



# Electrophysiological effects of CI-980, a tubulin binding agent, on guinea-pig papillary muscles

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**1** The electrophysiological effects of CI-980, a new tubulin-binding agent that inhibits assembly of cytoplasmic microtubules, on transmembrane action potential characteristics were studied in right ventricular papillary muscles from guinea-pig hearts.

**2** In papillary muscles driven at 1 Hz, CI-980 at concentrations  $\geq 10^{-5}$  M produced a concentration-dependent increase in the maximum upstroke velocity ( $V_{\max}$ ) and a lengthening of the action potential duration at 50% (APD<sub>50</sub>) and 90% (APD<sub>90</sub>) of repolarization without affecting the resting membrane potential. Prolongation of the APD<sub>90</sub> was accompanied by a parallel lengthening of the effective refractory period (ERP) so that the ERP/APD<sub>90</sub> ratio remained unaltered at all drug concentrations tested.

**3** CI-980 exhibits a reverse use-dependent effect on APD<sub>90</sub> values, that is, drug-induced APD<sub>90</sub> prolongation become exaggerated at slow rates and attenuated at fast rates.

**4** CI-980 at concentrations  $\geq 10^{-6}$  M lengthened the APD of the slow action potentials elicited by isoprenaline in papillary muscles depolarized by high K<sup>+</sup> (27 mM) solution.

**5** At  $10^{-5}$  M, CI-980 produced a small tonic  $V_{\max}$  block. However, in muscles driven at rates between 0.5 and 3 Hz it produced an exponential decline in  $V_{\max}$  (use-dependent  $V_{\max}$  block) which was augmented at higher rates of stimulation. At 3 Hz the onset kinetics of the use-dependent  $V_{\max}$  block was fitted by a monoexponential function with a  $K$  value  $0.07 \pm 0.01$  per AP. The recovery time constant ( $\tau_{\text{re}}$ ) from the use-dependent  $V_{\max}$  block was prolonged from  $21.6 \pm 2.6$  ms to  $3.5 \pm 0.2$  s.

**6** The curve relating membrane potential and  $V_{\max}$  was shifted by CI-980 ( $10^{-5}$  M) in the hyperpolarizing direction by  $2.3 \pm 1.1$  mV.

**7** It is concluded that in guinea-pig papillary muscles, CI-980 produces a use-dependent inhibition of  $V_{\max}$  and a reverse use-dependent prolongation of the ventricular action potential, thus exhibiting class I and class III antiarrhythmic actions, respectively. From the onset and offset kinetics of use-dependent  $V_{\max}$  block, CI-980 can be considered to be an intermediate kinetics (IA) Na<sup>+</sup> channel blocker.

**Keywords:** Tubulin binding agents; CI-980; action potential; ventricular muscle; use-dependent block;  $V_{\max}$

## Introduction

Tubulin-binding agents that effectively depolymerize microtubules (i.e., colchicine, vinca alkaloids, podophylotoxin) stimulate the rate of spontaneously beating cardiac cells cultured from newborn hearts (Lampidis *et al.*, 1992). However, this effect is not exhibited by taxol, a tubulin-binding drug that does not depolymerize microtubules (Schiff *et al.*, 1979; Schiff & Horwitz, 1980; Lampidis *et al.*, 1992). Furthermore, all these agents, including taxol, reversed adriamycin-induced arrhythmias in this *in vitro* model, which indicates that their antiarrhythmic effect does not necessarily depend on their ability to depolymerize microtubules. The mechanisms responsible for their antiarrhythmic activity are not known, although it has been hypothesized that, since cardiac myocytes contain tubulin on their cell surface, tubulin-binding agents might act at this site, thus affecting the activity of membrane transport proteins such as ion channels (Cantiello *et al.*, 1991; Lampidis *et al.*, 1992). CI-980 (ethyl(S)-(5-amino-1,2-dihydro-2-methyl-3-phenopyrido[3,4-b]pyrazin-7-yl)carbamate 2-hydroxyethane sulphonate (1:1) is a new tubulin-binding agent that inhibits assembly of cytoplasmic microtubules at the colchicine site (Ines *et al.*, 1994). It exhibits potent antitumour activity against several murine leukemic multidrug-resistant sublines in culture and *in vivo* (Waud *et al.*, 1990; Ines *et al.*,

