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Electrophysiological mechanisms for the antiarrhythmic action of mexiletine on digitalis-, reperfusion- and reoxygenation-induced arrhythmias

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1 The antiarrhythmic potency of mexiletine was evaluated on three groups of guinea-pig isolated hearts. Arrhythmias were induced (a) with digitalis intoxication, (b) with hypoxia followed by reoxygenation and (c) with ischaemia followed by reperfusion.

2 Mexiletine 10 μM was found to be very effective against all three types of arrhythmias in all three groups.

3 The electrophysiological effects of mexiletine were then studied on sheep cardiac Purkinje fibres manifesting oscillatory afterpotentials and triggered automaticity induced by barium or strophanthidin.

4 Mexiletine 10 μM consistently decreased the amplitude of oscillatory afterpotentials and blocked subsequent triggered activity in sheep Purkinje fibres.

5 In contrast, mexiletine 10 μM had no significant effect on $\dot{V}_{max}$ in normal, barium- and strophanthidin-treated preparations.

6 The results are discussed in relation to the mechanisms of antiarrhythmic action of mexiletine.

Introduction

Reperfusion-induced arrhythmias are observed in a number of clinical circumstances (Manning & Hearse, 1984). Increasing attention has been addressed to the definition of the underlying electrophysiological mechanisms and of their pharmacological control (Manning & Hearse, 1984).

The electrophysiological basis for dysrhythmia during reperfusion appears to be heterogeneous electrical recovery and possibly enhanced ventricular automaticity (Corr & Witkowsky, 1983; Manning & Hearse, 1984). It has been suggested that the rapid increase in idioventricular rate during coronary reperfusion (Penkoske *et al.*, 1978; Sheridan *et al.*, 1980; Kaplinsky *et al.*, 1981) may be related to calcium accumulation, resulting in oscillatory afterpotentials (delayed afterdepolarizations), similar to that induced by digitalis (Vassalle & Musso, 1976), leading to abnormal triggered automaticity. Accordingly, reperfusion- and digitalis-induced arrhythmias would be caused by the same electrophysiological mechanism. However, a direct demonstration of the presence of oscillatory afterpotentials (OAPs) during reperfusion has not yet been obtained, even though in an *in vitro* model it has been observed that OAPs can be generated in response to ischaemia and reperfusion (Ferrier *et al.*, 1985).

Thus, the suggestion of an analogy between the electrophysiological basis of digitalis- or reperfusion-induced arrhythmias is mainly based on indirect evidence, i.e. the presence of calcium accumulation in both conditions (Vassalle & Lin, 1979; Bourdillon & Poole-Wilson, 1981) and the demonstration that the same interventions or drugs attenuate both reperfusion- and digitalis-induced arrhythmias (Ferrier, 1977; Carbonin *et al.*, 1981; 1984; Clusin *et al.*, 1982; 1983; Di Gennaro *et al.*, 1983; 1984).

Further support for the above mentioned hypothesis could be obtained by demonstrating that antiarrhythmic drugs which are effective against digitalis-induced arrhythmias, also suppress reperfusion-induced arrythmias.

According to the present classification (Vaughan

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Williams, 1984) mexiletine is a class Ib antiarrhythmic agent, structurally related to lidocaine. Mexiletine has been found to be more active than the parent compound in controlling ventricular tachyarrhythmias following acute myocardial infarction (Horowitz *et al.*, 1981) and to suppress digitalis-induced arrhythmias (Allen *et al.*, 1972).

The antiarrhythmic effect of class I drugs is generally referred to as their property of interfering specifically with the sodium channels, an effect which is simply revealed by the reduction of the maximum rate of depolarization ($\dot{V}_{max}$) (Vaughan Williams, 1984). Consequently, we thought that it would be of interest to evaluate the antiarrhythmic potency of mexiletine on three different types of induced arrhythmias, thought to have a common underlying electrophysiological mechanism. Furthermore, in an attempt to gain insight into the electrophysiological mechanisms of the antiarrhythmic action of mexiletine, we evaluated its actions on the transmembrane potential properties of both normal and either barium- or strophanthidin-treated Purkinje fibres (Amerini *et al.*, 1985) manifesting oscillatory afterpotentials and triggered activity.

The two major aims of this study were: (1) to verify, on the basis of pharmacological sensitivity, the hypothesis of a common electrophysiological mechanism for digitalis-, reperfusion- and reoxygenation-induced arrhythmias; (2) to correlate the antiarrhythmic effect of mexiletine with its main electrophysiological actions.

