The combined electrophysiological effects of lignocaine and sotalol in canine isolated cardiac Purkinje fibres are rate-dependent

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- 1 The frequency-dependent electrophysiological effects of lignocaine, sotalol, and their combination were studied in dog isolated cardiac Purkinje fibres, both at various constant rates of stimulation and following abrupt changes in pacing cycle length.
- 2 The combined effect of 18 μM lignocaine and 30 μM sotalol selectively lengthened duration of premature action potentials evoked at a diastolic interval of 40 ms (from 172.2 ± 5.4 to 201.7 ± 4.9 ms, $n = 6$, $P < 0.01$) without significantly changing the durations of action potentials evoked at the basic cycle length of 500 ms (259.1 ± 7.7 vs 251.9 ± 3.9 ms, $n = 11$).
- 3 The combination of lignocaine with sotalol, like lignocaine alone, displayed a use-dependent depression of $\dot{V}_{\text{max}}$ and revealed a slow component for a recovery of $\dot{V}_{\text{max}}$ ($\tau = 173.5 \pm 16.0$ ms, $n = 5$).
- 4 The kinetics for restitution of action potential duration were also slowed by the combination of the two drugs ($\tau_r = 173.6 \pm 16.7$, before, vs 268.5 ± 8.5 ms, after; $n = 5$, $P < 0.01$), while the maximum action potential duration attained in this relation was not increased as it was by sotalol alone.
- 5 Lignocaine, therefore, appeared to inhibit the sotalol-induced lengthening of action potential duration at slow pacing rates and at long diastolic intervals. The combination of lignocaine with sotalol also completely abolished the occurrence of sotalol-induced early afterdepolarizations.
- 6 Finally, sotalol alone moderately increased the range of premature action potential durations, while the combination of the two drugs significantly decreased this parameter.
- 7 These findings indicate that the combination of lignocaine with sotalol may provide important, and unique, beneficial electrophysiological alterations that might be expected to provide enhanced antiarrhythmic efficacy in patients.

Introduction

Although many new antiarrhythmic drugs have been introduced into therapy during the last decade, the pharmacological treatment of cardiac arrhythmias with a single agent is not always successful (Anderson, 1985; Wagner *et al.*, 1987; Lévy, 1988). Simultaneous administration of antiarrhythmic drugs with different mechanisms of action may enhance their antiarrhythmic efficacy above that observed when either drug is administered alone and reduce their propensity toward arrhythmogenesis. Combination and the use of two or more antiarrhythmic drugs has therefore been increasingly used in the treatment of cardiac arrhythmias (Wagner *et al.*, 1987; Lévy, 1988).

Sotalol is a β -adrenoceptor blocker (Strauss *et al.*, 1970) which lengthens action potential duration and refractoriness in both cardiac Purkinje (Strauss *et al.*, 1970; Lathrop, 1985; Varró *et al.*, 1986) and ventricular muscle (Singh & Vaughan Williams, 1970; Strauss *et al.*, 1970; Lathrop, 1985) fibres without significantly affecting the rate of rise of the action potential upstroke or impulse conduction. Lignocaine, on the other hand, is a local anaesthetic type antiarrhythmic which produces a use-dependent depression of the fast sodium current (Lee *et al.*, 1981) and slows impulse conduction (Hoffman *et al.*, 1975). Lidocaine either shortens (Bigger & Mandel, 1970; Carmeliet & Saikawa, 1982; Colatsky, 1982; Kinnard & Man, 1984; Nattel & Zeng, 1984; Varró *et al.*, 1985) or does not change (Varró *et al.*, 1987) action potential duration, depending on its concentration and the type of cardiac tissue in which its effects are examined. These two

drugs also display distinctly different frequency-dependent electrophysiological effects (Varró *et al.*, 1986). Although, the cellular cardiac electrophysiological effects of both sotalol (Singh & Vaughan Williams, 1970; Strauss *et al.*, 1970; Lathrop, 1985; Varró *et al.*, 1986) and lignocaine (Bigger & Mandel, 1970; Carmeliet & Saikawa, 1982; Colatsky, 1982; Kinnard & Man, 1984; Nattel & Zeng, 1984; Varró *et al.*, 1985; 1987) alone have been extensively studied, no reports have appeared to describe the electrophysiological effects of these drugs when administered in combination with one another. We therefore examined the simultaneous electrophysiological effects of lignocaine and sotalol in isolated cardiac Purkinje fibres. These effects were examined over a wide range of constant pacing cycle lengths and following abrupt changes in diastolic interval, in order to mimic the heart rates of rhythm disturbances observed in patients requiring antiarrhythmic drug therapy.

