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Electrophysiological effects of diclofurime on rabbit and frog atrial heart muscle

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1 The effects of diclofurime on the electrical activity of the rabbit sinus node, rabbit atria and frog atrial fibres were studied using microelectrode and the double sucrose gap voltage-clamp techniques respectively.

2 In rabbit sinus node, diclofurime (10^{-7} M to 10^{-6} M) decreased the action potential (AP) amplitude and maximum rate of depolarization ($\dot{V}_{max}$), increased the AP duration and slowed the sinus rate.

3 In rabbit atria, the drug reduced the amplitude of the depolarizing phase and $\dot{V}_{max}$, lengthened the AP duration and decreased the resting membrane potential.

4 In frog atrial fibres, the drug (10^{-5} M) depolarized the resting membrane potential, decreased $\dot{V}_{max}$ as well as the plateau amplitude. It inhibited the sodium current (I_{Na}) with a dissociation constant of 3.7×10^{-6} M and a one to one relationship between the drug molecule and the Na channel. Diclofurime did not alter the apparent reversal potential for the fast Na current (E_{Na}) but it inhibited the sodium conductance (G_{Na}) in a frequency-dependent manner.

5 Diclofurime also blocked the slow inward current (I_{slow}) without alteration of E_{slow}. The block of I_{slow} occurred with a dissociation constant of 2×10^{-5} M and unity stoichiometry.

6 The data suggest that diclofurime might be effective in the control of cardiac arrythmias since it exhibited both local anaesthetic-like and calcium antagonistic properties.

Introduction

Diclofurime ((2,3-dichloro-4-methoxy-phenyl)-2-furyl-O-(2-diethylaminoethyl) -ketoxime) is a ketone oxime derivative (see Figure 1) with potent peripheral vasodilator properties in man (Letac *et al.*, 1980) which could be related to its calcium antagonistic effect (Thuillez & Guidicelli, 1981). According to Bessin & Thuillez (1975), diclofurime ($500\,\mu g\,kg^{-1}$) exerts a bradycardic effect on the pentobarbitone anaesthetized dog. Like verapamil and diltiazem, diclofurime is classified as a Class II calcium antagonist (Spedding, 1985). Class II calcium antagonists are chemically disparate although these compounds are basic and have a very similar lipophilicity. In the Class I group, there are dihydropyridines such as nifedipine and nitrendipine; the Class II/Class III group is composed of diphenylalkylamines like cinnarizine, perhexiline, lidoflazine. Very little is known about the pharmacological properties of diclofurime. In preliminary studies, Gautier *et al.* (1981) showed that 1×10^{-6} M diclofurime decreases the amplitude of the atrial action potential in rabbit heart. The aim of the present work was to extend the analysis of the action of diclofurime to cardiac transmembrane potentials and ionic currents by means of the classical microelectrode technique applied to rabbit atrial muscle and the double sucrose gap technique applied to frog atrial fibres.

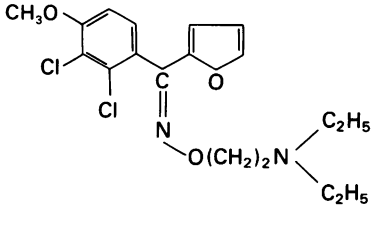

Figure 1 Structural formula of diclofurime.

