Electrophysiological effects of the combination of mexiletine and flecainide in guinea-pig ventricular fibres

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1 The effects of flecainide alone, mexiletine alone and their combination at the Na^+ channel level were studied in guinea-pig papillary muscles. The maximum upstroke velocity ($V_{\max}$) was used as an indirect index of the magnitude of the fast inward Na^+ current (I_{Na}).

2 In muscles driven at 0.02 Hz, neither mexiletine (10^{-5} M) nor flecainide (10^{-6} M) nor the combination of both drugs modified the action potential characteristics. Mexiletine, but not flecainide, increased the effective refractory period/action potential duration ratio; this enhancement was greater when flecainide was also present.

3 Mexiletine or flecainide alone produced a frequency-dependent $V_{\max}$ block. Although at 0.5 Hz the blockade induced by the combination of flecainide and mexiletine was similar to that produced by flecainide alone, in muscles driven at 1 and 2 Hz the combination increased the magnitude and the onset rate of the $V_{\max}$ block.

4 The time constant of recovery of $V_{\max}$ block was similar in the presence of flecainide or the combination mexiletine plus flecainide ($\tau_{\text{re}} = 16.4 \pm 2.3$ s and 16.7 ± 2.7 s, respectively), but the combination decreased the magnitude of the slow component of reactivation induced by flecainide ($93.8 \pm 1.5\%$ versus $68.9 \pm 1.7\%$). Moreover, the combination of both drugs was more effective in inhibiting the $V_{\max}$ of early test stimuli than either drug alone.

5 It is concluded that the combination of mexiletine and flecainide is synergistic at driving rates faster than 0.5 Hz without detracting from the characteristics of flecainide.

Keywords: Mexiletine; flecainide; $V_{\max}$; ventricular muscle; heart

Introduction

Flecainide, a class Ic drug, is a highly potent antiarrhythmic agent which exhibits both very slow onset kinetics and recovery from $V_{\max}$ block (Campbell & Vaughan Williams, 1983; Campbell, 1983a,b). It has been found effective in treatment and prophylaxis of ventricular arrhythmias and certain supraventricular arrhythmias (Anderson *et al.*, 1981; Duff *et al.*, 1981; Campbell, 1983b; Neuss *et al.*, 1983; Hodges & Heel, 1985; Somberg & Tepper, 1986). Like other class Ic agents, flecainide exhibits not only very potent antiarrhythmic but unfortunately also proarrhythmic properties (CAST, 1989). Recently, Anno & Hondeghem (1989, 1990) reported that most of the frequency-dependent $V_{\max}$ block produced by flecainide is associated with the activated state of the Na^+ channels. Moreover, they concluded that flecainide also could bind very slowly to slow inactivated Na^+ channels, or could have promoted the development of ultra slow inactivation. Mexiletine is a class Ib antiarrhythmic drug (Campbell, 1983a) which has been proved to be useful in the treatment of ventricular tachyarrhythmias (Sandoe *et al.*, 1978; Podrid, 1986; Campbell, 1987; Morganroth, 1987). It binds preferentially to the inactivated state of the Na^+ channel (Yatani & Akaike, 1985) but also to the activated state (Hering *et al.*, 1983). However, it dissociated faster from the Na^+ channel receptor during diastole than does flecainide (Campbell, 1983a). Recently it has been proposed that one possible way to reduce the cardiodepressant effects of class I antiarrhythmic drugs would be to combine drugs from different subgroups, i.e. Ia, Ib, Ic (Hondeghem, 1987; Tamargo *et al.*, 1989). According to the modulated receptor hypothesis (Hondeghem & Katzung, 1977; 1984) antiarrhythmic drugs with very different kinetics block Na^+ channels binding to a common receptor site at the Na^+ channel. Thus, if it is assumed that two drugs bind to the same receptor site, the frequency-dependent effects of the combination would be less pronounced than those induced by the drug with slower kinetics (Hondeghem, 1987; Tamargo *et al.*, 1989; Valenzuela *et al.*, 1989). However, it would be also possible that although both drugs bind to the same receptor site,

a fast (Ib) antiarrhythmic drug cannot displace a slow (Ic) drug from Na^+ channels when the drugs are given together. In this situation the combination would decrease the available I_{Na} at least at certain driving rates. Thus, the present work was undertaken to study in guinea-pig papillary muscles the electrophysiological effects of a combination of mexiletine (class Ib) plus flecainide (class Ic).