1994) and is presently being tested in clinical trials (Leopold *et al.*, 1993). In rat cultured ventricular myocytes, CI-980 increased the rate and the amplitude of both cell shortening and Ca<sup>2+</sup> transient, even when it decreased the spontaneous beating rate at high concentrations (Chevalier *et al.*, 1994). In dog Purkinje fibres, CI-980 decreased the amplitude, maximum upstroke velocity ( $V_{\max}$ ) and conduction velocity of the action potential without affecting resting membrane potential, effects which are consistent with an inhibitory effect on cardiac Na<sup>+</sup> channels (Chevalier *et al.*, 1994). Furthermore, it prolonged the action potential duration, reduced normal automaticity and suppressed the triggered activity associated with delayed afterdepolarizations (Chevalier *et al.*, 1994). On the basis of these results, Lampidis *et al.* (1992) and Chevalier *et al.* (1994) suggested that CI-980 represented a new and different antiarrhythmic strategy not included in the original classification of Vaughan Williams (1984). However, only limited information is available on the underlying mechanism for the antiarrhythmic activity of CI-980.

Therefore, the aim of this study was to characterize the electrophysiological effects of CI-980 on transmembrane action potentials recorded in guinea-pig ventricular muscles. The modulation of drug-induced inhibition of the maximum upstroke velocity ( $V_{\max}$ ) at different rates of stimulation and the membrane potentials were also studied in order to compare the characteristics of the Na<sup>+</sup> channel blocking properties of CI-980 with other class I antiarrhythmic drugs. All this information should help to understand further the mechanism of action of CI-980.

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## Methods

### General procedures

Guinea-pigs of either sex weighing 300–400 g were killed by cervical dislocation and their hearts were rapidly dissected 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 placed in a Lucite tissue bath and superfused at a constant rate of 7 ml min<sup>-1</sup> with Tyrode solution of the following composition (mM): NaCl 125, KCl 5.4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 24, NaH<sub>2</sub>PO<sub>4</sub> 0.42 and glucose 11. The solution was bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH = 7.4) and maintained at a temperature of 35 ± 0.5°C. 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 (1–2 ms in duration, 1.5–2 times the diastolic threshold) delivered from a multipurpose programmable stimulator (Cibertec CS-220). 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 and with tip resistances of 8–15 MΩ. The microelectrode was connected via Ag-AgCl wire to high-input impedance, capacity neutralizing amplifiers (WPI model 701, New Haven, CT, U.S.A.). The  $V_{\max}$  of the action potential was obtained by electronic differentiation as described by us previously (Valenzuela *et al.*, 1988; Delpón *et al.*, 1989). The suitable frequency filter for minimizing noise without reducing the  $V_{\max}$  was selected for each individual experiment. In order to avoid latency-induced alterations of  $V_{\max}$ , stimulus intensity and duration were adjusted throughout each experiment to maintain a constant latency (1–2 ms) from the stimulus artifact to the upstroke of the action potential (Valenzuela *et al.*, 1988). Both action potential and  $V_{\max}$  were displayed on a storage oscilloscope (model 4104N, 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.) or stored in a Hewlett-Packard 486/33 computer by use of Cibertec S.A. software. The following parameters of the transmembrane action potentials were measured: resting membrane potential, amplitude,  $V_{\max}$  and action potential duration at the 50% (APD<sub>50</sub>) and 90% (APD<sub>90</sub>) level of repolarization. The effective refractory period (ERP) was measured by introducing premature test-stimuli (S<sub>2</sub>) of twice the threshold strength at different intervals from the preceding basic action potential. S<sub>2</sub> were delivered every eighth basic stimulus (Delpón *et al.*, 1989). The values for the different parameters obtained in the absence of the drug were used as controls and compared with those obtained after each increment in drug concentration. All experimental results were obtained from a single continuous impalement throughout the whole experiment.

To study the use-dependent effect of CI-980 on  $V_{\max}$  following the equilibration period muscles were initially driven at a basal rate of 0.02 Hz. Then the preparations were driven by trains of stimuli at varying rates 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 use-dependent decreases in  $V_{\max}$ , were interpolated between the trains of stimuli (Valenzuela *et al.*, 1988; Delpón *et al.*, 1991). Under these circumstances two types of  $V_{\max}$  inhibition were detected, tonic and use-dependent  $V_{\max}$  block. Tonic blockade is the decrease of  $V_{\max}$  of the first action potential preceded by a rest period, whereas use-dependent blockade was defined as the decrease in  $V_{\max}$  during a train from the value of the first action potential to a new steady-state level. The recovery from use-dependent  $V_{\max}$  block was studied by applying a single test stimulus at various coupling intervals after a stimulation train for 12 s at 3 Hz. The length of the test stimuli was adjusted to obtain a constant latency (1–2 ms) from the stimulus artifact to the initiation of the action potential upstroke (Valenzuela *et al.*, 1988). The onset of and recovery from use-dependent block were fitted by exponential functions for calculations of the respective rate constants.

