Electrophysiological effects of CRE-1087 in guinea-pig ventricular muscles

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- 1 The electrophysiological effects of CRE-1087, a new antiarrhythmic drug, were studied in guinea-pig papillary muscles.
- 2 CRE-1087 (10^{-7} M– 10^{-4} M) produced a concentration-dependent decrease in the action potential amplitude and $V_{\max}$ of the upstroke without altering the action potential duration or the resting membrane potential.
- 3 At 5×10^{-6} M, CRE-1087 produced a $5.0 \pm 1.0\%$ tonic $V_{\max}$ block and this value was not modified at the different rates of stimulation tested. In the presence of CRE-1087, trains of stimuli at rates between 0.5 and 3 Hz led to an exponential decline in $V_{\max}$ (frequency-dependent $V_{\max}$ block) which augmented at higher rates of stimulation. At 2 Hz the onset kinetics of the frequency-dependent $V_{\max}$ block was fitted by a monoexponential function being the K value 0.099 ± 0.012 AP $^{-1}$. The time constant for the recovery of $V_{\max}$ from the frequency-dependent block was prolonged to 18.3 ± 1.5 s.
- 4 CRE-1087 (5×10^{-6} M) did not shift the membrane responsiveness curve.
- 5 CRE-1087 had no effect on the characteristics of the slow action potentials elicited by isoprenaline in ventricular fibres depolarized by 27 mM KCl, which suggested that CRE-1087 did not exhibit class IV (Ca antagonist) antiarrhythmic actions.
- 6 The slow onset and the slow offset kinetics of frequency-dependent $V_{\max}$ block during repetitive activity suggested that in guinea-pig ventricular muscle fibres CRE-1087 exhibited class Ic antiarrhythmic actions.

Keywords: CRE-1087; ventricular muscle; action potential; use-dependent $V_{\max}$ block; antiarrhythmics

Introduction

CRE-1087, 1-(2-methoxyphenoxy)-2,3-bis (2-diethylaminoethoxy) propane dicitrate, is a new antiarrhythmic drug which exhibits local anaesthetic properties (Priego *et al.*, 1984; Statkow *et al.*, 1985; Delpón *et al.*, 1991a). In preliminary experiments it has been shown that CRE-1087 is effective in suppressing cardiac arrhythmias induced by aconitine, digitalis and chloroform-adrenaline in dogs, cats and rats (Statkow *et al.*, 1985). However, CRE-1087 had no effect on the positive inotropic and chronotropic responses induced by isoprenaline in guinea-pig isolated atria (Delpón *et al.*, 1991a) which suggests that it did not exhibit class II (antisympathetic) antiarrhythmic actions. Moreover, preliminary microelectrode studies in guinea-pig ventricular muscle fibres indicated that CRE-1087 decreases the maximal rate of depolarization ($V_{\max}$) of the action potential, suggesting a class I mechanism of action, i.e., fast Na channel block (Delpón *et al.*, 1991a).

Thus the present experiments were undertaken to: (a) characterize the cellular electrophysiological effects of CRE-1087 in guinea-pig ventricular muscle fibres and (b) determine its effects on the onset and recovery kinetics of the sodium channel as reflected by its effects on $V_{\max}$. Moreover, since the prevailing classification of antiarrhythmic drugs is based on changes in action potential duration, refractoriness and onset and offset kinetics of the frequency-dependent block of fast sodium channels (Vaughan Williams, 1984), the present experiments were also carried out in order to (c) place this drug in the appropriate class I subgroup (i.e., class Ia, Ib, Ic).

Methods

General procedure

Guinea-pigs of either sex weighing 250–350 g were killed by a blow on the head. The hearts were rapidly removed and brought into a dissection chamber where papillary muscles (2–3 mm in length, 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 $_2$ PO $_4$ 0.42 and glucose 5.5. The solutions were bubbled with 95% O $_2$ and 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 a period of 1 h was allowed for equilibration during which a stable impalement was obtained. Driving stimuli were rectangular pulses (2 ms duration, twice threshold strength) delivered to the preparation from a multipurpose programmable stimulator (Cibertec CS-220, Cibertec S.A., Madrid, Spain). Electrical stimulation was applied to the surface of the preparation through Teflon-coated bipolar electrodes of silver wire.

