Differential effects of MS-551 and E-4031 on action potentials and the delayed rectifier K⁺ current in rabbit ventricular myocytes

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Abstract

Objective: The frequency-dependent effects of MS-551 on the action potential duration (APD) and the underlying ionic mechanisms were investigated in comparison with those of E-4031. Methods: Whole-cell clamp techniques were used to study action potentials and ionic currents in enzymatically isolated rabbit ventricular myocytes. Results: The frequency-response obtained within the range of 0.1 to 3.3 Hz was different for MS-551 and E-4031. The APD prolongation by MS-551 (10 μM) was significant at 0.5–3.3 Hz, whereas that by E-4031 (1 μM) was significant at 0.1–1.0 Hz. The prolongation by MS-551 (10 μM) of APD of a test action potential, which was preceded by a train of 1.0 Hz stimulation, decreased progressively as the rest duration increased, whereas that by E-4031 (1 μM) remained at the same level. Both MS-551 (10 μM) and E-4031 (1 μM) significantly decreased I_K, but showed no effects on the transient outward current (I_{Na}) and the inward rectifier K⁺ current (I_{K_{in}}). The development of the block on I_K and the recovery from the block by MS-551 were voltage dependent. At a holding potential of −50 mV, MS-551 reduced the tail current to a similar extent (21–34%, n = 6) across all the tested durations of the depolarizing pulses to +10 mV, whereas at −75 mV, the intensity of the block progressively increased as the durations of depolarizing pulses were prolonged. The recovery from the block by MS-551 was absent at −50 mV, but occurred at −75 mV with a time constant of 577 ± 179 ms (n = 6). The development of the block on I_K by E-4031 was voltage and time independent. No recovery from the block was observed for E-4031 at either −50 or −75 mV. Conclusions: These findings suggest that MS-551 produces frequency-dependent class III action, presumably due to the voltage dependent binding and unbinding to the I_K channels. The reverse frequency dependence of class III action by E-4031 cannot be explained by the effects on I_K.

Keywords: Antiarrhythmic drugs; MS-551; E-4031; Rabbit, ventricular myocytes; Membrane potential; Use dependence; Potassium channel, delayed rectifier

1. Introduction

Class I antiarrhythmic drugs (Na⁺ channel blockers) are the most frequently used agents in current clinical treatment of life threatening arrhythmias, but their use has always been limited by their proarrhythmic effect owing to the depression of conduction [1]. Furthermore, the cardiac arrhythmia suppression trial (CAST), which tested the effects of several potent class I agents, has shown that these drugs increase mortality in patients with recent myocardial infarction [2]. Therefore much concern has arisen regarding the efficacy and particularly the proarrhythmic effects of class I antiarrhythmic drugs, and attention has shifted to class III agents that exert their antiarrhythmic actions essentially by prolonging action potential duration (APD).

However, most recently developed class III antiarrhythmic agents (e.g., sotalol, E-4031, dofetilide, UK-66914 and almokalant) possess a common unfavorable feature: their APD prolonging effect diminishes at high stimulation frequencies [3–8]. This frequency-dependent variation in efficacy might reduce their usefulness in terminating tachyarrhythmias [3]. To be effective against tachyarrhythmias, a drug should preferentially prolong APD at high stimulation frequencies. For antiarrhythmic agents to exert such a
frequency-dependent effect, they must block the potassium channel in the activated or open state, and much attention has recently been focused on this aspect of research into the mechanism of action of antiarrhythmic agents. An open-state block on potassium channel has been observed for some class III antiarrhythmic agents such as dofetilide and almokalant in rabbit ventricular myocytes [9,10], and also for several class I antiarrhythmic agents such as quinidine [11], propafenone [12], flecainide and encainide [13].