Methods

Adult mongrel dogs of either sex, weighing 10 to 12 kg were utilized. Following sodium pentobarbitone (30 mg kg⁻¹ i.v.)-induced anaesthesia, their hearts were rapidly removed through a right lateral thoracotomy. The hearts were immediately rinsed in cold (0°C) oxygenated Tyrode solution containing (in mM): Na⁺ 153.0, K⁺ 5.4, Cl⁻ 162.9, Ca²⁺ 2.7, Mg²⁺ 1.05, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) buffer (Sigma Chemical, catalog No. H-3375) 5.4 and glucose 11.0. The pH of this solution was 7.35 to 7.45 when gassed with 100% oxygen at 37°C. Small diameter (<0.6 mm) Purkinje strands, obtained from either ven-

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tricle, were individually mounted in a small volume (0.5 ml) tissue chamber. Each preparation was initially stimulated (MECA, model STM-2 stimulator) at a basic cycle length of 500 ms (frequency = 2 Hz) using 1 ms long rectangular constant current pulses isolated from ground (WP-Instruments, model 830) and delivered across two platinum electrodes placed so as to not be in contact with the preparation (i.e., field stimulation). One hour was allowed for each preparation to equilibrate, while it was continuously superfused (flow = 4 to 5 ml min⁻¹) with Tyrode solution warmed to 37°C and gassed with 100% oxygen. Transmembrane potentials were recorded by conventional microelectrode techniques. Microelectrodes filled with 3 M KCl having tip resistances of 10 to 20 MΩ were connected to the input of a high impedance (10¹² Ω) electrometer (WP-Instruments, model 750), which was referenced to ground by a Ag-AgCl electrode connected to earth ground and placed in a 3 M KCl agar bridge within the superfusion chamber. The first derivative of transmembrane voltage with respect to time ($\dot{V}_{\max}$) was electronically derived by a MECA (model EP-0.3) amplifier which had a linear response over the range of 20 to 1000 V s⁻¹. The voltage outputs of all recording amplifiers were displayed on a single beam storage oscilloscope (Tektronix, model R-5115) with dual sweep speed capability (Tektronix, model 5B12N dual time base). Oscilloscope displays were photographed on Polaroid film (type 667) with a Tektronix camera (model C-59).

Experimental protocol

Following the 60 min equilibration period, while each preparation was stimulated at a constant frequency (2 Hz), control measurements of maximum diastolic potential (MDP), action potential amplitude (APA), action potential durations measured at 50 and 90% of repolarization (APD₅₀ and APD₉₀), and the maximum rising velocity of the action potential upstroke ($\dot{V}_{\max}$) were obtained. In one group of experiments designed to determine the recovery of $\dot{V}_{\max}$ and the restitution of action potential duration following abrupt changes in pacing cycle length, extra stimuli were used. Test action potentials were elicited by a single test pulse (S₂) which interrupted trains of 30 basic stimuli (S₁) delivered at a cycle length of 500 ms. The S₁-S₂ interval was progressively increased following each successive train of stimuli. The characteristics of action potentials evoked by each S₂ were determined. Effective refractory period (ERP), defined as the shortest S₁-S₂ interval which evoked a propagated action potential with a stimulus amplitude equal to twice the diastolic threshold intensity, was also determined. Diastolic intervals preceding test action potentials were measured from the point corresponding to 90% repolarization of the preceding basic action potential to delivery of S₂. Diastolic intervals were successively increased from 0 to 2.7 s.

In another group of experiments, 10 different constant cycle lengths (250, 300, 350, 400, 500, 700, 1000, 1500, 2000, and 3000 ms) were applied. Action potential parameters were measured at each cycle length following adaptation to the new pacing cycle length (usually after 2 min). In both protocols the preparation was superfused either for 20 min with 10 or 18 μM lidocaine, 60 min with 30 μM sotalol, or 60 min with 10 or 18 μM lignocaine in combination with 30 μM sotalol. Preliminary experiments with lignocaine indicated that after 20 min of superfusion no further electrophysiological changes occurred, while a 60 min period of superfusion with sotalol was required for steady-state effects to develop. Therefore, a 20 min superfusion period was used in the experiments involving lignocaine alone, while a 60 min period of superfusion was used in the experiments involving sotalol. All action potential parameters were again obtained following equilibration with either lignocaine, sotalol or the combination. These periods of superfusion were followed by a washout of the drug(s) for a period not exceeding 60 min.

