ELSEVIER

European Journal of Pharmacology 352 (1998) 317–327

ejp

Na^+ channel blocking effects of cibenzoline on guinea-pig ventricular cells

Ryoko Niwa, Haruo Honjo, Itsuo Kodama *, Kazuyasu Maruyama, Junji Toyama

Departments of Circulation and Humoral Regulation, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

Received 13 March 1998; revised 4 May 1998; accepted 5 May 1998

Abstract

The effects of cibenzoline on transmembrane action potentials were examined in right ventricular papillary muscles and in single ventricular myocytes isolated from guinea-pig hearts. In papillary muscles, cibenzoline ≥ 3 μM caused a significant decrease in the maximum upstroke velocity (V_{max}) of the action potential without affecting the action potential duration. The inhibition of V_{max} was enhanced at higher stimulation frequencies. In the presence of cibenzoline, trains of stimuli at rates ≥ 0.2 Hz led to a use-dependent inhibition of V_{max}. The time constant for V_{max} recovery (τ_R) from the use-dependent block was 26.2 s. The use-dependent block of V_{max} with cibenzoline was enhanced and τ_R was shortened when the resting potential was depolarized by high (8, 10 mM) $[K^+]_o$. The curve relating membrane potential and V_{max} in single myocytes was shifted by cibenzoline (10 μM) in a hyperpolarizing direction by 7.1 mV. In myocytes treated with cibenzoline (10 μM), a 10-ms conditioning clamp to 0 mV caused a significant decrease in V_{max} of the subsequent test action potential; the V_{max} inhibition was enhanced modestly in association with a prolongation of the 0 mV clamp pulse duration. In the presence of cibenzoline (3 μM), application of a train of depolarizing pulses (10 ms, 200 ms) to myocytes from the resting level (-80 mV) to 0 mV resulted in a progressive V_{max} reduction in a pulse number-dependent manner. Unlike glibenclamide (30 μM), cibenzoline (10 μM) did not prevent the hypoxia-induced shortening of action potential duration in papillary muscles. These findings indicate that the onset and offset kinetics of use-dependent Na^+ channel block by cibenzoline are slow. Given its state dependence, cibenzoline may be a blocker of activated Na^+ channels. The inhibitory action of this compound on the ATP-sensitive K^+ current ($I_{K,ATP}$) would be minimal or negligible at concentrations causing sufficient Na^+ channel block.

Keywords: Cibenzoline; Ventricular cell; Action potential; V_{max}; Use-dependent block

1. Introduction

Cibenzoline is a relatively new antiarrhythmic agent with a chemical structure quite different from that of other known antiarrhythmic drugs (Dangman and Miura, 1986). In vivo animal studies have demonstrated that this compound prevents tachyarrhythmias induced by acute ischemia, programmed electrical stimulation or digitalis intoxication (Harron et al., 1992). The high therapeutic potential of cibenzoline in oral as well as in parenteral use against both supraventricular and ventricular tachyarrhythmias has been confirmed in many clinical studies (Harron et al., 1992). The molecular and cellular pharmacodynamics of cibenzoline underlying such potent antiarrhythmic activity are multifaceted. In vitro experiments with atrial and ventricular tissues have shown that cibenzoline reduces the maximum upstroke velocity (V_{max}) of the action potential without affecting the resting membrane potential, and that the V_{max} inhibition is enhanced in a frequency-dependent manner (Millar and Vaughan Williams, 1982, 1983; Dangman, 1984; Satoh et al., 1987). Cibenzoline was also shown to prolong action potential duration in atrial and ventricular muscles in some animal species (Millar and Vaughan Williams, 1982, 1983; Dangman, 1984; Satoh et al., 1987). In voltage clamp studies, cibenzoline was shown to inhibit the fast Na^+ inward current (I_{Na}), the slow Ca^{2+} inward current (I_{Ca}), and several types of K^+ outward currents including the delayed rectifier current (I_K), the inward rectifier current (I_{K1}), and the muscarinic receptor-activated K^+ current ($I_{K,ACh}$) (Masse et al., 1984; Holck and Osterrieder, 1986; Kotake et al.,

* Corresponding author. Tel.: +81-52-789-3869; fax: +81-52-789-3890.

0014-2999/98/$19.00

1987; Matsuoka et al., 1991; Sato et al., 1994; Wang et al., 1996; Wu et al., 1994). It has been demonstrated in guinea-pig ventricular cells that cibenzoline also possesses an inhibitory action on the ATP-sensitive K^+ current ($I_{K,\ ATP}$) activated by 2,4-dinitrophenol or by diazoxide (Wu et al., 1992; Sato et al., 1993).

Among the multiple ionic currents affected, I_{Na} and $I_{K,\ ATP}$ are supposed to be the most important, as determined with effective concentrations of cibenzoline in experiments with guinea-pig ventricular cells (Sato et al., 1993, 1994). However, the kinetics and state dependence of the Na^+ channel block with cibenzoline are not known. The relative importance of I_{Na} and $I_{K,\ ATP}$ blockade also remains to be investigated. The K_{ATP} channel activity is known to be easily affected by intracellular metabolic cofactors including adenosine diphosphate, H^+, Mg^{2+}, and protein kinase C (Lederer and Nichols, 1995; Ferrero et al., 1996; Light et al., 1996), and these cofactors under conditions of whole-cell clamp are quite different from those in multicellular cardiac tissues. Intracellular nucleotide diphosphates were also shown to limit the efficacy with which sulfonylureas block cardiac K_{ATP} channels (Venkatesh et al., 1991; Weiss and Venkatesh, 1993; Brady et al., 1998). The findings of patch-clamp experiments (Wu et al., 1992; Sato et al., 1993), therefore, cannot be simply extrapolated to the intact heart. In the present study, we examined the effects of cibenzoline on the transmembrane action potential in right ventricular papillary muscles as well as in single ventricular myocytes isolated from guinea-pig hearts. The modulation of drug-induced V_{max} inhibition by stimulation frequencies and by membrane potential was studied in detail so as to compare their Na^+ channel blocking characteristics with those of other Class I drugs. The effects of cibenzoline against the hypoxia-induced shortening of action potential duration in ventricular muscle were also tested in order to obtain insight into the potential importance of its $I_{K,\ ATP}$ blocking action in the therapeutic concentration range.

2. Material and methods

2.1. Papillary muscle

Guinea-pigs of either sex weighing 200 to 250 g were killed by cervical dislocation under ether anaesthesia and the hearts were quickly removed. Papillary muscles, 2 to 3 mm in length and 0.3 to 0.4 mm in diameter, were dissected from the right ventricle. The preparation was mounted in a tissue bath and superfused continuously with Krebs–Ringer solution kept at 33°C and gassed with a mixture of 95% O_2 and 5% CO_2 to obtain $pO_2 > 600$ mmHg. The composition of the solution was as follows (in mM): NaCl 120.3, KCl 4.0, $CaCl_2$ 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 25.2 and glucose 5.5 (pH 7.4). The preparations were stimulated by a pair of 1.0 mm platinum wire electrodes placed 1 mm apart on either side of the muscles. Pulses used for stimulation were 0.5–1.0 ms in duration and 20% higher than the diastolic threshold in intensity unless otherwise specified. Equipment for recording transmembrane potential was the same as described previously (Kodama et al., 1985; Toyama et al., 1987). To study the use-dependent effect of cibenzoline on the maximum upstroke velocity (V_{max}) of action potentials, the preparation was stimulated repetitively at various rates ranging from 0.2 to 2.0 Hz. Resting periods of 90 s, which were sufficient to ensure full recovery from the rate-dependent decrease in V_{max}, were interposed between the trains of stimuli. The experimental protocol is able to detect the existence of two types of drug-induced V_{max} inhibition, tonic and use-dependent block. The tonic block was calculated from the first action potential preceded by the rest period. The use-dependent block was defined by the decrease in V_{max} during the trains (from the value of first action potential to the new steady-state level). The recovery in V_{max} from the use-dependent block was tested by applying a single test stimulus at various coupling intervals following a stimulation for 60 s at 1.0 Hz. The intensity of the test stimulus was adjusted to obtain a constant latency from the stimulus artifact to the initiation of action potential upstroke.