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 impedance, capacity neutralizing amplifiers (WPI model 701, New Haven, CT, U.S.A.). The action potential was differentiated electronically in order to measure the maximal rate of depolarization ($V_{\max}$) (Valenzuela *et al.*, 1988; 1989; Delpón *et al.*, 1989; 1990). The differentiator used had an upper limit of linearity of 1000 V s $^{-1}$ and variable input filters (3 Hz–260 kHz, E. Ehler, Homburg/Saar, Germany). The suitable frequency filter for minimizing noise without reducing the $V_{\max}$ was selected in

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each experiment. Another distortion of $V_{\max}$ can arise from the formation of the foot of the rising phase of the electrical stimulus. This phenomenon is accompanied by a reduced interval between stimulus artifact and the upstroke of the action potential particularly when strong electrical stimuli are applied. In order to diminish this artifact, stimulus intensity and duration were adjusted throughout each experiment to maintain a constant latency (1–2 ms) between stimulus and upstroke of the action potential (Valenzuela *et al.*, 1988; 1989). Both action potential and $V_{\max}$ were displayed on a storage oscilloscope (Tektronix Inc., Beaverton, OR, U.S.A.) and the oscilloscope traces were photographed with a kymographic camera (Grass C4, Grass Instrument Co., Quincy, MA, U.S.A.). All experimental results were obtained from a single continuous impalement through the whole experiment.

To study the rate-dependent effects of CRE-1087 on $V_{\max}$, preparations were driven at a basal rate of 0.02 Hz. Following the equilibration period, the preparations were driven by trains of stimuli at various rates ranging from 0.5 to 3 Hz for 40 s (0.5, 1 and 2 Hz) and 16 s (3 Hz). Rest periods of 5 min, which were sufficient to ensure full recovery from frequency-dependent decrease in $V_{\max}$, were interpolated between the trains of stimuli (Valenzuela *et al.*, 1988; Delpón *et al.*, 1989; 1990). This experimental protocol was repeated in the absence and presence of CRE-1087 and indicated that CRE-1087 at any given rate produced two types of $V_{\max}$ inhibition, a tonic and a frequency-dependent blockade. Tonic blockade is the decrease of $V_{\max}$ of the first action potential preceded by the rest period, whereas frequency-dependent blockade is the decrease of $V_{\max}$ during a train from the value of the first action potential to a new steady-state.

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 8 s at 3 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 artifact 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 (Delpón *et al.*, 1989).

Effects of CRE-1087 on the relationship between $V_{\max}$ and the resting membrane potential, i.e., membrane responsiveness, was studied in papillary muscles driven at 0.02 Hz and the resting membrane potential was depolarized by increasing the $[K]_o$ from 2.7 to 15 mM (Delpón *et al.*, 1990). The $V_{\max}$ was measured each time after an equilibration period of 8 min. Curves were obtained in the absence and after 30 min exposure to 5×10^{-6} M CRE-1087.

To study the effects of CRE-1087 on slow action potentials, papillary muscles were equilibrated in Tyrode solution and then rendered inexcitable by depolarizing the membrane potential with high (27 mM) K Tyrode solution (Delpón *et al.*, 1989). Under these conditions papillary muscle became inexcitable despite intense stimulation. Excitability was restored, i.e. slow action potentials, in muscles driven at a

basal rate of 0.1 Hz by addition of isoprenaline (10^{-6} M) to the bathing medium.

Drugs

The following drugs were used: CRE-1087 (Cermol S.A., Geneve, Switzerland) and isoprenaline hydrochloride (Sigma Chemical Co., London). CRE-1087 as a powder was dissolved in distilled deionized water. Further dilutions were carried out in Tyrode solution to obtain final concentrations between 10^{-7} M and 10^{-4} M.