Drug preparation

Drugs were prepared daily from aqueous stock solutions. Sotalol (Bristol-Myers, Wallingford, CT, U.S.A.) was diluted from a 30 mM stock solution to obtain a final concentration of 30 μM in Tyrode solution. Lignocaine (Sigma Chemical, St. Louis, MO, U.S.A.) was similarly diluted from a 1 mM stock solution to form final concentrations of either 10 or 18 μM in Tyrode solution.

Data analysis

Student's *t* tests for either paired or unpaired data were used to compare the results statistically. Results were considered significant when *P* was found to be less than 0.05.

For the purpose of analysis, each restitution of action potential duration relation was resolved into two exponential components (Elharrar & Surawicz, 1983). This was accomplished by normalizing APD₉₀ measured at each diastolic interval (APD_i) to a duration (APD_{pl}) 5% greater than that established at the longest diastolic interval used (2.7 s). The fast and slow time constants (τ_f and τ_s, respectively) were estimated by use of standard exponential curve fitting procedures. The curves for the relation between APD_i and diastolic interval were thus fitted to the following equation (Elharrar & Surawicz, 1983):

$$[1] \quad \text{APD}_i = \text{APD}_{pl} \cdot \{1 - [A_f \cdot e^{(-i/\tau_f)}] - [A_s \cdot e^{(-i/\tau_s)}]\}$$

where *i* represents the diastolic interval preceding APD_i, and *A* and τ the amplitudes and the time constants of the fast (A_f and τ_f) and the slow (A_s, τ_s) exponential components.

The relations for the recovery of $\dot{V}_{\max}$ were either fitted to a single or to a double exponential relation, similar to equation 1, after $\dot{V}_{\max}$ was normalized to the greatest value obtained at long diastolic intervals.

Results

Effects of lignocaine, sotalol and their combination at the basic cycle length (500 ms)

The effects of 10 and 18 μM lignocaine, 30 μM sotalol, and their combination on the electrophysiological parameters of dog Purkinje fibres paced at a basic cycle length of 500 ms are summarized in Table 1. None of these interventions significantly altered MDP. Sotalol (30 μM) also did not significantly change APA and $\dot{V}_{\max}$; but, it did significantly lengthen action potential durations measured both at 50 and 90% of repolarization. Both 10 and 18 μM lidocaine slightly decreased APA, $\dot{V}_{\max}$ and markedly shortened APD₅₀ and APD₉₀. The combination of 10 or 18 μM lignocaine with 30 μM sotalol elicited changes similar to those produced by lignocaine alone on APA, APD₅₀ and $\dot{V}_{\max}$; while the combination did not significantly change APD₉₀.

The effect of 10 μM lignocaine, 30 μM sotalol and their combination on effective refractory period (ERP) was proportional to the effects produced on APD so that there was no significant change in the ERP/APD₉₀ ratio. The higher lignocaine concentration (18 μM) and its combination with 30 μM sotalol significantly increased the ERP/APD₉₀ ratio.

Effects of lignocaine, sotalol and their combination at different constant pacing cycle lengths

The effects of 18 μM lignocaine, 30 μM sotalol, and their combination were examined at 10 different constant pacing cycle lengths ranging from 250 to 3000 ms (Figure 1). Sotalol (30 μM) lengthened APD at every cycle length examined. The sotalol-induced increase in APD₉₀ was gradually increased to a proportionally greater extent as the cycle length was increased

Table 1 The electrophysiological effects of lignocaine, sotalol and their combination in dog Purkinje fibres at basic cycle length of 500 ms