MS-551 (1,3-dimethyl-6-[2-N-(2-hydroxyethyl)-3-(4-nitrophenyl)propylaminoethylamino]-2,4(1H,3H)-pyrimidinedione hydrochloride) is a newly synthesized antiarrhythmic agent having a pyrimidinedione structure. It differs from most methylsulfonamide class III antiarrhythmic agents (e.g., sotalol, E-4031, dofetilide) in having a nitro-rather than a methylsulfonamide group in the p-position of the benzene ring. In previous studies, MS-551 was reported to prolong APD in canine Purkinje fibers without affecting the maximum upstroke velocity of phase 0 depolarization (V_{max}), and thus was classified as a class III antiarrhythmic agent [14]. MS-551 was also found to have an inhibitory effect on the transient outward K+ current (I_{to}), the inward rectifier K+ current (I_{irr}), and the delayed rectifier K+ current (I_{k}) in rabbit ventricular myocytes [15]. However, modulation of its class III action under various modes of stimulation and the underlying mechanisms have not been evaluated. In the present study, we investigated the frequency-dependent mode of the class III action of MS-551. We also analyzed the effects of MS-551 on the delayed rectifier K+ current in terms of voltage-dependence and recovery from block, and compared these properties with those of the other class III antiarrhythmic agent E-4031 [16].

2. Methods

2.1. Isolation of rabbit ventricular myocytes

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). Single ventricular myocytes from rabbit hearts were obtained by the enzymatic dissociation procedure [17]. Rabbis of either sex weighing 1.5 to 2.0 kg were anesthetized with thiaymol sodium after being heparinized, and the hearts were rapidly excised and mounted via the aorta on a Langendorff retrograde perfusion apparatus. The hearts were firstly perfused with normal Tyrode's solution (gassed with 100% O_2 at 37°C) for 3-5 min, secondly with calcium-free Tyrode's solution for 10-15 min, and finally with 0.12 mg/ml collagenase (Yakult, Japan) containing calcium-free Tyrode's solution to block the calcium and sodium currents, respectively. The hearts were subsequently washed with high-potassium solution (KB solution) for 5 min, the myocytes were separated and minced with a pair of surgical scissors, and the tissue was then passed through a 200 μm stainless steel mesh. The filtrate was washed two times with KB solution by centrifuging at a speed of 1000 rpm for 5 min. The obtained cells were stored in KB solution at 4°C before use.

2.2. Electrophysiological recordings

The single-pipette whole-cell clamp method was used for recording the membrane potentials and currents [18]. An aliquot of the cell suspension was placed in the recording chamber on the stage of an inverted microscope (Diaphot, Nikon Co., Tokyo). A brief period was allowed for cell adhesion to the coverslip at the bottom of the chamber, and then the cells were superfused with normal Tyrode's solution at 3 ml/min. The bath temperature in all experiments was maintained at 34°C. The glass pipette has a resistance of 3-5 MΩ after filling with the pipette solution. The pipette was connected to a patch-clamp amplifier (List-medical, Darmstadt, Germany). Cell membrane capacitance averaged 203 ± 27 pf (n = 12); this value was obtained by calculating the area under the capacitive transient evoked by applying a depolarization pulse (5 mV) from a holding potential of -50 mV. The cell capacitance and series resistance were electronically compensated by about 70%. Command potentials were generated by a multichannel stimulus (Nihon-Kohden, Tokyo, Japan). Action potentials were recorded in the current clamp mode. The action potential was elicited by injecting 5 ms long rectangular pulses of depolarizing current through the pipette. The elicited action potentials were displayed on an oscilloscope (Tektronix, 5111A, USA) and photographed. The action potential duration at 90% repolarization was analyzed. For the current studies, data acquisition was performed with a NEC computer (NEC9801DA Tokyo, Japan). Current signals were filtered at 3 kHz, and digitized at sampling frequencies of 200 Hz and 2 kHz, respectively, depending on the pulse duration.

