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ELECTROPHYSIOLOGICAL ACTIONS OF MEXILETINE (Kö1173) ON CANINE PURKINJE FIBRES AND VENTRICULAR MUSCLE

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1 The effects of mexiletine (Kö1173) were investigated in canine isolated cardiac Purkinje fibres and ventricular muscle with microelectrodes. Some Purkinje fibres were depolarized by mechanical stretch to induce spontaneous activity with slow upstroke velocity. The preparations were stimulated at rates of 1, 2, 3 and 4 Hz. The drug concentrations tested were 0.4, 2 and 10 μg/ml in Tyrode solution (KCl = 5.4 mM).

2 The 'therapeutic' drug concentration (2 μg/ml) shortened action potential duration and effective refractory period of Purkinje fibres, the effect being pronounced at lower stimulation rates. In ventricular fibres, action potential duration changes were not consistent while the effective refractory period was prolonged.

3 In depolarized Purkinje fibres showing automatic activity, the drug (0.4 or 2 μg/ml) depressed phase 4 depolarization and reduced the firing rate without changing maximum diastolic potential. However, when depolarized Purkinje fibres were electrically driven at a constant rate, the maximum diastolic potential became more negative with a concomitant decrease of pacemaker slope and increase of maximum rate of rise ($\dot{V}_{max}$) of action potentials.

4 Moderate (2 μg/ml) to high (10 μg/ml) concentrations of the drug depressed $\dot{V}_{max}$ in Purkinje fibres stimulated at 2 Hz by 12 and 42% respectively and depressed 'membrane responsiveness'. The decrease in $\dot{V}_{max}$ depended upon the stimulation rate, being minimum at the lowest (1 Hz) and maximum at the highest (4 Hz) stimulation rate.

5 The drug (2 μg/ml) improved $\dot{V}_{max}$ of the earliest propagated premature action potentials by shifting the takeoff potential to more negative levels in both Purkinje and ventricular fibres.

6 Membrane conductance in fibres mounted in a single sucrose gap chamber was increased by the drug (2 μg/ml) in both fibre types in normal and in Na^+-deficient solutions. This increase was attributed to an increase in membrane K^+ permeability produced by the drug.

7 All these effects are similar to those of lignocaine, diphenylhydantoin or aprindine, and can explain the antiarrhythmic action of mexiletine.

Introduction

Mexiletine (Kö1173), 1-(2',6'-dimethyl-phenoxy)-2-amino-propane, is a primary amine with certain structural similarities to lignocaine. It was originally introduced as an anticonvulsant agent, but subsequent studies revealed that the drug was also effective in suppressing ventricular arrhythmias both in experimental animals (Allen, Kofi-Ekue, Shanks & Zaidi, 1970; Singh & Vaughan-Williams, 1972; Okuma, Sugiyama, Wada, Sugenoya, Niimi, Oguri, Toyama & Yamada, 1976) and in man (Talbot, Clark, Nimmo, Neilson, Julian & Prescott, 1973; Campbell, Chaturvedi, Kelly, Strong, Shanks & Pantridge, 1973). From the clinical point of view, the drug is advantageous because of its long half-life and its effectiveness when given orally (Talbot *et al.*, 1973). There have been only a few electrophysiological studies of the drug on single fibres. In one of these, Singh & Vaughan Williams (1972) found that the drug reduced the maximum rate of rise of action potentials without affecting resting membrane potential, or the duration of the action potential in rabbit atrial and ventricular fibres. This is a typical 'class I' antidysrhythmic action (Vaughan Williams, 1970). The paucity of electrophysiological studies of mexiletine, especially on the fibres of the conducting tissue prompted us to investigate the effects of the drug in canine Purkinje fibres, and compare them with those in ventricular muscle. We

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used standard microelectrode and superfusion techniques to determine the effect of therapeutic and toxic concentrations of mexiletine on ventricular muscle and Purkinje fibres exhibiting both a normal fast channel-dependent response (Wit, Rosen & Hoffman, 1974a), and a slow channel-dependent response (Wit, Rosen & Hoffman, 1974b; Wit, Rosen & Hoffman, 1974c). The results obtained are discussed in relation to the reported antiarrhythmic actions of the drug.

Methods

Mongrel dogs, weighing 8 to 15 kg, were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.). The hearts were quickly removed and dissected in cool oxygenated Tyrode solution. Strands of Purkinje fibres (0.8 to 1 mm in diameter, 4 to 6 mm long) and small papillary muscles or trabeculae (1 to 5 mm in diameter, 4 to 6 mm long) were excised from both ventricles.