The relationship between  $V_{\max}$  and the resting membrane potential from which the action potential was elicited was studied in papillary muscles driven at 0.05 Hz. The resting membrane potential was depolarized by increasing in a step-wise manner the extracellular K<sup>+</sup> concentration, [K<sup>+</sup>]<sub>o</sub>, from 2 to 16 mM (Pérez *et al.*, 1994) and the  $V_{\max}$  was measured after the resting membrane potential had reached a steady-state at each [K<sup>+</sup>]<sub>o</sub>.

Slow, Ca<sup>2+</sup>-dependent, action potentials were elicited in papillary muscles rendered unexcitable by depolarizing with 27 mM K<sup>+</sup> Tyrode solution. Under these conditions, the fast inward Na<sup>+</sup> current was voltage-inactivated and excitability, i.e. slow action potentials, was restored in depolarized muscles driven at 0.1 Hz by adding isoprenaline (10<sup>-6</sup> M) to the perfusate (Pérez *et al.*, 1994). After control values for each parameter had been obtained, incremental concentrations of each drug were added to the bath to obtain a complete concentration-response curve. The values for the different parameters obtained in the absence of drug were used as a control and compared with those obtained 30 min after each increment in drug concentration.

### Drugs used

CI-980 (Parke-Davis, Ann Arbor, U.S.A.) as a powder, was initially dissolved in distilled deionized water as a 10<sup>-3</sup> M stock solution. Further dilutions were carried out in Tyrode solution. Ascorbic acid (10<sup>-4</sup> M) was added to prevent oxidation of isoprenaline. Throughout the paper data are given as the means ± 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 to be significant.

## Results

### Effects of CI-980 on transmembrane action potentials

The electrophysiological effects of CI-980 (10<sup>-7</sup> M–5 × 10<sup>-5</sup> M) on the action potential characteristics were studied in 10 guinea-pig papillary muscles driven at the basal rate of

**Table 1** Effects of CI-980 on transmembrane action potentials in guinea-pig papillary muscles driven at 1 Hz

Concentration (M)	RMP (mV)	APA (mV)	$V_{\max}$ (V s <sup>-1</sup> )	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)
0	85.5 ± 1.7	126.8 ± 1.1	181.0 ± 7.2	209.6 ± 15.7	238.2 ± 16.4
10 <sup>-6</sup>	84.8 ± 1.9	126.2 ± 0.8	172.3 ± 5.5	215.7 ± 15.2	245.7 ± 15.4
10 <sup>-5</sup>	85.2 ± 1.7	126.6 ± 0.9	162.2 ± 6.2*	249.8 ± 16.3*	283.4 ± 16.9*
5 × 10 <sup>-5</sup>	83.8 ± 3.2	125.7 ± 1.2	141.3 ± 10.6*	333.6 ± 39.1*	372.8 ± 38.7*
10 <sup>-4</sup>	83.3 ± 3.4	122.0 ± 2.1*	108.0 ± 17.1**	417.9 ± 54.5**	463.7 ± 54.5**

Values are mean ± s.e.mean, *n* = 10. RMP: resting membrane potential. APA: amplitude of the action potential.  $V_{\max}$ : maximal upstroke of the action potential. APD<sub>50</sub> and APD<sub>90</sub>: action potential duration at the 50% and 90% level of repolarization. \**P* < 0.05; \*\**P* < 0.01.

**Table 2** Effects of CI-980 on slow action potentials elicited by  $10^{-6}$  M isoprenaline in guinea-pig papillary muscles depolarized with KCl 27 mM and driven at a basal rate of 0.1 Hz

Concentration (M)	RMP (mV)	APA (mV)	$V_{max}$ ( $V s^{-1}$ )	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)
0	44.2 ± 2.7	90.2 ± 0.8	15.4 ± 0.7	291.4 ± 11.0	317.8 ± 11.7
$10^{-7}$	44.7 ± 2.6	91.2 ± 1.0	15.3 ± 0.8	299.9 ± 20.5	317.4 ± 19.8
$10^{-6}$	44.0 ± 2.5	90.0 ± 0.9	14.7 ± 0.5	296.8 ± 22.5	309.1 ± 18.6
$10^{-5}$	44.0 ± 2.6	89.7 ± 0.7	14.8 ± 0.8	366.7 ± 27.5*	382.7 ± 26.3*
$5 \times 10^{-5}$	43.9 ± 2.6	89.5 ± 0.5	14.1 ± 0.1	446.7 ± 15.4**	467.4 ± 15.6**