2.3. Solutions and drugs

Tyrode's solution used in the isolation of the myocytes and in the experiment was composed of (in mM): NaCl, 143; KCl, 5.4; CaCl_2, 1.8; MgCl_2, 0.5; NaH_2PO_4, 0.25; HEPES, 5.0; and glucose, 5.6, pH adjusted to 7.4 with NaOH. The calcium-free Tyrode's solution was the same as above except that it lacked CaCl_2. The high-potassium storage solution contained (in mM): KOH, 82; l-glutamic acid, 50; KCl, 40; KH_2PO_4, 20; taurine, 20; HEPES, 10; MgCl_2, 3; glucose, 10; EGTA, 0.5; pH adjusted to 7.4 with KOH. To measure the transient outward current (I_{to}), 2 mM CoCl_2 and 10 μM TTX were added to Tyrode's solution to block the calcium and sodium currents, respectively. When the delayed rectifier potassium currents (I_{k}) were measured, the superfusate was changed to a solution...
composed of the following (in mM): N-methyl-\(\alpha\)-glucosamine, 149; MgCl\(_2\), 5; HEPES, 5; and nisoldipine, 0.003, pH adjusted to 7.4 with HCl. In this Na\(^+\)-free, K\(^+\)-free and nisoldipine (3 \(\mu\)M)-containing external solution, \(I_n\) and \(I_{\text{Kt}}\), the electrogenic Na\(^+\)/Ca\(^{2+}\) exchange current and the electrogenic pump current were blocked, respectively [19]. The internal pipette solution contained (in mM): KOH, 60; KCl, 80; aspartate, 40; HEPES, 5; EGTA, 10; MgATP, 5; sodium creatinine phosphate, 5; and CaCl\(_2\), 0.65 (pH 7.2, pCa 8.0).

MS-551 was obtained from Mitsui Pharmaceuticals Incorporated (Tokyo, Japan). E-4031 (N-[4-[[1-[[2-(6-methyl-2-pyridinyl)ethyl][4-piperidinyl]carbonyl]phenyl] methanesulfonamide dihydrochloride dihydrate) was obtained from Eisai Pharmaceuticals (Tokyo, Japan). They were dissolved in distilled water as stock solutions and diluted in superfusates to the desired final concentrations immediately before each experiment.

2.4. Statistics

Data were expressed as mean ± s.e. The curve-fitting program Igor (Wave Metrics, USA) was used in data analysis. Statistical analysis was performed using Student's paired \(t\)-test.

Fig. 1. Frequency-dependent effects of MS-551 and E-4031 on APD. Upper panel: Examples of action potentials recorded before (○) and after exposure to 10 \(\mu\)M of MS-551 (△) or 1 \(\mu\)M of E-4031 (▲) at stimulation frequencies of 0.1, 1.0 and 3.3 Hz. Lower panel: APD before and during application of MS-551 or E-4031 was plotted as a function of stimulation frequency. MS-551 prolonged APD significantly at higher stimulating frequencies from 0.5 to 3.3 Hz, and the maximum prolongation appeared at 1.0 Hz. This effect attenuated at lower or higher stimulation frequencies. E-4031 was more effective at lower stimulating frequencies from 0.1 to 1.0 Hz. * \(P < 0.05\), ** \(P < 0.01\) vs each control value. Data at different frequencies were obtained from the same cell.
3. Results

3.1. Frequency-dependent effects on APD

The effects of MS-551 and E-4031 on APD were examined at different stimulation frequencies. Stimulation frequency was changed in steps from 0.1 to 3.3 Hz, and the steady-state action potentials were recorded before and during the application of MS-551 (10 μM) or E-4031 (1 μM). The results are shown in Fig. 1. In untreated control groups, when the stimulation frequency was changed in steps from 0.1 to 3.3 Hz, APD initially increased and then decreased, showing a biphasic frequency response. In the presence of MS-551 (10 μM), maximum prolongation of APD appeared at 1.0 Hz stimulation, and this effect was attenuated at lower or higher stimulation frequencies, resulting in a bell-shaped frequency-response. In contrast, prolongation of APD by E-4031 (1 μM) was more effective at lower stimulating frequencies (from 0.1 to 1.0 Hz), resulting in reverse frequency-dependence.