The preparation was mounted in a 0.8 ml volume tissue bath through which Tyrode solution, gassed with 95% O_2 and 5% CO_2, flowed continuously at a constant rate of 1.8 ml/min. In this perfusion system, the solution that filled the bath could be completely replaced by other test solutions within 2 min. Temperature in the bath was maintained at 36°C. The composition of the Tyrode solution (mmol/l) was: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.0.

The transmembrane potentials were measured by means of 3 M KCl-filled glass microelectrodes (resistance 15 to 25 MΩ). The microelectrode was coupled to the input of Nihonkohden MZ-3B cathode-follower amplifier and the recorded potential was displayed and photographed on a Nihonkohden VC-9 oscilloscope, or recorded on a Nihonkohden ink-writing Recticorder. The maximum rate of rise ($\dot{V}_{max}$) of action potentials was obtained by an electronic differentiator equipped with an operational amplifier (Filbrick 100901). The differentiator was calibrated by means of a sawtooth wave generator, and was linear within the 0 to 800 V/s range.

In most experiments, the preparation was electrically driven at a frequency of 2 Hz by rectangular pulses of 2 ms duration and twice diastolic threshold strength delivered by a Nihonkohden stimulator MSE-3R and isolation unit. The stimuli were applied to the surface of the preparation through a pair of silver wire electrodes (diameter 200 μm, 0.7 mm apart) insulated, except at their tips, by Teflon.

The effective refractory period was determined by applying premature test stimuli of 2 ms duration and 4 times diastolic threshold through the same pair of electrodes using a second stimulator. 'Membrane responsiveness' curves were obtained by introducing premature stimuli at various times during phase 3 of the action potentials. In those experiments where the stimulation rate was changed stepwise from 1 to 2, 3 and 4 Hz, the records were made approximately fifty action potentials after each change in rate.

To measure the change of input resistance (Figure 6), preparations of both fibre types were mounted in a single, three compartment sucrose gap chamber. The central compartment, which was separated from the other compartments by thin acrylic plates, was filled with isotonic sucrose solution containing 10^{-5} M $CaCl_2$ (New & Trautwein, 1972). The distal compartment contained non-oxygenated Tyrode solution while the proximal compartment (test chamber) was perfused with oxygenated Tyrode solution with or without the drug. The length of preparation in the test chamber was $\leqq 1$ mm to minimize cable complication and to attain fairly uniform polarization throughout the tissue (Deck & Trautwein, 1964). Transmembrane action potentials were recorded from the tissue in the test chamber. Hyper- or depolarizing constant current pulses of 50 to 80 ms duration and small amplitude were applied across the sucrose gap. The resulting voltage deflection was used to calculate membrane input resistance. When necessary, the test chamber was perfused with a sodium-deficient solution in which NaCl was totally replaced by isosmolar choline chloride containing atropine sulphate (1 μg/ml) to prevent any possible cholinergic effects. In these experiments, current pulses of 500 ms duration and various intensities were introduced via the sucrose gap to obtain the current-voltage relationship (Figure 7).

Purkinje strands were stretched and maintained under slight tension to study slow response action potentials (Rosen, Danilo, Alonso & Pippenger, 1976). The technique usually resulted in action potentials having maximum diastolic potentials less negative than −65 mV and $\dot{V}_{max}$ less than 30 V/s. In most of these stretched fibres with low maximum diastolic potentials, automatic and repetitive action potential discharges were recognized. Drug effects on automaticity were examined in preparations firing at a stable rate for at least 20 min before application of the drug.

Stock solutions of mexiletine hydrochloride (250 μg/10 ml) were diluted in the Tyrode solution immediately before use to give test solutions of 0.4, 2 and 10 μg/ml. To ensure equilibration of the preparation with its environment, the experiments were initiated at least 1 h after mounting the preparation in the bath.

The duration of the plateau phase of action potentials was measured at 40% and 30% of full repolarization in Purkinje and ventricular fibres respectively. The total action potential duration was measured at 90% of full repolarization. Experiments were per-