Throughout the paper data are expressed as the mean $\pm$ s.e.mean and paired 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 of CRE-1087 on transmembrane action potentials

The effects of a wide range of concentrations of CRE-1087 (10^{-7} M to 10^{-4} M) were studied on 9 papillary muscles. The onset of action of CRE-1087 was approximately 5 min after beginning the perfusion and reached steady-state values within 30 min. Therefore, results obtained under control conditions and 30 min after each increment in drug concentration are shown in Table 1. In ventricular muscle fibres CRE-1087, at concentrations higher than 10^{-7} M produced a significant decrease ($P < 0.01$) in $V_{\max}$, which at concentrations $\geq 5 \times 10^{-5}$ M was accompanied by a significant decrease in action potential amplitude ($P < 0.01$). However, at all concentrations tested, CRE-1087 had no effect on either the resting membrane potential or the action potential duration at both 50% (APD₅₀) and 90% (APD₉₀) of repolarization.

In 6 papillary muscles the ventricular ERP averaged 199.3 ± 8.5 ms. CRE-1087, 10^{-7} M– 10^{-4} M, produced a dose-dependent lengthening of the ERP. Since the lengthening of the ERP was greater than that observed in the APD₉₀ values, CRE-1087 also increased the ERP/APD₉₀ ratio at 5×10^{-5} M and 10^{-4} M from 1.03 ± 0.02 to 1.12 ± 0.03 ($P < 0.05$) and 1.20 ± 0.02 ($P < 0.01$), respectively.

Frequency-dependent effects of CRE-1087

The effectiveness of many class I antiarrhythmic drugs in inhibiting the fast inward sodium current (I_{Na}) is strongly dependent on the frequency of stimulation. Thus, the influence of stimulation frequency on the depressant effect of CRE-1087 on $V_{\max}$ was studied in papillary muscles by applying trains of pulses at different rates (0.5, 1, 2 and 3 Hz), separated from one another by a rest period of 5 min.

Table 1 Electrophysiological effects of CRE-1087 on transmembrane action potentials in guinea-pig ventricular muscles driven at 1 Hz

Drug concentration (M)	RMP (mV)	APA (mV)	$V_{\max}$ (V s ⁻¹)	APD ₅₀ (ms)	APD ₉₀ (ms)
0	81 $\pm$ 2	122 $\pm$ 2	218 $\pm$ 7	156 $\pm$ 6	197 $\pm$ 8
10 ⁻⁷	81 $\pm$ 2	123 $\pm$ 2	218 $\pm$ 7	167 $\pm$ 9	203 $\pm$ 5
10 ⁻⁶	81 $\pm$ 2	122 $\pm$ 1	166 $\pm$ 9 ^b	160 $\pm$ 5	197 $\pm$ 2
10 ⁻⁵	81 $\pm$ 2	120 $\pm$ 1	137 $\pm$ 9 ^b	172 $\pm$ 7	210 $\pm$ 7
5 $\times$ 10 ⁻⁵	81 $\pm$ 2	114 $\pm$ 2	58 $\pm$ 8 ^c	175 $\pm$ 9	211 $\pm$ 12
10 ⁻⁴	81 $\pm$ 2	110 $\pm$ 1 ^b	40 $\pm$ 4 ^c	178 $\pm$ 7	220 $\pm$ 11

$x \pm$ s.e.mean, *n* = 9

^a*P* < 0.05; ^b*P* < 0.01; ^c*P* < 0.001.

In the presence of CRE-1087 this experimental protocol demonstrated the existence of two types of $V_{\max}$ inhibition, i.e. tonic and frequency-dependent $V_{\max}$ block. In the experiment shown in Figure 1, following perfusion with 5×10^{-6} M CRE-1087 the $V_{\max}$ of the first action potential of each train preceded by a rest period was reduced from 200 V s^{-1} to 212 V s^{-1} , i.e. it produced a 3.6% tonic $V_{\max}$ block. In 5 experiments the degree of tonic $V_{\max}$ block induced by 5×10^{-6} M CRE-1087 averaged $5.0 \pm 1.0\%$ and this value was not modified at the different rates of stimulation tested. These data indicated that CRE-1087 exhibits a low affinity for the resting state of the sodium channel.

