.93 ms, and that of $\bar{T}$ (mean cycle length) from 9.95 ± 0.86 ms to 16.4 ± 1.0 ms. The increase of the T_n/T_1 ratio (columns on the

extreme right) from 0.76 to 0.95 indicates that bencyclane significantly reduced, or even reversed in some experiments spontaneous acceleration of the volley.

Effect of bencyclane on slow oscillations of membrane potential

Cevadine (10^{-4} mol/l), similarly as veratrine, induced slow oscillations of membrane potential lasting for hours (Varga et al. 1972). The rhythmical changes of the membrane potential have mainly been attributed to function of Na channels modified by the alkaloid. Two typical experiments are illustrated in Fig. 5, showing the effect of bencyclane (5×10^{-6} mol/l in A and 10^{-5} mol/l in B) on slow oscillations of membrane potential. Both records were taken in the 60th minute of cevadine treatment. The addition of 10^{-5} mol/l bencyclane abolished SMPO within a few minutes without any significant change of the baseline ($n = 7$), whereas 5×10^{-6} mol/l bencyclane failed to terminate SMPO but reduced the oscillation frequency ($n = 5$).

Discussion

The present paper is concerned with the effects of bencyclane on the parameters of repetitive electrical activity evoked in three different ways. Both with the train and the volley 10^{-5} mol/l bencyclane diminished the overshoot potential and dV/dt values of the action potential in a use-dependent manner. The inhibition of Na channels by bencyclane was characterized as a decrease in the V^{+}_{max} values during the consecutive spikes, ignoring the possible nonlinearity in the $V^{+}_{max} - \bar{G}_{Na}$ relationship (Hondeghem 1978; Strichartz and Cohen 1978). It is important to note that the individual V^{+}_{max} values within a volley were determined by the function of Na channels with normal gating machinery, since cevadine-modified Na channels can close neither by deactivation nor by inactivation during the 50 ms recording interval (Honerjäger 1983; Leibowitz et al. 1987). The background inward current flowing through these channels depolarizes the membrane to the threshold level triggering, in this way, the repetitive activity. With the above explanation, the bencyclane-induced inhibition of V^{+}_{max} can be assumed to be the consequence of non-modified Na channel blockade, whereas the prolongation of cycle length can be mainly attributed to inhibition of cevadine-modified channels. As far as SMPO is considered, a considerable fraction of the channels may exist in the modified state, and the abolishment of SMPO by bencyclane may also be due to the inhibition of the modified channel population. Our results indicate that bencyclane, in a similar way as lidocaine, quinidine and verapamil (Nánási and Dankó 1989), inhibits both normal and cevadine-modified Na channels in the skeletal muscle. On the other hand,

Ulbricht and Stoye-Herzog (1984) reported that the neutral anesthetic benzocaine failed to inhibit Na channels in the frog node of Ranvier when their inactivation was abolished by veratridine, but inhibited them if chloramine-T was used for inactivation removal. Khodorov and Zaborovskaya (1983) studied the effects of ajmaline and N-propyl ajmaline on Na channels modified by veratridine, batrachotoxin, aconitine and ATX-II in the same preparation. They found that batrachotoxin and aconitine strongly reduced the binding of ajmaline and N-propyl ajmaline, while veratridine and ATX-II did not. This finding is especially interesting because veratridine, batrachotoxin and aconitine are supposed to share a common receptor site in the Na channel (Catterall 1979; 1980). In skeletal muscle, however, bencyclane and the previously studied antiarrhythmics (lidocaine, quinidine, verapamil) inhibited the cevadine-modified Na channels. The question remains open whether the heterogeneity of the published data reflect a significant specificity of the drug, species or the tissue applied, or whether further differences in the experimental conditions might be responsible for the diversity.

The present results suggest that the inhibitory effect of bencyclane on normal Na channels is voltage dependent. The use-dependent action of the antiarrhythmic drugs was described by the modulated receptor theory (Hille 1977; Hondeghem and Katzung 1984) and the guarded receptor theory of Starmer (1984). Either explanation may be accepted: both of them predict some voltage dependence of the block induced by a charged molecule either through voltage dependence of channel gating or by a direct action of the transmembrane electric field on the molecule, or both. The bencyclane-induced suppression of V^{+}_{max} in Fig. 2*C* was significantly stronger with the volley than with the train, presumably due to the less negative membrane potential during the former (compare Figs. 1*A* with 1*C* and 1*B* with 1*D*).

In studying the use-dependent action of 10^{-5} mol/l bencyclane on the dV/dt values of the action potentials, the inhibition of V^{+}_{max} and V^{-}_{max} was found to be proportional. Similar results were obtained with the use-dependent action of other antiarrhythmic drugs at low concentrations, or in the case of TTX-induced tonic block. It was concluded that the proportional reduction of V^{+}_{max} and V^{-}_{max} was the consequence of Na channel inhibition, and only the fraction of the V^{-}_{max} block that exceeds the magnitude of the V^{+}_{max} block can be ascribed to inhibition of K channels. As shown in Fig. 2 the reduction of V^{+}_{max} and V^{-}_{max} was proportional, suggesting that K channels were hardly affected by 10^{-5} mol/l bencyclane. At the higher concentration used (5×10^{-5} mol/l) a moderate inhibition of K channels can be supposed.

In a earlier report (Nánási and Dankó 1985) two calcium channel blocking drugs, verapamil and D-600, were demonstrated to inhibit Na channels in the skeletal muscle in the same way as class I antiarrhythmic agents (quinidine or

lidocaine). Similar results were obtained in the present experiments with bencyclane, which also showed Ca-antagonistic action. Though the inhibition of Na channel by these drugs develops at somewhat higher concentrations than sufficient to inhibit Ca channels, the question arises whether the use-dependent Na channel blockade by verapamil or bencyclane can be an additive component of the therapeutic action of these drugs.

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