Methods

Guinea-pigs of either sex weighing 350–450 g were killed by a blow on the neck. The hearts were rapidly removed and placed in a dissection chamber where papillary muscles of 2–3 mm in length and less than 1 mm in diameter were excised from the right ventricle. The muscles were pinned to the bottom of a Lucite chamber and superfused continuously at a constant rate of 7 ml min^{-1} with Tyrode solution of the following composition (mM): NaCl 137, KCl 5.4, CaCl_2 1.8, MgCl_2 1.05, NaHCO_3 11, NaH_2PO_4 0.42, and glucose 5.5. Solutions were bubbled with 95% O_2 :5% CO_2 and maintained at $34 \pm 0.5^\circ\text{C}$ (pH = 7.4). The preparations were initially driven at 1 Hz and equilibrated for at least 1 h while a stable impalement was obtained. Driving stimuli were rectangular pulses (2 ms in duration and twice diastolic threshold) delivered via a multipurpose programmable stimulator (Cibertec-CS220).

Transmembrane action potentials were conventionally recorded through glass microelectrodes filled with 3 M KCl (tip resistance of 8–15 M Ω). The microelectrode was connected via Ag-AgCl wire to high-input impedance capacity neutralizing amplifiers (WPI model 701). The maximum upstroke velocity ($V_{\max}$) of the action potential was obtained by electronic differentiation (Valenzuela *et al.*, 1988; Delpón *et al.*, 1989). The differentiator used had an upper limit of linearity of 1000 V s^{-1} and variable input filters (3 Hz–260 kHz). The suitable frequency filter for minimizing noise without reducing the $V_{\max}$ was selected for each individual experiment. Another distortion of $V_{\max}$ can arise from the deformation of the foot of

the rising phase by the electrical stimulus. In order to diminish this artefact, stimulus intensity and duration were adjusted throughout each experiment to maintain a constant latency (1–2 ms) from the stimulus artefact to the initiation of the upstroke of the action potential (Valenzuela *et al.*, 1989). Both action potential and $V_{\max}$ were displayed on a storage oscilloscope (Tektronix 5104N) and photographed with a kymograph Grass camera (Model C4).

To study the influence of stimulation frequency on the depressant effects of mexiletine and flecainide on $V_{\max}$ block, papillary muscles were stimulated after a rest period of 3 min with trains of pulses of 40 s in duration at 0.5, 1 and 2 Hz. The preparations were then exposed to various solutions in the following sequence: (1) control Tyrode solution; (2) solution with mexiletine alone (30 min); (3) control solution (50 min); (4) solution with flecainide (30 min); (5) solution with mexiletine plus flecainide (30 min). The concentrations of mexiletine and flecainide tested were 10^{-5} M and 10^{-6} M, respectively, and were chosen because in previous studies they had been used to study onset and recovery kinetics (Courtney, 1981; Campbell & Vaughan Williams, 1983; Campbell, 1983a,b). In this way we were able to compare the individual effects of each drug alone with those induced by the combination. This experimental protocol demonstrated that both mexiletine and flecainide produced two types of $V_{\max}$ inhibition, a tonic and a frequency-dependent block. The tonic block was defined as the decrease of $V_{\max}$ of the first action potential preceded by a rest period, while the frequency-dependent block was defined by the decrease of the $V_{\max}$ observed during a train of stimuli.