Following a train of pulses in the presence of CRE-1087, there was a gradual decrease of $V_{\max}$ from beat to beat until a new steady-state occurred (frequency-dependent $V_{\max}$ block), which depended on the stimulation frequency. Figure 1 shows a typical experiment in the presence of 5×10^{-6} M of CRE-1087. The percentage decrease of $V_{\max}$ from the first action potential of the train to a new steady-state level in the absence and presence of 5×10^{-6} M CRE-1087 at different stimulation frequencies (0.5–3 Hz) is summarized in Table 2. At all stimulation rates tested CRE-1087 significantly increased ($P < 0.001$) the frequency-dependent block. Moreover, it can be observed that the degree of frequency-dependent $V_{\max}$ block increased when the rate of stimulation increased from 0.5 to 2 Hz, while at 3 Hz the degree of frequency-dependent $V_{\max}$ block was similar to that observed at 2 Hz. This result suggests that at frequencies higher than 2 Hz the $V_{\max}$ block apparently achieved a steady-state value.

Onset kinetics of frequency-dependent $V_{\max}$ block can be defined either in terms of a time- or a rate-dependent process. In both cases, the decline of $V_{\max}$ during stimulation trains represents a first or second order process and can be well fitted by a single or biexponential function (Courtney, 1980; Grant *et al.*, 1984; Delpón *et al.*, 1991b). The time-constant (τ_{on} , s) and the onset rate 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 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

Table 2 Frequency-dependent $V_{\max}$ block induced by 5×10^{-6} M CRE-1087 in papillary muscles driven at different stimulation rates

Frequency of stimulation (Hz)	Frequency-dependent $V_{\max}$ block (%) Control (%)	Frequency-dependent $V_{\max}$ block (%) CRE-1087 (%)
0.5	0.5 ± 0.4	19.3 ± 3.3^a
1.0	2.9 ± 0.6	28.4 ± 4.9^a
2.0	11.3 ± 0.9	42.0 ± 4.0^a
3.0	18.7 ± 1.0	43.1 ± 2.0^a

$X \pm \text{s.e.mean}$, $n = 5$.

$^a P < 0.001$.

The percentage of frequency-dependent $V_{\max}$ block was calculated as indicated in Figure 1.

action potential (K), was dependent on the stimulation frequency, being always higher at lower stimulation frequencies at any drug concentration tested. This is shown in Table 3. Thus, at 0.5, 1 and 2 Hz, τ_{on} values progressively decreased in the presence of CRE-1087 from 9.4 ± 0.6 s to 5.2 ± 0.7 s, respectively. On the other hand, at frequencies of stimulation between 0.5 and 2 Hz the rate-constants (K) decreased from $0.216 \pm 0.013 \text{ AP}^{-1}$ to $0.099 \pm 0.012 \text{ AP}^{-1}$. However, at 3 Hz the onset kinetics of the frequency-dependent $V_{\max}$ block was better fitted by a biexponential function the K values of the fast and slow components being $0.393 \pm 0.08 \text{ AP}^{-1}$ and $0.070 \pm 0.02 \text{ AP}^{-1}$, respectively (Table 3).

Recovery kinetics of frequency-dependent $V_{\max}$ block

The effects of CRE-1087 on the recovery kinetics of frequency-dependent $V_{\max}$ block were also studied in 6 papillary muscles. In this group of experiments, muscles were driven every 3 min by a train of stimuli at a frequency of 3 Hz for 8 s and a test-stimulus was applied at variable coupling intervals from 500 ms to 36 s. Recovery from frequency-dependent $V_{\max}$ block occurred as a monoexponential process for which a single time constant (τ_{re}) can be estimated by regression analysis of the data. Under control conditions, the τ_{re} was 28.5 ± 3.2 ms whereas in the presence of 5×10^{-6} M CRE-1087 (Figure 2) the τ_{re} was prolonged to 18.3 ± 1.5 s ($P < 0.001$). The y-intercept of this slow phase of recovery can be taken as the fraction of sodium channels blocked by CRE-1087, which rose to a value of $85.4 \pm 2.0\%$. Thus, these results indicated that CRE-1087 must be considered as belonging to the kinetically slow group of class I antiarrhythmic drugs (Campbell, 1983a,b; Trump *et al.*, 1989).

