Frequency and Voltage-Dependent Depression of Maximum Upstroke Velocity of Action Potentials by Pirmenol in Guinea Pig Ventricular Muscles

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Abstract—The frequency-dependency and voltage-dependency of the suppressing effect of pirmenol, a novel antiarrhythmic agent, on the maximum upstroke velocity (V_max) of action potentials were examined and compared with those of disopyramide in guinea pig papillary muscles. Pirmenol in concentrations higher than 3 μM decreased V_max with a slight increase in action potential duration. The reduction of V_max by pirmenol was enhanced in a frequency-dependent manner over the range of 0.1–2.0 Hz. Pirmenol (30 μM) produced a small resting block (5.5%), whereas disopyramide (100 μM) produced a greater one (25.8%). The onset of frequency-dependent V_max reduction at 2.0 Hz followed a monoexponential function with a slow rate constant (0.308±0.055 AP^1). The time constant for the recovery from the frequency-dependent block by pirmenol was also slow (33.5±5.4 sec), but faster than that of disopyramide (82.5±12.3 sec). At 1.0 Hz, pirmenol caused a shift (9.5 mV) of the curve relating the resting membrane potential and V_max along the voltage axis in the hyperpolarizing direction. Thus, pirmenol is a Class 1a drug that has frequency- and voltage-dependent inhibitory actions on V_max, and its onset and offset kinetics are relatively slow.

It is well-established that Class I antiarrhythmic drugs can be subclassified into 3 groups based on their effect on the action potential duration (APD) and electrocardiographic parameters (1, 2). Recently, it has become apparent that there are also marked differences among Class I drugs in their onset and offset kinetics of the frequency-dependent (use- or rate-dependent) inhibition of the maximum upstroke velocity (V_max) of action potentials. From their kinetic spectrum, Class I drugs are now divided into three groups: fast, intermediate, and slow kinetic drugs (3–5). Frequency-dependency as well as voltage-dependency of the V_max depression by Class I drugs are suggested to contribute to their antiarrhythmic profile in treating various clinical arrhythmias (6–8).

Pirmenol is a new Class I antiarrhythmic drug with chemical structures somewhat similar to disopyramide, but has less potent anticholinergic action than disopyramide (9). This drug is reportedly effective against various arrhythmias in experimental animals (10) and humans (11–15). It has been demonstrated that pirmenol depresses the V_max of action potentials in Purkinje fibers (16, 17), atrial muscles and ventricular muscles (17), but has little effect on action potentials in the sinoatrial and ativoventricular nodes (17). However, the frequency-dependency and voltage-dependency of V_max depression by this drug have not been thoroughly evaluated. Therefore, the present study was undertaken to determine whether pirmenol-induced V_max depression was modulated by stimulation rate and membrane potential. Guinea pig papillary muscle was chosen to facilitate comparison of our experimental results with previously published studies on the frequency-dependent block of V_max by Class I antiarrhythmic drugs in the same tissue.

Materials and Methods
Guinea pigs, weighing 250–400 g, were...
killed by a blow on the head, and their hearts were rapidly removed. The heart was immersed in an oxygenated Tyrode’s solution, and papillary muscles less than 1 mm in diameter were dissected from the right ventricle. The preparations were pinned to the bottom of a 5-ml tissue chamber and continuously superfused with the modified Tyrode’s solution equilibrated with 95% O₂ and 5% CO₂. The composition of the solution was as follows: 132 mM NaCl, 4 mM KCl, 1.0 mM MgCl₂, 1.8 mM CaCl₂, 0.4 mM NaH₂PO₄, 12 mM NaHCO₃ and 10 mM glucose. The temperature of the tissue bath was maintained at 36.0±1.0°C.

The preparation was electrically stimulated with pulses of 1 msec duration at twice the diastolic threshold with a pair of bipolar electrodes. The rate of stimulation was 1.0 Hz during the equilibration period; other stimulation rates when used were mentioned in the text. The stimuli were delivered from an electronic stimulator (Nihon Kohden SEN 6100, Tokyo, Japan) through an isolation unit (Nihon Kohden SS-302J). Transmembrane action potentials were recorded using glass microelectrodes filled with 3 M KCl, which had a tip resistance of 10–30 MΩ. The microelectrode was coupled with an Ag-AgCl junction and connected to the input stage of a high impedance amplifier with capacitance neutralization (Nihon Kohden MEZ 8201). An agar bridge containing 3 M KCl was used as a reference electrode. The first time derivative of the transmembrane action potential was obtained using an electronic differentiator with a linearity up to 800 V/sec. The amplified signals were displayed on a dual-beam oscilloscope (Nihon Kohden VC-9) and photographed by a camera (Nihon Kohden RLG-6101). Film analysis was used to measure action potential parameters including Vmax.