Methods

Studies in the isolated heart

Guinea-pigs (body weight 350–500 g) fed *ad libitum* were used. The animals were injected with heparin, 100 u, intraperitoneally, and killed by a sharp blow at the base of the skull. The heart was immediately removed and mounted on a double-reservoir non-recirculating Langendorff apparatus perfused in a retrograde manner according to the technique described elsewhere (Carbonin *et al.*, 1981). The hydrostatic aortic perfusion pressure was 8 kPa. The control medium (composition, mM: NaCl 117, KCl 4.6, $CaCl_2$ 2, $NaHCO_3$ 20, NaH_2PO_4 0.8, $MgCl_2$ 1 and glucose 5) was equilibrated at 37°C with 95% O_2 + 5% CO_2. The CO_2 and O_2 partial pressure and the pH value of the perfusion fluid were monitored by means of a gas analyzer (Instrumentation Laboratory model 213).

Epicardial electrograms were recorded by means of an atraumatic electrode connected to an amplifier (E&M Instrument model V 1205). The left ventricular pressure was measured by inserting a 12 cm polyethylene catheter (0.5 mm diameter) into the left ventricle via the mitral valve. Left ventricular pressure and dP/dt were recorded by means of a pressure transducer (Statham P23) connected to a pressure amplifier (E&M Instrument model V 2203). All data were recorded on paper with an E&M Instrument model VR 12 Simultrace recorder. The coronary flow rate was measured by collecting the effluent.

Rhythm disturbances were subdivided into: (a) conduction disturbances (sinoatrial and atrioventricular blocks) and (b) ventricular tachyarrhythmias (VTAs): (1) total VTAs (ventricular premature beats (VPBs) + ventricular tachycardia (VT) + ventricular fibrillation (VF)), and (2) VF. A large and aberrant QRS complex and the absence of a preceding P wave identified VPBs. More than 5 consecutive VPBs were considered VT. Complete morphological irregularity of at least 10 complexes was considered VF.

After 20 min of control perfusion, in order to obtain the stabilization of heart rate and ventricular function, the hearts, in one group, were exposed to ischaemia followed by reperfusion, in another group, to hypoxia followed by reoxygenation and, in a third group, to perfusion with digitalis.

Ischaemia was produced by reducing the perfusion pressure from 8 kPa to 1 kPa (reduction of coronary flow rate 85%). After an ischaemic period of 40 min the control perfusion pressure was rapidly restored and reperfusion was maintained for 10 min.

Hypoxia was achieved by gassing the medium with a mixture of 95% N_2 + 5% CO_2 ($Po_2 < 40$ mmHg). During hypoxia the hearts were perfused with a glucose-free medium. After 15 min of hypoxia, the perfusion with the oxygenated medium was rapidly restored and maintained for 10 min (reoxygenation phase).

Digitalis intoxication of the guinea-pig isolated hearts was obtained by means of perfusion with a medium containing β-methyl-digoxin 1 μM. This glycoside concentration induces VPBs within 5–10 min and VF within 13–26 min.

Cellular electrophysiological studies on sheep isolated cardiac Purkinje fibres

Sheep hearts were brought from the slaughterhouse to the laboratory in cool oxygenated Tyrode solution. Purkinje strands were excised from the ventricles and kept in oxyenated Tyrode solution at room temperature until used. One strand was mounted in a tissue bath and superfused with Tyrode solution at a rate of 8 ml min^{-1}. The Tyrode solution had been equilibrated with a mixture of 97% O_2 and 3% CO_2 and warmed to 37°C; the pH of the solution was 7.3–7.4. The composition of the Tyrode solution was as follows (mM): NaCl 137, KCl 4, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, $MgCl_2$ 0.5, $CaCl_2$ 2.7, glucose 5. The concentration of $CaCl_2$ was changed in some ex-

periments, as indicated. The preparations were stimulated with rectangular pulses (0.5 to 1 ms in duration and 1.5 times the threshold) through bipolar silver electrodes that were electrically insulated except for the tip. Transmembrane action potentials were recorded by means of two glass microelectrodes filled under vacuum with 3 M KCl (resistance about 10 MΩ). The membrane potential was measured differentially by means of two high-input impedance guard electrometer amplifiers (Bigongiari, Firenze). The potential was displayed on a Tektronix model 5113 dual beam storage oscilloscope and recorded on a FM tape recorder (Racal 14 DS) at 7.5 inches s^{-1}. The records were played back into a chart recorder (Gould Brush 2400). For studies in normal Purkinje fibres, the preparations were stimulated at constant rate (1 or 2 Hz) and an automated analysis of the action potential was performed as previously described (Fusi *et al.*, 1984). The evaluation of the following parameters was carried out: action potential amplitude (AP), overshoot (OS), maximum diastolic potential (MDP), maximum upstroke velocity ($\dot{V}_{max}$), action potential duration at -60 mV (APD_{-60}) and at 90% repolarization (APD_{90}). Oscillatory afterpotentials (OAPs) were induced by exposing the preparations to a low barium concentration or to strophanthidin as previously described (Mugelli *et al.*, 1983; Amerini *et al.*, 1985). The drive stimulus was interrupted periodically (usually every min for 30 s) to assess the presence of oscillatory afterpotentials and triggered activity. The amplitude of the oscillatory potentials was measured from the peak to the maximum membrane potential immediately following the oscillatory afterpotentials (Amerini *et al.*, 1985).