Methods

Rabbit heart

Ten young albino rabbits of either sex weighing between 2 and 3 kg were killed by a blow on the neck. The hearts were removed and placed in warm oxygenated Tyrode solution of the following composition (mM): NaCl 137, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, glucose 11.5. Solutions were gassed with O_2 (95%) and CO_2 (5%) and the pH was 7.35. Experiments were performed at 36.0 ± 0.5°C. The right atrium including the superior vena cava and right atrial appendage (1 × 2 cm), but without the AV node, was isolated and mounted in a tissue bath with its endocardial surface uppermost. The preparation beats spontaneously in superfused Tyrode solution. A fine bipolar recording Ag-$AgCl_2$ electrode, insulated except at the tip, was placed on the crista-terminalis to record a surface electrogram. Transmembrane potentials in the sinus node area and in the crista-terminalis were recorded through glass microelectrodes filled with 3 M KCl (electrical resistance ranged from 10 to 20 MΩ; tip potential $< \pm 3$ mV). A Ag-$AgCl_2$ plate served as indifferent electrode. After allowing 60 min for the preparation to equilibrate with the superfusate, the action potential (AP) of the sinus node was recorded; it was associated with crista-terminalis AP or with crista-terminalis surface electrogram. This method allowed us to measure the pacemaker-crista terminalis conduction time (29.7 ± 4.1 ms; $n = 10$) and to detect pacemaker shift. The parameters measured on sinus node were sinus rate, maximum diastolic potential (the most negative voltage reached during diastole), slope of diastolic depolarization (phase 4), maximum rate of depolarization ($\dot{V}_{max}$), amplitude and duration (determined to 50% repolarization, APD_{50}) of sinus node AP. On atrial AP, we measured membrane resting potential (RP), total amplitude of the AP (AP amplitude), maximum rate of depolarization ($\dot{V}_{max}$) and duration (determined to 90% repolarization, APD_{90}). After recording of control values, diclofurime was added to Tyrode solution and AP were recorded from the same cell 20–30 min after the drug superfusion onset. The statistically significant differences between means of the AP parameters were calculated by a Student's paired *t*-test.

Frog heart

Current clamp and voltage clamp experiments were performed at 8–10°C on fine atrial trabeculae (75 to 150 μm in diameter, 2 to 4 mm in length) isolated from the heart of *Rana esculenta*. The double sucrose gap technique with vaseline seals was used (Rougier *et al.*, 1968). The experimental set up was as described

Table 1 Effects of diclofurime on the electrophysiological properties of rabbit sinus node

	(n)	*Control*	*Diclofurime* 1×10^{-7} M	*Control*	*Diclofurime* 1×10^{-6} M
Sinus rate (impulses min^{-1})	(10)	122.6 ± 8.8	110.5 ± 10.6**	121.0 ± 9.1	96.7 ± 6.2***
Slope of phase 4 ($mV\,s^{-1}$)	(6)	42.5 ± 19.8	34.2 ± 15.0	45.0 ± 21.5	21.7 ± 16.1*
AP amplitude (mV)	(6)	80.3 ± 4.7	62.3 ± 14.9*	73.5 ± 9.5	41.2 ± 17.3**
$\dot{V}_{max}$ ($V\,s^{-1}$)	(6)	3.6 ± 1.2	2.0 ± 1.3**	3.6 ± 1.9	1.5 ± 0.8**
APD_{50} (ms)	(6)	98.7 ± 4.0	110.0 ± 15.6	98.6 ± 18.3	139.6 ± 27.1*
Maximum diastolic potential (mV)	(6)	− 71.8 ± 4.5	− 65.8 ± 7.7*	− 69.4 ± 6.6	− 58.4 ± 14.6*

Sinus rate: spontaneous frequency of pacemaker; slope of phase 4; AP amplitude: amplitude of the action potential; $\dot{V}_{max}$: rate of rise of depolarization; APD_{50}: duration of the AP measured at 50% of the repolarization. The data represent mean values ± confidence intervals. n = number of experiments. The significance of difference from control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

previously by Sauviat & Suchaud (1981). The composition of the Ringer solution was (mM): NaCl 110.5, KCl 2.5, $CaCl_2$ 2, the pH of the solution was maintained at 7.3 with HEPES buffer (5 mM). In the present study, the effect of diclofurime on the fast Na conductance (G_{Na}) was studied. Thus, the control solution, in voltage clamp experiments, contained cadmium ions (1 mM) used as $CdCl_2$ to inhibit the slow inward current (I_{slow}) mainly carried by Ca ions. The effect of the drug on I_{slow} was studied in the presence of tetrodotoxin (TTX, 5.7×10^{-7} M) at a concentration that entirely inhibits G_{Na} (Sauviat, 1981).