After an equilibration period of 1–2 hr, control recordings were made under 1.0 Hz stimulation. The preparations were then exposed to a solution containing the lowest concentration of pirmenol or disopyramide, and their concentration was increased in a stepwise fashion at intervals of 20 min until it reached 100 μM for pirmenol and 300 μM for disopyramide. Preliminary experiments revealed that a 20-min superfusion period was sufficient for action potential changes to reach a steady state. The recording of transmembrane potential was repeated at the end of each drug superfusion period.

In order to study the frequency-dependent block of Vmax, the preparations were stimulated repetitively at varying rates ranging from 0.1 to 2.0 Hz for 30 beats. Rest periods of 5 min were interposed between trains of stimuli to ensure full recovery from the frequency-dependent decrease in Vmax.

The recovery from the block of Vmax that developed during a train of stimuli was also studied. This process was quantified by determining the time course of the recovery from Vmax depression produced by 30 pulses delivered at 1.0 Hz. Following a 3 min pulse-free period, the train of stimuli was delivered; after a variable pause, a single test pulse was then given, and the Vmax for this pulse was compared to the first pulse in the stimulus train. This procedure was repeated 10–15 times while varying the coupling interval.

In these experiments in which the onset or offset kinetics from the rate-dependent block was evaluated, measurements were repeated before and after the 30-min drug superfusion period, during which the stimulator was switched off and the tissue was allowed to rest. The onset of and recovery from the frequency-dependent block by pirmenol and disopyramide were fitted by monoexponential functions for calculation of rate constants and time constants.

The relationship between Vmax and resting membrane potential was analyzed at a rate of 0.01 Hz or 1.0 Hz. In order to change the resting membrane potential over a wide range, [K+]o was increased from 3 mM to 20 mM in 1 mM increments by adding small aliquots of 2.0 M KCl to the superfusion solution. After high K⁺ Tyrode’s solution caused failure of conduction of action potentials, the perfusate was then changed back to 3 mM K⁺ Tyrode’s solution. Following reattainment of the control value for Vmax, the perfusate was changed to a solution containing a drug for a period of 30 min, during which Vmax fell to a new steady level. Subsequently, the preparations were again depolarized slowly by raising [K⁺]o in the presence of the drug.
Pirmenol was used as the hydrochloride salt, supplied from Warner Lambert (Tokyo, Japan). Disopyramide phosphate was obtained from Chugai Pharmaceutical Company (Tokyo, Japan). Stock solutions of these drugs were prepared as 10 mM solutions in distilled water and diluted in Tyrode’s solution as required.

Only experiments in which a single impalement was maintained from the control period through the drug application were used for data analysis. Statistical analyses of drug-induced changes of action potential parameters were performed using Student’s t-test. Significance was established when the probability value was less than 0.05. All data are presented as means±standard errors.

**Results**

Effects of pirmenol and disopyramide on the transmembrane action potentials: Action potentials recorded from 10 guinea pig papillary muscles at a stimulation rate of 1.0 Hz in the drug-free Tyrode’s solution had the following characteristics: resting membrane potential (RMP), -92.3±0.3 mV; the maximum rate of rise (Vmax), 240.5±14.2 V/sec; action potential amplitude (APA), 127.5±0.7 mV; action potential duration at 20% repolarization (APD20), 116.5±3.1 msec; APD at 50% repolarization (APD50), 178.0±4.5 msec; and APD at 90% repolarization (APD90), 207.0±5.2 msec.

Figure 1 illustrates typical effects of pirmenol (3 and 30 μM, panel A) and disopyramide (10 and 100 μM, panel B) on the action potential configuration and Vmax in preparations constantly driven at 1.0 Hz. Either pirmenol or disopyramide decreased Vmax in a concentration-dependent manner. Lower concentrations of these drugs prolonged APDs, especially at the 90% repolarization level, whereas higher concentrations of these drugs shortened APD20 and APD50.