Values are mean ± s.e.mean,  $n = 8$ . RMP: resting membrane potential. APA: amplitude of the action potential.  $V_{max}$ : maximal upstroke of the action potential. APD<sub>50</sub> and APD<sub>90</sub>: action potential duration at the 50% and 90% level of repolarization. \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 3** Effects of CI-980 ( $10^{-5}$  M) on the use-dependent  $V_{max}$  in guinea-pig papillary muscles

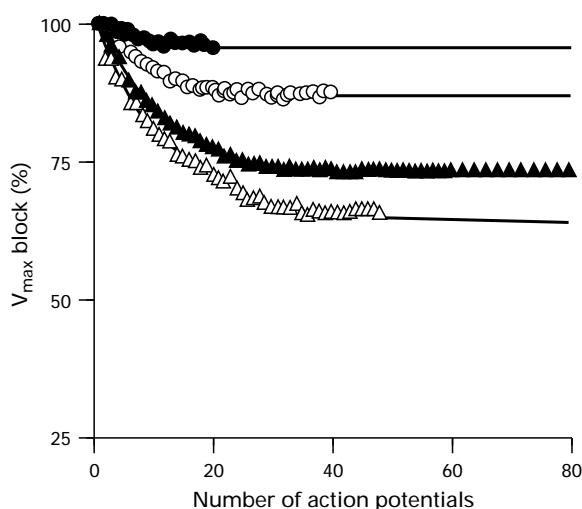
Frequency of stimulation (Hz)	Use-dependent $V_{max}$ Control	block (%) CI-980
0.5	1.5 ± 0.4	4.5 ± 0.8*
0.1	3.4 ± 0.3	10.6 ± 0.9***
2.0	6.6 ± 0.4	22.6 ± 2.0***
3.0	9.8 ± 0.9	33.3 ± 2.6***

Values are mean ± s.e.mean of 9 experiments. \* $P < 0.05$ , \*\*\* $P < 0.001$ .

**Table 4** Rate constants of development of use-dependent  $V_{max}$  block ( $K$ ) in the presence of CI-980 ( $10^{-5}$  M)

Frequency of stimulation (Hz)	Onset rate per AP $K$ (per AP)
0.5	0.25 ± 0.05
1.0	0.14 ± 0.02
2.0	0.08 ± 0.04
3.0	0.07 ± 0.01

Values are mean ± s.e.mean of 9 experiments.



**Figure 1** Onset of use-dependent decrease of  $V_{max}$  induced by CI-980,  $10^{-5}$  M, in guinea-pig papillary muscles driven by trains of stimuli at various rates (0.5–3 Hz). Ordinate scale: percentage of  $V_{max}$  block. Frequency-dependent  $V_{max}$  block (%) results from  $(1 - V_{max(ss, drug)} / V_{max(first\ beat, drug)})$ , where  $V_{max(ss)}$  is the steady-state value attained during continuous stimulation and  $V_{max(first\ beat)}$  the value of the first beat of each train of stimuli. Abscissae: number of action potentials. Frequencies of stimulation were 0.5 Hz (●), 1 Hz (○), 2 Hz (▲) and 3 Hz (△).

1 Hz. Results obtained under control conditions and 30 min after drug concentrations are shown in Table 1. At concentrations  $> 10^{-6}$  M, CI-980 significantly ( $P < 0.05$ ) decreased the  $V_{max}$  of the action potential and prolonged the APD<sub>50</sub> and APD<sub>90</sub>. At  $10^{-4}$  M, CI-980 also decreased the amplitude of the action potential ( $P < 0.05$ ), but these changes were not accompanied by changes in the resting membrane potential. Prolongation of the APD<sub>90</sub> was accompanied by a parallel lengthening of the ERP and hence, even in the presence of CI-980  $10^{-4}$  M the ERP/APD<sub>90</sub> ratio remained unaltered (from  $1.03 \pm 0.03$  to  $1.10 \pm 0.01$ ;  $n = 6$ ;  $P > 0.05$ ).