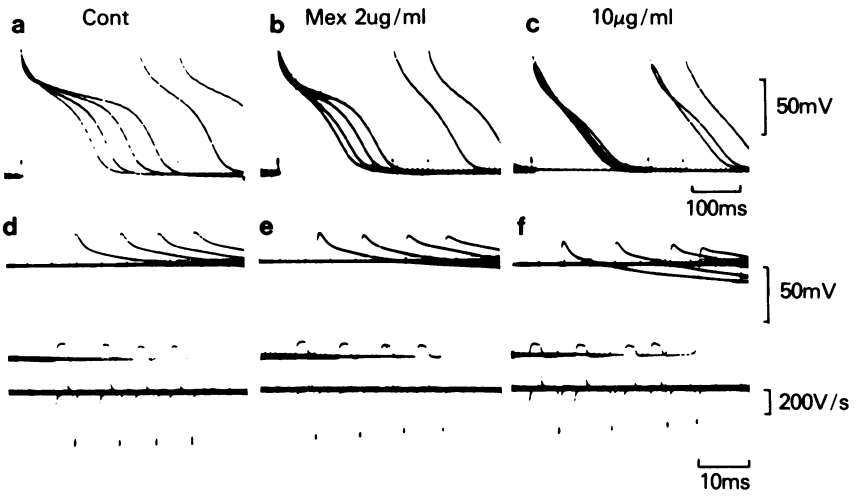

Figure 1 Effects of different stimulation rates on the action potential configuration (a–c) and upstroke phase (d–f) in a normal Purkinje fibre before (a and d) and during action of mexiletine (Mex) at a concentration of 2 μg/ml (b and e) and 10 μg/ml (c and f). In panels (a–c), the stimulation rate was increased from right to left traces 1, 2, 3 and 4 Hz. In panels (d–f), the top trace is the zero reference potential; the middle is the action potential upstroke; and the bottom is the 1st derivative of the upstroke phase, $\dot{V}_{max}$ (downward positive). The tips of $\dot{V}_{max}$ were retouched.

formed in 42 Purkinje fibres and 38 ventricular fibres obtained from 24 dogs. Statistical evaluation was performed using Student's *t* test. All average values were expressed as mean ± standard error of the mean.

Results

Effects on repolarization of normal Purkinje fibres and ventricular muscle

Figure 1a–c shows the steady state control and effects of mexiletine (2 and 10 μg/ml) on the repolarization phase of the action potentials of a normal Purkinje fibre stimulated at rates of 1, 2, 3 and 4 Hz. The steady state effect was usually attained after 10 to 15 min of drug application. The drug at concentrations used in the present study did not change the resting potential but decreased the level of plateau and shortened the action potential duration at each stimulation rate. The effects depended on the concentrations and the stimulation rates, viz., the shortening became more marked either when the drug concentration was increased, or when the stimulation rate was decreased. The effects induced by a low (0.4 μg/ml) or moderate (2 μg/ml) concentration were almost completely reversed after washout periods of about 30 min, while the effects produced by high concentration (10 μg/ml) were only partially reversed.

Table 1 summarizes the drug effect (2 μg/ml) on the duration of repolarization phases in both Purkinje and ventricular fibres stimulated at 2 Hz in experiments in which the microelectrode remained in the same cells throughout the experiment. In Purkinje fibres, the drug shortened both the plateau and total action potential duration. However, in the ventricular fibres the drug shortened only the plateau duration, while the change of total action potential duration was not consistent. It consisted of slight shortening, no change, and in 3 out of 8 preparations slight prolongation (by less than 6%).

Table 1 Percentage shortening of action potential duration attained after 10 to 15 min of application of mexiletine (2 μg/ml)

	Purkinje fibre (n = 5)	*Ventricular muscle* (n = 8)
Plateau duration	31.3 ± 7.9*	6.5 ± 4.4***
Total duration	14.8 ± 8.5**	4.0 ± 6.1

Control action potential durations were 235 ± 8 ms in Purkinje fibres and 142 ± 6 ms in ventricular muscle at the stimulation rate of 2 Hz.
*$P < 0.001$ as compared with the control.
**$P < 0.02$ as compared with the control and the % shortening of plateau duration.
***$P < 0.01$ as compared with the control.

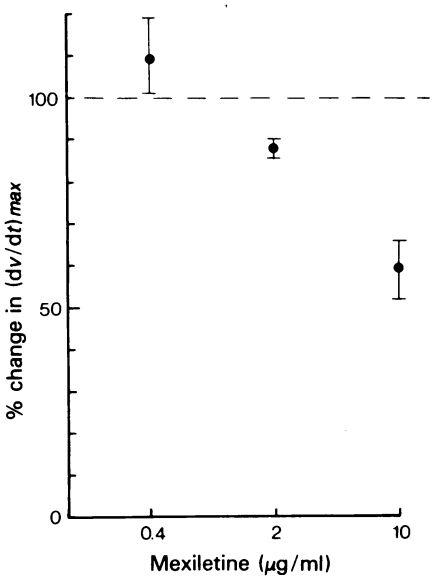

Figure 2 The effect of different concentrations of mexiletine on the maximum rate of rise, $(dv/dt)_{max}$, of Purkinje fibres driven at 2 Hz. Ordinate scale is % of the control without the drug. The effect was evaluated after 10 to 15 min of the drug application. Vertical bars indicate s.e. mean. The number of preparations studied was 4 at concentrations of 0.4 and 2 μg/ml, and 3 at 10 μg/ml. Mean control $\dot{V}_{max}$ of these 11 fibres was 443 ± 9 V/s.