Recovery of $V_{\max}$ from the frequency-dependent block was studied by applying a single test stimulus at various coupling intervals after a stimulation train for 7 s at 2 Hz (Valenzuela *et al.*, 1988; Delpón *et al.*, 1989). The intensity and duration of the test and conditioning stimuli were adjusted to obtain a constant latency from the stimulus artefact to the initiation of the upstroke of the action potential. The effective refractory period (ERP) was measured by introducing premature test-stimuli of twice threshold strength at different intervals from the preceding basic action potential (Tamargo, 1980). In another group of experiments the first 1000 ms of the recovery process of $V_{\max}$ were analyzed in muscles driven at a basic rate of 1 Hz by introducing a test stimulus (S_2) every 10th basic pulse (S_1). The test interval was defined as the interval between 90% repolarization of the conditioning action potential and the onset of the test action potential (Sánchez-Capula, 1985; Delpón *et al.*, 1990).

All experimental results were obtained from a single continuous impalement throughout the whole experiment.

Drugs

Flecainide hydrochloride (Laboratorios Dr. Esteve S.A.) and mexiletine (Boehringer Ingelheim S.A.) as a powder were initially dissolved in distilled deionized water. Further dilutions were carried out in Tyrode solution to obtain the desired concentrations.

Throughout the paper data are given as means $\pm$ s.e.mean and Student's *t* test was used to estimate the significance of

differences from control values. For statistical comparison of more than two groups, a one-way analysis of variance was performed (Wallenstein *et al.*, 1980). A *P* value of less than 0.05 was considered as significant. More details on each procedure are given under Results.

Results

Effects on transmembrane action potentials

The electrophysiological effects of mexiletine alone (10^{-5} M), flecainide alone (10^{-6} M) and a combination of mexiletine plus flecainide were studied in 10 guinea-pig papillary muscles driven at the basal rate of 0.02 Hz. As is shown in Table 1 neither mexiletine nor flecainide alone nor the combination of both drugs had an effect on the resting membrane potential, action potential amplitude or the $V_{\max}$ values. Also the action potential duration measured at 50% and 90% of repolarization remained unchanged. However, whereas flecainide alone did not modify the ERP/APD₉₀ ratio, mexiletine significantly increased this parameter ($P < 0.01$), and this enhancement of ERP/APD₉₀ ratio was potentiated when both drugs were present.

Frequency-dependent blockade of $V_{\max}$

Flecainide alone (10^{-6} M), mexiletine (10^{-5} M) or the combination of both drugs did not decrease the $V_{\max}$ of the first action potential preceded by a rest period and thus, the tonic block averaged $1.3 \pm 0.5\%$, $1.7 \pm 3.5\%$ and $3.4 \pm 1.2\%$, respectively. These results suggested that flecainide and mexiletine exhibited a low affinity for the rested state of the Na⁺ channel. The influence of stimulation frequency on the depressant effect of mexiletine and flecainide alone and the combination of mexiletine plus flecainide on $V_{\max}$ was studied in papillary muscles driven by trains of stimuli at 0.5, 1 and 2 Hz for 40 s, separated from one another by a rest period of 3 min. As is shown in Table 2, under control conditions the $V_{\max}$ values were reduced by $1.7 \pm 0.7\%$, $5.4 \pm 2.8\%$ and $7.0 \pm 1.4\%$ during stimulation trains at a rate of 0.5, 1 and 2 Hz, respectively. Mexiletine alone did not induce a significant frequency-dependent $V_{\max}$ block when trains of action potentials at 0.5 Hz or 1 Hz were applied, whereas at 2 Hz, mexiletine induced a frequency-dependent $V_{\max}$ block of $19.8 \pm 2.4\%$ ($P < 0.01$). As is shown in Table 2 and Figure 1, the frequency-dependent $V_{\max}$ block induced by flecainide was of the same order of magnitude as that induced by mexiletine when trains of action potentials at a rate of 2 Hz were applied ($18.0 \pm 2.2\%$, $P < 0.01$). In contrast, when trains of action potentials at a rate of 0.5 and 1 Hz were applied, flecainide induced a significant frequency-dependent $V_{\max}$ block and the steady-state $V_{\max}$ values were reduced by $5.4 \pm 0.8\%$ ($P < 0.01$) and $10.4 \pm 1.4\%$ ($P < 0.01$) respectively. When both mexiletine and flecainide were perfused, the frequency-dependent $V_{\max}$ block observed at 1 and 2 Hz was significantly