Fig. 2. Recovery from prolongation on APD by MS-551 and E-4031. The experimental protocol: a train of stimulation at 1.0 Hz was interrupted by a test pulse with rest durations from 1.0 to 30 s. Upper panel: Examples of action potentials recorded before (○) and during administration of 10 μM MS-551 (∆) or 1 μM E-4031 (▲) at steady-state 1 Hz stimulation as well as after 5 or 20 s of rest. Lower panel: APD before and during application of MS-551 or E-4031 was plotted as a function of rest duration. The APD prolongation by MS-551 decreased progressively as the rest duration increased, whereas that by E-4031 was maintained almost unchanged at rest durations ranging from 1.0 to 30 s. * P < 0.05; ** P < 0.01 vs each control value.
3.2. Recovery from prolongation on APD

The relationship between APD prolongation and the rest duration was examined. Fig. 2 shows the experimental protocol and the results obtained. A train of 10 pulses of basic stimulation at 1.0 Hz was applied for APD to reach steady-state value, the cell was then kept resting for varying durations ranging from 2.0 s to 30 s and this was followed by a test pulse. The effects of MS-551 and E-4031 on the APD of the test action potential were studied in comparison with their effects on the APD at the end of the 1.0 Hz stimulation train. In myocytes treated

![Graph showing experimental protocol and results.](image-url)

**Fig. 3. Effects of MS-551 on total membrane current.** Currents were elicited by applying 300-ms hyperpolarizing or depolarizing voltage steps between −100 and +40 mV from a holding potential of −40 mV. Upper panel: The representative recordings recorded before and during the application of 10 μM MS-551 are shown. Cell capacitance: 170 pF. Lower panel: Current–voltage relations for the steady-state current (I∞) and the peak current (I, peak) in the absence and presence of 10 or 100 μM MS-551 are shown. At 10 μM, MS-551 showed no significant effects on I∞ or I, peak, whereas 100 μM MS-551 significantly decreased both I∞ and I, peak. *P < 0.05; **P < 0.01 vs each control value.
with MS-551 (10 μM), APD prolongation was largest at the end of 1.0 Hz stimulation train (24.7% ± 2.3%, n = 6). This effect decreased gradually as the rest duration was increased, and disappeared after 20 s of rest. In myocytes treated with E-4031 (1 μM), the APD at the end of 1.0 s stimulation train was prolonged by 35.0% ± 2.3% (n = 5) compared with the untreated control value. This APD prolongation remained at nearly the same level even when the rest duration was increased to 30 s.

3.3. Effects on total current–voltage (I–V) relationship

Effects of MS-551 and E-4031 on instantaneous and steady-state total current were examined. Membrane current was elicited by applying 300-ms hyperpolarizing or depolarizing voltage steps between -100 and +40 mV from a holding potential of -40 mV. The steady-state currents at the end of the clamp pulses up to 0 mV were measured as the inward rectifier K+ current (I_{K1}). I_{K1} is considered to be responsible for the final repolarization in rabbit ventricular cells [20]. 10 μM MS-551 hardly affected I_{K1}, whereas 100 μM MS-551 significantly reduced I_{K1} and the slope of the I–V relation, as shown in Fig. 3. At -60 mV, the current amplitudes before and during the application of 10 μM and 100 μM MS-551 were 736 ± 78 pA, 704 ± 63 pA (n = 5, P > 0.05) and 497 ± 70 pA (n = 5, P < 0.05), respectively. The instantaneous peak inward current measured upon depolarization to potentials from -30 to +40 mV was considered as an index of the calcium current (I_{Ca}). 10 μM MS-551 slightly decreased I_{Ca}, while 100 μM MS-551 caused a significant decrease in I_{Ca}. At 0 mV, the current amplitudes before and during the application of 10 and 100 μM MS-551 were 1276 ± 81 pA, 1143 ± 99 pA (n = 5, P > 0.05) and 861 ± 99 pA (n = 5, P < 0.05), respectively (Fig. 3). The same protocol was used to study the effect of E-4031, and neither I_{K1} nor I_{Ca} was affected by 1 μM E-4031 (results not illustrated).