Current and potential measurements

Starting from a holding potential (HP), the potential of the test node was displaced in rectangular steps. The fast inward Na current recorded in Ringer Cd solution (I_{Na}) and the slow inward current recorded in TTX-containing solution (I_{slow}) were measured as net inward currents if not otherwise specified. In current-voltage relationships (*I*-V curves), inward currents correspond to negative currents and depolarizations to positive potentials applied from a holding potential = − 80 mV. The apparent reversal potential for the fast Na current (E_{Na}) and for the slow inward current (E_{slow}) were determined from *I*-V curves as the point of intersection of the curves drawn before and after addition of the corresponding inhibitor i.e. TTX and Cd respectively. Preparations were stimulated with square pulses at a rate of 0.2 Hz. The limitation of the voltage clamp method as applied to frog atrial fibres as far as the fast Na current measurement is concerned have been discussed previously as well as the experimental procedure used to test the quality of the preparation and the control of the membrane potential during voltage clamp experiments (Sauviat, 1980; 1981; Sauviat & Suchaud, 1981). In the present study, the external series resistance of the equivalent circuit applicable to frog atrial bundles (Tarr & Tranck, 1971; Johnson & Liebermann, 1971) was not compensated for in view of the difficulty in making adequate correction, particularly in the case of series resistances (R_s) located in the vicinity of numerous deeper cells. The voltage distortion due to R_s was of minor interest since this work refers to relative change in Na current rather than to its absolute magnitude. Results are expressed as mean values of (n) experiments ± s.e.mean. Dose-response curves were calculated by the following modified Langmuir equation (Yeh & Narahashi., 1976): $Y = Y_{max}(\times^m/(K_D + (\times)^m))$ where Y is the percentage of inhibition of the ionic current, × the concentration of drug, m the stoichiometric parameter and K_D the dissociation constant, Y_{max} being taken as 100%.

Results

Effect on rabbit atria

The mean data from 6 to 10 experiments in which the effects of 1×10^{-7} and 1×10^{-6} M diclofurime on various electrophysiological parameters of the sino-atrial node function, primary and latent pacemaker cells as defined by Kreitner (1985), were determined,

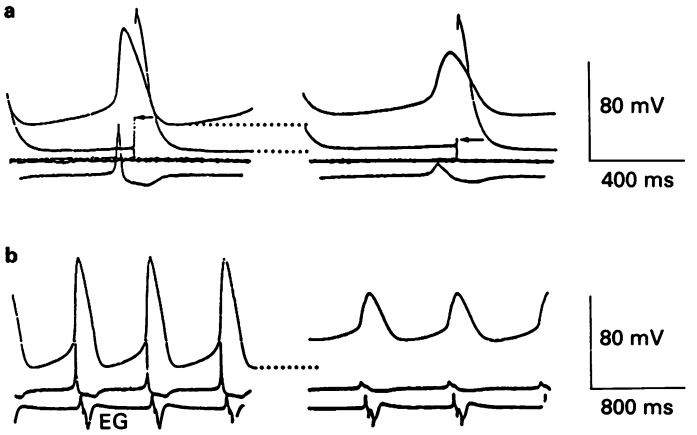

Figure 2 Effect of diclofurime 1×10^{-7} (a) and 1×10^{-6} M (b) on action potentials (AP) of two rabbit right atrial preparations. On the left: control; on the right: 25 min after exposure to the drug. From top to bottom, traces in (a) are pacemaker AP, crista terminalis AP, crista terminalis $\dot{V}_{max}$ (110 Vs^{-1}) and pacemaker $\dot{V}_{max}$ (2.6 Vs^{-1}. Arrows indicate $\dot{V}_{max}$ amplitude of atrial AP. Traces in (b) are pacemaker AP its corresponding $\dot{V}_{max}$ (5 Vs^{-1}) and the surface electrogram (EG) of crista terminalis.