Effects on depolarization in normal Purkinje fibres and ventricular muscle

Figure 1d–f shows the upstroke phase of action potentials and corresponding first derivatives ($\dot{V}_{max}$) in a normal Purkinje fibre before and after drug application. The control $\dot{V}_{max}$ was not altered (about 440 V/s) by the stimulation rate (d); however, during action of the drug (e and f), $\dot{V}_{max}$ was decreased at each stimulation rate, but the decrease was greater when the stimulation rate was increased. The decrease in $\dot{V}_{max}$ was also more pronounced when the concentration was increased.

Figure 2 shows mean percentage change in $\dot{V}_{max}$ during perfusion with 3 different concentrations of the drug on Purkinje fibres when the stimulation rate was 2 Hz. At the lowest concentration (0.4 μg/ml), $\dot{V}_{max}$ increased in 2 out of 4 fibres tested and slightly decreased in the other 2 fibres, i.e., the effect was not consistent. At moderate (2 μg/ml) and high concentrations (10 μg/ml), there were statistically significant decreases in $\dot{V}_{max}$ of 12% and 43% respectively.

Effects on effective refractory period and membrane responsiveness

The effective refractory period was determined in 2 Purkinje fibres and 2 ventricular fibres before and after application of the drug (2 μg/ml). The effective

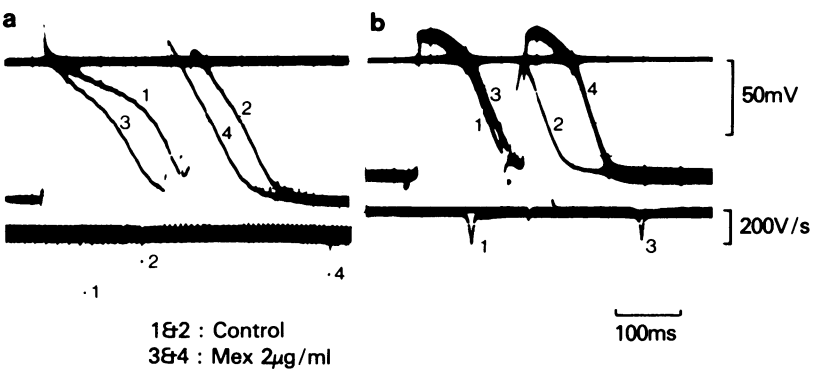

Figure 3 Effect of mexiletine (Mex, 2 μg/ml) on a pair of action potentials, i.e., the last basic action potential (1) driven at a rate of 2 Hz and subsequent premature action potential (2) that was elicited by the earliest effective premature stimulus, before (1 and 2) and during (3 and 4) application of the drug. (a) Purkinje fibre, (b) ventricular muscle. In each panel, the top trace is zero reference potential; the middle trace is action potential; and the bottom is 1st derivative of the action potential upstroke. The number beside each $\dot{V}_{max}$ in the bottom trace corresponds to the number on the action potentials in the middle trace. The tips of $\dot{V}_{max}$ in (a) were retouched. The $\dot{V}_{max}$ in (b) is recorded at 10 times the sweep speed of the middle trace.

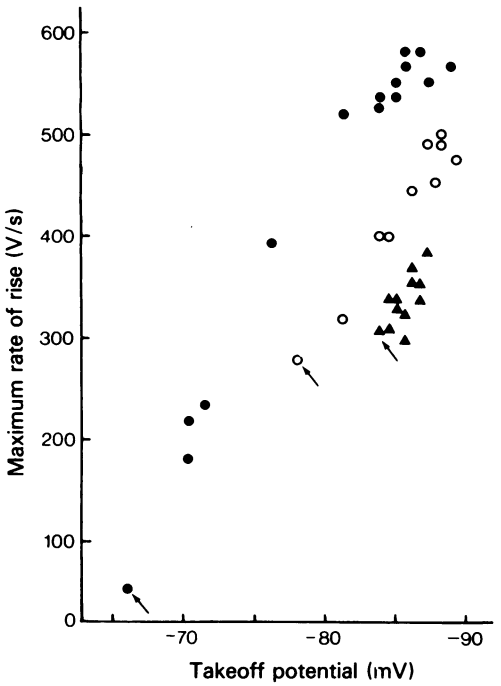

Figure 4 Relation between the maximum rate of rise ($\dot{V}_{max}$) and the membrane potential at the onset of depolarization (takeoff potential) in a Purkinje fibre stimulated at a frequency of 1 Hz, before (●) and after treatment with mexiletine at the concentrations of 2 μg/ml (○) and 10 μg/ml (▲).