In the absence and presence of 1 μM E-4031, the amplitudes of I_{K1} at -60 mV were 720 ± 61 pA and 709 ± 58 pA (n = 5, P > 0.05), respectively, and the values of I_{Ca}

Fig. 4. Concentration-dependent effects of MS-551 and E-4031 on I_{K1} were elicited by a series of 300 ms depolarizing pulses applied at 0.1 Hz from a holding potential of -60 mV to +60 mV. Upper panel shows the protocol and the representative recordings. Membrane capacitances for the left and right cells were 168 pF and 175 pF, respectively. Lower panel gives the effects of MS-551 (left) and E-4031 (right) on the I–V curve of I_{K1}. The amplitudes of the peak current (I_{p}) and the steady-state current (I_{ss}) were plotted as a function of the test potential. At concentrations of 1 and 10 μM, neither MS-551 nor E-4031 decreased the amplitude of the peak current and the steady-state current, 100 μM of MS-551 caused a significant decrease in the amplitude of the peak current. * P < 0.05 vs control.
at 0 mV were 1130 ± 71 pA and 1094 ± 78 pA (n = 5, P > 0.05), respectively.

3.4. Effects on transient outward K⁺ current

It is well known that ionic currents responsible for the repolarization of the cardiac action potential show important species dependence. In rabbit ventricular myocytes, Iₒ was considered to be of predominant importance in repolarization [21–23].

In our experiment, because Iₒ current was recorded in the presence of Co²⁺, it represented the 4-aminopyridine sensitive component of Iₒ [22]. The concentration-dependent effect of MS-551 and E-4031 on the I–V curve of Iₒ

![Diagram](image-url)

Fig. 5. Effects of MS-551 and E-4031 on the voltage-dependent activation of Iₒ. Activation of Iₒ was elicited by applying the voltage clamp steps from a holding potential of -40 mV to depolarizing potentials ranging from -30 to +60 mV. Upper panel: Effects of MS-551 (left) and E-4031 (right) on the activation curve of Iₒ. Tail current amplitude was plotted as a function of the test potential. Both MS-551 (10 μM) and E-4031 (1 μM) inhibited Iₒ significantly without greatly altering the voltage dependence of activation. *** P < 0.05, ** P < 0.01 vs control. Membrane capacitances for the left and right representative recordings were 188 pF and 173 pF, respectively. Lower panel: Voltage dependence of Iₒ block by MS-551 and E-4031. The original data are the same as in the upper panel. Ratios of the tail current amplitude in the presence of the drugs to those under control conditions were plotted as a function of the test potential. In the presence of either MS-551 or E-4031, Iₒ tails decreased almost to the same extent at all depolarizing levels tested.
Table 1

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<th></th>
<th>( n )</th>
<th>( \tau_1 ) (ms)</th>
<th>( \tau_2 ) (ms)</th>
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<tr>
<td>Control</td>
<td>6</td>
<td>14.6 ± 1.7</td>
<td>94.7 ± 5.0</td>
</tr>
<tr>
<td>MS-551 (10 ( \mu )M)</td>
<td>6</td>
<td>16.2 ± 1.6</td>
<td>99.1 ± 6.1</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>12.1 ± 1.8</td>
<td>93.3 ± 5.4</td>
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<tr>
<td>E-4031 (1 ( \mu )M)</td>
<td>6</td>
<td>12.9 ± 2.0</td>
<td>100.1 ± 4.8</td>
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is shown in Fig. 4. \( I_{\text{Na}} \) was elicited by a series of 300-ms step depolarizing pulses applied at 0.1 Hz from −60 mV to test potentials between −50 mV to +60 mV. At concentrations of 1 and 10 \( \mu \)M, MS-551 had no significant effect on \( I_{\text{Na}} \). At the highest concentration (100 \( \mu \)M) applied, MS-551 reduced \( I_{\text{Na}} \) significantly, as shown in the decrease of the peak current in Fig. 4. Neither 1 \( \mu \)M nor 10 \( \mu \)M of E-4031 caused any significant change in \( I_{\text{Na}} \) over the entire range of depolarizing voltages tested.