	MDP (mV)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)	$\dot{V}_{max}$ (V s ⁻¹)	ERP/APD ₉₀
Control	-84.2 ± 0.6	122.4 ± 1.0	180.9 ± 7.4	267.3 ± 8.9	595.0 ± 27.5	0.958 ± 0.015
Lignocaine (10 µM)	-84.2 ± 0.5 (11)	121.1 ± 0.9 (11)	129.1 ± 4.4** (11)	216.8 ± 4.4** (11)	559.1 ± 31.6 (11)	0.976 ± 0.023 (5)
Control	-84.0 ± 0.4	124.2 ± 0.5	170.0 ± 5.8	254.2 ± 6.8	565.0 ± 27.4	0.943 ± 0.018
Sotalol (30 µM)	-84.5 ± 0.4 (12)	123.5 ± 0.7 (12)	205.8 ± 4.6** (12)	330.4 ± 7.3** (12)	541.7 ± 28.0 (12)	0.962 ± 0.009 (6)
Control	-84.4 ± 0.8	122.7 ± 0.9	181.4 ± 8.2	267.2 ± 7.4	600.9 ± 23.1	0.925 ± 0.024
Lignocaine (10 µM) + sotalol (30 µM)	-84.5 ± 0.8 (11)	121.8 ± 1.0 (11)	155.9 ± 6.4** (11)	275.9 ± 6.9 (11)	545.4 ± 23.9** (11)	0.962 ± 0.029 (6)
Control	-83.8 ± 0.5	121.8 ± 0.8	182.1 ± 5.1	263.3 ± 7.2	599.6 ± 30.4	0.945 ± 0.005
Lignocaine (18 µM)	-84.2 ± 0.5 (12)	119.8 ± 0.7* (12)	119.2 ± 5.6** (12)	203.3 ± 2.9** (12)	529.6 ± 29.7** (12)	0.993 ± 0.009** (6)
Control	-85.1 ± 0.4	124.0 ± 0.8	173.2 ± 6.9	259.1 ± 7.7	575.2 ± 20.6	0.963 ± 0.026
Lignocaine (18 µM) + sotalol (30 µM)	-83.8 ± 0.6 (11)	119.1 ± 1.4** (11)	132.7 ± 6.4** (11)	251.9 ± 3.9 (11)	481.3 ± 31.1** (11)	1.010 ± 0.006* (6)

Data shown are means ± s.e. of *n* (number in parentheses) observations. * *P* < 0.05, ** *P* < 0.01. MDP, maximum diastolic potential; APA, action potential amplitude; APD₅₀, action potential duration at 50% of repolarization; APD₉₀, action potential duration at 90% of repolarization; $\dot{V}_{max}$, maximum rising velocity of the action potential upstroke; ERP, effective refractory period.

(Figure 1a). The effect of 18 µM lignocaine on the cycle length-dependent changes in APD₉₀ was opposite; i.e., lignocaine shortened APD₉₀ more at long cycle lengths than at short ones. Because each drug alone produced opposite effects on APD, the combination of the two drugs did not result in a significant change in APD₉₀ at any pacing frequency examined. Sotalol alone also did not significantly influence $\dot{V}_{max}$ at any applied pacing frequency (Figure 1b). Lignocaine (18 µM) and its combination with 30 µM sotalol, however, did produce a use-dependent depression of $\dot{V}_{max}$. At cycle lengths longer than 700 ms, the $\dot{V}_{max}$ depression was no longer dependent on pacing frequency, reflecting only a frequency-independent or 'tonic' block. At longer cycle lengths (2000 and 3000 ms, not

shown in Figure 1) there was a further decrease in $\dot{V}_{max}$ in some fibres due to spontaneous diastolic depolarization, with a concomitant decrease in take-off potential. In these fibres, at these especially long pacing cycle lengths, $\dot{V}_{max}$ was decreased both before and after administration of the drugs.

Effects of lignocaine, sotalol and their combination following abrupt changes in pacing cycle length

The effects of 10 and 18 µM lignocaine, of 30 µM sotalol, and of their combination on APD₉₀ and $\dot{V}_{max}$ were also examined after producing abrupt changes in pacing cycle length. The effects of lignocaine, sotalol, and their combination on early premature action potentials that occur shortly after final repolarization of the preceding basic action potential (i.e., at a diastolic interval = 40 ms) are summarized in Table 2, with examples illustrated in Figure 2.

Sotalol (30 µM) exerted effects on early premature action potentials similar to those produced on basic action potentials. The only notable effect was lengthening of APD₅₀ and APD₉₀. Lignocaine (10 and 18 µM) shortened early premature action potential APD₅₀ and APD₉₀ as it did those of the basic action potential. In addition, lignocaine alone significantly (*P* < 0.05) decreased action potential amplitude (APA) and $\dot{V}_{max}$ of premature action potentials more than those of action potentials evoked at the basic cycle length of 500 ms. The combination of 10 or 18 µM lignocaine with 30 µM sotalol also depressed APA and $\dot{V}_{max}$ of the early premature action potential significantly (*P* < 0.05) more than those of the basic action potential. Duration of the premature action potential measured at 90% of repolarization (APD₉₀) was significantly increased when lignocaine and sotalol were applied in combination. This was an important finding which was in contrast to the effects observed on the basic action potential, where APD₉₀ was not significantly changed.