refractory periods in Purkinje fibres were shortened by an average of 12.1% whereas that of ventricular fibres was lengthened by an average of 17.9%. An example of this is shown in Figure 3a (Purkinje fibre) and in Figure 3b (ventricular fibre). As shown in Figure 1b, the drug shortened the action potential duration of the Purkinje fibre in the basic beat (compare trace 1 and 3), and this may be the cause of the shortening of the effective refractory period in the Purkinje fibres. In the ventricular fibre (Figure 3b), the drug prolonged the action potential duration of the basic beat (compare trace 1 and 3). This lengthening may explain, at least in part, the prolongation of the effective refractory period in the ventricular fibres.

Premature excitations elicited during phase 3 of the basic action potential in a Purkinje fibre provided a relationship between the takeoff potential and $\dot{V}_{max}$, i.e., the membrane responsiveness curve (Figure 4). This figure shows the concentration-dependent depression of membrane responsiveness. However, $\dot{V}_{max}$ of the earliest premature beat (indicated by arrows) was increased during superfusion with both concentrations of the drug. Similar experiments were undertaken in 2 other Purkinje fibres. The average membrane potential at which the earliest premature action potentials could be elicited was -62.8 ± 6.35 mV before, and -79.8 ± 1.75 mV after, the drug application (2 μg/ml). Thus the more negative takeoff potentials (by 17 mV) during the drug action, may be the main cause of increased amplitude of the upstroke phase in the earliest premature action potentials.

Effects on automaticity and pacemaker potentials in depolarized Purkinje fibres

In the extracellular K^+ concentration (5.4 mM) used in the present study, normal Purkinje fibres whose resting potentials were more negative than −85 mV were not spontaneously automatic. However, fibres depolarized by mechanical stretch developed automatic pacemaker activity even at this K^+ concentration, probably due to increased specific membrane resistance (R_m) or decreased K^+ conductance (Deck, 1964; Kaufmann & Theophile, 1967). We examined the drug effect on such action potentials which exhibited pacemaker activity, probably of the 'slow response' type. Figure 5a–d shows the effect of mexiletine on such action potentials, in a fibre in which spontaneous discharge continued at constant cycle length of 490 ms and maximum diastolic potential of −45.8 mV (a). Application of 0.4 μg/ml of mexiletine decreased the slope of phase 4 depolarization and prolonged the cycle length to 840 ms without measurable change in the maximum diastolic potential (b). This effect was partially reversed by reperfusion with normal Tyrode solution (c). Comparable effects were obtained in 3 other automatic Purkinje fibres perfused with the drug solution at concentrations of 0.4 or 2.0 μg/ml. After washing out the drug for more than 10 min, the cycle length returned to a value close to the control.

Figure 5e–g shows a typical drug effect on a similar automatic response when the preparation was driven at a cycle length of 620 ms. Before the drug application (e), the fibre developed stable pacemaker activity with a maximum diastolic potential of −61 mV. After the drug application, the maximum diastolic potential became more negative and the slope of phase 4 decreased in association with slight shortening of action potential duration (f). After prolonged drug application, the maximum diastolic potential increased still further up to −68 mV, the pacemaker activity virtually ceased, and the $\dot{V}_{max}$ increased about three fold compared to the control. Similar effects were observed in other 2 fibres tested.

These results show considerable difference in the effect of the drug on spontaneous (Figure 5a–d) and driven (Figure 5e–g) action potentials, namely, no