The drugs used in this study were chemically pure: strophanthidin (Sigma); β-methyl-digoxin (Boehringer-Biochemia); mexiletine hydrochloride (Boehringer Ingelheim); $BaCl_2$.

Analysis of data

All data are presented as means ± s.e.mean. The analysis of data for significance was performed by means of Student's *t* test for paired or grouped data and χ^2 (2×2 or $2 \times k$ contingency tables were calculated by standard procedures). Differences with *P* values of < 0.05 were considered significant.

Results

Effect of mexiletine on reoxygenation- and reperfusion-induced ventricular arrhythmias

Perfusion with a hypoxic glucose-free medium or ischaemia caused, as previously demonstrated (Carbonin *et al.*, 1981), a rapid decrease in ventricular rate; during reoxygenation or reperfusion VTAs were present in 80–90% of the control hearts, while VF was induced in almost half of the preparations. Figure 1 shows one typical experiment: during the hypoxic phase, the systolic pressure fell to zero and the diastolic pressure progressively increased, as contracture develops during anoxia (Ganote & Sims, 1983). During this period, conduction disturbances without

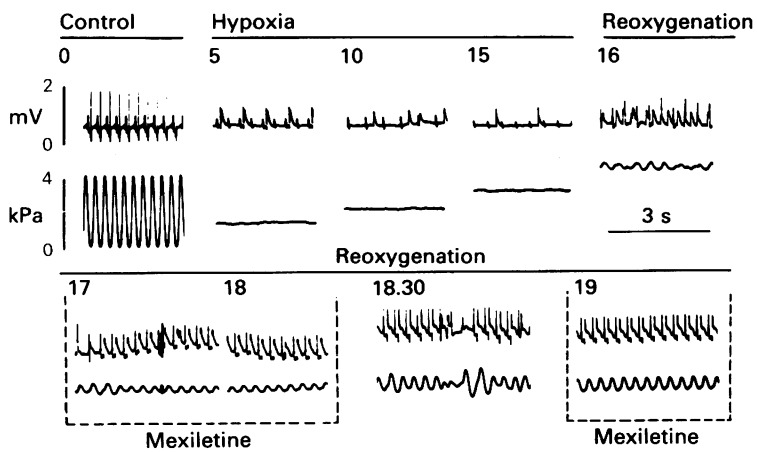

Figure 1 Effect of mexiletine 10 μM on reoxygenation-induced arrhythmias in guinea-pig isolated hearts. The traces are arranged in pairs with top trace an ECG recording and bottom trace a recording of ventricular pressure. Numbers that identify the pairs indicate the time (min) during hypoxia or reoxygenation when the traces were recorded. The traces within the dashed line indicate the time when mexiletine was added to the medium.

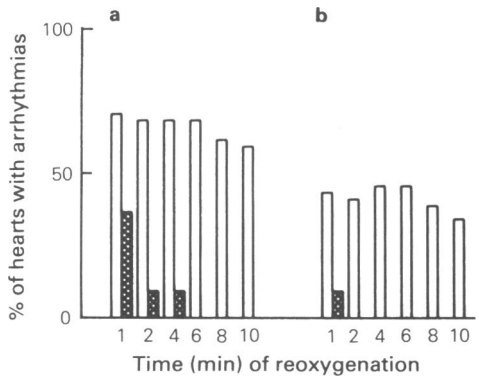

Figure 2 The number (%) of hearts developing (a) ventricular tachyarrhythmias or (b) ventricular fibrillation during the reoxygenation phase. Open and hatched columns indicate control and mexiletine (10 μM) perfused hearts, respectively.

VTAs were present. During the reoxygenation, VTAs rapidly developed.

Mexiletine 10 μM was found to be the lowest effective concentration able to abolish VTAs; sinus rhythm was restored in about 40 s. Washout of mexiletine was followed by reappearance of VTAs, which were again abolished by the readmission of the drug. Similar results were obtained in 5 out of 5 experiments.

The antiarrhythmic effect of mexiletine (10 μM) was clearly demonstrable when the drug was added at the beginning of the reoxygenation phase. In fact Figure 2 shows that the arrhythmias duration (incidence of arrhythmias in each minute of reoxygenation calculated by means of the 2 × k contingency table test) was significantly reduced by mexiletine ($P < 0.0001$ for both total VTAs and VF).

The overall incidence of arrhythmias (2 × 2 contingency table test) was also significantly reduced by mexiletine: 4 out of 10 hearts perfused with mexiletine developed VTAs vs 27 out of 30 controls during reoxygenation (χ^2: 8.85, $P < 0.02$), and 1 out of 10 hearts developed VF with mexiletine vs 21 out of 30 controls (χ^2: 8.42, $P < 0.02$).