and presented in Table 1. The control AP parameters for the sino-atrial node agree with those previously obtained on the same preparation by Kreitner (1981, 1985), Op'T Hof *et al.* (1983) and Nathan (1986). Diclofurime decreased the AP amplitude of the sinus node; the maximum rate of depolarization was also reduced (Figure 2a). It increased the AP duration and produced a decrease in maximal diastolic potential and in the slope of the slow diastolic depolarization phase (phase 4). Diclofurime slowed the sinus rate. This effect of the drug increased with the drug concentration as shown in Table 1 and in Figure 2. Table 2 presents the data relative to the effect of the drug (1×10^{-7} and 1×10^{-6} M) on the various parameters of atrial transmembrane potentials. Diclofurime reduced the amplitude of the depolarizing phase and $\dot{V}_{max}$; it lengthened the AP duration and decreased the membrane resting potential. The effect of the drug on pacemaker crista-terminalis conduction time were variable; Figure 2 shows that 1×10^{-6} M diclofurime did not significantly change the normal conduction time (28.5 ms in Figure 2b) while 1×10^{-7} M diclofurime increased the conduction time from 57 to 71 ms (Figure 2a). These variable results are due to large changes in the sino-atrial node and crista-terminalis action potential recorded in the presence of the drug. No shift in the true pacemaker was recorded with diclofurime. The present experiments show that diclofurime alters the electrical activity of the sinus node more than the activity of the auricule. On this last tissue, the main effect of the drug (1×10^{-7} M) on the AP was a reduction in $\dot{V}_{max}$.

Effect on frog atrial fibres

Effect on the resting (RP) and action potential The addition of diclofurime (1×10^{-6} M) to the Ringer solution decreased both the RP (5.0 ± 1.4 mV ($n = 5$)) and the amplitude of the AP. Figure 3 shows that, 4 min after drug exposure, the amplitude of the initial depolarizing phase was reduced, the corresponding $\dot{V}_{max}$ decreased by $56.1 \pm 6.6\%$ ($n = 5$). Diclofurime decreased the magnitude of the plateau, the plateau duration (measured in comparison with the zero potential line) was reduced by $35.6 \pm 18\%$ ($n = 5$) while the AP duration (measured at membrane potential 10 mV higher than the resting potential) was unchanged.

Effect on voltage clamp currents Figure 4a shows that diclofurime (5×10^{-6} M) constantly reduced the amplitude of the peak I_{Na} by 36% within 3 min. The time to peak of the current (T_p); the time needed for I_{Na} to reach one half of its peak value (t_i), used as a measure of the activation phase of the current, and the time constant of the inactivation phase of I_{Na} (τ_h; measured by semi-log plot of the falling phase of the

Table 2 Effects of diclofurime on the electrophysiological properties of rabbit atria

	(n)	*Control*	*Diclofurime* 1×10^{-7} M	(n)	*Control*	*Diclofurime* 1×10^{-6} M
AP amplitude (mV)	(7)	112.3 ± 5.9	105.1 ± 7.9**	(8)	109.4 ± 5.6	101.6 ± 10.1**
$\dot{V}_{max}$ (V s^{-1})	(7)	117.7 ± 33.9	86.1 ± 36.2**	(8)	126.2 ± 49.2	94.4 ± 43.4*
APD_{90} (ms)	(7)	95.3 ± 14.4	97.9 ± 16.9	(8)	96.2 ± 13.5	105.5 ± 13.8*
RP (mV)	(7)	− 89.7 ± 2.5	− 88.0 ± 2.9*	(8)	− 91.2 ± 3.5	− 88.2 ± 6.2*

AP amplitude: amplitude of the action potential; $\dot{V}_{max}$: upstroke velocity of the AP; APD_{90}: duration of the AP measured at 90% of the repolarization; RP: resting membrane potential. The data represent the mean values ± confidence intervals. n = number of experiments. The significance of differences from control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