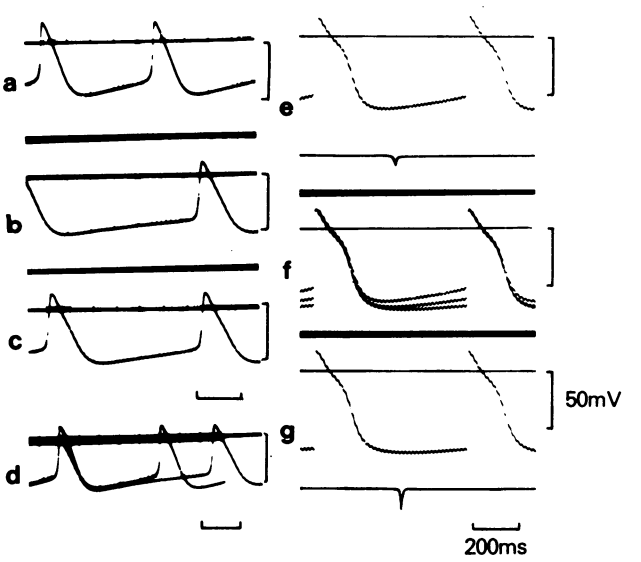

Figure 5 Effects of mexiletine (0.4 μg/ml) on slow response action potentials with pacemaker activity in depolarized Purkinje fibres; (a–d) were recorded from a spontaneously beating fibre: (a) is control; (b) 5 min after the drug application; (c) 6 min after washing out the drug; (d) is superimposition of (a) and (b), but the photograph was enlarged with a slightly smaller magnification; (e–g) were recorded from an electrically driven fibre: (e) is control; (f) superimposition of the control, 3 min and 6 min after the drug application; (g) 9 min after the drug application. Bottom trace in panels (e) and (g) shows the $\dot{V}_{max}$ of corresponding action potential.

change in the maximum diastolic potential without pacing and a more negative potential during pacing. However, both procedures were associated with similar suppression of the phase 4 depolarization.

Effects on input resistance

Marked shortening of action potential duration in normal Purkinje fibres (Figure 1a–c) and the suppression of phase 4 in depolarized Purkinje fibres (Figure 5) suggested to us that the drug might have increased membrane K^+-conductance. Hence we investigated the drug effect on the membrane slope resistance in both fibre types. An example of such an experiment in a Purkinje fibre is shown in Figure 6. In this fibre the maximum diastolic potential was −80.4 mV (i.e. slightly depressed) before drug application (a). During drug application, the takeoff potential increased to −89.1 mV but the voltage deflections produced by the current pulses decreased (b), suggesting decreasing membrane input resistance and an increase in K^+ permeability. In spite of this increase in the resting potential by 8 mV, the $\dot{V}_{max}$ decreased slightly probably due to simultaneous reduction in membrane responsiveness (see Figure 4). These effects disappeared completely after washing out the drug (c). In the ventricular fibre, the membrane input resistance decreased also during the drug application but without a change in the resting potential.

Another possible approach to the estimate of the drug action on the resting K^+ conductance is by testing the effect in Na^+-deficient solutions (Hall, Hutter & Noble, 1963). We found that Na^+-deficient solution caused depolarization (up to 15 mV; reason unknown) and that the action potential could not be elicited even by strong electrical stimuli. In Figure 7, the membrane potential at the end of current pulses (500 ms duration) was plotted against the strength of current applied to a Purkinje fibre before, and during, the drug action. In the fibre shown in this figure, the membrane conductance at the resting potential (chord conductance) increased by a factor of 1.2 after the drug application, while in the other two Purkinje fibres, by 1.9 and 2.9 respectively. In two ventricular fibres the conductance increased by a factor of 1.2 and 1.5 respectively. These increases in membrane conductance were partially reversible by washing out the drug in all preparations. These results suggest that mexiletine increased membrane permeability to potassium ions.

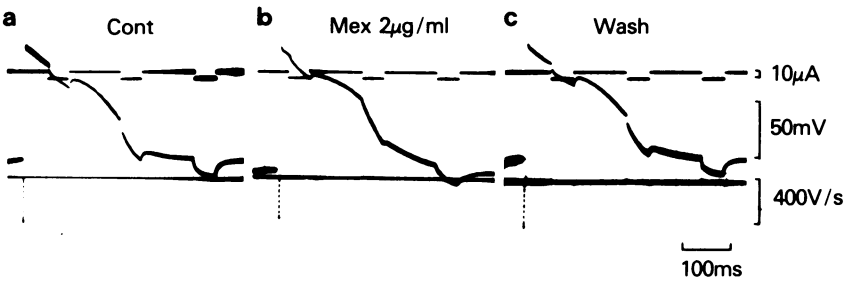

Figure 6 Effect of mexiletine (2 μg/ml) on the input resistance measured as a voltage deflection produced by a passage of 50 ms hyperpolarizing constant current pulses over the membrane of a Purkinje fibre: (a) is control; (b) during drug action, and (c) after washout. In each panel, top traces indicate zero reference potential as well as the intensity of the current applied; middle, action potential; and bottom, $\dot{V}_{max}$ of the action potential upstroke (retouched).