Mexiletine (10 μM) was also effective against reperfusion VTAs. Figure 3 shows the records from a representative experiment of a series of 7 hearts in which the same procedure was followed. During the 40 min of ischaemia, the ventricular rate progressively decreased, as a consequence of the increasing impairment of conduction, and the systolic pressure fell to zero. During the reperfusion phase, the diastolic pressure increased and VTAs rapidly developed resulting in VF. Sinus rhythm was restored by perfusion with mexiletine within 30 s. VTAs reappeared immediately after the return to the drug-free solution. The antiarrhythmic effect of mexiletine was reproduceable in the same heart.

Effect of mexiletine on digitalis-induced arrhythmias

As shown in Figure 4, mexiletine 10 μM was found to be the lowest but fully effective concentration able to suppress VTAs induced by perfusing the hearts with β-methyl-digoxin.

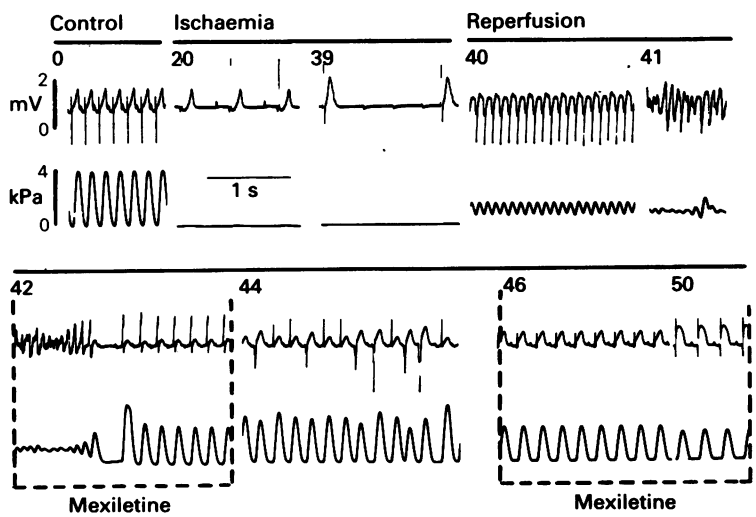

Figure 3 Effects of mexiletine (10 μM) on reperfusion-induced arrhythmias in the guinea-pig isolated heart. For explanation of arrangement of traces and use of numbers see legend to Figure 1.

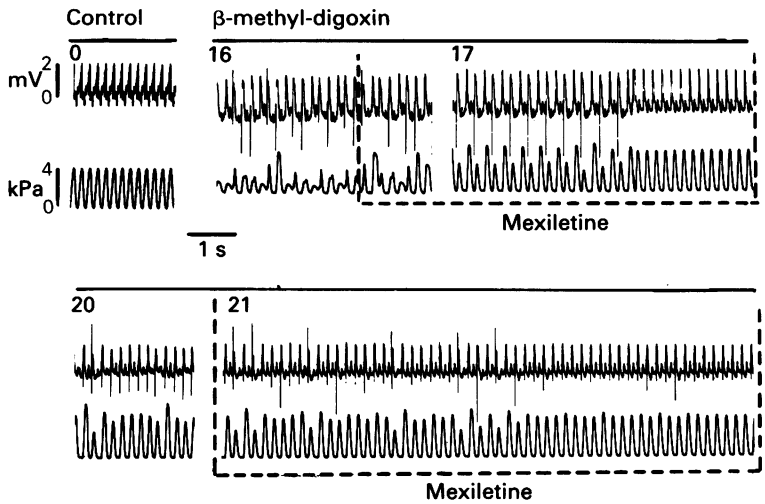

Figure 4 Effect of mexiletine (10 μM) on digitalis (β-methyl-digoxin 1 μM)-induced arrhythmias in the guinea-pig isolated hearts. For explanation of arrangement of traces and use of numbers see legend to Figure 1.

In fact, multiple ventricular premature beats were present after 16 min perfusion with the cardiac glycoside. Mexiletine rapidly restored the sinus rhythm; the return to a mexiletine-free medium caused the VTAs to reappear (20 min). Readmission of mexiletine (21 min) progressively reduced the frequency of VPBs and again restored the sinus rhythm. Similar results were obtained in 7 out of 7 experiments.

It is worth noting that in all these different models of arrhythmias, concentrations of mexiletine lower than 10 μM were devoid of any significant antiarrhythmic effect.

Effects on normal action potential characteristics in sheep Purkinje fibres

The effects of mexiletine (0.1–100 μM) were then studied on sheep Purkinje fibres driven at 1 or 2 Hz. The results thus obtained are presented in Tables 1 and 2, respectively.

Concentrations lower than 10 μM did not have any significant effect on the action potential characteristics. Mexiletine (10 μM) caused only slight effects, mainly a few mV reduction in AP amplitude, due to a reduction in the overshoot, and a tendency to reduce the $\dot{V}_{max}$, which was not statistically significant (Tables 1 and 2). The most significant effect of mexiletine was on the action potential duration, which was shortened at all levels of repolarization (Tables 1 and 2). Mexiletine, however, significantly affected all the action potential characteristics at a higher concentration (100 μM). The results were qualitatively similar at both driving rates (Tables 1 and 2).