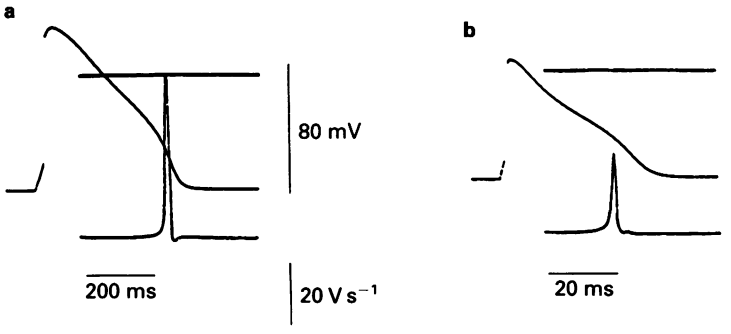

Figure 3 Alteration in frog atrial resting membrane and action potential (upper traces) and the corresponding $\dot{V}_{max}$ (lower traces) during 4 min of diclofurime (1×10^{-6} M) treatment. Horizontal line: zero membrane potential line. (a) Control, (b) in the presence of diclofurime.

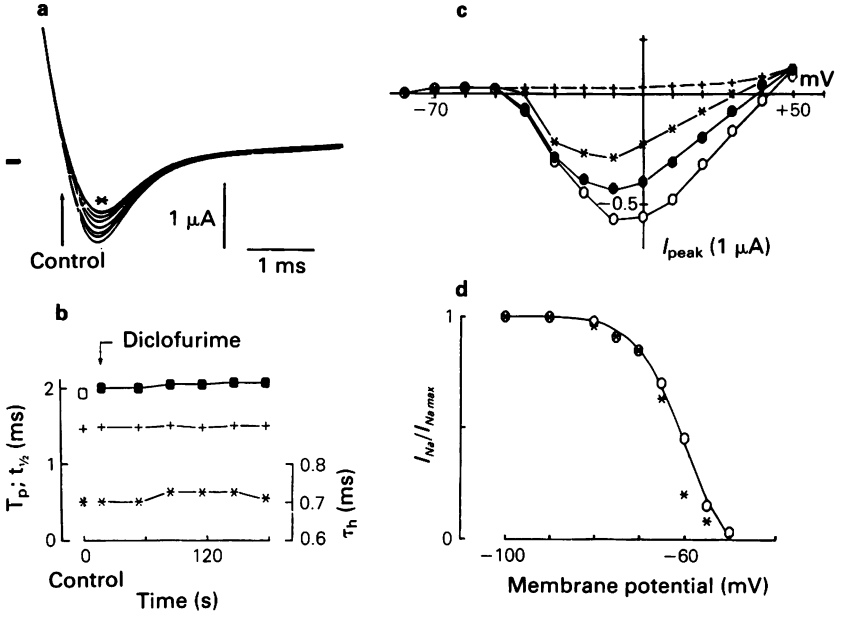

Figure 4 (a) Effect of diclofurime on the fast inward Na current (I_{Na}) elicited by a 80 mV depolarizing pulse applied from a holding potential = −80 mV, in the control solution and during diclofurime (★) (5×10^{-6} M) treatment; each trace corresponds to 35, 60, 90, 120, 150 and 180 s of drug application respectively. (b) Representation of: (●) time to peak (T_p) of I_{Na}; (+) time needed for I_{Na} to reach one half of its peak value ($t_{1/2}$); (★) time constant of the inactivation phase of I_{Na} (τ_h: determined by semi-log plot of the falling phase of the current) as a function of the peak I_{Na} blockage during diclofurime (5×10^{-6} M) treatment (abscissa scale). (c) Current-voltage relationships plotted for peak fast inward Na current before (○), after (4 min) of diclofurime 3×10^{-6} M (●); 5×10^{-6} M (★) treatment and after the addition of tetrodotoxin (5.7×10^{-7} M) to the drug containing solution (+). (d) Curve for the steady-state inactivation of the Na system plotted before (○) and during (5×10^{-6} M, ★) drug application. Ordinate scale: relative magnitude of the Na current $I_{Na}/I_{Na\,max}$ (h_∞) which developed under a 80 mV depolarizing test pulse. Abscissa scale: membrane potential (mV) during the conditioning pulse. Holding potential = −80 mV.