In experiments to assess the influence of the resting membrane potential on the tonic and use-dependent V_{max} inhibition by the drug, the K^+ concentration in the superfusate was elevated from 4.0 up to 8.0 and 10.0 mM by adding KCl.

When the effects of cibenzoline on hypoxia-induced shortening of action potential duration were examined, the tissue bath was perfused with hypoxic glucose-free Krebs–Ringer solution for three times in 15 min. The hypoxic solution was gassed with a mixture of 95% N_2 and 5% CO_2 to obtain a pH of 7.4 and pO_2 of 10 to 20 mmHg. Each exposure to the hypoxic solution was followed by 60 min of reoxygenation with normal Krebs–Ringer solution. The effects of cibenzoline or glibenclamide were determined during the third hypoxic period with reference to the second period as control.

2.2. Single ventricular myocytes

Single ventricular myocytes were isolated enzymatically from guinea-pig hearts by essentially the same procedure as described in our previous paper (Kodama et al., 1990). The cells were suspended in the modified KB solution of the following composition (mM): KCl 25, KH_2PO_4 10, $MgCl_2$ 3, taurine 20, glutamic acid 70, glucose 10, EGTA 0.5, HEPES 10 (pH was adjusted to 7.4 by adding ~70 mM KOH). A few drops of cell suspension were placed in a recording chamber attached to an inverted microscope.

The chamber was perfused at a rate of 2 ml/min with normal Tyrode solution of the following composition (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHPO_4$ 0.33, HEPES 5.0 and glucose 5.0; pH was adjusted to 7.4 by adding NaOH, and the solution was equilibrated by gassing with 100% O_2. The temperature was maintained at 35°C. After the calcium concentration of the medium was increased to 1.8 mM (normal Tyrode solution), 30 to 40% of myocytes deteriorated into round-shaped cells due to irreversible contracture. The remaining cells were tolerant of calcium; their intact rod-shape was maintained without spontaneous beating, and the experiments were carried out with these myocytes.

In experiments to investigate the voltage dependence of V_{max} inhibition by cibenzoline, the whole-cell clamp technique was used with a patch pipette (2–3 MΩ) containing a solution of (mM): KCl 120.0, NaH_2PO_4 10.0, EGTA 1.0, MgATP 5.0 and HEPES 10.0; the pH was adjusted to 7.2 by adding KOH. Single cells were voltage-clamped with an Axoclamp-2A (Axon, Burlingame, CA, USA) in a single-electrode discontinuous mode (sampling frequency: 8–11 kHz). To elicit an action potential, the voltage clamp was switched to the current clamp mode by computer-operated digital signals, during which a square pulse (2 ms) for stimulation was applied. The upstroke velocity (V_{max}) was sampled at 70 kHz by an on-line analysis system (Anno and Hondeghem, 1990).

Three different conditioning clamp protocols were used. The first one examined the relationship between V_{max} and the resting membrane potential from which the action potential originates. Following a rest period of 90 s at −80 mV, which was long enough to eliminate the use-dependent depression of V_{max} by the drug, the membrane potential was clamped for 10 s to various levels (from −110 mV to −55 mV). The voltage clamp was then released and a stimulus was applied to elicit the test action potential.

The second protocol was to determine whether the use-dependent V_{max} inhibition by the drug was due to the blockade of an activated or inactivated sodium channel. Following a rest period of 90 s at −80 mV, the membrane potential was clamped from the resting level to 0 mV for 10 to 5000 ms. At the end of step depolarization, the membrane potential was clamped back to −80 mV for 100 ms, which is long enough for a drug-free channel to reactivate fully (Carmeliet and Vereecke, 1979; Ebihara and Johnson, 1980), but short enough for the drug to dissociate only partially from the blocked channel (Grant et al., 1984). The voltage clamp was then released and stimulus was applied to elicit the test action potential.

The third protocol was to compare the extent of use-dependent block during trains of a short or a long depolarization pulse. Following a rest period of 90 s to allow full recovery of V_{max} at −80 mV, a train of 10 ms or 200 ms depolarization to 0 mV was applied with an interpulse interval of 500 ms. Each depolarizing pulse was preceded by a 4-ms window of a current clamp, during which the action potential upstroke to measure V_{max} was elicited (the membrane potential was voltage-clamped for the remainder of the period).

2.3. Drugs and data analysis

Cibenzoline succinate (a kind gift from Fujisawa Pharmaceutical, Osaka, Japan) was dissolved in deionized water and diluted with superfusate (Krebs–Ringer solution or Tyrode solution) to achieve the final concentration required (1–30 μM). Glibenclamide (Sigma) was dissolved in dimethylsulfoxide (DMSO) as 30-mM stock solution and it was added to the Krebs–Ringer solution before use to produce the final concentration required. Final DMSO concentrations did not exceed 0.1%.

Values are presented as means ± S.E.M. unless otherwise stated. Analysis of variance (ANOVA) with Scheffe's test was used for critical differences among multiple means, and Student's *t*-test was used from comparison between the two means. Differences were considered significant at $P < 0.05$. All fitting of the exponential curves to the data was accomplished with a nonlinear least-squares algorithm.

3. Results

3.1. Action potentials of papillary muscle

The effects of cibenzoline (1 μM to 10 μM) on the action potential configuration were examined in five papillary muscles constantly stimulated at 0.2 Hz or 1.0 Hz (Table 1). Treatment with cibenzoline at 1 μM for 60 min resulted in no significant changes in the action potential configuration (data not shown). Cibenzoline ≥ 3 μM caused a significant decrease in the maximum upstroke velocity (V_{max}). The V_{max} reduction was greater at the higher stimulation frequency and at the higher drug concentration. The resting membrane potential, action potential amplitude and the action potential duration at 30% and 80% repolarization were unaffected even at 10 μM. When cibenzoline was washed out, the action potential configuration returned slowly toward the control. A full recovery of V_{max} was obtained 120 min after wash-out of the drug (Table 1).