Changes of Vmax and APDs produced by pirmenol and disopyramide are summarized in Fig. 2. Pirmenol at concentrations higher than 3 μM significantly decreased Vmax. Disopyramide also caused a concentration-dependent decrease in Vmax. However, the inhibitory action on Vmax was about one-third less potent than that of pirmenol on a molar basis. APDs were prolonged by lower concentrations of these drugs, but shortened by higher concentrations. APA was also significantly decreased by high concentrations of pirmenol (100 μM) and disopyramide.

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**Fig. 1.** Effects of pirmenol (A) and disopyramide (B) on the action potential and Vmax in guinea pig papillary muscles driven at 1.0 Hz. In panel A, control action potential (left), action potential recorded 20 min after 3 μM (middle) and 30 μM pirmenol (right) are shown. In panel B, control action potential (left), action potential recorded 20 min after 10 μM (middle) and 100 μM disopyramide (right) are depicted. The upper, middle and lower tracings in each record indicate 0 potential, the transmembrane potential and dV/dt (on expanded time base), respectively. Calibrations in panel B are applicable to panel A.
No statistically significant changes in RMP were observed on addition of these drugs. All the changes of action potential parameters caused by these drugs reverted toward control levels after a 30-min washout period.

Fig. 2. Concentration-response curves for $V_{\text{max}}$ changes and APD (action potential duration) changes with pirmenol (open circles) and disopyramide (open triangles) in guinea pig papillary muscles. The ordinate scale represents changes of $V_{\text{max}}$ and APDs from the control, expressed as % changes ($V_{\text{max}}$) and $\mu$sec (APD$_{20}$, APD$_{50}$, APD$_{90}$). Concentrations of drugs are on the abscissae. Points are means±S.E. of five experiments. *Significant change (P<0.05) from the control value.

(30, 100, 300 $\mu$M). No statistically significant changes in RMP were observed on addition of these drugs. All the changes of action potential parameters caused by these drugs reverted toward control levels after a 30-min washout period.

**Frequency-dependent effects of pirmenol and disopyramide on $V_{\text{max}}$:** In the following experiments in which the frequency- and voltage-dependency of the drug-induced $V_{\text{max}}$ depression were investigated, we used 30 $\mu$M pirmenol and 100 $\mu$M disopyramide. These concentrations were chosen because of equipotent depression of $V_{\text{max}}$ by approximately 40% at a stimulation rate of 1.0 Hz (see Fig. 2). The rate of block development was studied by applying stimulation trains at different rates to the muscle after a pulse-free period that was long enough to allow complete recovery from the frequency-dependent block of $V_{\text{max}}$ (5 min for both drugs).

The $V_{\text{max}}$ of the first action potential obtained after a long period of quiescence (more than 5 min) was slightly depressed by 30 $\mu$M pirmenol. The decrease in the $V_{\text{max}}$ of the first beat of an induced train of action potentials from the control value, which is designated as a resting block (18, 19), was 5.5±0.7% for pirmenol. Disopyramide produced greater depression of the $V_{\text{max}}$ of the first beat, and the resting block with 100 $\mu$M disopyramide was 25.8±0.8%.

The $V_{\text{max}}$ reduction caused by 30 $\mu$M pirmenol was frequency-dependent over the range of 0.1–2.0 Hz (Fig. 3). The maximum reduction (42.8±1.0%) occurred at the highest frequency, and the $V_{\text{max}}$ decrease at 2.0 Hz was significantly greater than those at 0.1–0.5 Hz. However, the frequency-dependency of $V_{\text{max}}$ depression with 100 $\mu$M disopyramide was less overt than that with pirmenol at these stimulation rates. In disopyramide-treated preparations, the $V_{\text{max}}$ decrease at 2.0 Hz was not significantly different from those at 0.1–1.0 Hz. During the control period, $V_{\text{max}}$ did not show a frequency-dependent decrease, apart from a small reduction at 2.0 Hz (4.5±0.8%, P<0.05, n=10).