Effect on membrane responsiveness

The relationship between $V_{\max}$ and the membrane resting potential from which the action potential arises, i.e. membrane responsiveness, was analyzed in 5 papillary muscles driven at a basal rate of 0.02 Hz. The resting membrane potential was depolarized stepwise to approximately -60

Figure 1 Onset of frequency-dependent depression of $V_{\max}$ induced by 5×10^{-6} M CRE-1087 in guinea-pig papillary muscles driven by trains of stimuli at various rates (0.5, 1, 2 and 3 Hz). Stimulation caused a frequency-dependent blockade which disappeared, whereas the tonic $V_{\max}$ block persisted. The tonic block (percentage) results from $[1 - V_{\max}(\text{first beat, drug})/V_{\max}(\text{rested, drug free})] \times 100\%$ and the frequency-dependent block (percentage) from $[1 - V_{\max}(\text{ss, drug})/V_{\max}(\text{first beat, drug})]$. $V_{\max}(\text{ss})$ is the steady-state value attained during continuous stimulation and $V_{\max}(\text{first beat})$ is the value of the first beat of each train of stimuli. (●) 0.5 Hz, (○) 1 Hz, (▲) 2 Hz, (△) 3 Hz.

Table 3 Effect of 5×10^{-6} M CRE-1087 on the onset kinetics of frequency-dependent $V_{\max}$ block

Frequency of stimulation (Hz)	τ_{on} (s)	Onset rate per AP (AP^{-1})
0.5	9.4 ± 0.6	0.216 ± 0.013
1.0	8.6 ± 0.5	0.117 ± 0.006
2.0	5.2 ± 0.7	0.099 ± 0.012
3.0	2.8 ± 0.5	0.393 ± 0.080
	12.2 ± 2.3	0.070 ± 0.020

$X \pm \text{s.e.mean}$, $n = 5$.

Figure 2 Effects of CRE-1087 (5×10^{-6} M) on the recovery process of $V_{\max}$ block after trains of action potentials at 3 Hz for 8 s. ΔT : $V_{\max}$ 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. Time = interval (s) between the last action potential of the train and the test response. First-order regressions of the slow component were fitted by the method of least squares.

mV by increasing the $[K]_o$ from 2.7 to 15 mM and the $V_{\max}$ value was measured when the resting membrane potential reached steady-state values at each $[K]_o$. Results of a typical experiment are shown in Figure 3. It can be observed that 5×10^{-6} M CRE-1087 did not shift the membrane-responsiveness curve.

Effect on the slow action potentials

The effects of CRE-1087 on the slow action potentials were studied in papillary muscles perfused with 27 mM K Tyrode solution and driven at a basal rate of 0.1 Hz. Under these conditions the resting membrane potential depolarized from -81.5 ± 2.4 mV to -45.7 ± 1.6 mV ($n = 6$) and ventricular fibres became inexcitable. Excitability, i.e. slow action potentials, was restored by adding isoprenaline (10^{-6} M) to the perfusate. CRE-1087, 10^{-4} M, had no effect on the amplitude (83.5 ± 2.1 mV vs 81.3 ± 2.4 mV), $V_{\max}$ (8.9 ± 1.2 V s $^{-1}$ vs 8.3 ± 1.1 V s $^{-1}$) and duration of the action potential measured at 90% of repolarization (356.0 ± 32.1 vs 336.5 ± 34.5 ms) or the resting membrane potential (-45.6 ± 1.4 mV vs -45.8 ± 1.2 mV).