3.2. Use-dependent effects on V_{max}

The effects of cibenzoline on V_{max} were examined with stimulation trains at different rates separated from each other by a 90-s rest period. In control preparations, the value of V_{max} was almost unchanged with stimulation trains at rates from 0.2 to 2.0 Hz. After treatment with cibenzoline, the V_{max} of the first action potential in each

Table 1
Effects of cibenzoline on action potential characteristics

		Resting potential (mV)	Amplitude (mV)	Duration (30%) (ms)	Duration (80%) (ms)	V_{max} (Vs^{-1})
1.0 Hz	Control ($n=5$)	-92.7 ± 0.4	126.6 ± 1.1	240 ± 10	300 ± 9	196 ± 4
	Cibenzoline					
	3 μM	-92.1 ± 0.6	125.8 ± 0.5	234 ± 9	299 ± 10	170 ± 5[a]
	10 μM	-92.7 ± 0.4	125.0 ± 0.8	219 ± 10	293 ± 10	124 ± 6[a]
	Wash-out	-92.3 ± 0.7	124.8 ± 0.8	226 ± 11	301 ± 11	194 ± 6
0.2 Hz	Control ($n=5$)	-93.7 ± 0.5	130.3 ± 0.7	247 ± 12	310 ± 11	208 ± 4
	Cibenzoline					
	3 μM	-94.0 ± 0.3	129.4 ± 0.4	$234 + 9$	302 ± 8	193 ± 7[a]
	10 μM	-93.7 ± 0.3	127.8 ± 0.4	213 ± 8	292 ± 7	162 ± 3[a]
	Wash-out	-93.8 ± 0.4	127.5 ± 0.5	218 ± 10	301 ± 9	203 ± 5

Values are means $\pm$ S.E.M. of five experiments.
The preparations were constantly stimulated at 1.0 Hz or 0.2 Hz.
Data were obtained before (control), 50 min after application of cibenzoline at a given concentration, and 120 min after wash-out of the drug.
V_{max}: maximum upstroke velocity of the action potential.
Action potential duration was measured from the upstroke to 30% and 80% repolarization.
[a] Significantly different from control at $P < 0.05$.

train was slightly decreased, indicating a minimal tonic block ($3.1 \pm 0.2\%$ at 3 μM and $4.4 \pm 1.4\%$ at 10 μM, $n = 5$). A further decline of V_{max} during the repeated pulses (use-dependent block) was dependent on the stimulation frequency: the higher the frequency, the greater the block (Fig. 1).

The beat-to-beat decline of V_{max} during stimulation trains at ≥ 0.5 Hz fitted a single exponential curve (Fig. 1), so that the rate of onset of each action potential at which V_{max} fell to the new steady-state level could be calculated in each experiment (Table 2). The rate of onset of the use-dependent block by cibenzoline was greater at the higher drug concentration and at the lower stimulation frequency (Table 2).

The recovery of V_{max} from the use-dependent block was studied by applying a single test stimulus at various coupling intervals following a stimulation train for 60 s at 1.0 Hz. Before the application of the drug, the V_{max} of the test action potential recovered almost completely within 100 ms of the diastolic interval (the interval from the end of the last action potential of the train to the beginning of the test action potential). The recovery process was approximated by a single exponential function with a mean time constant of 23.9 ± 2.8 ms ($n = 6$). After treatment

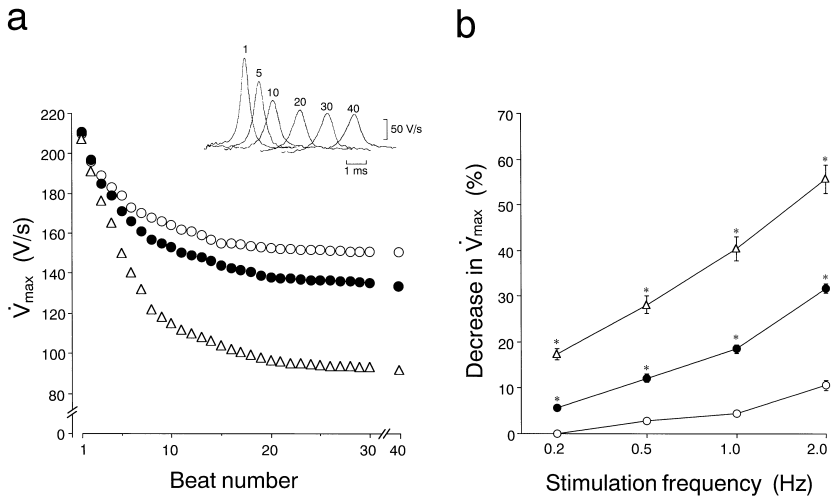

Fig. 1. Use-dependent decrease of the maximum upstroke velocity (V_{max}) of the action potential in papillary muscles. (a) Beat-to-beat changes in V_{max} at the onset of stimulation trains at 0.5 Hz (○), 1.0 Hz (●), and 2.0 Hz (△) in the presence of cibenzoline (10 μM). Ordinate scale: V_{max}. Abscissa scale: number of beats from initiation of the stimulation train. Inset shows superimposed records of differentiated upstroke spikes of action potentials from the first to the 40th beats at 1.0 Hz. (b) Relation between stimulation frequency and intensity of the use-dependent block. Ordinate scale: percent decrease of V_{max} from the first action potential to the new steady-state level. Abscissa scale: stimulation frequency. Data were obtained before (control ○) and 50–60 min after application of cibenzoline at 3 μM (●) and 10 μM (△). Values are means $\pm$ S.E.M. ($n = 5$). $*$Changes were statistically significant from control at $P < 0.05$.

Table 2
Onset rate of use-dependent block of V_{max}

Cibenzoline	n	0.5 Hz	1.0 Hz	2.0 Hz
3 μM	(6)	0.13 ± 0.01	0.11 ± 0.01	0.07 ± 0.01
10 μM	(6)	0.19 ± 0.02	0.14 ± 0.01	0.12 ± 0.01

Values are means ± S.E.M.
n = number of preparations.
The onset rate per action potential at which V_{max} fell to the new steady-state level during the stimulation train was calculated by fitting the beat-to-beat decline of V_{max} to a single exponential function.

with cibenzoline (10 μM), a much slower recovery of V_{max} was observed. Representative results are shown in Fig. 2, where the V_{max} of the test action potentials was plotted against the diastolic interval. In the presence of cibenzoline, the time course of recovery when diastolic interval was longer than 100 ms was approximated by a single exponential function. The average time constant (τ_R) was 26.2 ± 2.3 s (n = 6).

3.3. Influence of resting membrane potential on the tonic and use-dependent V_{max} inhibition

Tonic and use-dependent block of V_{max} by cibenzoline was also examined in partially depolarized papillary muscles in the presence of high extracellular K^+ concentrations. In the medium with 8 and 10 mM K^+, the resting membrane potential of the preparation was decreased to −78.1 ± 0.3 mV (n = 4) and −69.8 ± 0.6 mV (n = 4), respectively. In such preparations, cibenzoline (10 μM) caused a decrease in V_{max} of the first action potential following a long (90 s) rest period by 7.8 ± 1.8% (n = 4) with 8 mM $[K^+]_o$, and 12.1 ± 2.0% (n = 4) with 10 mM of $[K^+]_o$. The resting potential was unaffected by the drug treatment. Thus, the tonic block of V_{max} by cibenzoline was augmented in partially depolarized preparations.