Effects on oscillatory afterpotentials and on 'Triggered activity' in sheep Purkinje fibres

Typically, superfusion of Purkinje fibres with low barium concentrations or strophanthidin (Figure 5) caused the appearance of oscillatory afterpotentials (a,b), which, when they reached threshold (i.e. c,d), gave rise to a 'triggered rhythm'. Such an activity might terminate after a few spontaneous action potentials with subthreshold oscillatory afterpotentials or may last longer.

Mexiletine (10 μM) consistently blocked the 'triggered activity' induced by barium or by strophanthidin. Figure 6 shows a representative experiment of a series of 4 experiments in which similar results were obtained.

In the presence of barium (10 μM) and high Ca^{2+} (7.2 mM), a triggered rhythm ensued at the interruption of the stimulation (Figure 6a). Mexiletine (10 μM) immediately blocked such an activity, as shown in (b). The interruption of the stimulation was associated with a subthreshold oscillatory afterpotential, when mexiletine was present in the superfusate. Washout of the drug resulted in the reappearance of the triggered activity (Figure 6c). Similar results were obtained in the presence of strophanthidin. Figure 7 shows triggered activity in the presence of strophanthidin (a) and that mexiletine 10 μM abolishes the triggered activity: after the interruption of the stimulation, an oscillatory afterpotential, which does not reach the threshold and which is followed by oscillatory potentials of decreasing amplitude, is recorded after 1 min of contact (b). If the superfusion with mexiletine is continued, the

Table 1 Effect of mexiletine on transmembrane action potentials of sheep Purkinje fibres (driving rate 1 Hz)

	AP (mv)	*MDP* (mv)	*OS* (mv)	APD_{-60} (ms)	APD_{90} (ms)	$\dot{V}_{max}$ ($V\,s^{-1}$)
Control ($n = 6$)	127.3 ± 1.4	92.5 ± 1.4	36.5 ± 1.1	246.6 ± 16.2	282.7 ± 17.1	828 ± 61
Mexiletine 0.1 μM	126.6 ± 1.3	92.0 ± 1.3	36.1 ± 1.5	252.2 ± 18.2	288.5 ± 20.0	826 ± 61
Control ($n = 7$)	127.1 ± 1.2	90.9 ± 2.0	37.6 ± 1.5	260.5 ± 19.5	296.7 ± 20.2	827 ± 60
Mexiletine 1 μM	126.2 ± 1.3	90.6 ± 2.1	37.0 ± 1.9	243.7 ± 16.0	281.4 ± 17.3	822 ± 59
Control ($n = 7$)	126.9 ± 1.3	89.7 ± 1.7	37.2 ± 1.5	277.1 ± 23.3	312.0 ± 24.4	772 ± 61
Mexiletine 10 μM	123.6 ± 1.3***	88.5 ± 1.9*	35.0 ± 1.7**	207.6 ± 10.8***	237.0 ± 11.3***	743 ± 59
Control ($n = 5$)	124.8 ± 2.6	91.2 ± 1.0	33.6 ± 1.8	261.1 ± 27.4	295.5 ± 29.9	712 ± 65
Mexiletine 100 μM	114.4 ± 2.7***	88.4 ± 1.2**	25.9 ± 1.9***	156.3 ± 18.2*	190.7 ± 18.5*	580 ± 70**

AP = amplitude, OS = overshoot, MDP = maximum diastolic potential, APD_{-60} and APD_{90} = action potential duration at − 60 mV and at 90% of repolarization respectively, $\dot{V}_{max}$ = maximum rate of upstroke. Data are presented as means ± s.e.means * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Table 2 Effect of mexiletine on transmembrane potentials of Purkinje fibres (driving rate 2 Hz)

	AP (mv)	*MDP* (mv)	*OS* (mv)	APD_{-60} (ms)	APD_{90} (ms)	$\dot{V}_{max}$ ($V\,s^{-1}$)
Control ($n = 5$)	127.3 ± 1.7	92.1 ± 1.2	35.3 ± 1.7	214.0 ± 16.3	251.2 ± 16.5	787 ± 68
Mexiletine 0.1 μM	126.7 ± 1.8	91.6 ± 1.2	35.1 ± 1.9	220.8 ± 15.7	256.6 ± 17.7	780 ± 69
Control ($n = 6$)	125.6 ± 2.2	90.3 ± 2.1	35.3 ± 1.6	225.2 ± 17.4	262.5 ± 17.5	784 ± 60
Mexiletine 1 μM	125.3 ± 2.2	90.5 ± 2.1	34.8 ± 1.6	217.0 ± 16.1	254.2 ± 15.3	776 ± 65
Control ($n = 7$)	127.0 ± 1.2	90.3 ± 1.7	36.7 ± 1.8	235.1 ± 17.7	274.1 ± 18.9	759. ± 62
Mexiletine 10 μM	123.3 ± 1.2***	88.9 ± 2.0	34.3 ± 1.9***	183.1 ± 9.2***	213.5 ± 9.5***	722 ± 58
Control ($n = 5$)	123.2 ± 3.0	91.6 ± 1.0	31.5 ± 2.2	222.8 ± 21.3	261.6 ± 24.0	711 ± 67
Mexiletine 100 μM	112.7 ± 2.6***	88.5 ± 1.2**	24.2 ± 2.0***	149.2 ± 16.2*	182.8 ± 15.1*	526 ± 80**

Abbreviations as in Table 1.