The effects of CI-980 ( $10^{-7}$  M– $5 \times 10^{-5}$  M) were also studied on the slow action potentials elicited by isoprenaline ( $10^{-6}$  M) in 8 papillary muscles depolarized in high  $K^+$  (27 mM) Tyrode solution and driven at 0.1 Hz. Table 2 shows that CI-980 had no effect on resting membrane potential, amplitude or  $V_{max}$  of the slow action potentials, but at concentrations  $\geq 10^{-6}$  M produced a significant ( $P < 0.05$ ) concentration-dependent prolongation of the APD<sub>50</sub> and APD<sub>90</sub> values.

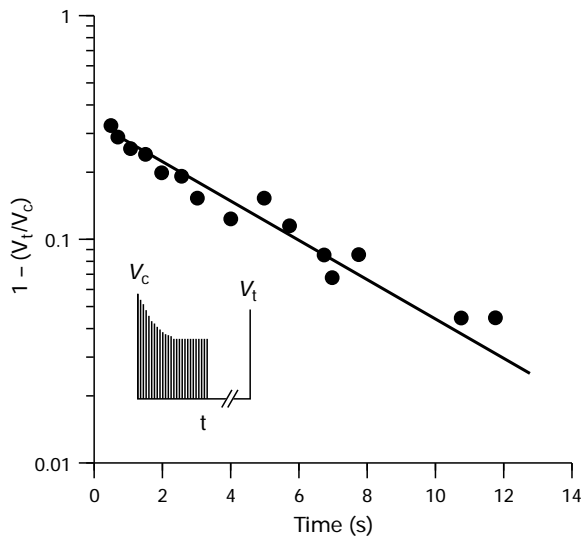
#### Use-dependent effects on $V_{max}$

The influence of stimulation frequency on the inhibitory effect of CI-980 on  $V_{max}$  was studied in papillary muscles by applying trains of pulses at different rates (0.5–3 Hz), separated from one another by a rest period of 5 min. Following the perfusion with  $10^{-5}$  M CI-980, the  $V_{max}$  of the first action potential in each train was reduced (e.g. tonic block) by  $2.1 \pm 0.9\%$  ( $n = 9$ ), which indicated that, even at this high concentration, the drug exhibited a low affinity for the resting state of the  $Na^+$  channels.

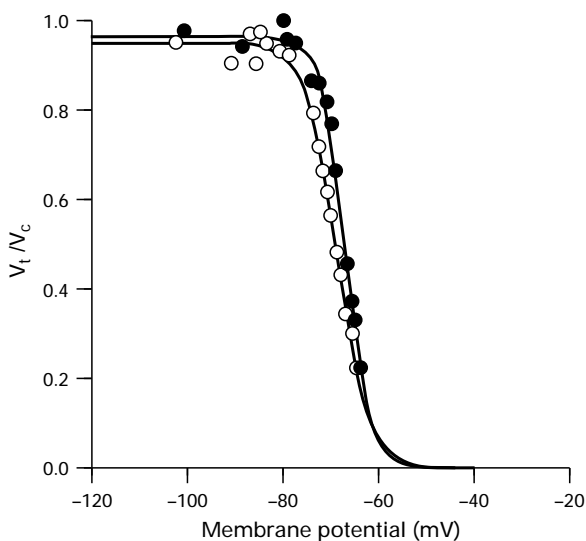
When applying a train of pulses in the presence of CI-980 there was a gradual decrease of  $V_{max}$  from beat to beat to a new steady-state, which depended on the stimulation frequency. Table 3 shows a summary of the percentage decreases in  $V_{max}$  from the first action potential of the train to a new steady-state level in the absence and in the presence of  $10^{-5}$  M CI-980 in muscles driven at 0.5–3 Hz and Figure 1 shows the results from a typical experiment. Under control conditions an increase in driving rate progressively decreased  $V_{max}$  by 10% during stimulation trains at a rate of 3 Hz. In the presence of CI-980, the degree of frequency-dependent  $V_{max}$  block significantly increased with the driving rate, this increase was more marked at fast (1, 2 and 3 Hz,  $P < 0.001$ ) than at slow (0.5 Hz,  $P < 0.05$ ) stimulation frequencies. The onset kinetics of use dependent  $V_{max}$  block can be defined in terms of a rate-dependent process. In muscles driven at 0.5–3 Hz, the onset kinetics of CI-980 was best fitted by a single exponential, from which the onset rate constant per action potential [ $K$ , (per AP)] at which  $V_{max}$  fell to a new steady-state was calculated. The value of  $K$  depended on stimulation frequency, decreasing at faster driving rates in the presence of CI-980 (Table 4).