Discussion

Low and moderate concentrations of mexiletine (0.4 and 2 μg/ml) used in the present study are within the range of plasma concentrations attained in patients treated with the drug, while the high concentration (10 μg/ml) may be in the toxic range (Talbot *et al.*, 1973; Campbell *et al.*, 1973). In dogs with ouabain-induced ventricular extrasystoles and tachycardia, sinus rhythm was restored after intravenous injection of 1.37 mg/kg mexiletine (Allen *et al.*, 1970). This dose may be equivalent to the effective clinical dose (30 to 250 mg, i.v.) given to patients with digitalis-induced ventricular arrhythmias (Talbot *et al.*, 1973). Thus the data obtained in the present animal experiments appear pertinent to the discussion of the mechanism of the drug action on human hearts.

Effects on repolarization

A moderate concentration (2 μg/ml) of mexiletine produced shortening of the effective refractory period in Purkinje fibres. The change was opposite to that observed in the ventricular muscle, where the period was lengthened. This means that the drug may reduce the difference in effective refractory period between Purkinje and ventricular fibres. This different action on repolarization may be due to a different ionic basis in maintenance and termination of action potential plateau in the two fibre types (Noble & Tsien, 1969).

Mexiletine shortened plateau duration in both Purkinje and ventricular fibres. The effect was more pronounced at the slow rate of stimulation and resulted in a decrease of rate-induced difference in action potential duration (Figure 1b and c). A similar phenomenon has been described in frog ventricular fibres in the presence of metabolic inhibitors (McFarlane, 1960) and in canine ventricular and Purkinje fibres treated with phenothiazines (Arita & Surawicz, 1973).

The precise ionic mechanism of the drug-induced shortening of the action potential plateau is uncertain, but the increase of resting or maximum diastolic potential, as well as suppression of slope of phase 4 in depolarized Purkinje fibres, suggest that the drug increased membrane potassium permeability. In support of this assumption are the findings that membrane slope and chord conductance were increased during perfusion with mexiletine (Figures 6 and 7).

Effects on pacemaker potential

Mexiletine suppressed automatic activity in depolarized Purkinje fibres (Figure 5). This automaticity occurred at membrane potentials less negative than −60 mV. At these membrane potentials, the pacemaker current i_{K2} is probably fully activated, and therefore the pacemaker activity may be attributed to time and voltage-dependent deactivation of i_{x1} current that could have been modified by mexiletine.

However, it is also possible that the inhibition of phase 4 depolarization was due, at least in part, to the decrease of background or slow inward current occurring simultaneously with an increase of outward K^+ currents.

In the spontaneously beating Purkinje fibres of slow response type, mexiletine decreased the firing frequency with no change in the maximum diastolic potential (Figure 5a–d). This contrasts to the drug effect on the driven Purkinje fibres of slow response type in which significant hyperpolarization ensued (Figure 5e–g). The lack of drug-induced hyperpolarization in the spontaneously beating preparation may be attributed a concomitant decrease in the contribution of overdrive hyperpolarization. That is, slowing

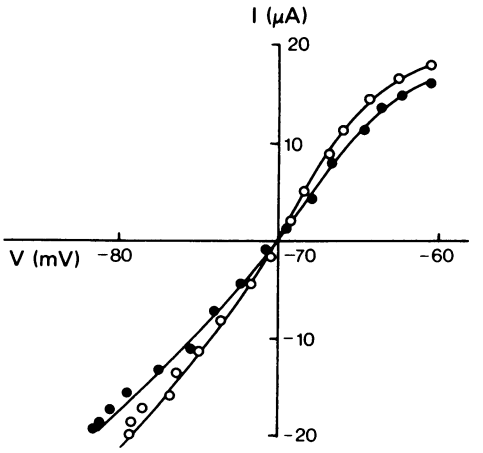

Figure 7 Current-voltage relationship measured in a Purkinje fibre perfused with Na^+-deficient (11.9 mM) solution in the absence (●) and in the presence (○) of mexiletine (2 μg/ml).

of firing rate may produce secondarily a decrease in electrogenic Na^+-pump activity (Vassalle, 1970). As a result, the hyperpolarizing effect of mexiletine (due to increased K^+ permeability) might have been mostly offset and resulted in little change in the maximum diastolic potential.