The effects of 18 µM lignocaine, 30 µM sotalol and their combination on restitution of action potential duration (APD₉₀ vs diastolic interval) are illustrated in Figure 3. Figure 3a shows the entire range of diastolic intervals studied; in (b) the relation established for the early extrasystoles (diastolic intervals between 0 and 250 ms) is shown. Sotalol (30 µM) did not alter the time constants for restitution (Table 3), while it did produce an increase in action potential duration throughout the restitution curve. Lignocaine (18 µM) and the combination of 18 µM lignocaine with 30 µM sotalol significantly slowed the kinetics of the fast component without changing the slow one.

Sotalol (30 µM) thus increased APD₉₀ over the entire range of diastolic intervals studied, while 18 µM lignocaine shortened

Figure 1 Rate-dependent effect of 18 µM lignocaine (○, *n* = 5), 30 µM sotalol (●, *n* = 5), and their combination (□, *n* = 5) on action potential duration (a) and on $\dot{V}_{max}$ (b). Control values (■, *n* = 15) were combined for all preparations examined. The mean values are indicated at each point; vertical lines show s.e. The abscissa scales represent the various pacing cycle lengths used. The ordinate scale in (a) shows the action potential duration at the 90% of repolarization (APD₉₀) while the normalized values of $\dot{V}_{max}$ are shown in (b).

Table 2 The effects of lignocaine, sotalol and their combination on the premature action potential at 40 ms of diastolic interval in dog Purkinje fibres

	TOP (mV)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)	$\dot{V}_{max}$ (V s ⁻¹)
Control	-82.4 ± 0.7	123.2 ± 0.8	109.0 ± 6.8	187.0 ± 9.4	550.0 ± 37.7
Lignocaine (10 μ M)	-83.6 ± 0.7 (5)	118.4 ± 1.9*	80.0 ± 7.4** (5)	156.0 ± 3.7** (5)	440.0 ± 60.6** (5)
Control	-81.3 ± 0.8	121.5 ± 1.9	110.8 ± 7.0	178.3 ± 4.8	516.7 ± 15.0
Sotalolol (30 μ M)	-81.0 ± 0.9 (6)	119.0 ± 1.7** (6)	133.3 ± 10.7** (6)	238.3 ± 12.8** (6)	471.7 ± 26.1* (6)
Control	-82.7 ± 1.3	121.7 ± 1.3	103.3 ± 2.5	185.8 ± 6.6	561.7 ± 34.5
Lignocaine (10 μ M) + sotalol (30 μ M)	-81.0 ± 1.1 (6)	113.0 ± 3.3** (6)	95.0 ± 4.5 (6)	198.3 ± 7.8 (6)	428.3 ± 54.2** (6)
Control	-83.3 ± 0.8	121.3 ± 1.0	122.5 ± 4.6	190.8 ± 5.1	601.7 ± 46.8
Lignocaine (18 μ M)	-83.3 ± 1.0 (6)	114.3 ± 2.3** (6)	95.0 ± 7.8** (6)	160.8 ± 7.2** (6)	398.3 ± 43.5** (6)
Control	-82.7 ± 1.2	120.3 ± 6.6	105.0 ± 2.9	179.2 ± 5.4	515.0 ± 28.9
Lignocaine (18 μ M) + sotalol (30 μ M)	-83.7 ± 0.8 (6)	104.0 ± 4.5** (6)	111.7 ± 8.5 (6)	201.7 ± 4.9** (6)	325.3 ± 37.9** (6)

Data shown are means $\pm$ s.e. of n (number in parentheses) observations. * $P < 0.05$, ** $P < 0.01$. TOP, take off potential; APA, action potential amplitude; APD₅₀, action potential duration at 50% of repolarization; APD₉₀, action potential duration at 90% of repolarization; $\dot{V}_{max}$, maximum rising velocity of the action potential upstroke.

APD₉₀ (Figure 3). The combined effect of 18 μ M lignocaine and 30 μ M sotalol was to increase selectively APD₉₀ of the early premature beats without significantly affecting APD₉₀ of action potentials evoked at longer diastolic intervals.