Table 1 Electrophysiological effects of mexiletine (10^{-5} M), flecainide (10^{-6} M) and the combination of both drugs in guinea-pig papillary muscles ($n = 10$)

	RMP (mV)	$V_{\max}$ (V s ⁻¹)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)	ERP/APD ₉₀
Control	85.7 $\pm$ 1.5	202.5 $\pm$ 5.9	124.0 $\pm$ 0.6	211.1 $\pm$ 13.9	240.3 $\pm$ 14.4	1.02 $\pm$ 0.005
Mexiletine	85.1 $\pm$ 2.0	197.7 $\pm$ 4.0	123.7 $\pm$ 0.7	214.7 $\pm$ 13.6	245.8 $\pm$ 6.8	1.04 $\pm$ 0.005*
Control	85.1 $\pm$ 1.9	200.0 $\pm$ 3.1	123.8 $\pm$ 0.6	213.6 $\pm$ 13.7	243.4 $\pm$ 8.1	1.02 $\pm$ 0.006
Flecainide	83.8 $\pm$ 1.7	200.0 $\pm$ 2.9	123.8 $\pm$ 0.5	224.6 $\pm$ 17.1	256.4 $\pm$ 17.1	1.02 $\pm$ 0.006
Flecainide + mexiletine	84.5 $\pm$ 1.4	196.1 $\pm$ 2.9	123.4 $\pm$ 0.3	213.9 $\pm$ 35.4	248.6 $\pm$ 33.9	1.06 $\pm$ 0.004**

Values are mean $\pm$ s.e.mean ($n = 10$). * $P < 0.05$; ** $P < 0.001$.
APD: action potential duration. ERP: effective refractory period.

Table 2 Effects of mexiletine (10^{-5} M) alone, flecainide (10^{-6} M) alone and the combination of both drugs on the tonic and frequency-dependent $V_{\max}$ block in guinea-pig papillary muscles

	Tonic block (%)	Frequency-dependent block (%)		
		0.5 Hz	1 Hz	2 Hz
Control	0	1.7 ± 0.7	5.4 ± 2.8	7.0 ± 1.4
Mexiletine	1.3 ± 0.5	4.7 ± 1.0	9.1 ± 1.7	$19.8 \pm 2.4^*$
Flecainide	1.7 ± 3.5	$5.4 \pm 0.8^*$	$10.4 \pm 1.4^*$	$18.0 \pm 2.2^*$
Flecainide + mexiletine	3.4 ± 1.2	$6.7 \pm 0.7^*$	$14.7 \pm 1.3^{**}$	$27.3 \pm 1.4^{**}$

Values are mean $\pm$ s.e.mean ($n = 10$). * $P < 0.01$; ** $P < 0.001$.

more pronounced ($14.7 \pm 1.3\%$ and $27.3 \pm 1.4\%$, $P < 0.01$) than that induced by flecainide alone.

Figure 2 shows the quantitative analysis of the frequency-dependent $V_{\max}$ block produced by flecainide alone and flecainide plus mexiletine as a function of the interstimulus interval. The relationship fits well (correlation coefficients being 0.985 and 0.986, respectively) into the equation $y = ax^{-b}$, where y is the % strength of frequency-dependent $V_{\max}$ block, a is a constant (dimension per time unit), x the interstimulus interval (s) and b a dimensionless constant (Kohlhardt & Seifert, 1985). This kind of relationship has been described previously for other antiarrhythmic drugs (Kohlhardt & Seifert, 1985; Valenzuela *et al.*, 1988). The a parameter calculated from the experimental values was augmented from 24.8 in the presence of flecainide alone to 41.2 when both drugs were present, which indicated that the $V_{\max}$ block induced by flecainide plus mexiletine will be more pronounced at higher frequencies.