peak inward current) were plotted as a function of I_{Na} reduction i.e. of the duration of the drug application. Figure 4b shows that the drug treatment did not change T_p, t_i or τ_h values suggesting that diclofurime did not alter the kinetic parameters of I_{Na}. Current-voltage relationships plotted in Figure 4c for drug concentrations ranging from 3 to 5×10^{-6} M show three important features. (i) The magnitude of the peak inward current was reduced when the drug concentration increased. (ii) The minimum of the *I*-V curves was not appreciably shifted as the drug inhibition increased. (iii) The difference in the slopes of the positive region of the *I*-V curves drawn for each solution suggests that the drug decreased the maximum Na conductance. The apparent reversal potential for Na ions (E_{Na}) was not altered by diclofurime; this suggests that the selectivity of Na channels was not modified by the drug. Diclofurime (5×10^{-6} M) did not markedly change the steady-state inactivation curve (h_∞) versus membrane potential (Figure 4d), half inactivation was only shifted by 1.7 ± 0.7 mV ($n = 4$) towards a more negative membrane potential.

Dose-response relationship Figure 5 illustrates the inhibition of the peak inward Na current as a function of drug concentration. The half-maximal response was reached at a drug concentration of 3.5×10^{-6} M. The Hill plot of the data gives a stoichiometric parameter value close to unity (0.97 ± 0.07 ($n = 5$)) suggesting a one to one relationship between the drug molecule and the Na channel with a dissociation constant $K_D = 3.7 \pm 0.8 \times 10^{-6}$ M ($n = 5$).

Frequency-dependence of the block The inhibition of I_{Na} by the drug was frequency-dependent. The behaviour of the peak inward current partially inhibited after drug (5×10^{-6} M) treatment as a function of the stimulation rate is illustrated in Figure 6. Increasing the frequency of the clamp increased the inhibition of the peak current, while the same increase in stimulation rate only moderately altered I_{peak} recorded in the absence of drug.

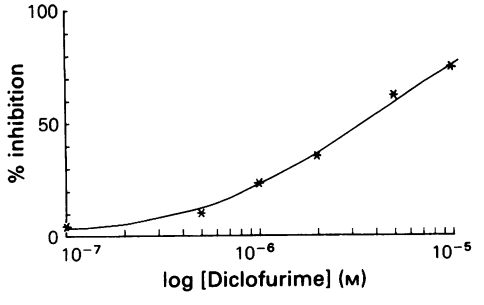

Figure 5 Log concentration-response relationship for the effect of diclofurime on the peak inward Na current amplitude (ordinate scale). Results are expressed as % of the current recorded in the absence of drug. The curve fitting experimental data was drawn according to Langmuir equation with $K_D = 3.5 \times 10^{-6}$ M and m = 1.

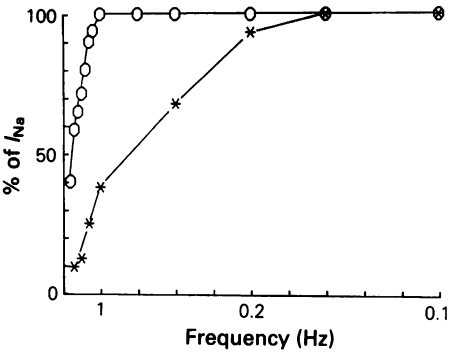

Figure 6 Effect of stimulation frequency on the block of peak inward Na current (I_{Na}) by diclofurime (5×10^{-6} M) (★). Abscissa scale: frequency of stimulation; ordinate scale: % of peak current inhibition. The holding potential was −80 mV, the depolarizing test potential was 70 mV; 25 ms duration. The preparation was driven for 1 min at the chosen rate before recording the current. (○) Same experiment performed in the control solution.