#### Recovery kinetics of use-dependent $V_{max}$ block

To study the effects of CI-980 on the recovery kinetics of frequency-dependent  $V_{max}$  block, papillary muscles were driven every 5 min by a train of stimuli at a frequency of 3 Hz for 12 s



**Figure 2** Effects of CI-980,  $10^{-5}$  M, on the recovery process of  $V_{\max}$  from use-dependent block. Ordinate scale: normalized  $V_{\max}$  values ( $V_t/V_c$ ), where  $V_t$  is the  $V_{\max}$  of the test action potential and  $V_c$  is the  $V_{\max}$  of the first action potential of the train. Abscissae: test interval defined as the interval between the  $V_{\max}$  of the last action potential of the train and the  $V_{\max}$  of the test action potential.

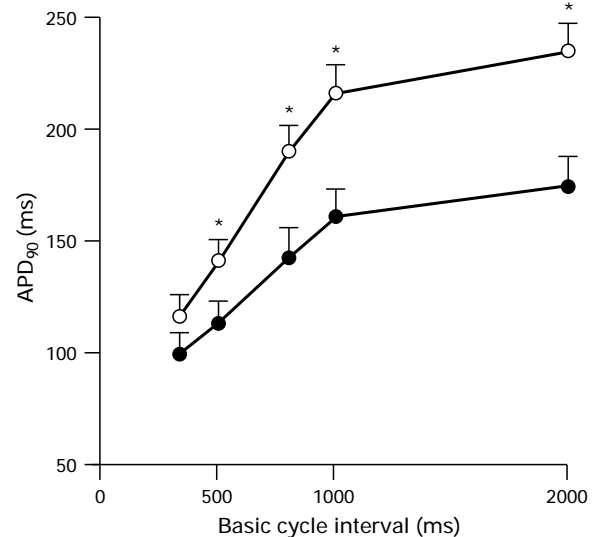


**Figure 3** Effect of CI-980,  $10^{-5}$  M, on the relationship between  $V_{\max}$  and the resting membrane potential at which the action potential is generated (i.e., membrane responsiveness). Ordinate scale: normalized  $V_{\max}$  values ( $V_s^{-1}$ ). Abscissa scale: membrane potential (mV). Control (●), CI-980 (○).

and a test-stimulus was applied at variable coupling intervals from 500 ms to 14 s. Under control conditions, the recovery from inactivation occurred as a monoexponential process with a time constant ( $\tau_{re}$ ) which averaged  $21.6 \pm 2.2$  ms ( $n=7$ ). Figure 2 shows that in the presence of CI-980 ( $10^{-5}$  M) the recovery from  $V_{\max}$  block was also well fitted by a monoexponential function and the value of the  $\tau_{re}$  averaged  $3.5 \pm 0.2$  s ( $n=6$ ). The y-intercept of the exponential, which can be taken as the fraction of  $Na^+$  channels blocked by CI-980, rose to  $24.1 \pm 2.9\%$ .

#### Voltage-dependence of the effects of $V_{\max}$

The relationship between  $V_{\max}$  and the membrane resting potential from which the action potential takes off was analysed



**Figure 4** Rate-dependent effects of CI-980,  $10^{-5}$  M, on the action potential duration to 90% of repolarization ( $APD_{90}$ ). Ordinate scale: percentage of change in  $APD_{90}$  values. Abscissa scale: basic cycle length (s). Control (●), CI-980 (○). \* $P < 0.05$  vs. control values.

in 6 papillary muscles driven at a basal rate of 0.05 Hz which was long enough to eliminate the use-dependent inhibition of  $V_{\max}$  by CI-980. The resting membrane potential was depolarized stepwise by increasing the  $[K^+]_o$  in the bathing media from 2 to 16 mM. Figure 3 shows that CI-980, in  $10^{-5}$  M, only slightly shifted the relationship along the voltage axis in the hyperpolarizing direction, so that the membrane potential at which  $V_{\max}$  was reduced to half of its maximal value was shifted by  $2.3 \pm 1.1$  mV ( $n=6$ ,  $P > 0.05$ ). These results suggest that CI-980 is not a blocker of the  $Na^+$  channel in the inactivated state.