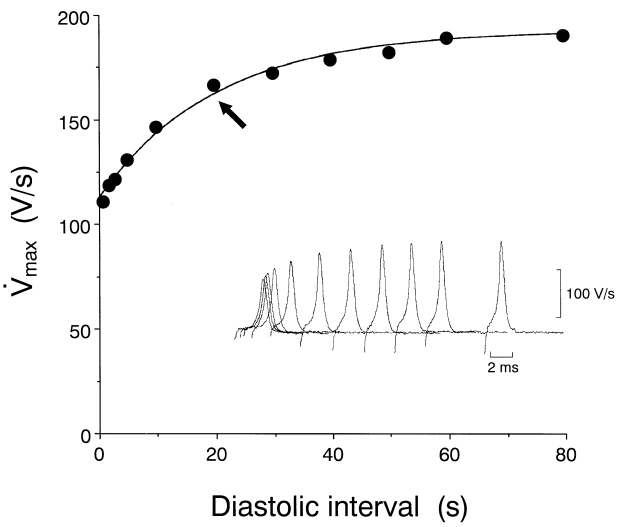

Fig. 2. Recovery of V_{max} from the use-dependent block produced by cibenzoline in a papillary muscle. Inset shows differentiated upstroke spikes of test action potentials after application of cibenzoline (10 μM). Following 1.0 Hz stimulation for 60 s, a single test stimulus was applied with various coupling intervals. The graph shows V_{max} recovery in the presence of cibenzoline (10 μM). Ordinate scale: V_{max}, Abscissa scale: diastolic interval (interval from the end of the last conditioning action potential to the upstroke of the test action potential). The V_{max} recovery after cibenzoline was approximated by a single exponential function at a time constant (τ_R) of 23.7 s (solid arrow) for the data with diastolic intervals of over 100 ms.

The intensity of use-dependent block with cibenzoline (10 μM) during the stimulation train at 1.0 Hz was increased significantly from 40.2 ± 2.4% (n = 6) at 4 mM of $[K^+]_o$ to 51.5 ± 2.5% (n = 5) at 8 mM of $[K^+]_o$ and to 55.0 ± 1.5% (n = 4) at 10 mM of $[K^+]_o$. The recovery time constants (τ_R) of the use-dependent block with cibenzoline (10 μM) at 8 mM (18.3 ± 1.5 s, n = 5) and at 10 mM (15.7 ± 1.3 s, n = 4) were significantly shorter than the value at normal (4 mM) $[K^+]_o$.

3.4. Voltage dependence of V_{max} inhibition

The baseline characteristics of the action potential elicited in single ventricular cells when there was a long interstimulus interval (90 s) were as follows: resting membrane potential, −80.5 ± 0.2 mV; V_{max}, 571 ± 17 V s^{-1}; action potential duration at 80% repolarization, 160 ± 9 ms (n = 8).

First, the relationship between V_{max} and the resting membrane potential from which the action potential originates was examined. Test action potentials to measure V_{max} were elicited with a conditioning potential ranging between −110 mV and −55 mV. Cibenzoline (10 μM) caused a greater V_{max} reduction at less negative conditioning membrane potentials. A fraction of V_{max} was calculated in each experiment before (control) and after application of the drug by normalizing the data with the value at −100 mV (Fig. 3). The data points fitted the Boltzmann equation:

$$y = 1/\{1 + \exp(V_m - V_h)/k\} \quad (1)$$

where V_m is the conditioning clamp potential and V_h is the membrane potential showing a half maximal V_{max}. V_h was changed from −60.1 ± 1.6 mV in control to −67.2 ± 2.1 mV after cibenzoline, indicating a hyperpolarizing shift of 7.1 ± 0.9 mV (n = 4). The k values were almost identical before (4.9 ± 0.2 mV, control) and after cibenzoline (5.1 ± 0.2 mV).

In the second protocol, a single conditioning 0 mV clamp of varying duration was applied from the resting level (−80 mV), and a test action potential to measure V_{max} was elicited 100 ms after the potential returned to the resting level (Fig. 3). In untreated control myocytes, such a pulse clamp with a duration less than 1000 ms had no

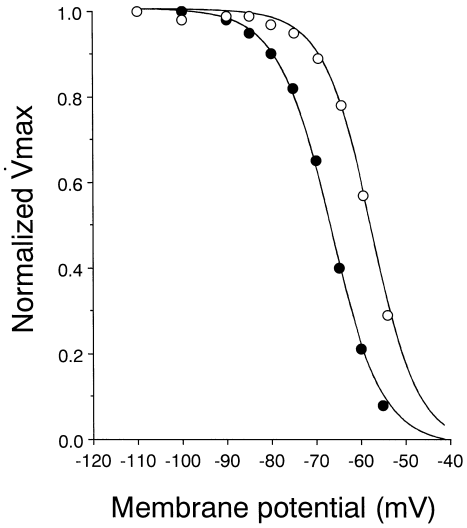

Fig. 3. Voltage dependence of V_{max} inhibition by cibenzoline in single ventricular myocytes. Ordinate scales: V_{max} of test action potential normalized by the value at a conditioning membrane potential of -110 mV. Abscissa scales: conditioning membrane potential from which a test action potential was elicited. Following a rest period of 90 s at a holding potential of -80 mV, the membrane potential was clamped for 10 s to various conditioning levels (from -110 to -55 mV). At the end of the conditioning clamp, the voltage clamp was released, and a test action potential was elicited to measure V_{max}. Data were obtained before (○) and 10–20 min after application of cibenzoline (10 μM) (●).

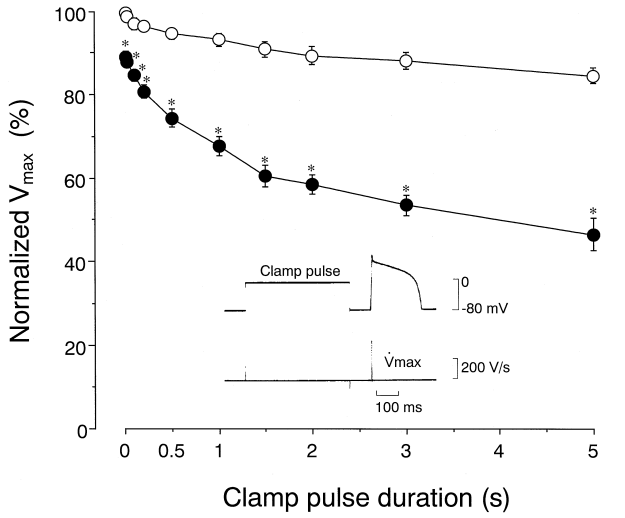

Fig. 4. Effects of single 0 mV conditioning clamp pulse on the V_{max} inhibition induced by cibenzoline. Ordinate scale: V_{max} of test action potential normalized by the value of action potential without clamp pulse (taken at the tonic block subtracted value). Abscissa scale: duration of the conditioning 0 mV clamp. Values are presented as means ± S.E.M. for four cells. Data were obtained before (control, ○) and 15–20 min after application of cibenzoline at 10 μM (●). ∗Significantly different from control at $P < 0.05$.

significant effect on the V_{max} of the test action potential. However, further prolongation of the 0 mV clamp resulted in a slight but significant V_{max} reduction, probably due to slow inactivation of Na^+ channels (Saikawa and Carmeliet, 1982; Clarkson et al., 1984). A clamp pulse lasting 5000 ms decreased V_{max} by $15.5 \pm 1.9\%$ ($n = 4$) from the value of the action potential without the conditioning clamp (reference level). Treatment of the myocytes with cibenzoline (10 μM) for 15 min did not affect the resting potential. The V_{max} of the reference action potential was decreased by $6.1 \pm 2.8\%$ with cibenzoline ($n = 4$) from the value before drug application. In such myocytes, a conditioning 0 mV clamp caused a much greater reduction in the V_{max} of the test action potential. In the presence of cibenzoline, the shortest clamp pulse (10 ms) caused a significant V_{max} reduction by $11.0 \pm 1.4\%$ ($n = 4$) from the reference level. When the clamp pulse duration was prolonged, the V_{max} reduction was enhanced gradually, and the V_{max} reduction reached $53.5 \pm 3.9\%$ at 5000 ms ($n = 4$).