Effect on the slow inward current The addition of diclofurime 2×10^{-5} M to the TTX-containing Ringer solution decreased the magnitude of I_{slow} by 60% while a further addition of Cd (1 mM) entirely suppressed the remaining current (Figure 7a). Current-voltage relationships plotted in Figure 7b for a drug concentration of 2×10^{-5} M show four important features. (i) The magnitude of I_{slow} was reduced. (ii) The current ratio (diclofurime treated/control) did not appreciably change over the entire range of membrane potential investigated. (iii) The minimum of the *I*-V curve was not shiffed by the drug inhibition. (iv) The difference in slope of the positive region of the *I*-V curves drawn in the absence and in the presence of drug suggests that diclofurime decreased the maximum slow conductance. After drug effect on I_{slow} was complete, a further addition of Cd (1 mM) to the drug-containing solution completely suppressed I_{slow}. The apparent reversal potential for I_{slow} (E_{slow}) was not changed by diclofurime; E_{slow} was $+62.4 \pm 0.8$ mV ($n = 5$). The dose-response curve plotted in Figure 7c shows that the I_{slow} is inhibited by half at a drug concentration of about 2×10^{-5} M. The stoichiometry unity of the reaction which best fits experimental data required the reaction between one molecule of diclofurime and one slow channel.

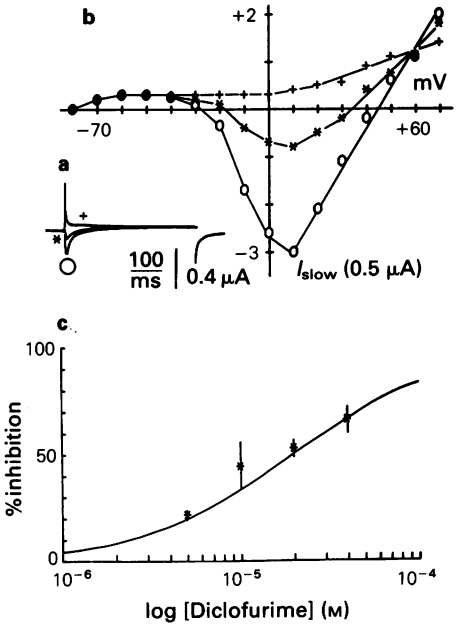

Figure 7 (a) Effect of diclofurime (★; 2×10^{-5} M) treatment (for 3 min) on the slow inward current recorded in tetrodotoxin (TTX) -containing Ringer solution (O) under a 90 mV depolarizing pulse applied from a holding potential = −80 mV. (+) Further addition of Cd (1 mM) to the control solution. (b) Current-voltage relationships plotted for I_{slow}: (O) TTX-containing control solution; (★) diclofurime (2×10^{-5} M)-containing solution; (+) Cd (1 mM)-containing-drug solution. (c) Log concentration-response curve of the effect of diclofurime on I_{slow} (ordinate scale). Results are expressed as % of the current recorded in the absence of drug. The curve fitting experimental data was drawn according to modified Langmuir equation with $K_D = 2 \times 10^{-5}$ M and m = 1. Vertical lines indicated s.e.mean ($n = 5$).

Discussion

Diclofurime at concentrations ranging between 1×10^{-7} and 1×10^{-6} M shows effects on membrane action potentials of rabbit sinus node. It causes a marked decrease in the amplitude of the AP and $\dot{V}_{max}$. These parameters, in sino-atrial node are known to depend on the slow inward current carried by Ca and/or Na (Noble, 1984) and inhibited by calcium antagonists. Our results show that the effects of diclofurime on the AP of sinus node are similar to those of verapamil (Wit & Cranefield, 1974; Gautier & Guiraudou, 1978), diltiazem and nifedipine (Kawai *et al.*, 1981). Our voltage clamp study on frog atrial fibres also shows that diclofurime decreases the slow inward current. However, the sensitivity of I_{slow} to the drug appears to be less than that of sinus node cells. The blockage of I_{slow} by diclofurime is only clearly detectable for drug concentrations larger than 1×10^{-6} M. The effect of the drug develops without alteration of E_{slow} and strongly suggests that the drug decreases the maximal slow conductance. Half inhibition of I_{slow} by the drug occurs at a concentration one order of magnitude larger (10^{-5} M) than that observed for the fast Na conductance (10^{-6} M), while the stoichiometry of the reaction indicates a one to one reaction between the drug molecule, the fast channel and the slow channel. Diclofurime decreases the slope of phase 4. Since a slow inward current is activated during diastole depolarization (Yanagihara & Irisawa, 1980), the inhibitory action of diclofurime on the slope of phase 4 can also be explained by its calcium antagonistic properties.