Under *in vivo* conditions, the differences in action potential durations evoked at short diastolic intervals may be due to non-uniform propagation of early premature impulses; an effect which may enhance the dispersion of repolarization. Therefore, in our study we compared the differences in premature action potential durations that were observed at diastolic intervals between 0 and 100 ms. As previously defined, this difference denoted the range of premature action potential durations (Varró *et al.*, 1985). Sotalol (30 μ M) moderately increased

this range while 18 μ M lignocaine and the combination of 18 μ M lignocaine with 30 μ M sotalol significantly decreased the range of premature action potential durations (Table 3).

The recovery kinetics of $\dot{V}_{max}$ during control and in the pre-

Figure 2 Effect of sotalol (30 μ M), lignocaine (18 μ M) and their combination on premature Purkinje fibre action potential characteristics. The top pair of recordings illustrate the effects of 30 μ M sotalol alone on premature action potentials evoked at a diastolic interval equal to 40 ms. The middle pair of recordings illustrate the effect of 18 μ M lignocaine alone at the same premature diastolic interval. The bottom pair of recordings show the combined effects of 30 μ M sotalol and 18 μ M lignocaine at a diastolic interval = 40 ms. The left panel in each row was recorded in the absence of drug (control); the right panel shows the effect of the drug or the combination of 2 drugs. The basic pacing cycle length was in all cases equal to 500 ms. Voltage and time calibrations for the action potentials are indicated on the left of the figure while amplitude and time scales for the first derivative of transmembrane voltage with respect to time are indicated to the right. Note that the derivatives have been inverted.

Figure 3 Effects of 18 μ M lignocaine ($\circ$, $n = 6$), 30 μ M sotalol ($\bullet$, $n = 6$), and their combination ($\square$, $n = 5$) on restitution of action potential duration (APD₉₀). As in the previous figure, ($\blacksquare$) represent control measurements combined from all experiments ($n = 17$). All curves were fitted to equation 1, described in the Methods section. Means are indicated with vertical lines show s.e. In (a), the abscissa scale shows the diastolic intervals (in s) and in (b) these values are presented with an expanded time scale (expressed in ms). The ordinates indicate APD₉₀ values.

Table 3 The effect of lignocaine, sotalol and their combination on the kinetics of restitution, recovery of $\dot{V}_{\max}$ and premature APD range in dog Purkinje fibres

	τ_f (ms)	APD_{90} τ_s (s)	τ_f (ms)	$\dot{V}_{\max}$ τ_s (ms)	Range of premature APDs (ms)
Control	171.3 $\pm$ 3.5	2.394 $\pm$ 0.128	23.4 $\pm$ 2.9	—	55.8 $\pm$ 7.9
Lignocaine (18 μ M)	258.3 $\pm$ 15.1** (6)	2.541 $\pm$ 0.161 (6)	22.0 $\pm$ 4.4 (6)	188.3 $\pm$ 32.6 (6)	14.2 $\pm$ 3.0** (6)
Control	159.0 $\pm$ 9.3	2.525 $\pm$ 0.269	37.3 $\pm$ 5.5	—	59.2 $\pm$ 7.9
Sotalol (30 μ M)	154.3 $\pm$ 14.8 (6)	1.806 $\pm$ 0.575 (6)	31.0 $\pm$ 4.4 (6)	— (6)	75.0 $\pm$ 10.2* (6)
Control	173.6 $\pm$ 16.7	2.393 $\pm$ 0.205	24.2 $\pm$ 3.3	—	45.8 $\pm$ 5.8
Lignocaine (18 μ M) + sotalol (30 μ M)	268.5 $\pm$ 8.5** (5)	2.142 $\pm$ 0.119 (5)	20.1 $\pm$ 3.3 (5)	173.5 $\pm$ 16.0 (5)	18.3 $\pm$ 3.1** (5)

Data shown are means $\pm$ s.e. of n (number in parentheses) observations. * $P < 0.05$; ** $P < 0.01$. APD_{90} , action potential duration at 90% of repolarization; $\dot{V}_{\max}$, maximum rising velocity of the action potential upstroke; τ_f , time constants of the fast component; τ_s , time constants of the slow component.