In the third protocol, short (10 ms) or long (200 ms) depolarizing trains of pulses were applied from the resting level (-80 mV) to 0 mV with an interpulse interval of 500 ms (Fig. 4). Such trains of depolarizing pulses caused no significant decrease in V_{max} before drug application (control). In the presence of cibenzoline (3 μM), both short and long pulse trains caused a progressive V_{max} reduction in a pulse number-dependent manner. The use-dependent V_{max} reduction elicited by 20 pulses was $15.1 \pm 2.8\%$ ($n = 4$) for short pulse trains and $19.8 \pm 3.1\%$ ($n = 4$) for long pulse trains. The block increase at the longer pulse (by 31%) was small but statistically significant.

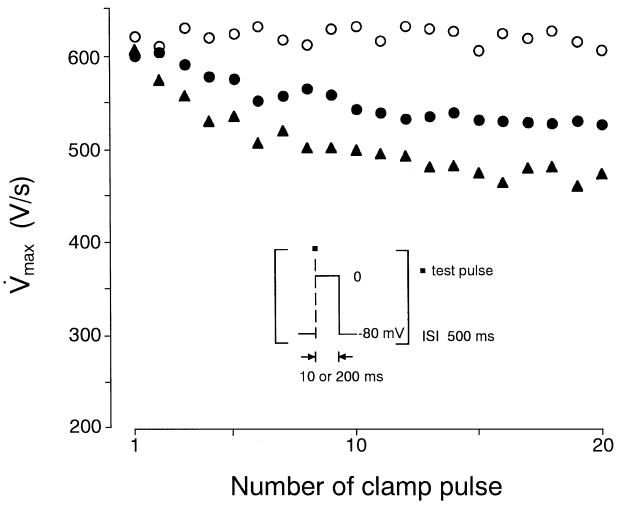

Fig. 5. Use-dependent decrease of the maximum upstroke velocity (V_{max}) of the action potential in single ventricular cells. Following a rest period of 90 s, a train of short (10 ms) or long (200 ms) depolarization from the resting potential level (-80 mV) to 0 mV was applied in voltage-clamp mode with an interpulse interval of 500 ms. Each depolarizing pulse was preceded by a 4-ms window of current clamp mode, during which the action potential upstroke to measure V_{max} was elicited. Ordinate scale: V_{max}, Abscissa scale: number of pulses from the initiation of the train. Data were obtained before (control ○, 10 ms pulses), and 15–20 min after application of cibenzoline at 3 μM (●, 10 ms pulses; ▲, 200 ms pulses).

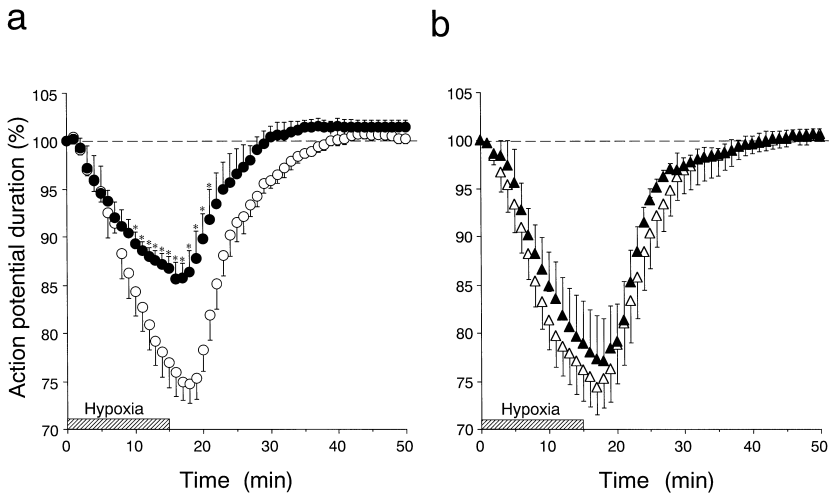

Fig. 6. Effects of glibenclamide and cibenzoline on the hypoxia-induced shortening of action potential duration in papillary muscles. The superfusion medium was switched from normoxic Krebs–Ringer solution to hypoxic, glucose-free Krebs–Ringer solution for 15 min, and then returned to the normal solution (reoxygenation). Ordinate scales: action potential duration at 80% repolarization normalized by the value before the hypoxia. Abscissa scales: time after the initiation of hypoxia. Data were obtained before (control ○, △) and after pretreatment with glibenclamide (30 μM, ●, a) or cibenzoline (10 μM, ▲, b). Values are means ± S.E.M. ($n = 4$). ∗Significantly different from control at $P < 0.05$.

3.5. Effects of cibenzoline on the action potential shortening in hypoxic papillary muscle

It was found in our preliminary experiments that exposure of guinea-pig papillary muscle to hypoxic glucose-free Krebs–Ringer solution for 15 min resulted in a substantial shortening of the action potential duration, which was reversed during subsequent reoxygenation with normal Krebs–Ringer solution. The shortening of the action potential duration with the first hypoxic period was often variable, but the shortening with the second and third hypoxic periods was reproducible. We, therefore, examined the drug effects in each muscle during the third hypoxic period reference to the second hypoxic period as control.

The results obtained in eight muscles are summarized in Fig. 5. The control hypoxia (without drugs) caused a progressive shortening of the action potential duration (at 80% repolarization) of guinea-pig papillary muscles constantly stimulated at 1.0 Hz. The shortening reached a peak of 2–4 min after switching to the normal oxygenated Krebs–Ringer solution. The action potential duration then recovered gradually during the subsequent period of reoxygenation; full recovery was obtained at 22–25 min of reoxygenation.

Treatment of the muscle with 30 μM glibenclamide for 50 min did not affect the action potential configuration under the normoxic condition. During hypoxia, glibenclaminde significantly prevented the shortening of action potential duration (Fig. 6): the maximum shortening at 80% repolarization was 26.6 ± 3.4% and 15.2 ± 2.7% in the absence and presence of glibenclamide, respectively ($n = 4$, $P < 0.05$).

Treatment of the muscle with 10 μM cibenzoline for 50 min caused a significant reduction of V_{max} by 35.3 ± 4.0% under the normoxic condition ($n = 4$). Unlike glibenclamide, however, cibenzoline did not significantly prevent the shortening of the action potential duration during hypoxia (Fig. 6): the maximum shortening at 80% repolarization was 24.6 ± 2.8% and 23.3 ± 5.2% in the absence and presence of cibenzoline, respectively ($n = 4$).

4. Discussion

The present experiments on guinea-pig papillary muscles revealed that cibenzoline ≥ 3 μM causes a significant concentration-dependent decrease in V_{max} of the action potential without affecting the resting membrane potential and the action potential duration. The decrease in V_{max} by cibenzoline reflects an inhibitory effect of the drug on I_{Na}, since the entire muscle was excited simultaneously and there was no conduction within the preparation. Furthermore, a similar V_{max} inhibition was observed in single ventricular myocytes. The probable convex-shaped nonlinear relationship between V_{max} and peak I_{Na} in cardiac cells (Cohen et al., 1984; Sheets et al., 1988) might introduce variable errors in estimating the precise extent of Na^+ channel block. Nevertheless, such a limitation does not invalidate V_{max} as a qualitative index. The voltage clamp techniques currently available for I_{Na} measurement require more artificial experimental conditions (low temperature and low extracellular Na^+ concentrations) than those for V_{max} measurement.