Diclofurime causes a concentration-dependent decrease in the spontaneous firing cycle length of the sinus node. These results are attributable to the slowing of the rate of diastolic depolarization and to a lengthening of the AP duration of the pacemaker cell. However, we cannot be sure that this last effect is not due to the bradycardia. In atrial muscle fibres, diclofurime (10^{-7} to 10^{-5} M) reduced the amplitude and $\dot{V}_{max}$ of the action potential of rabbit and frog heart. The voltage-clamp studies on frog atrial fibres confirm that these effects are due to the inhibition of the peak inward Na current by the drug. As proposed in the Hodgkin-Huxley (1952) theory, the fast sodium conductance (G_{Na}) is governed by the product of three parameters: the activation (m); the inactivation (h) and the maximal Na conductance (G_{Na}); $G_{Na} = \bar{G}_{Na}$ (m^3h). This allows several possibilities for the reduction of the peak transient current. Analysis of our data shows that diclofurime did not noticeably alter the activation (m) or inactivation (h) parameters of the peak current (Figure 4a,b). The shift in h_∞-membrane potential curve towards hyperpolarizations cannot account for the important decrease in peak current observed. Moreover, the drug treatment did not alter E_{Na} which suggests that the selectivity of the Na channel was not changed. These results lead to the conclusion that diclofurime decreases the fast Na conductance. Diclofurime also inhibited I_{peak} in a frequency-dependent manner (Figure 6) suggesting that the affinity of the drug for the receptor changes with the channel state (Grant *et al.*, 1984): as I_{Na} diminution increases with the frequency of atrial cell stimulation, diclofurime may, like Class I antiarrhythmic agents, interact with open or inactived channels (Hondeghem & Katzung, 1977).

Thus, the present observations demonstrate that diclofurime inhibits the slow inward current in atrial fibres and behaves like a calcium antagonist. Our results also show that diclofurime possesses the

additional ability to inhibit the rapid inward current at the atrial level; and has local anaesthetic-like properties like the other Class II calcium antagonists of Spedding's classification: verapamil (Rosen *et al.*, 1974; Gautier, 1980) and diltiazem (Hirth *et al.*, 1983). Verapamil and diltiazem are more potent at inhibiting I_{slow} than the fast inward current. A decrease in $\dot{V}_{max}$ in mammalian cardiac cells is obtained with concentrations larger that 1×10^{-5} M for verapamil (Gautier, 1980) and diltiazem (Hirth *et al.*, 1983) while their calcium antagonist activity is recorded for concentrations ranging between 1×10^{-7} to 1×10^{-6} M. In the case of diclofurime, our study shows that local anaesthetic activity was obtained with drug concentrations larger than (frog atrium) or equal to (rabbit atrium) those inducing calcium antagonist activity. This particular property of diclofurime in Class II calcium antagonists may be a disadvantage (decrease in inotropism and conduction velocity) in the treatment of angina pectoris or hypertension. But, as with bepridil (Schwartz *et al.*, 1985), the rapid inward current inhibition recorded at concentrations corresponding to the range of therapeutic plasma concentrations may confer Class I antiarrhythmic activity on diclofurime, whose activity can be added to Class IV antiarrhythmic properties of calcium antagonists (Gilmour & Zipes, 1985).

In conclusion, our results demonstrate that diclofurime exerts a marked inhibitory effect on the slow Ca conductance of rabbit sinus node while the blocking action of the drug appears to affect mainly the fast Na conductance in both rabbit and frog atrial muscle.

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