The onset kinetics of the frequency-dependent $V_{\max}$ block can be defined in terms of an event-dependent process, that

can be well-fitted by a single exponential curve (Courtney, 1980; Campbell, 1983a,b; Grant *et al.*, 1984). The onset rate constant per action potential [$K(AP^{-1})$] at which $V_{\max}$ decreased to a new steady-state level was calculated from the regression lines in semilogarithmic plots. As is shown in Table 3, the onset rate constant of $V_{\max}$ block was dependent on the stimulation frequency, being always higher at lower stimulation frequencies. In muscles driven at 0.5–2.0 Hz the onset kinetics of flecainide were fitted by a single exponential curve ($K = 0.05 \pm 0.004 AP^{-1}$ at 2 Hz, $n = 10$). At 0.5 and 1 Hz the onset kinetics of mexiletine was fitted by a single exponential curve, whereas at 2 Hz this process was better described by a biexponential curve (see Campbell, 1983b), the K values of the

Figure 1 Frequency-dependent $V_{\max}$ block obtained under control conditions (●), in the presence of mexiletine alone (○, 10^{-5} M), flecainide alone (△, 10^{-6} M) and a combination of mexiletine and flecainide (▲) during stimulation trains at a frequency of 2 Hz after a rest period of 3 min. The percentage of $V_{\max}$ normalized by the value of the first action potential of the train [V_t/V_c (%), $V_t = V_{\max}$ of the action potentials of the train, $V_c = V_{\max}$ of the first action potential of the train] was plotted against the number of consecutive beats of the train.

Figure 2 Dependence of frequency-dependent $V_{\max}$ block prior to (●) and after (○) mexiletine admixture to the flecainide-containing perfusate on the interstimulus interval. The curves have been drawn from $y = 24.8x^{-0.45}$ ($r^2 = 0.985$) in the presence of flecainide alone and from $y = 41.2x^{-0.39}$ ($r^2 = 0.986$) in the presence of flecainide plus mexiletine.

Table 3 Rate constants of development of frequency-dependent $V_{\max}$ block (K) in the presence of mexiletine alone (10^{-5} M), flecainide alone (10^{-6} M) and a combination of both drugs

	$K (AP^{-1})$		
	0.5	1	2 (Hz)
Mexiletine	0.19 ± 0.02	0.17 ± 0.03	2.04 ± 0.30
	—	—	0.12 ± 0.01
Flecainide	0.14 ± 0.01	0.07 ± 0.005	0.05 ± 0.004
Flecainide + mexiletine	0.14 ± 0.01	0.10 ± 0.01	1.19 ± 0.20
	—	—	0.07 ± 0.20

Values are mean $\pm$ s.e.mean ($n = 10$).

fast and slow components being $2.04 \pm 0.30 \text{ AP}^{-1}$ and $0.12 \pm 0.01 \text{ AP}^{-1}$, respectively. In the presence of mexiletine plus flecainide the onset kinetics at 0.5 and 1 Hz was also a monoexponential process, while at 2 Hz was better fitted by a biexponential curve, the K values being $1.19 \pm 0.30 \text{ AP}^{-1}$ and $0.07 \pm 0.02 \text{ AP}^{-1}$, respectively. These results indicated that when compared to flecainide alone the combination of mexiletine plus flecainide increased the development kinetics of frequency-dependent $V_{\max}$ block.

Effect on recovery process of $V_{\max}$

The effects of mexiletine alone, flecainide alone and a combination of both drugs were also studied on the recovery kinetics of frequency-dependent $V_{\max}$ block. In this group of experiments, muscles were driven every 3 min by a train of stimuli at a frequency of 2 Hz for 7 s and a test-stimuli was applied at variable coupling intervals.