The inhibition of V_{max} by cibenzoline was enhanced at the higher stimulation frequency. In papillary muscles, cibenzoline at concentrations ranging from 1 to 10 μM caused a minimal tonic V_{max} inhibition despite their marked use-dependent V_{max} inhibition. Cibenzoline, like most other

Class I antiarrhythmic drugs, may bind to the Na^+ channel receptor mainly in an activated and/or inactivated state (Grant et al., 1995). This characteristic would lead to an accumulation of blocked channels during stimulation trains above a certain rate.

The use-dependent block of V_{max} by cibenzoline in guinea-pig papillary muscle was observed during stimulation trains at rates ≥ 0.2 Hz. The onset rates of use-dependent block with cibenzoline were slow (0.11 per action potential at 1.0 Hz with 3 μM). The offset kinetics of use-dependent block by cibenzoline were also slow: the average time constant of V_{max} recovery (τ_R) was 26.2 s. The τ_R value for cibenzoline was comparable to that for diprafenone, moricizine, flecainide and disopyramide in our experiments on guinea-pig papillary muscles (Table 3). These facts may indicate that cibenzoline belongs to the group of Class I drugs with slow kinetics in terms of binding to and unbinding from cardiac Na^+ channels.

The tonic block of V_{max} with cibenzoline was more pronounced in partially depolarized papillary muscles with high $[K^+]_o$. In experiments to examine the relationship between V_{max} and the membrane potential following a rest period of 90 s, V_h was shifted in a hyperpolarizing direction by 7.1 mV. These observations are most likely explained by a higher affinity of cibenzoline for inactivated Na^+ channels than for resting ones (Grant et al., 1984).

We further investigated the state dependence of the Na^+ channel block produced by cibenzoline in single cell experiments by using single and multiple 0 mV conditioning clamp pulses. The single pulse protocol was essentially the same as in our previous studies (Kodama et al., 1990, 1996). In the presence of cibenzoline (10 μM), the shortest (10 ms) conditioning clamp caused a substantial V_{max} reduction of the test action potential. The V_{max} reduction was enhanced moderately by prolongation of the conditioning clamp duration (especially when the duration was longer than 0.5 s). The approximate extent of the activated Na^+ channel block (ACB), which was defined as the percent reduction of V_{max} elicited by a 10-ms clamp pulse from the reference level (tonic block subtracted value), was estimated to be on average 11.0%. The approximate extent of the inactivated Na^+ channel block (ICB), which was defined by the additional percent decrease in V_{max} when the clamp pulse duration was prolonged from 10 ms to 500 ms, was 16.4%. The ratio of ICB/ACB for cibenzoline (1.5) was comparable to that of quinidine, disopyramide and diprafenone, but appreciably lower than that of aprindine, mexiletine, lidocaine, and moricizine (Table 4).

The index obtained in single conditioning voltage clamp experiments (ICB/ACB) should be interpreted with caution because of several drawbacks as acknowledged in our previous report (Kodama et al., 1990). First, the decrease in V_{max} following a 10-ms clamp pulse may reflect not only ACB but also a part of ICB, since the Na^+ channel inactivates nearly completely within several milliseconds at 0 mV. Second, a small but significant fraction of Na^+ channels remain open during depolarization at 0 mV (Grant et al., 1995). The progressive decrease of V_{max} during the prolonged depolarization may, therefore, include not only ICB but also part of ACB. Third, during the 100-ms coupling interval a variable block dissipation may occur, leading to an attenuation of the change in V_{max}. This effect would be greater for drugs with faster dissociation kinetics. In addition, a part of ACB is supposed to occur during the upstroke phase of the reference action potential, leading to a decrease of their V_{max} values (Grant et al., 1984). Despite these limitations, this index is useful to estimate roughly the state dependence of Na^+ channel block by individual drugs when action potentials are elicited from the normal resting membrane potential.

Table 3
Onset and offset kinetics of the use-dependent block of V_{max} with Class I antiarrhythmic drugs

Drugs	Concentration (μM)	Stimulation frequency (Hz)	Use-dependent block	
			Onset rate (per action potential)	Recovery time constant (s)
Lidocaine	20	2.0	Very rapid	0.19
Mexiletine	20	2.0	Very rapid	0.35
Tocainide	100	2.0	Very rapid	0.46
Phenytoin	100	2.0	Very rapid	0.52
Aprindine	3	1.0	0.28	5.1
Procainamide	200	1.0	0.23	5.6
Quinidine	10	1.0	0.19	6.8
Propafenone	3	1.0	0.14	8.8
Diprafenone	3	1.0	0.09	15.5
Cibenzoline	10	1.0	0.14	26.2
Moricizine	3	1.0	0.06	26.4
Flecainide	3	1.0	0.06	29.0
Disopyramide	30	1.0	0.27	43.0

The onset rate and the recovery time constant of the use-dependent block elicited by 13 Class I drugs were calculated by exponential analysis of the V_{max} changes of guinea-pig papillary muscles during and after stimulation trains at 1.0 Hz or 2.0 Hz.
Values are means of four to eight experiments for each drug.
Data for the drugs other than cibenzoline are from Kodama et al. (1996).

Table 4
Activation- and inactivation-dependent block of cardiac Na^+ channels with Class I antiarrhythmic drugs estimated from the V_{max} change of guinea-pig ventricular myocytes

Drugs	Concentration (μM)	ACB (%)	ICB (%)	ICB/ACB
Aprindine	3	5.2	49.6	9.5
Mexiletine	40	5.0	31.8	6.4
Lidocaine	40	6.1	36.1	5.9
Moricizine	3	2.0	8.2	5.5
Propafenone	10	6.8	21.1	3.1
Quinidine	30	8.8	14.5	1.6
Cibenzoline	10	11.0	16.4	1.5
Disopyramide	100	9.4	14.1	1.5
Diprafenone	10	10.1	12.8	1.3

Values are means of four to six experiments for each drug.
ACB: activated channel block defined as a percent decrease in V_{max} from the action potential without conditioning clamp pulse to that preceded by a 10-ms clamp pulse.
ICB: inactivated channel block defined as the additional percent decrease in V_{max} when the clamp pulse duration was prolonged from 10 to 500 ms.
ACB, ICB and ICB/ACB values are means for each drug.
Data for the drugs other than cibenzoline are from Kodama et al. (1996).

In our single cell experiments using multiple 0 mV conditioning clamps, both the short (10 ms) and the long (200 ms) pulse trains applied to myocytes treated with cibenzoline caused a marked use-dependent V_{max} reduction, and the enhancement of the V_{max} reduction by the prolongation of the clamp pulse duration was 31%. This may suggest that the use-dependent Na^+ channel block elicited by cibenzoline with repeated ventricular action potentials from normal resting membrane potential occurs mainly during the activated state of the channel.

In experiments to change the resting membrane potential of papillary muscles by altering $[K^+]_o$, the intensity of the use-dependent block of V_{max} with cibenzoline was enhanced and the time constant of V_{max} recovery (τ_R) was shortened at less negative membrane potentials. These properties could be explained by the high affinity of cibenzoline for the activated state of the sodium channel.