#### Effect on the action potential duration

Changes in  $APD_{90}$  depended on the rate of stimulation. Figure 4 shows the effects of CI-980,  $10^{-5}$  M, on the  $APD_{90}$  at different basic cycle lengths. At each cycle length, five minutes was allowed for  $APD_{90}$  to reach a steady state before measurements were made. In control conditions, decreasing the basic cycle length from 1 s to 330 ms progressively shortened the  $APD_{90}$ . In the presence of CI-980 the prolongation of the  $APD_{90}$  values was reduced as the driving rate increased. Thus, CI-980 significantly increased the  $APD_{90}$  by  $35.0 \pm 10.7\%$  at a cycle length of 2 s ( $P < 0.01$ ), while at a cycle length of 500 ms and 330 ms its effect was reduced to a  $24.3 \pm 9.0\%$  ( $P < 0.05$ ) and  $17.0 \pm 9.3\%$  ( $n=6$ ,  $P > 0.05$ ) increase, respectively.

#### Discussion

The present paper studied the electrophysiological effects of CI-980, a new tubulin-binding agent, in guinea-pig isolated ventricular muscle fibres. We have documented that: (a) CI-980 inhibited the  $V_{\max}$  of the action potential without affecting the resting membrane potential, which indicated that it exhibited  $Na^+$  channel blocking (class I antiarrhythmic) properties; (b) the inhibition of  $V_{\max}$  was increased at higher driving frequencies and from the onset and offset kinetics of the use-dependent  $V_{\max}$  block, CI-980 can be included as an intermediate kinetics  $Na^+$  channel blocker; and (c) at the same range of concentrations CI-980 markedly prolonged the ventricular APD values, i.e., it exhibited class III antiarrhythmic actions according to the Vaughan Williams classification (1994).

In the present study,  $V_{\max}$  values were used as an indirect estimate of the magnitude of the  $I_{Na}$ . The  $V_{\max}$  is a monotonic

but non-linear index of peak  $I_{Na}$  (Sheets *et al.*, 1988), but there is little doubt that  $V_{max}$  is mainly generated by this current (Sheets *et al.*, 1988; Fozzard & Hanck, 1992), so that it is possible that non-linearities between  $V_{max}$  and  $Na^+$  conductance may affect the present estimation of the amount of  $I_{Na}$  block. Nevertheless, patch-clamp experiments currently available for  $I_{Na}$  measurement require more artificial experimental conditions (low temperature and external  $Na^+$  concentrations) than those used for  $V_{max}$  measurement (Kodama *et al.*, 1995).

$Na^+$  channel blockers are characterized by their ability to depress the  $V_{max}$  of cardiac action potentials (Hondegheem & Katzung, 1984; Tamargo *et al.*, 1992). The  $V_{max}$  block produced by CI-980 can be interpreted with the framework of the modulated receptor hypothesis (Hondegheem & Katzung, 1984; Hondegheem, 1987), which assumes that  $Na^+$  channel blockers bind to a specific binding site located within or functionally associated with the  $Na^+$  channel and their affinity increases with a transition from the resting to the activated/inactivated state of the channel. CI-980 produced two types of  $V_{max}$  block, i.e. tonic and use-dependent. Like other  $Na^+$  channel blockers (Campbell, 1983a,b; Hondegheem & Katzung, 1984; Tamargo *et al.*, 1989; 1992), high concentrations of CI-980 caused little tonic  $V_{max}$  block in normally polarized ventricular fibres, which indicates that it exhibits little affinity for the resting state of the channel. Use-dependent  $V_{max}$  block can be explained by a preferential binding of the drugs to the activated and/or inactivated state of the  $Na^+$  channel during a train of action potentials, when the diastolic interval between pulses is too short to allow complete recovery of  $Na^+$  availability (Hondegheem & Katzung, 1984; Hondegheem, 1987; Tamargo *et al.*, 1989; 1992). This would lead to an accumulation of blocked, non-conducting, channels following stimulation of progressive higher driving rates. In the presence of CI-980, the onset rate of use-dependent  $V_{max}$  block was faster at lower stimulation frequencies. At  $10^{-5}$  M the  $K$  value was  $0.07 \pm 0.01$  per AP in muscles driven at 3 Hz which is similar to that obtained for quinidine, an intermediated kinetics (class IA) drug (Campbell, 1983a,b). However, the onset kinetics of use-dependent  $V_{max}$  block are affected by changes in drug concentration and stimulation rate (Hondegheem & Katzung, 1984; Grant *et al.*, 1984; Tamargo *et al.*, 1989). The  $\tau_{re}$ , which represents the rate of unbinding of the drug from blocked  $Na^+$  channels, is independent of changes in drug concentration or the stimulation rate and, therefore, has been considered to be one of the most reliable parameters to differentiate  $Na^+$  channel blockers (Campbell, 1983a,b; Hondegheem & Katzung, 1984; Tamargo *et al.*, 1992). CI-980 prolonged the  $\tau_{re}$  to 3.5 s, a value close to that previously described for intermediate kinetics (class IA drugs)  $Na^+$  channel blockers such as quinidine (3.7–4.7 s, Grant *et al.*, 1982; Sánchez-Chapula, 1985), procainamide (2.3 s, Courtney, 1987) and disopyramide (2.2 s, Campbell, 1983a) and, therefore, it may be classified as a drug of the same subgroup. This prolongation of the  $\tau_{re}$  explains why the diastolic interval was short enough during stimulation trains  $\geq 0.5$  Hz to prevent complete recovery of  $V_{max}$ .