In 7 papillary muscles the recovery process of $V_{\max}$ exhibits a time constant (τ_{re}) which averaged $24.5 \pm 2.4 \text{ ms}$. In the presence of mexiletine alone the τ_{re} increased to $314.8 \pm 51.6 \text{ ms}$, whereas flecainide alone (Figure 3) induced the slow component of recovery of $V_{\max}$ with a τ_{re} of $16.4 \pm 2.3 \text{ s}$. The y-intercept of this slow phase of recovery can be taken as the fraction of Na^+ channels blocked by flecainide, which rose to a value of $93.8 \pm 1.5\%$. Figure 3 also shows that when both flecainide and mexiletine were present, two components in the reactivation process can be observed, a fast initial component followed by a second slow component of similar kinetics to that observed in the presence of flecainide alone ($\tau_{re} = 16.7 \pm 0.7 \text{ s}$). However, in the presence of the combination, the fraction of sodium channels blocked (y-intercept of the slow component) significantly decreased to $68.9 \pm 1.7\%$ ($P < 0.001$). This result suggests that mexiletine decreased the fraction of sodium channels blocked by flecainide without changing the time constant of the recovery process.

In order to study the fast component of the recovery process, another experimental procedure was carried out. Preparations were driven at a basal rate of 1 Hz, and a test stimuli (S_2) was interpolated at different time intervals every 10th basic pulse (S_1). Figure 4 shows a typical experiment. Under control conditions the recovery process was completed within 30 ms. Mexiletine depressed the $V_{\max}$ of early test action potentials more than flecainide and lengthened the τ_{re} to

Figure 3 Effects of flecainide ($\circ$, 10^{-6} M) and flecainide plus mexiletine ($\blacktriangle$, 10^{-5} M) on the recovery process of $V_{\max}$ block (mexiletine data were omitted for clarity) after trains of action potentials at 2 Hz. $\Delta T = V_{\max}$ of the first action potential of the train minus $V_{\max}$ of the test response; $\Delta X = V_{\max}$ of the first action potential minus steady-state value of $V_{\max}$ reached during the train; t = interval between the last action potential of the train and the test response. First-order regressions of the slow component of recovery were fitted by the method of least squares.

Figure 4 First 1000 ms of the time course of recovery of $V_{\max}$. Data are plotted as percentage of the maximal value of $V_{\max}$ under control conditions. The recovery of $V_{\max}$ was determined in muscles driven at 1 Hz by applying test stimuli (S_2) every 10 basic stimuli (S_1). ($\bullet$) Controls; ($\circ$) mexiletine, 10^{-5} M ; (Δ) flecainide, 10^{-6} M ; ($\blacktriangle$) flecainide plus mexiletine.

206 ms. A further $V_{\max}$ inhibition of early test action potentials was observed with mexiletine plus flecainide, a finding which agrees with the results shown in Figure 2 where the combination increased the $V_{\max}$ block at frequencies of stimulation faster than 0.5 Hz. In the presence of flecainide alone or flecainide plus mexiletine the recovery process of $V_{\max}$ block exhibits two components, a fast initial followed by a slow component. In the presence of flecainide alone, the kinetics of the fast component were similar to those obtained under control conditions ($\tau_{re} = 36 \text{ ms}$), whereas in the presence of the combination this initial component was almost similar to that obtained in the presence of mexiletine alone ($\tau_{re} = 243 \text{ ms}$). Both in the presence of flecainide or of the combination this initial component was followed by the late slow component described in Figure 3.

Discussion

In the present paper the possible interactions between two antiarrhythmic drugs, mexiletine (class Ib) and flecainide (class Ic), in depressing the $V_{\max}$ of ventricular action potentials were studied in guinea-pig papillary muscles. The results obtained suggest that at high frequencies of stimulation this combination increased the frequency-dependent $V_{\max}$ block without modifying the time course of the slow component of reactivation produced by flecainide alone. However, the magnitude of the slow component of the reactivation of $V_{\max}$ block (y-intercept) in the presence of the combination of both drugs was less than that obtained in the presence of flecainide alone.