V_{max} recovery from the use-dependent block with lidocaine and mexiletine is known to be accelerated at more negative membrane potentials (hyperpolarization). This has been attributed to the higher affinity of these drugs for the inactivated state of the Na^+ channel than the resting state. Under such conditions, hyperpolarization would enhance drug dissociation from channel receptors by increasing the resting fraction of drug-associated channels at the expense of their inactivated fraction. The voltage dependence of V_{max} recovery for cibenzoline is opposite to that of these drugs and similar to that reported for penticainide (Carmeliet, 1988), disopyramide (Gruber and Carmeliet, 1989), pirmenol (Nakaya et al., 1992) and diprafenone (Kodama et al., 1992). This reverse voltage dependence of V_{max} recovery has been interpreted as 'activation trapping' (Carmeliet, 1988; Gruber and Carmeliet, 1989). According to the hypothesis, drug molecules, which bind to and unbind from Na^+ channels primarily during the activated state, are trapped in the channel when it returns from the activated to the resting state. Since the probability of having the activation gate in the open position will be smaller the more negative the membrane potential, dissociation of the drugs from channel receptors would be accelerated by depolarization. Dissociation (unbinding) of drugs from Na^+ channels during the activated state (activation unblock) is enhanced by hyperpolarization of the resting membrane potential (Anno and Hondeghem, 1990), leading to an attenuation of the extent of block during stimulation trains. In fact, as for the present data for cibenzoline, the extent of use-dependent block of V_{max} or I_{Na} elicited by penticainide, disopyramide and flecainide is attenuated by hyperpolarization and enhanced by depolarization of the membrane potential within the range of -90 to -70 mV (Carmeliet, 1988; Gruber and Carmeliet, 1989; Anno and Hondeghem, 1990).

It was shown in whole-cell voltage clamp experiments in single guinea-pig ventricular cells by Wu et al. (1992) and Sato et al. (1993) that the time-independent outward current induced by 2,4-dinitrophenol or by diazoxide was inhibited almost completely by 5–10 μM cibenzoline. They, therefore, concluded that cibenzoline may have a potent inhibitory action against ATP-sensitive K^+ (K_{ATP}) channels at concentrations close to the therapeutic plasma level (1–5 μM). In support of this hypothesis, Sato et al. (1993) demonstrated that cibenzoline (10 μM) significantly reversed the 2,4-dinitrophenol-induced shortening of the action potential duration in guinea-pig ventricular cells. Millar and Vaughan Williams (1982) also reported that cibenzoline (2.63 μM) prevented the shortening of action potential duration induced by hypoxia in isolated rabbit atria. However, no information is available about the potential protective effects of cibenzoline against the hypoxia-induced shortening of the action potential duration in ventricular muscles at concentrations that cause substantial Na^+ channel block. We, therefore, tested the effects of cibenzoline on the action potential duration of guinea-pig papillary muscles exposed to hypoxia in comparison with glibenclamide, a standard K_{ATP} channel blocker. The shortening of the action potential duration during superfusion with hypoxic glucose-free solution was prevented significantly by pretreatment with 30 μM glibenclamide, as reported previously by other investigators (Wilde et al., 1990; Deutsch et al., 1991; Nakaya et al., 1991). Pretreatment with 10 μM cibenzoline, which caused V_{max} reduction by 35%, however, had no significant effect on the hypoxia-induced shortening of the action potential duration. These results suggest that the K_{ATP} channel blocking action of cibenzoline at therapeutic concentrations may be minimal or negligible in multicellular ventricular muscle. K_{ATP} channel activity is known to be affected by various intracellular cofactors including adenosine diphosphate, acidosis, and protein kinase C (Weiss, 1995; Light et al., 1996). The efficacy of sulfonylureas to block cardiac K_{ATP}

channels is also influenced by intracellular nucleotide diphosphates (Venkatesh et al., 1991; Weiss and Venkatesh, 1993; Brady et al., 1998). Different levels of these cofactors could be involved in the discrepancy between the present data and those of whole-cell clamp experiments (Wu et al., 1992; Sato et al., 1993).

Based on the results obtained in the present study, we conclude that cibenzoline may block the Na^+ channel mainly during the activated state. From the onset and offset kinetics of use-dependent block, cibenzoline belongs to the group of Class I drugs with slow kinetics. The inhibitory action of this compound on the ATP-sensitive potassium current ($I_{K,ATP}$) would be minimal or negligible in intact hearts at concentrations causing sufficient Class I action.

References

Anno, T., Hondeghem, L.M., 1990. Interaction of flecainide with guinea-pig cardiac sodium channels: importance of activation unblocking to the voltage dependence of recovery. Circ. Res. 66, 789–803.

Brady, P.A., Alekseev, A.E., Terzic, A., 1998. Operative condition-dependent response of cardiac ATP-sensitive K^+ channels toward sulfonylureas. Circ. Res. 82, 272–278.

Carmeliet, E., 1988. Activation block and trapping of penticainide, a disopyramide analogue, in the Na^+ channel of rabbit cardiac Purkinje fibers. Circ. Res. 63, 50–60.

Carmeliet, E., Vereecke, J., 1979. Electrogenesis of the action potential and automaticity. In: Berne, R.M., Geiger, S.R. (Eds.), Handbook of Physiology, The Cardiovascular System, I. American Physiological Society, Bethesda, MD, pp. 269–334.

Clarkson, C.W., Matsubara, T., Hondeghem, L.M., 1984. Slow inactivation of V_{max} in guinea-pig ventricular myocardium. Am. J. Physiol. 247, H645–H654.

Cohen, C.J., Bean, B.P., Tsien, R.W., 1984. Maximal upstroke velocity as an index of available sodium conductance: comparison of maximal upstroke velocity and voltage clamp measurements of sodium current in rabbit Purkinje fibers. Circ. Res. 54, 635–641.

Dangman, K.H., 1984. Cardiac effects of cibenzoline. J. Cardiovasc. Pharmacol. 6, 300–311.

Dangman, K.H., Miura, D.S., 1986. Cibenzoline. In: Scriabine, A. (Ed.), New Cardiovascular Drugs. Raven Press, New York, pp. 107–128.

Deutsch, N., Klitzner, T.S., Lamp, S.T., Weiss, J.N., 1991. Activation of cardiac ATP-sensitive K^+ current during hypoxia: correlation with tissue ATP levels. Am. J. Physiol. 261, H671–H676.

Ebihara, L., Johnson, E.A., 1980. Fast sodium current in cardiac muscle: a quantitative description. Biophys. J. 32, 779–790.

Ferrero, J.M. Jr., Saiz, J., Ferrero, J.M., Thakor, N.V., 1996. Simulation of action potentials from metabolically impaired cardiac myocytes. Role of ATP-sensitive K^+ current. Circ. Res. 79, 208–221.

Grant, A.O., Starmer, C.F., Strauss, H.C., 1984. Antiarrhythmic drug action: blockade of the inward sodium current. Circ. Res. 55, 427–439.

Grant, A.O., Whalley, D.W., Wendt, D.J., 1995. Pharmacology of the cardiac sodium channel. In: Zipes, D.P., Jalife, J. (Eds.), Cardiac Electrophysiology. From Cell to Bedside. Saunders, Philadelphia, pp. 247–259.