Effects on depolarization

It has been reported that therapeutic concentrations of lidocaine (Bigger, Basset & Hoffman, 1968; Davis & Temte, 1969; Bigger & Mandel, 1970) and diphenylhydantoin (Bigger *et al.*, 1968) enhanced rather than depressed the membrane responsiveness in cardiac fibres. However the drug effects on depolarization are also known to be dependent upon the K^+ concentration in the perfusing media. Thus, when K^+ concentrations of 5 to 6 mM were used, these agents were found to decrease membrane responsiveness (Jensen & Katzung, 1970; Singh & Vaughan Williams, 1971). In the present study we used a rather high K^+ concentration (5.4 mM), and found that the therapeutic concentration of the drug (2 μg/ml) decreased the $\dot{V}_{max}$ only slightly (12%). This may explain the absence of significant change in intraventricular conduction (H–V interval in the His electrogram) reported both in man (Roots, Paalman & Dunning, 1976) and in dogs (Okuma *et al.*, 1976) treated with mexiletine.

The depressant action of the drug on $\dot{V}_{max}$ depended on the stimulation frequency (Figure 1e and f). The mechanism of the decrease in $\dot{V}_{max}$ which followed an increase in driving frequency may be attributed to: (1) a small decrease in membrane potential due to K^+ accumulation immediately outside the fibre; (2) resetting of the Na^+–K^+ pump, and (3) incomplete reactivation of the rapid Na^+ system (Arita & Surawicz, 1973; Chen & Gettes, 1976). In the present experiments, the first possibility could be ruled out because all action potentials driven at different frequencies took off from exactly the same membrane potentials (see Figure 1d–f). However, we cannot distinguish between the second and the third possibilities, because there are no studies of the effect of mexiletine on sodium-potassium mediated ATPase (Na^+–K^+ pump), or on the recovery kinetics for the rapid Na^+ current.

In the mexiletine-treated Purkinje fibres, the effective refractory period was shortened less than the action potential duration (Figure 3a). As a result, the earliest effective test stimulus elicited a response in which the amplitude and the $\dot{V}_{max}$ of the action potential were greater than under control conditions. A similar effect has already been reported in Purkinje fibres treated with diphenylhydantoin (Bigger *et al.*, 1968). This has been proposed as one of the mechanisms which could suppress ventricular arrhythmias by improving conduction of premature excitation.

In contrast, in the ventricular fibres, the effective refractory period was lengthened but the lengthening exceeded any prolongation of the action potential duration. This resulted in the same effect, i.e., an increase of the amplitude and $\dot{V}_{max}$ of the earliest premature action potentials (Figure 3b).

Possible mechanism of antidysrhythmic action of mexiletine

Dysrhythmias may be produced by: (1) enhanced automaticity; (2) depressed conduction velocity combined with a reduced refractory period; or (3) a combination of these two factors (Antoni, 1971).

Mexiletine may be expected to exert an antidysrhythmic activity in cases where the arrhythmia is provoked by a parasystolic automatic focus, because the drug suppressed effectively this type of pacemaker activity (Figure 5a–d). A possible model for such pathological automaticity may be the slow repetitive discharges recorded from subendocardial Purkinje fibres surviving myocardial infarction (Friedman, Stewart, Fenglio & Wit, 1973).

On the other hand, if the depressed Purkinje fibres exhibiting action potentials of slow response type were excited by impulses from another pacemaker site firing at higher frequency, e.g., impulses from the sinus node, the drug may be expected to increase diastolic membrane potential, and thereby increase $\dot{V}_{max}$ and conduction velocity. Such an effect could reduce vulnerability to ventricular arrhythmias due to reentrant excitation. The mexiletine-induced increase in $\dot{V}_{max}$ of the earliest premature action potential may

be also of advantage in protecting against ventricular fibrillation following 'R on T' phenomenon (Surawicz, 1971).

Mexiletine reduced the $\dot{V}_{max}$ of Purkinje fibres (Figures 1e and f). An appropriate decrease in the conduction velocity might block the re-entrant excitation by changing a unidirectional block into a bidirectional one. However, in the present study, the reduction of $\dot{V}_{max}$ was only about 12% at a rate of 2 Hz and at the therapeutic concentration of 2 μg/ml (Figure 2). This may not be sufficient to play a significant role in suppression of re-entrant type arrhythmias. However, the reduction of $\dot{V}_{max}$ might be more pronounced under the following circumstances: (1) high doses of mexiletine (Figure 2), and (2) rapid heart rate (Figure 1f).

In conclusion the main antidysrhythmic actions of mexiletine may derive from: (1) suppression of automaticity and acceleration of conduction in depolarized Purkinje fibres, and (2) disproportionate lengthening of the effective refractory period in relation to the action potential duration in both Purkinje and ventricular muscle fibres. All these effects appear primarily due to an increase in membrane K^+ conductance.

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