Although $V_{\text{max}}$ was only slightly decreased during a train of stimuli under the control conditions, in the drug-treated muscles, the
Fig. 3. Relationship between stimulation rate and the intensity of the frequency-dependent block. The ordinate indicates the percentage of decrease of $V_{\text{max}}$ from the first action potential of the stimulation train to the new steady state level. Abscissa shows the stimulation rate (Hz). Data were obtained before (solid circles) and 30 min after 30 $\mu$M pirmenol (open circles) or 100 $\mu$M disopyramide (open triangles). Values are expressed as means±S.E. of 10 preparations for the control and 5 preparations for each drug. *P<0.05, compared with the control $V_{\text{max}}$ value at the corresponding rate.

Fig. 4. Frequency-dependent decrease of $V_{\text{max}}$ by 30 $\mu$M pirmenol (A) and 100 $\mu$M disopyramide (B) under a stimulation of 2.0 Hz. In upper panels, the actual values of $V_{\text{max}}$ are indicated on the ordinates, and the beat numbers are on the abscissae. Closed and open symbols indicate the data in the absence and presence of drug, respectively. In lower panels, onset of the frequency-dependent block of $V_{\text{max}}$ after drug treatment is presented on a semilogarithmic scale. The difference of $V_{\text{max}}$ from the new steady state level during the stimulation train ($\Delta V_{\text{max}}$) is on the ordinate, and the beat numbers are on the abscissae. A regression line and a rate of onset are shown in the lower panels.
$V_{\text{max}}$ decreased gradually beat after beat and reached a new steady state level within 20 beats (Fig. 4, upper panels). The decrease of $V_{\text{max}}$ observed in these papillary muscles at 2.0 Hz stimulation were replotted on a semilogarithmic scale (Fig. 4, lower panels), and a regression analysis allowed the rate of onset of frequency-dependent block to be expressed in terms of the slope of that exponential. The onset rates for 30 $\mu$M pirmenol and 100 $\mu$M disopyramide at 2.0 Hz were 0.308±0.055 AP$^{-1}$ (n=5) and 0.423±0.050 AP$^{-1}$ (n=5), respectively. Thus, the onset of the frequency-dependent block was somewhat slower with pirmenol than with disopyramide when these concentrations were compared. The onset rate per action potential was larger at a lower stimulus frequency for the respective concentrations of these drugs. For example, in the presence of 30 $\mu$M pirmenol, the onset rate increased from 0.308±0.055 AP$^{-1}$ at 2.0 Hz to 0.545±0.049 AP$^{-1}$ at 0.1 Hz.

The recovery process of $V_{\text{max}}$ from the frequency-dependent block by pirmenol or disopyramide was studied by applying a single extra stimulus at various coupling intervals following a stimulus train of 1.0 Hz for 30 sec in 9 preparations. Semilogarithmic plots of the recovery time course of $V_{\text{max}}$ were made, and the recovery time courses for these drugs were found to fit well to a single exponential curve. Representative data obtained from preparations treated with 30 $\mu$M pirmenol and 100 $\mu$M disopyramide are

Fig. 5. Recovery of $V_{\text{max}}$ from the frequency-dependent block by pirmenol (A) and disopyramide (B). Upper panels: the actual value of the $V_{\text{max}}$ of the test action potential, which was introduced following a stimulation train at 1.0 Hz for 30 sec. The abscissa indicates the diastolic interval, which was measured from the end of the last action potential (APD$_{90}$) induced by the stimulation train to the upstroke of the test action potential. Data were obtained before (solid symbols) and 30 min after application of 30 $\mu$M pirmenol (open circles) or 100 $\mu$M disopyramide (open triangles). Lower panels: semilogarithmic plots of the time course of recovery of $V_{\text{max}}$ from the rate-dependent block. Ordinates indicate the fractional $V_{\text{max}}$ reduction to the test action potential as compared with $V_{\text{max}}$ in the first action potential of the stimulation train: $1 - [(V_{\text{max}})\text{ test}/(V_{\text{max}})\text{ first}]$. The straight line in the figure indicates the regression line and is the time constant of recovery.
shown in Fig. 5. The time constant for the recovery process from the pirmenol-induced block was 33.5±5.4 sec (n=5), whereas that from the disopyramide-induced block was 82.5±12.3 sec (n=4). Thus, the recovery time constant for pirmenol was relatively slow, but faster than that for disopyramide.