Discussion

In this study the effects of CRE-1087, a new antiarrhythmic drug, were analyzed on the electrophysiological properties of guinea-pig ventricular muscle fibres. Also, the onset and recovery kinetics of frequency-dependent $V_{\max}$ block were studied in order to determine the subclassification of this compound. The present results indicated that in ventricular muscle fibres, CRE-1087 produced a concentration-dependent decrease in $V_{\max}$ values which occurred even at concentrations that had no effect on other action potential characteristics or resting membrane potential. Therefore, these findings suggested that the depressive effect on $V_{\max}$ is probably due to an inhibition of the I_{Na} , and the rate-dependent effect of the drug on $V_{\max}$ suggests that CRE-1087 has an effect compatible with that of class I antiarrhythmic drugs having slow kinetics (Ic).

In the present experiments the $V_{\max}$ values were used as an

Figure 3 Effect of CRE-1087 (5×10^{-6} M) on the relationship between $V_{\max}$ and the resting membrane potential at which the action potential is generated (i.e. membrane responsiveness). Ordinate scale: normalized $V_{\max}$ values (V s $^{-1}$). Abscissa scale: membrane potential (mV). Controls (●), CRE-1087 (○).

indirect index of the I_{Na} . Although there is no doubt that most of the ionic current crossing the cell membrane at the $V_{\max}$ time is the I_{Na} (Hondeghe, 1978; Sheets *et al.*, 1988), there is also evidence that the relationship between the Na conductance (G_{Na}) and the $V_{\max}$ values is a monotonic but not a linear index (Walton & Fozzard, 1979). Thus, the use of $V_{\max}$ can introduce a variable error in the quantitative measurements which can overestimate the I_{Na} block induced by the drug. 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 sodium concentration 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 sodium channels. The characteristics of the Na-channel block can be interpreted within the framework of the 'modulated receptor hypothesis' proposed by Hondeghe & Katzung (1977, 1984) to explain the interaction between local anaesthetic-type antiarrhythmic drugs with cardiac Na channels. According to this hypothesis, reduction of I_{Na} is due to the accumulation of drug-associated nonconducting channels (blocked channels). Accumulation results from the binding of drugs to a channel receptor that changes state or conformation as the channel cycles through the resting, activated and inactivated states (Hondeghe & Katzung, 1977; 1984; Hondeghe, 1987; Tamargo *et al.*, 1989). The affinity of the drug receptor differs in the three different receptor conformations. At therapeutic concentrations most class I antiarrhythmic drugs exhibit little or no tonic $V_{\max}$ block (Campbell, 1983a,b; Hondeghe & Katzung, 1984; Valenzuela *et al.*, 1988; 1989; Delpón *et al.*, 1989; 1990) and CRE-1087 was no exception. The fact that little resting block was found with normally polarized ventricular fibres suggests that CRE-1087 exhibits little affinity for the resting state of the sodium channel.

In contrast, during stimulation trains, sodium channels spend proportionately more time in the activated state during the upstroke and in the activated state during the plateau. If CRE-1087 has a higher affinity for the receptor of an activated or inactivated channel than for a resting channel receptor, an accumulation of the blocked channels during the

stimulation train leading to a use-dependent block of $V_{\max}$ would be expected. The decrease in $V_{\max}$ increased progressively when the preparations were stimulated at progressively increasing driving rates between 0.5 and 2 Hz and the onset rate of frequency-dependent block decreased as the stimulation rate was increased. At 2 Hz the onset rate value of the frequency-dependent $V_{\max}$ block was 0.099 AP^{-1} , a value which is similar to that of drugs which had been proposed as slow kinetics class I drugs by Campbell (1983a,b). The recovery from frequency-dependent $V_{\max}$ block (reactivation) induced by CRE-1087 was also a slow process. In fact, the value of τ_{re} found in this study is quite similar to that described with other class Ic antiarrhythmic drugs, like propafenone (Kohlhardt & Seifert, 1983; Valenzuela *et al.*, 1989) or flecainide (Campbell & Vaughan Williams, 1983; Delpón *et al.*, 1991b). Thus, CRE-1087 can be categorized as a slow kinetic class I drug, according to the classification of Vaughan Williams (1984), judging from the value of τ_{re} . The τ_{re} is one of the most reliable parameters for subdivision of the class I antiarrhythmic drugs because it is little affected by changes in drug concentration and stimulation frequencies (Courtney, 1980; Campbell, 1983a,b).