We also studied the effect of CI-980 on the relationship between  $V_{max}$  and membrane potential by increasing the  $[K^+]_o$  in muscles driven at 0.05 Hz. Under these conditions, only a slight shift of the curve in a hyperpolarizing direction was

observed, which suggested that even at high concentrations, CI-980 exhibits a low affinity for inactivated  $Na^+$  channels. Thus, as previously described for other class IA antiarrhythmic drugs (i.e. quinidine and disopyramide; Komada *et al.*, 1990), CI-980 may block  $Na^+$  channels primarily when they are in the activated state which corresponds to the upstroke of the action potential.

At concentrations  $>10^{-6}$  M, CI-980 not only caused a significant decrease in  $V_{max}$  but also prolonged the APD<sub>50</sub> and APD<sub>90</sub> values, i.e. it also exhibits class III antiarrhythmic actions. Furthermore, as previously described with most of the available drugs that prolong repolarization, CI-980 exhibited reverse use-dependence, that is, drug-induced APD prolongation became exaggerated at slow rates and attenuated at fast rates (Hondegheem & Snyders, 1990). As previously described with other class IA and III antiarrhythmics, reverse use-dependent prolongation of APD would reduce the effectiveness of CI-980 against tachycardia and has been associated with polymorphic ventricular tachycardias (torsades de pointes), especially after long diastolic intervals (Hondegheem & Snyders, 1990; Woosley, 1991; Morganroth, 1993; Roden, 1993).

The repolarization of the cardiac action potential is the result of a delicate balance between inward  $Na^+$  and/or  $Ca^{2+}$  currents flowing during the plateau of the action potential and outward  $K^+$  currents responsible for repolarization. Block of  $Na^+$  channels induced by CI-980 is expected to shorten, but not to lengthen, the APD. To assess whether CI-980 inhibited the slow inward  $Ca^{2+}$  current ( $I_{Ca}$ ), its effects were analysed on the slow action potentials induced in  $K^+$ -depolarized papillary muscles. Under these conditions,  $I_{Na}$  is voltage-inactivated by partial depolarization and isoprenaline induces slowly rising action potentials by increasing the  $I_{Ca}$  (Reuter, 1984). CI-980 had no effect on the amplitude and  $V_{max}$  of the slow action potentials, a fairly good approximation of the  $I_{Ca}$  (Malecot & Trautwein, 1987), which indicates that it has no effect on this ionic current. Thus, the effect of CI-980 in prolonging the APD (class III action) is most likely caused by a reduction of repolarizing  $K^+$  currents. Since the transient outward current is not present in guinea-pig ventricular myocytes (Sorota & Boyden, 1991) and CI-980 had no effect on resting membrane potential, prolongation of the ventricular APD could be due to a decrease in the delayed rectifier  $K$  current ( $I_K$ ). Since class III antiarrhythmic drugs that block the rapidly activating component of this current ( $I_{Kr}$ ) exhibit reverse use-dependence (Carmeliet, 1993; Delpón *et al.*, 1995), it is tempting to speculate that CI-980 blocks  $I_{Kr}$ . However, definitive identification of the mechanisms underlying rate-dependent APD changes awaits detailed voltage-clamp studies of the effects of CI-980 on ventricular  $K^+$  currents.

In conclusion, at the same range of concentrations CI-980 inhibited the  $V_{max}$  and prolonged the APD in guinea-pig papillary muscles, thus exhibiting class I and III antiarrhythmic actions, respectively. From the onset and offset kinetics of the use-dependent  $V_{max}$  block, CI-980 can be considered as an intermediate kinetics (class IA)  $Na^+$  channel blocker.

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