Voltage-dependent effects of pirmenol and disopyramide on V$_{\text{max}}$: The relationship between V$_{\text{max}}$ and the resting membrane potential (V$_{\text{m}}$), from which the action potential took off, was examined in papillary muscles constantly driven at 0.01 Hz and 1.0 Hz. The resting membrane potential was depolarized

![Graph](image-url)
in steps from its original resting level to around -60 mV by increasing the K⁺ concentration in the modified Tyrode's solution. The results obtained from a preparation stimulated at 0.01 Hz in the absence and presence of 30 μM pirmenol or 100 μM disopyramide are shown in Fig. 6. This infrequent stimulation was used in order to eliminate the frequency-dependent depression of Vₓₐₓₓ by the drugs. It can be seen that absolute value of Vₓₐₓₓ was reduced at all levels of membrane potential by pirmenol (Fig. 6A, left panel). When the curves were normalized, the curve obtained with pirmenol was still slightly shifted in the hyperpolarizing direction as compared with the drug-free curve (Fig. 6A, right panel). In the four preparations stimulated at 0.01 Hz, pirmenol slightly but significantly changed the Vₚ₀ value, the membrane potential at which the Vₓₐₓₓ value became 50% of that in 3 mM [K⁺]₀ solution, from -63.6±0.3 to -66.1±0.9 mV (P<0.05).

In contrast, disopyramide shifted the normalized Vₓₐₓₓ-Vₓₐₓ curve at 0.01 Hz up-

Fig. 7. Effects of pirmenol (A) and disopyramide (B) on the relationship between the membrane potential and Vₓₐₓₓ in preparations stimulated at 1.0 Hz. Symbols are the same as in Fig. 6. Absolute values are shown in the left panel and normalized values are in the right panel. Values for Vₚ₀ and S were (respectively): control: -61.8 mV, 3.25; 30 μM pirmenol: -74.4 mV, 6.16 in panel A, and control: -65.6 mV, 4.77; 100 μM disopyramide: -71.7 mV, 8.90 in panel B.
ward at $V_m$ between $-90$ and $-70$ mV, and the $V_{50}$ values were $-63.9\pm0.4$ and $-62.5\pm0.5$ mV in the absence and presence of 100 $\mu$M disopyramide ($n=3$), respectively (Fig. 6B). Such an upward shift of the normalized $V_{\text{max}}-V_m$ curve by disopyramide was also reported by other investigators (20).

When the papillary muscles were stimulated at 1.0 Hz, the decrease in $V_{\text{max}}$ by 30 $\mu$M pirmenol became more pronounced at less negative potential, and the normalized curve was greatly shifted in the hyperpolarizing direction (Fig. 7A). In 4 preparations, the shift at 50% $V_{\text{max}}$ level (i.e., $V_{50}$) after 30 $\mu$M pirmenol was 9.5±1.7 mV ($P<0.05$). Disopyramide also shifted the normalized $V_{\text{max}}-V_m$ curves along the voltage axis in the hyperpolarizing direction without any upward shift when the preparation was stimulated at 1.0 Hz (Fig. 7B). However, the voltage shift at $V_{50}$ level by 100 $\mu$M disopyramide was 5.7±0.6 mV, and it was smaller than that by 30 $\mu$M pirmenol.

**Discussion**

In this study, we found that in the guinea pig papillary muscles, pirmenol at concentrations higher than 3 $\mu$M causes a dose-dependent decrease in $V_{\text{max}}$ of the action potential accompanied by a prolongation of APD, especially in the late repolarization phase. At the highest concentration (100 $\mu$M), APA was also decreased, while the resting membrane potential was not affected by this drug. Other investigators have also reported qualitatively similar changes of action potential configurations in canine Purkinje fibers (16) and rabbit cardiac tissues (17). These findings, taken together with previous reports (16, 17), suggest that pirmenol is a Class la antiarrhythmic drug.

The $V_{\text{max}}$ depression with pirmenol was approximately three times more potent than that with disopyramide on a molar basis, although both drugs produced very similar action potential configuration changes. The anticholinergic activity of some Class la drugs such as quinidine and disopyramide is considered not only to influence their cardiac effects but also to produce several undesirable side effects including constipation, dry mouth and urinary retention (21). It has been reported that the anticholinergic action of pirmenol in the guinea pig ileum is four times less potent than that of disopyramide (9). Therefore, such characteristics of pirmenol, i.e., potent sodium channel blocking action in spite of weak anticholinergic action, may give pirmenol a distinct advantage in a clinical situation.