Gruber, R., Carmeliet, E., 1989. The activation gate of the sodium channel controls blockade and deblockade by disopyramide in rabbit Purkinje fibers. Br. J. Pharmacol. 97, 41–50.

Harron, W.G., Brogden, R.N., Faulds, D., Fitton, A., 1992. Cibenzoline: a review of its pharmacological properties and therapeutic potential in arrhythmias. Drugs 43, 734–759.

Holck, M., Osterrieder, W., 1986. Inhibition of the myocardial Ca^{2+} inward current by the Class I antiarrhythmic agent, cibenzoline. Br. J. Pharmacol. 87, 705–711.

Kodama, I., Kondo, N., Shibata, S., Yamada, K., 1985. Effects of dimethylpropranolol (UM-272) on the electrophysiological properties of guinea-pig ventricular muscles. J. Pharmacol. Exp. Ther. 234, 507–514.

Kodama, I., Honjo, H., Kamiya, K., Toyama, J., 1990. Two types of sodium channel block by Class I antiarrhythmic drugs studied by using V_{max} of action potential in single ventricular myocytes. J. Mol. Cell. Cardiol. 22, 1–12.

Kodama, I., Suzuki, R., Honjo, H., Toyama, J., 1992. Electrophysiological effects of diprafenone, a dimethyl congener of propafenone on guinea-pig ventricular cells. Br. J. Pharmacol. 107, 813–820.

Kodama, I., Suzuki, R., Honjo, H., Miyawaki, N., Toyama, J., 1996. Electropharmacology of SD-3212, a new antiarrhythmic agent. Cardiovasc. Drug Rev. 14, 135–147.

Kotake, H., Matsuoka, S., Ogino, K., Takami, T., Hasegawa, J., Mashiba, H., 1987. Electrophysiological study of cibenzoline in voltage-clamped rabbit sinoatrial node preparations. J. Pharmacol. Exp. Ther. 241, 982–986.

Lederer, W.J., Nichols, C.G., 1995. Regulation and function of adenosine triphosphate-sensitive potassium channels in the cardiovascular system. In: Zipes, D.P., Jalife, J. (Eds.), Cardiac Electrophysiology. From Cell to Bedside. Saunders, Philadelphia, pp. 103–115.

Light, P.E., Sabir, A.A., Allen, B.G., Walsh, M.P., French, R.J., 1996. Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K^+ channels: a possible mechanistic link to ischemic preconditioning. Circ. Res. 79, 399–406.

Masse, C., Cazes, M., Sassine, A., 1984. Effects of cibenzoline, a novel antiarrhythmic drug, on action potential and transmembrane currents in frog atrial muscle. Arch. Int. Pharmacodyn. 269, 219–235.

Matsuoka, S., Nawada, T., Hisatome, I., Miyamato, J., Hasegawa, J., Kotake, H., Mashiba, H., 1991. Comparison of Ca^{2+} channel inhibitory effects of cibenzoline with verapamil on guinea-pig heart. Gen. Pharmacol. 22, 87–91.

Millar, J.S., Vaughan Williams, E.M., 1982. Effects on rabbit nodal, atrial, ventricular and Purkinje cell potentials of a new antiarrhythmic drug, cibenzoline, which protects against action potential shortening in hypoxia. Br. J. Pharmacol. 75, 469–478.

Millar, J.S., Vaughan Williams, E.M., 1983. Pharmacological mapping of regional effects in the rabbit heart of some new antiarrhythmic drugs. Br. J. Pharmacol. 79, 701–709.

Nakaya, H., Takeda, Y., Tohse, N., Kanno, M., 1991. Effects of ATP-sensitive K^+ channel blockers on the action potential shortening in hypoxic and ischaemic myocardium. Br. J. Pharmacol. 103, 1019–1026.

Nakaya, H., Shishido, Y., Oyama, Y., Kanno, M., 1992. Voltage-dependent modification of V_{max} recovery from use-dependent block by pirmenol in guinea-pig papillary muscles: comparison with other Class I drugs. J. Cardiovasc. Pharmacol. 19, 140–147.

Saikawa, T., Carmeliet, E., 1982. Slow recovery of the maximum rate of rise (V_{max}) of the action potential in sheep cardiac Purkinje fibers. Pflüg. Arch. 349, 90–93.

Sato, T., Wu, B., Nakamura, S., Kiyosue, T., Arita, M., 1993. Cibenzoline inhibits diazoxide- and 2,4-dinitrophenol-activated ATP-sensitive K^+ channels in guinea-pig ventricular cells. Br. J. Pharmacol. 108, 549–556.

Sato, T., Wu, B., Kiyosue, T., Arita, M., 1994. Effects of cibenzoline, a new Class Ia antiarrhythmic drug, on various membrane ionic currents and action potentials of guinea-pig ventricular cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 167–173.

Satoh, H., Ishii, M., Hashimoto, K., 1987. Effect of cibenzoline, a Class I antiarrhythmic drug, on action potential in canine ventricular muscle. Jpn. J. Pharmacol. 44, 113–119.

Sheets, M.F., Hank, D.A., Fozzard, H.A., 1988. Nonlinear relation between V_{max} and I_{Na} in canine cardiac Purkinje cells. Circ. Res. 63, 386–398.

Toyama, J., Kamiya, K., Kodama, I., Yamada, K., 1987. Frequency- and voltage-dependent effects of aprindine on the upstroke velocity of action potential in guinea-pig ventricular muscles. J. Cardiovasc. Pharmacol. 9, 165–172.

Venkatesh, N., Lamp, S.T., Weiss, J.N., 1991. Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. Circ. Res. 69, 623–637.

Wang, D.W., Kiyosue, T., Sato, T., Arita, M., 1996. Comparison of the effects of Class I anti-arrhythmic drugs, cibenzoline, mexiletine and flecainide, on the delayed rectifier K^+ current of guinea-pig ventricular myocytes. J. Mol. Cell. Cardiol. 28, 893–904.

Weiss, J.N., 1995. ATP-sensitive K^+ channels in myocardial ischemia and hypoxia. In: Toyama, J., Hiraoka, M., Kodama, I. (Eds.), Recent Progress in Electropharmacology of the Heart. CRC Press, Boca Raton, New York, pp. 49–61.

Weiss, J.N., Venkatesh, N., 1993. Metabolic regulation of cardiac ATP-sensitive K^+ channels. Cardiovasc. Drugs Ther. 7, 499–505.

Wilde, A.A.M., Escande, D., Schmacher, C.A., Thuringer, D., Mestre, M., Fiolet, J.W.T., Janse, M.J., 1990. Potassium accumulation in the globally ischemic mammalian heart: a role for the ATP-sensitive potassium channel. Circ. Res. 67, 835–843.

Wu, B., Sato, T., Kiyosue, T., Arita, M., 1992. Blockade of 2,4-dinitrophenol induced ATP sensitive potassium current in guinea-pig ventricular myocytes by Class I antiarrhythmic drugs. Cardiovasc. Res. 26, 1095–1192.

Wu, S.-N., Nakajima, T., Yamashita, T., Hamada, E., Hazama, H., Iwasaka, K., Omata, M., Kurachi, Y., 1994. Molecular mechanism of cibenzoline-induced anticholinergic action in single atrial myocytes: comparison with effect of disopyramide. J. Cardiovasc. Pharmacol. 23, 618–624.