In this study the $V_{\max}$ values were used as an indirect estimative index of the magnitude of the fast inward Na^+ current (I_{Na}). Thus, even when $V_{\max}$ is a monotonic but not a linear index of the peak I_{Na} (Sheets *et al.*, 1988), there is little doubt that $V_{\max}$ is mainly generated by this current. Whether or not possible non-linearities between $V_{\max}$ and g_{Na} affect the present results remains to be seen in reliable voltage clamp experiments under the same conditions of temperature and external Na concentrations as used in the present experiments.

Class I antiarrhythmic drugs are characterized by their ability to depress the $V_{\max}$ of the cardiac action potentials as a consequence of interaction of these drugs with Na^+ channels. On the basis of the modulated receptor hypothesis (Hondegheem & Katzung, 1977; 1984; Hondegheem, 1987; Tamargo *et al.*, 1989) class I antiarrhythmics bind to a receptor site located within or functionally associated with the Na^+ channel. In this model, the affinity of the receptor for the drugs is modulated by the state of the channel (resting-R, activated-A, inactivated-I). In addition, cardiac Na^+ channels also exhibited slow inactivation (SI) and ultra slow inac-

tivation (USI) (Saikawa & Carmeliet, 1982; Clarkson *et al.*, 1984). When these states are drug-associated they are termed RD, AD, ID, SID and USID, respectively.

The results obtained in the presence of individual drugs are in line with those previously described elsewhere (Courtney, 1981; Hohnloser *et al.*, 1982; Campbell, 1983a,b; Hering *et al.*, 1983; Varro *et al.*, 1985; Valenzuela *et al.*, 1989; Valenzuela & Sánchez-Chapula, 1989). Therefore while mexiletine produced a fast development of and fast recovery from $V_{\max}$ block, flecainide caused a very slow development and a very slow recovery from block. In fact, in the presence of mexiletine the recovery of $V_{\max}$ was completed in less than 800 ms after a conditioning action potential. This explains why mexiletine only produced frequency-dependent $V_{\max}$ block when trains of stimuli at 2 Hz were applied, but not at 0.5 or 1 Hz, while flecainide-induced $V_{\max}$ block became evident during repetitive stimulation at 0.5, 1 and 2 Hz. This is due to the fact that in the presence of flecainide the recovery kinetics from $V_{\max}$ block is a very slow process ($\tau_{re} = 16.4 \pm 2.3$ s), the diastolic interval during the trains not being long enough to bring about a complete reactivation of $V_{\max}$. As a consequence, flecainide would produce an accumulation of blocked Na^+ channels during each successive action potential until a new steady-state is reached. Furthermore, the percentage of frequency-dependent $V_{\max}$ block produced by mexiletine plus flecainide significantly increased over that induced by flecainide alone as the rate of stimulation increased. Thus, while at 2 Hz the $V_{\max}$ block produced by the combination markedly increased over that induced by flecainide alone, this increment exponentially decreased at slower rates of stimulation, so that at 0.5 Hz both flecainide and the combination produced a similar amount of $V_{\max}$ block.

As can be predicted from the equation for the two-state model (Courtney, 1983) the onset rate of the frequency-dependent $V_{\max}$ block, expressed as a fractional decrease per action potential (K , AP^{-1}), was faster at slower stimulation frequencies at any drug concentrations tested. When 2 Hz-trains were applied, the development kinetics of $V_{\max}$ block induced by mexiletine consisted of a very fast initial process followed by a slower one ($K = 2.04 \pm 0.30$ AP^{-1} and 0.12 ± 0.01 AP^{-1} , respectively), whereas that induced by flecainide was a very slow process (0.05 ± 0.004 AP^{-1}). The development kinetics of flecainide plus mexiletine also exhibited two components ($K = 1.20 \pm 0.30$ AP^{-1} and 0.08 ± 0.02 AP^{-1} , respectively). The K values of the fast component were of similar magnitude both in the presence of mexiletine alone or in the presence of the combination, whereas the K values of the slow component obtained in the presence of the combination were similar to that obtained in the presence of flecainide alone. Thus, it can be concluded that the first component observed in the presence of the combination is probably due to the binding of mexiletine to the Na^+ channels, while the slow one reflects the access of flecainide to its receptor site.