The relationship between $V_{\max}$ and the resting membrane potential was studied in muscles driven with an interstimulus interval of 50 s, which is three times longer than the τ_{re} value. Since this diastolic interval is much longer than the τ_{re} , under these conditions an inhibition of $V_{\max}$ may reflect only the tonic block induced by CRE-1087. In the present study CRE-1087 did not shift this relationship, thus suggesting that the drug exhibits little or no affinity for the inactivated state of the Na channel. On the other hand, it has been proposed that class I antiarrhythmic drugs with slow or intermediate onset of frequency-dependent $V_{\max}$ block (like CRE-1087) may have a significant affinity for the open-activated channel state only (Campbell, 1989; Snyders & Hondeghem, 1990), and that they also exhibit a long τ_{re} because the primary route for unblocking is associated with activation (Snyders & Hondeghem, 1990). In fact, in the present paper the degree of frequency-dependent $V_{\max}$ block induced by CRE-1087 at frequencies of stimulation higher than 2 Hz apparently achieved a steady-state value. This result can be explained because with this experimental approach, very high frequencies of stimulation not only increase the number of activations per time unit, but also, and more importantly, increase the percentage of Na channels in the inactivated state. Furthermore, the small voltage-shift induced by CRE-1087 suggests that at any potential, there will be more activation unblock than desired for optimal sequestration of depolarized tissue into the drug-bound inactivated state. Unfortunately our experiments do not allow us to estimate the relative contribution of block of activated vs. inactivated sodium channels.

Class Ic antiarrhythmic drugs characterized by a slow onset of and recovery from $V_{\max}$ block are often very effective in suppressing both early and late premature beats since they

will find a significant fraction of non-conducting sodium channels (Hondeghem & Katzung, 1977; 1984). Moreover, in the presence of tachyarrhythmias, sodium channels are in activated and/or inactivated states for a longer period and there is less time to recover from block between beats. Because in the presence of CRE-1087 the recovery kinetics from $V_{\max}$ block is a very slow process (τ_{re} about 18 s), the diastolic interval during the trains is not long enough to bring about a complete reactivation of $V_{\max}$. As a consequence, during a train of pulses this drug would produce an accumulation of blocked sodium channels during each successive action potential until a new steady-state is reached.

The present results indicate that CRE-1087 had no effect on ventricular action potential duration. This can be due to the fact that it had no effect and/or exhibited opposite effects on the ionic currents responsible for repolarization of ventricular muscle fibres, i.e. the slow inward Ca current, the outward repolarizing potassium current (I_K) and the late sodium current flowing during the plateau (Carmeliet & Vereecke, 1979; Kiyosue & Arita, 1989).

To ascertain whether CRE-1087 may inhibit the slow inward Ca current, its effects were analyzed on the slow action potentials elicited by isoprenaline in K-depolarized fibres. CRE-1087 had no effect on the electrophysiological characteristics of these slow action potentials, which suggests that it did not exhibit class IV activity in guinea-pig papillary muscles. Thus, even though in the present experiments a direct analysis of the outward K current (I_K) was not performed, it seems unlikely that CRE-1087 exerts a significant effect on this current.

The results of the Cardiac Arrhythmia Suppression Trial (CAST, 1989) demonstrated that antiarrhythmic drugs with class Ic actions (flecainide and encainide) produced an approximately threefold increase in arrhythmic deaths in patients with prior myocardial infarction and asymptomatic ventricular arrhythmias compared to their respective placebo group. Even though caution should be exercised in extrapolating the CAST data to other class I antiarrhythmics or for other patient groups, it seems that class Ic drugs, like CRE-1087, should be restricted to the treatment of cardiac arrhythmias in patients with no structural heart disease (Pratt *et al.*, 1990). In patients with structural heart disease the risk-benefit ratio is critical in clinical decision, particularly when the benefit of class Ic therapy may be low and the risk extremely high (Morganroth, 1992).

On the basis of the present results obtained in guinea-pig ventricular muscle fibres, it can be concluded that CRE-1087 is a class I antiarrhythmic agent which can be classified as a kinetically slow class Ic agent.

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