In this study, we used $V_{\text{max}}$ as an approximate index of sodium channel availability since a major ionic current crossing the cardiac cell membrane at the time of $V_{\text{max}}$ is the sodium current (22). However, there is still a controversy as to the validity of $V_{\text{max}}$ as an index of sodium conductance. Cohen and co-workers (23) have suggested that $V_{\text{max}}$ is a nonlinear indicator of sodium conductance when voltage-clamp techniques were used in rabbit Purkinje fibers. Despite such limitations, $V_{\text{max}}$ is usually used as an indirect measure of sodium conductance, because direct measurement of peak sodium current in cardiac muscles under physiological conditions is not yet possible.

Many Class I antiarrhythmic drugs show frequency- and voltage-dependence of their effects on $V_{\text{max}}$ (3-8, 19, 20), and Hondeghem and Katzung proposed a modulated receptor hypothesis to explain this (5, 24, 25). In this model, the binding affinity of drugs for sodium channels depends on the channel state, i.e., resting, open (activated), and inactivated, and the drug-associated channels do not conduct sodium ions even when activated.

In the present study, frequency-dependency and voltage-dependency of $V_{\text{max}}$ inhibition with pirmenol was examined and compared with those of disopyramide. Although the $V_{\text{max}}$ of the first action potential after a long period of quiescence was only slightly decreased by pirmenol, the $V_{\text{max}}$ inhibition with this drug was enhanced by increasing stimulus frequency. According to the above hypothesis, it can be interpreted that pirmenol has a higher affinity for open and/or inactivated sodium channels than for resting ones, as do other Class I antiarrhythmic drugs. Repetitive stimulation produced repetitive opening and inactivation, and therefore the binding of pirmenol to the open and/or inactivated channels might be accelerated,
leading to an accumulation of drug-bound nonconducting channels during stimulation. In contrast, disopyramide at a concentration of 100 \( \mu \text{M} \) produced a greater resting block, and the frequency-dependent block was less overt at stimulation rates from 0.1 to 2.0 Hz, as shown in Fig. 3. These findings are consistent with previous reports (19, 26) and suggest that disopyramide has a relatively high affinity for resting channels as well as for open and/or inactivated channels.

The onset rate of the frequency-dependent block with 30 \( \mu \text{M} \) pirmenol was 0.308±0.055 AP\(^{-1}\) at 2.0 Hz stimulation. Although it is well-known that the onset kinetics vary with drug concentrations and stimulation rates, this value seems to be comparable to those for quinidine and disopyramide, which has been proposed as intermediate drugs (ref. 26 and this study).

The recovery time constant from the frequency-dependent block of pirmenol was 33.5±5.4 sec and slower than those of other Class 1a drugs, (e.g., quinidine, 10 sec (24), 4.0–4.7 sec (27); procainamide, 4.4–6.3 sec (28)). From this point of view, pirmenol resembles Class 1c slow kinetic drugs such as encainide (recovery time constant 20.3 sec), flecainide (15.5 sec) and lorcanide (13.2 sec) (19, 26, 29). The slow recovery process from the frequency-dependent block may reflect a very low dissociation rate constant of pirmenol from blocked sodium channels.

The recovery time constant of disopyramide was 82.5±12.3 sec and much slower than that of pirmenol. However, Campbell (26) reported a relatively faster recovery time constant of disopyramide (12.2 sec), which is apparently inconsistent with the present findings. This inconsistency might stem from differences in experimental conditions. Kojima et al. (20) have reported that the recovery time constant from the frequency-dependent block of disopyramide varies with the extracellular K\(^+\) concentration (197 sec in 2.7 mM [K\(^+\)]\(_o\) and 28 sec in 8.1 mM [K\(^+\)]\(_o\)). Therefore, such an apparent discrepancy may be due to differences in the [K\(^+\)]\(_o\) of the superfused solution. In the present study, we used 4.0 mM [K\(^+\)]\(_o\) solution, whereas Campbell (26) used 5.6 mM [K\(^+\)]\(_o\) solution. The slower recovery process of disopyramide in low [K\(^+\)]\(_o\) solution may reflect that recovery from the frequency-dependent block during rest may be voltage-dependent, and the recovery time constant might be increased by hyperpolarization. Supporting this concept is the finding that at a stimulation rate of 0.01 Hz, the normalized \( V_{\text{max}}-V_m \) curve was shifted upward by disopyramide in a mildly-depolarized voltage range (see Fig. 6B). In 3 mM [K\(^+\)]\(_o\) solution, the recovery time constant might be so long that the frequency-dependent block might contribute to the depression of \( V_{\text{max}} \).