Recently, it has been described (Anno & Hondeghem, 1989; 1990) that in the presence of flecainide the recovery process of I_{Na} block showed two different components: (1) a very fast initial component which results from unblocking associated with activation (AD – A), and (2) a very slow component attributed to the transition from RD and SID to the R and SI states of the Na^+ channels (RD – R; SID – SI). These two components were observed both in the presence of flecainide alone and of the combination flecainide plus mexiletine. Therefore, the first fast component of $V_{\max}$ reactivation observed in the presence of flecainide alone was probably due to the unblocking of flecainide bound activated channels, whereas the component observed in the presence of the combination of mexiletine plus flecainide is probably due to the

unblocking process of mexiletine. In fact, the time constant of this fast component was similar to that obtained in the presence of mexiletine alone. The interaction between mexiletine and flecainide could be understood in terms of the proposal of Clarkson & Hondeghem (1985), in which they adapted the modulated receptor hypothesis (Hondeghem & Katzung, 1977; 1984) to simulate the behaviour of $V_{\max}$ when two anti-arrhythmic drugs are combined. In these simulations the following assumptions were made: (1) in the absence of drugs, Na^+ channels can exist in three primary states (R, A, I); (2) each drug competes for a single common receptor associated with the Na^+ channel; and (3) drug-associated channels do not conduct and have the inactivation parameters offset by two different voltage shifts. Therefore, in the presence of the combination of two drugs, each one could compete for a limited number of receptor sites during each action potential. If both drugs have similar affinities for the different states of the Na^+ channel as well as similar rate constants of binding and dissociation, then the competition between them will be of little electrophysiological consequence. In contrast, when both drugs exhibit substantially different characteristics, as in the case of mexiletine and flecainide, then the competitive interaction becomes quite noticeable at least at certain driving rates. In fact, at stimulation frequencies higher than 0.5 Hz the $V_{\max}$ block induced by mexiletine plus flecainide increased over that induced by flecainide alone; this combination can be considered as synergistic according to what is predicted by the model of Clarkson & Hondeghem (1985).

It seems that there is an apparent contradiction in these results since the magnitude of the slow component of $V_{\max}$ reactivation was significantly less in the presence of the combination than in the presence of flecainide alone even when the time constant of this slow component was similar in both conditions. However, because mexiletine binds and unbinds faster from the Na^+ channels, it may decrease the number of channels available to be blocked by a drug with much slower binding kinetics as flecainide. Therefore, the slow component of reactivation, which represents the unblocking process of flecainide-bound channels, would be lowered in the presence of the combination. The synergistic effect observed at rapid frequencies of stimulation in the presence of the combination can also be explained by the additive effect of the slow binding kinetics of flecainide to the population of Na^+ channels available; that is, those which were not previously blocked by mexiletine.

Mexiletine and flecainide have been found effective in treating or preventing ventricular arrhythmias (Sandoe *et al.*, 1978; Anderson *et al.*, 1981; Duff *et al.*, 1981; Hodges & Heel, 1985; Somberg & Tepper, 1986; Podrid, 1986; Campbell, 1987). In the present study mexiletine produced a more marked depression of the $V_{\max}$ of early action potentials than flecainide alone, while the combination of both drugs produced more depression of early test action potentials than mexiletine alone. These results explain why the ERP/APD₉₀ ratio significantly increased in the presence of mexiletine or the combination, but not in the presence of flecainide alone. Thus, this combination could be effective in preventing and/or treating ventricular tachyarrhythmias resistant to either drug alone. On the other hand, the present results also demonstrated that at frequencies higher than 0.5 Hz the combination of mexiletine plus flecainide produced a greater depression of $V_{\max}$ of the ventricular action potentials, thus suggesting that this combination may be accompanied by a higher depression of intraventricular conduction velocity and a higher incidence of proarrhythmic effects.

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