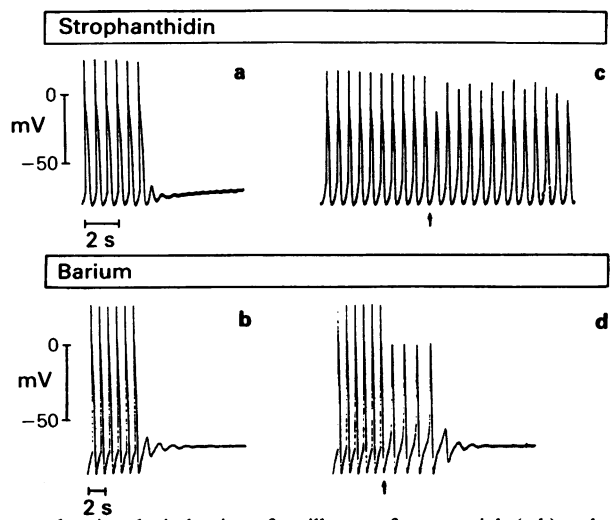

Figure 5 Typical experiment showing the induction of oscillatory afterpotentials (a,b) and triggered activity (c,d), in sheep Purkinje fibres, in the presence of barium and of strophanthidin. Each panel shows the last driven action potentials and the electrical activity during the interruption of the stimulation. The first non-driven action potential is indicated by an arrow in (c) and (d).

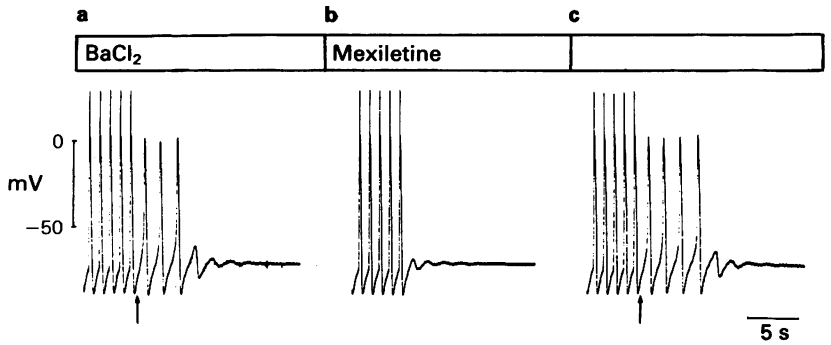

Figure 6 Effect of mexiletine (b) on barium-induced triggered activity in sheep Purkinje fibres. Each panel shows the last 5 driven action potentials and the electrical activity during the interruption of the stimulation. Arrows indicate the first non-driven action potential. (c) Recorded after washing out mexiletine.

amplitude of the OAP becomes reduced (c). After washout of mexiletine as recovery occurs the triggered rhythm is resumed (d). Similar results were obtained in 4 experiments.

In order to decide which electrophysiological action of mexiletine was responsible for the termination of triggered activity, we evaluated the effect of mexiletine on $\dot{V}_{max}$, OAP amplitude and action potential duration of Purkinje fibres superfused with barium and strophanthidin.

Figure 8 summarizes the results obtained with mexiletine. In 11 experiments in the presence of barium, and in 7 other experiments in the presence of strophanthidin, mexiletine 10 μM reduced APD as it did in normal Tyrode solution. However, $\dot{V}_{max}$ was not significantly modified under both experimental conditions; but OAP amplitude was greatly reduced.

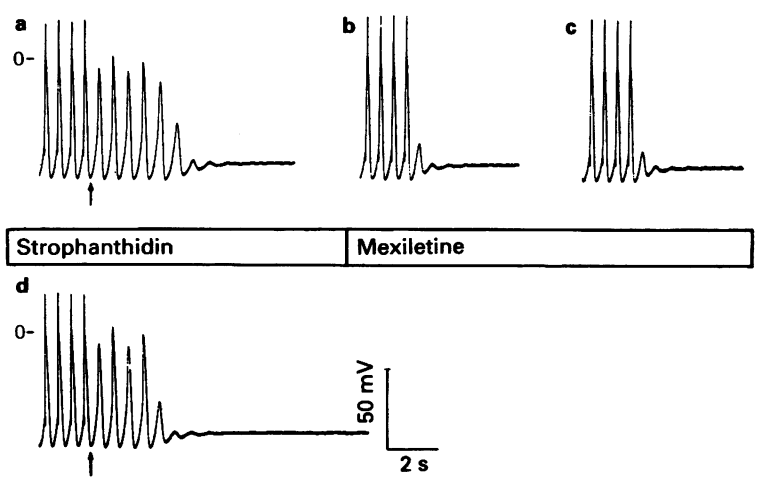

Figure 7 Effect of mexiletine (b and c) on triggered activity induced by strophanthidin in sheep Purkinje fibres. Each panel shows the last 4 driven action potentials and the electrical activity during the interruption of the stimulation. Arrows indicate the first non-driven action potential. (d) Recorded after washing out mexiletine.