The effects of pirmenol on the \( V_{\text{max}}-V_m \) relationship were investigated in preparations stimulated at 0.01 Hz. With an interstimulus interval (100 sec) that is approximately three times the recovery time constant of pirmenol, the contribution of the frequency-dependent block is considered to be negligible. In the experiments, the decrease in \( V_{\text{max}} \) by pirmenol was slightly pronounced at less negative potential, resulting in an appreciable shift of the normalized curve toward the hyperpolarizing direction. These results can be interpreted as indicating a slightly higher affinity of this drug for inactivated channels than for resting ones. When the stimulus frequency was increased to 1.0 Hz, a physiological stimulation rate, pirmenol caused a more marked shift of the normalized curve along the voltage axis in the hyperpolarizing direction. At this stimulation rate, the fraction of channels trapped in the drug-associated inactivated state might be increased with membrane depolarization. These findings imply a selective depression of \( V_{\text{max}} \) (and hence of conduction) in depolarized myocardium.

In conclusion, the present study revealed that pirmenol is a Class 1a antiarrhythmic drug possessing frequency-dependent and voltage-dependent inhibitory action on fast sodium channels. The onset and offset kinetics of the frequency-dependent block by this drug are relatively slow in comparison with other Class 1a drugs. These properties of this drug would lead to a selective depression of \( V_{\text{max}} \) in depolarized cardiac tissues caused by ischemia and other pathological conditions, and in rapidly firing cardiac tissues where tachyarrhythmias or premature
beats with shorter coupling intervals occur. Thereby, pirmenol may suppress the development or the maintenance of various cardiac arrhythmias.