sence of 30 μ M sotalol were fitted to a single exponential relation (Table 3). At 37°C the time constant for recovery of $\dot{V}_{\max}$ was fast during control and recovery was complete before the end of the final repolarization of the action potential. Sotalol (30 μ M) did not change the recovery time constant for recovery of $\dot{V}_{\max}$ (Table 3). In the presence of 18 μ M lignocaine and of 18 μ M lignocaine in combination with 30 μ M sotalol the recovery kinetics of $\dot{V}_{\max}$ were best described as having 2 exponential components. The fast component, which may reflect effects on drug free sodium channels (Hondeghe & Katzung, 1984), was not significantly changed from control by either lignocaine alone or in combination with sotalol. However, lignocaine (18 μ M) and the combination of 18 μ M lignocaine with 30 μ M sotalol revealed a slow component for the recovery of $\dot{V}_{\max}$, which may reflect effects upon drug modified channels (Table 3). The recovery time constants of 188.3 ms and 173.5 ms with 18 μ M lignocaine and the combination of 18 μ M lignocaine with 30 μ M sotalol, respectively, indicated that the $\dot{V}_{\max}$ depression is predominant over the range of early extrasystoles.

Effects of lignocaine on sotalol-induced early afterdepolarizations

In 62.5% of the experiments (5 out of 8 preparations) utilizing 30 μ M sotalol alone, early afterdepolarizations were observed at long (3 s) constant pacing cycle intervals. When pacing cycle length was increased beyond 3 s, these early afterdepolarizations became repetitive while the membrane potential remained within the range of plateau potentials. In these preparations, addition of 10 μ M lignocaine abolished the sotalol-induced early afterdepolarizations and produced a concomitant shortening of action potential duration in each of these 5 preparations. Figure 4 illustrates an example of such activity and the effect of lignocaine, observed while one preparation was stimulated at an extremely long basic cycle length (7 s) in order to emphasize the effect of sotalol on induction of early afterdepolarizations at slow pacing frequencies.

Discussion

The concentrations of lignocaine and of sotalol utilized were chosen to produce effects similar to those described earlier (Bigger & Mandel, 1970; Strauss *et al.*, 1970; Carmeliet & Saikawa, 1982; Coltasky, 1982; Lathrop, 1985). These concentrations fall within the upper range of therapeutic plasma concentrations observed in patients (Hoffman *et al.*, 1975; Neuvonen *et al.*, 1982).

Electrophysiological effects of lignocaine, sotalol and their combinations

The effects of lignocaine (Bigger & Mandel, 1970; Varró *et al.*, 1986) and of sotalol (Strauss *et al.*, 1970; Lathrop, 1985) alone that we observed at the various constant pacing frequencies confirm the results of earlier investigations. The effects of sotalol on restitution of action potential duration and recovery of $\dot{V}_{\max}$ are similar to those we previously obtained in isolated cardiac Purkinje fibres (Lathrop *et al.*, 1989). The effects of lignocaine on the recovery kinetics of $\dot{V}_{\max}$ (Hondeghe & Katzung, 1984; Elharrar, 1988) and the restitution of action potential duration (Varró *et al.*, 1985; Elharrar, 1988) also agree with the results of previous investigations.

The combination of lignocaine with sotalol resulted in an additive effect of the two antiarrhythmic agents. Action potential durations observed while pacing at different constant cycle lengths, or at long diastolic intervals, changed in a manner that may have been expected by summing the effects of lignocaine (shortening) and sotalol (lengthening) alone on APD together. However, the significant increase in the duration of the early premature action potentials observed with the combination of the two drugs was somewhat of an unexpected finding. Examination of the data presented in Tables 1 and 2 reveals that the durations of the early premature action potentials were shortened less by 18 μ M lignocaine and lengthened

Figure 4 Effect of 10 μ M lignocaine on sotalol-induced early afterdepolarizations in dog isolated Purkinje fibre. The left panel illustrates the development of early afterdepolarizations 45 min after beginning superfusion with Tyrode solution containing 30 μ M sotalol. In the right panel a recording of transmembrane potential from the same impalement, in the same fibre, is shown after adding 10 μ M lignocaine to the sotalol-containing superfusate. Voltage and time calibrations for the action potential recordings are indicated on the left of the figure. The basic cycle length in this experiment was 7000 ms (0.143 Hz).

more by 30 μM sotalol than were the durations of the basic ones. The effect of the lignocaine-sotalol combination on the early premature action potential duration, therefore, also represented the summed effect of the two drugs. The use-dependent effect of the combination of the two drugs on $\dot{V}_{\text{max}}$ was very similar to the effect produced by lignocaine alone.