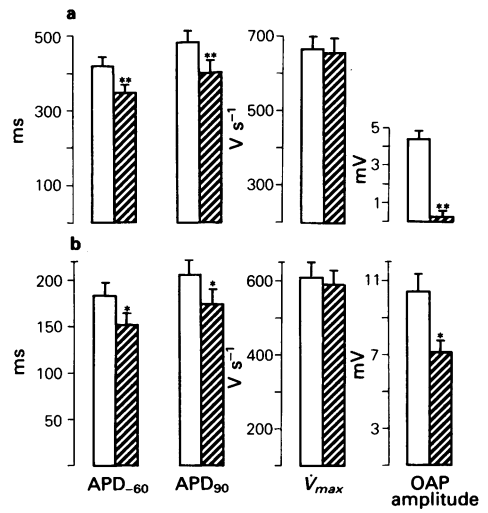

Figure 8 Effect of mexiletine on action potential duration (at -60 mV (APD_{-60}) and at 90% repolarization (APD_{90})), $\dot{V}_{max}$ and oscillatory afterpotential (OAP) amplitude in Purkinje fibres superfused with barium (a) or strophanthidin (b). Open columns = control; hatched columns = mexiletine 10 μM. * $P<0.02$, ** $P<0.001$.

Discussion

The present results demonstrate that: (1) in the guinea-pig isolated heart, mexiletine abolishes reoxygenation-, reperfusion- and digitalis-induced VTAs, the minimum effective concentration being 10 μM in all of these three experimental conditions; (2) in sheep Purkinje fibres, mexiletine 10 μM suppresses strophanthidin- or barium-induced triggered activity, an effect caused by a reduction of the amplitude of oscillatory afterpotentials; (3) mexiletine 10 μM has no statistically significant effect on the maximum upstroke velocity ($\dot{V}_{max}$) of the phase 0 of the action potential in all experimental conditions.

The antiarrhythmic action of mexiletine is generally attributed to its depressant effect on the Na^+ current. Even if mexiletine does not significantly reduce $\dot{V}_{max}$ in sheep Purkinje fibres, it is likely that a greater effect of the drug may occur in the isolated heart. Mexiletine in fact depresses $\dot{V}_{max}$ of cardiac action potentials in a rate-dependent (Campbell, 1983) and voltage-dependent (Hohnloser *et al.*, 1982) manner. Arrhythmias suppressed by mexiletine in the guinea-pig isolated heart were of a high frequency (i.e. >6 Hz during reperfusion) and it is well known that a partial depolarization of cardiac cells is caused by hypoxia or ischaemia (Moréna *et al.*, 1980) and digitalis (Vassalle & Musso, 1976). Thus mexiletine will directly and rapidly decrease the Na^+ current and consequently the excitability of the cell.

However, it is possible that mexiletine may act not only by decreasing the excitability of the cell, but also by decreasing the arrhythmogenic stimulus.

The precise electrophysiological mechanism of reoxygenation and reperfusion arrhythmias remains unclear (Corr & Witkowsky, 1983; Manning & Hearse, 1984). An interesting association between contracture and development of these arrhythmias has been recently proposed (Pahor *et al.*, 1985). Recent evidence suggests that a mechanism similar to that described for digitalis-induced arrhythmias, i.e. oscillatory afterpotentials (delayed afterdepolarizations), may be involved.

In fact, calcium overload seems to be a common underlying mechanism for both digitalis intoxication and reperfusion and reoxygenation arrhythmias (Vassalle & Lin, 1979; Sharma *et al.*, 1983; Poole-Wilson *et al.*, 1984). Interventions interfering with cell calcium movements abolish digitalis arrhythmias and significantly reduce the incidence and the severity of reperfusion- and reoxygenation-induced VTAs (Ferrier, 1977; Carbonin *et al.*, 1981; 1984; Clusin *et al.*, 1982; Di Gennaro *et al.*, 1983; 1984). Furthermore, calcium-overload is associated with a marked increase in the rate of idioventricular rhythm during reperfusion and reoxygenation as well as during digitalis toxicity (Sheridan *et al.*, 1980; Di Gennaro *et al.*, 1983). The increase in ventricular automaticity could be due to OAPs which appear to be an expression of intracellular calcium-overload. It is worth noting that the same interventions which suppress reperfusion and digitalis arrhythmias in the isolated heart (Di Gennaro *et al.*, 1983; Carbonin *et al.* 1984) are also able to abolish OAPs and triggered automaticity in cardiac fibres (Di Gennaro *et al.*, 1984).