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References
1 Vaughan Williams, E.M.: Antiarrhythmic Action and the Puzzle of Perhexiline. Academic Press, London (1980)
2 Harrison, D.C., Winkle, R.A., Sami, M. and Mason, J.W.: Encainide: a new and potent antiarrhythmic agent. In Cardiac Arrhythmias: A Decade of Progress. Edited by Harrison, D.C., p. 315–330, G.K. Hall, Boston (1981)
3 Campbell, T.J.: Kinetics of onset of rate-dependent effects of Class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. Cardiovasc. Res. 17, 344–352 (1983)
4 Courtney, K.R.: Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. J. Mol. Cell. Cardiol. 12, 1273–1286 (1980)
5 Hondeghem, L.M. and Katzung, B.G.: Antiarrhythmic agents: The modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. Annu. Rev. Pharmacol. Toxicol. 24, 387–423 (1984)
6 Vaughan Williams, E.M.: A classification of antiarrhythmic action reassessed after a decade of new drugs. J. Clin. Pharmacol. 24, 129–147 (1984)
7 Bigger, J.T.: Anti-arrhythmic treatment: an overview. Am. J. Cardiol. 53, 88–16B (1984)
8 Kodama, I., Toyama, J., Takanaka, C. and Yamada, K.: Block of activated and inactivated sodium channels by class-I antiarrhythmic drugs studied by using the maximum upstroke velocity ($V_{\text{max}}$) of action potential in guinea-pig cardiac muscles. J. Mol. Cell. Cardiol. 19, 367–377 (1987)
9 Kaplan, H.R., Mertz, T.E. and Steffe, T.J.: Preclinical pharmacology of pirmenol. Am. J. Cardiol. 59, 2H–9H (1987)
10 Steffe, T.J., Mertz, T.E., Hastings, S.G., Potoczak, R.E. and Kaplan, H.R.: CI-845 (Pirmenol hydrochloride): a new orally effective long acting antiarrhythmic agents. J. Pharmacol. Exp. Ther. 214, 50–57 (1980)
11 Hammill, S.C., Shand, D.G., Routledge, P.A., Hindman, M.C., Baker, K.T. and Pritchett, E.C.L.: Pirmenol, a new antiarrhythmic agent: initial study of efficacy, safety and pharmacokinetics. Circulation 65, 369–375 (1982)
12 Lee, T.G., Goldberg, D.A., Chang, T., Serkland, M.T., Yakata, G.J., Johnson, E.L., Toole, J.G. and Goldstein, S.: Pharmacokinetics and efficacy of pirmenol hydrochloride in treatment of ventricular dysrhythmia. J. Cardiovasc. Pharmacol. 5, 632–637 (1983)
13 Toivonen, L.K., Nieminen, M.S., Manninen, V. and Frick, H.: Pirmenol in the termination of paroxysmal supraventricular tachycardia. Am. J. Cardiol. 59, 35H–38H (1987)
14 Toivonen, L.K., Nieminen, M.S., Manninen, V. and Frick, H.: Conversion of paroxysmal atrial fibrillation to sinus rhythm by intravenous pirmenol. Am. J. Cardiol. 59, 39H–42H (1987)
15 Farnham, D.J.: A multicenter dose-response study of pirmenol hydrochloride in patients with ventricular premature contractions. Am. J. Cardiol. 59, 43H–47H (1987)
16 Reder, R.F., Danilo, P., Jr. and Rosen, M.R.: Effects of pirmenol HCI on electrophysiologic properties of cardiac Purkinje fibers. Eur. J. Pharmacol. 61, 321–333 (1980)
17 Dukes, I.D., Vaughan Williams, E.M. and Dennis, P.D.: Electrophysiological and cardiovascular effects of pirmenol, a new Class I antiarrhythmic drug. J. Cardiovasc. Pharmacol. 8, 227–234 (1986)
18 Sada, H. and Ban, T.: Time-dependent effects on cardiac action potential upstroke velocity (resting block) and lipid solubility of beta adrenergic blockers. Experimentia 37, 171–172 (1981)
19 Campbell, T.J.: Resting and rate-dependent depression of maximum rate of depolarisation ($V_{\text{max}}$) in guinea pig ventricular action potentials by mexiletine, disopyramide, and encainide. J. Cardiovasc. Pharmacol. 5, 291–296 (1983)
20 Kojima, M., Ban, T. and Sada, H.: Effects of disopyramide on the maximum rate of rise of action potential ($V_{\text{max}}$) in guinea-pig papillary muscles. Japan. J. Pharmacol. 32, 91–102 (1982)
21 Bigger, J.T. and Hoffman, B.F.: Antiarrhythmic drugs. In The Pharmacological Basis of Therapeutics, Seventh edition. Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., p. 748–783, Macmillan Publishing Company, New York (1985)
22 Walton, M. and Fozzard, H.A.: The relation of $V_{\text{max}}$ to $I_{\text{Na}}$, $G_{\text{Na}}$, and h in a model of the cardiac Purkinje fiber. Biophys. J. 25, 407–420 (1979)
23 Cohen, C.J., Bean, B.P. and Tsien, R.W.: Maximum 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, 636–651 (1984)

24 Hondeghem, L.M. and Katzung, B.G.: Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochim. Biophys. Acta 472, 373–398 (1977)

25 Hondeghem, L.M. and Katzung, B.G.: Test of a model of antiarrhythmic drug action. Effect of quinidine and lidocaine on myocardial conduction. Circulation 61, 1217–1224 (1980)

26 Campbell, T.J.: Importance of physico-chemical properties in determining the kinetics of the effects of Class I antiarrhythmic drugs on maximum rate of depolarization in guinea-pig ventricle. Br. J. Pharmacol. 80, 30–40 (1980)

27 Grant, A.O., Trantham, J.L., Brown, K.K. and Strauss, H.C.: pH-dependent effects of quinidine on kinetics of dV/dtmax in guinea pig ventricular myocardium. Circ. Res. 50, 210–217 (1982)

28 Sada, H., Kojima, H. and Ban, T.: Effect of procainamide on transmembrane action potential in guinea pig papillary muscles affected by external potassium concentration. Naunyn Schmiedebergs Arch. Pharmacol. 309, 179–190 (1979)

29 Campbell, T.J. and Vaughan Williams, E.M.: Voltage- and time-dependent depression of maximum rate of depolarisation of guinea-pig ventricular action potentials by two new antiarrhythmic drugs, flecainide and lorcainide. Cardiovasc. Res. 17, 251–258 (1983)