Arnsdorf (1977) has demonstrated that lignocaine blocks spontaneously occurring early afterdepolarizations in sheep isolated Purkinje fibres bathed in solutions containing 4 mM potassium. In the present study, we demonstrated that sotalol-induced early afterdepolarizations are completely suppressed by addition of 10 μM lignocaine. Such a finding may have an important clinical implication in the treatment of sotalol intoxication.

Possible ionic mechanisms

The specific ionic mechanisms by which lignocaine and sotalol affect action potential configuration have been examined in studies utilizing voltage and patch-clamp techniques. Sotalol was found to decrease both the inward and delayed rectifier potassium currents without affecting the inward calcium current (Carmeliet, 1985). Lidocaine decreased peak fast sodium current (Lee *et al.*, 1981) as well as the sodium 'window' current (Carmeliet & Saikawa, 1982; Colatsky, 1982).

Recently both lignocaine and mexiletine were demonstrated to slow recovery of $\dot{V}_{\text{max}}$ while shortening APD and lengthening the time course of restitution of action potential duration. Because both these drugs reduce the magnitude of the sodium 'window' current, which contributes to maintenance of the action potential plateau, an effect on this current was suggested to have a role in controlling restitution of action potential duration in cardiac Purkinje fibres (Elharrar, 1988).

Clinical implications

The results presented in this study may have clinical implications. The combination of lignocaine with sotalol increased early premature action potential duration without producing a significant effect on the duration of the basic action potential. The ERP/APD₉₀ ratio was also increased. These effects might be expected to reduce the likelihood of a second conducted impulse from occurring at short premature intervals and thereby lessen the likelihood of developing tachycardia.

The significant enhanced depressant effect on $\dot{V}_{\text{max}}$ of the combination of lignocaine and sotalol, albeit slight, observed at early premature coupling intervals, presumed to have resulted primarily from the effect of lignocaine, might also tend to block conduction of early premature impulses selec-

tively. This effect might be expected to be further amplified if the myocardium was depolarized (Hondeghe & Katzung, 1984) as in myocardial ischaemia. The use-dependent effect on $\dot{V}_{\text{max}}$ produced by lignocaine or by the combination was only apparent at short cycle lengths. This effect might also be expected to depress impulse conduction during tachycardia while not affecting conduction at normal heart rates when recovery of $\dot{V}_{\text{max}}$ would be complete. On the other hand, lignocaine prevented the sotalol-induced lengthening of APD at the basic pacing cycle length; and, this class III effect represents the supposed basis of the antiarrhythmic effect of sotalol. Whether the preferential lengthening of premature action potential duration and depression of $\dot{V}_{\text{max}}$ at short coupling intervals, produced by the combination, actually produces enhanced antiarrhythmic efficacy remains to be tested in intact animal models and patients.

Differences between the durations of early premature action potentials occurring at various short coupling intervals may reflect dispersion of repolarization when non-uniform impulse propagation occurs (Kuo *et al.*, 1983a,b). A decrease in the differences between premature action potential durations, defined as the range of premature action potential durations, has therefore also been suggested to represent an important antiarrhythmic mechanism (Varró *et al.*, 1985). Sotalol alone slightly increased the range of premature action potential durations, while lignocaine and lignocaine combined with sotalol significantly decreased it. This effect might also be expected to reduce the likelihood of development of arrhythmias related to enhanced regional dispersion of action potential duration and refractoriness (Kuo *et al.*, 1983b).

One side-effect of sotalol administration is the development of *torsade de pointes* (Kontopoulos *et al.*, 1981; Rakovec *et al.*, 1984) and other bradycardia-induced tachyarrhythmias (Neovonen *et al.*, 1981). The occurrence of early afterdepolarizations has been implicated in the production of these tachyarrhythmias (El-Sherif *et al.*, 1988). Sotalol, as demonstrated, markedly lengthens action potential duration at slow heart rates, or following long diastolic intervals; and, early afterdepolarizations have often been observed under these conditions in the presence of sotalol in isolated cardiac preparations (Hiromasa *et al.*, 1988). Therefore, an additional clinically beneficial effect of combining lignocaine with sotalol might be to decrease the sotalol-induced increase in action potential duration, the occurrence of early afterdepolarizations, and possibly the increased incidence of 'triggered' activity produced by sotalol alone.

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