Finally, in a canine false tendon-papillary muscle preparation exposed to ischaemic conditions followed by a return to non-ischaemic conditions, oscillatory afterpotentials were regularly observed during the reperfusion phase (Ferrier *et al.*, 1985). Thus the arrhythmogenic stimulus possibly decreased by mexiletine could be represented by the oscillatory afterpotentials. Our results further support this view, since mexiletine, at the same concentration at which it significantly reduced the incidence of digitalis-, reoxygenation- and reperfusion-arrhythmias, also blocked triggered activity and reduced OAP amplitude in isolated Purkinje fibres.

The reduction in the amplitudes of oscillatory afterpotentials caused by mexiletine makes it likely that oscillatory afterpotentials will not reach threshold and consequently the tachyarrhythmias will not occur.

OAPs are transient depolarizations which occur after a normal action potential under conditions of calcium-overload (Hoffman & Rosen, 1981; Amerini

et al., 1985). Interventions, known to increase cellular calcium, increase the transient inward oscillatory current (Vassalle & Mugelli, 1981) which underlies the oscillatory afterpotentials.

The transient inward current, as proposed by Tsien *et al.* (1979) is carried by Na^+ and perhaps other cations through a non-specific membrane channel (Kass *et al.*, 1978a,b).

An alternative possibility is that calcium released from the myofilaments at the relaxation of the tonic tension, is taken up by an overloaded sarcoplasmic reticulum and this, in turn, triggers an oscillatory release of calcium (Vassalle & Mugelli, 1981). The released calcium may stimulate the Na^+-Ca^{2+} exchange process which extrudes calcium ions in exchange for an electrically greater quantity of Na^+ ions (Noble, 1984). This electrogenic Na^+-Ca^{2+} exchange mechanism could well explain why high levels of intracellular calcium ions are required to generate the transient inward current. Furthermore, in support of this mechanism, is the observation that the transient inward current disappears in Na-free solution and is reduced by lidocaine through a reduction in intracellular sodium activity (Eisner *et al.*, 1983).

According to these models, a drug may conceivably reduce the OAP amplitude by several mechanisms, such as by reducing inward background currents or increasing outward background currents and by blocking directly or indirectly the ionic conductances responsible for oscillatory afterpotential.

An indirect mechanism for depressing the OAP amplitude would be through blockade of sodium or calcium conductance, thus reducing cellular sodium or calcium loading (Bhattacharyya & Vassalle, 1981; Amerini *et al.*, 1985).

In sheep Purkinje fibres, mexiletine did not have any significant effect on $\dot{V}_{max}$ while significantly reducing the OAP amplitude. Since the relationship between $\dot{V}_{max}$ measurements and fast sodium current is controversial (Cohen *et al.*, 1984), we cannot exclude the fact that an effect on inward sodium current may contribute to the action of mexiletine on the OAP. Furthermore, sodium may also enter through the so-called 'window' current (Attwell *et al.*, 1979) and mexiletine, under the same conditions, significantly reduced action potential duration. Recently it has been demonstrated (Henning & Wit, 1984) that there is a marked effect of action potential duration on OAP amplitude, at least in coronary sinus. As the action potential duration decreased, the OAP also decreased in amplitude. This agrees with the observation that, on increasing the duration of the depolarization used to elicit the transient inward current in voltage clamp experiments, this current increases (Vassalle & Mugelli, 1981; Karagueuzian & Katzung, 1982). Thus one must keep in mind the mexiletine-induced shortening of APD, that is, its effect on the underlying plateau currents, when evaluating its effect on OAP amplitude.

Regardless of the intimate mechanism by which mexiletine decreases OAP amplitude, this appears to be the main electrophysiological effect of the drug, when studied in Purkinje fibres, at the concentrations at which it exerts its antiarrhythmic effect on digitalis-, reoxygenation- and reperfusion-induced arrhythmias.

According to Hewett *et al.* (1983), the identification of a drug, or a concentration of drug, that has specific effects on OAP without affecting other electrophysiological variables is of value in identifying mechanisms causing arrhythmias. Therefore, these findings further support the view that OAP may be the common electrophysiological mechanism leading to digitalis-, reperfusion- and reoxygenation-arrhythmias, even if the possibility that the effect of mexiletine on these arrhythmias is due only to a reduction of $\dot{V}_{max}$, that is of the excitability of the cells, cannot be excluded. In both cases antiarrhythmic action of mexiletine depends ultimately on its interaction with the Na channel. In one case. the interaction will cause a direct decrease of the Na^+ current and hence the excitability of the tissue; in the other, the reduction of the Na^+ current will cause a decrease in intracellular sodium activity, a reduction of the transient inward current and of the oscillatory afterpotential and thereby abnormal automatic activity will be abolished.

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