The inactivation time course of \( I_{\text{Na}} \) could be fitted by double exponential. Under control conditions, the time constants \( \tau_1 \) and \( \tau_2 \) at +30 mV depolarization were 14.6 ± 1.7 ms and 94.7 ± 5.0 ms (\( n = 6 \)), respectively. These values were consistent with those reported by Hiraoka et al. [22]. Neither MS 551 nor E 4031 caused significant changes in the time course of \( I_{\text{Na}} \) inactivation. The time constants before and during applications of MS 551 and E 4031 are listed in Table 1.

3.5. Effects on delayed rectifier \( K^+ \) current

Block of \( I_{\text{K}} \) has been shown to prolong APD and thus produce an antiarrhythmic effect by class III antiarrhythmic agents [24]. In our experiments, \( I_{\text{K}} \) was activated by applying voltage clamp steps for 3000 ms from a holding potential of −40 mV (to inactivate \( I_{\text{Na}} \)) to different depolarizing levels. Depolarization elicits a family of slowly developed time-dependent outward currents. On clamping...
Table 2

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<thead>
<tr>
<th></th>
<th>$V_{1/2}$ (mV)</th>
<th>k</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-4.3 ± 0.2</td>
<td>10.8 ± 0.2</td>
</tr>
<tr>
<td>MS-551 (10 μM)</td>
<td>-2.0 ± 0.5</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>-7.1 ± 0.4</td>
<td>10.1 ± 0.4</td>
</tr>
<tr>
<td>E-4031 (1 μM)</td>
<td>-8.8 ± 0.7</td>
<td>10.2 ± 0.6</td>
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Data were obtained by fitting each activation curve to the Boltzmann equation: $I_x = I_{max}/[1 + \exp(V_{1/2} - V)/k]$, where $I_{max}$ is the maximum tail current amplitude, $V_{1/2}$ is the half maximum activation voltage, and $k$ is the slope factor.

back to the holding potential, outward tail currents ($I_x$ tail) could be detected. These tail currents have been shown to represent the deactivation of $I_x$ [25], and their peak value amplitude measured in reference to holding current provides an estimate of the current activated during the depolarizing steps. Thus, in our experiment the effects of these drugs were investigated on $I_x$ tail current.

The effects of MS-551 and E-4031 on the voltage-dependent activation of $I_x$ were studied, and the results are shown in Fig. 5. Activation of $I_x$ was elicited by applying voltage clamp steps from a holding potential of −40 mV to different depolarizing levels ranging from −30 to +60 mV. In the upper panel, the activation curves were drawn by plotting the tail amplitude to test potential. Before the drugs were applied, the $I_x$ tail increased with depolarization reaching saturation at about +30 mV. Both MS-551 (10 μM) and E-4031 (1 μM) depressed the amplitude of the tail currents without significantly altering the voltage dependence of activation. The activation parameters obtained before and after the application of drugs are listed in Table 2.

Voltage dependence of $I_x$ block by MS-551 and E-4031 is further analyzed in the lower panel of Fig. 5. The original data are the same as those obtained in the activation curve study. The amplitude of the tail current elicited at each testing potential in the presence of the drug is normalized to the amplitude of the tail current observed under control conditions at the same depolarization level. In the presence of either MS-551 (10 μM) or E-4031 (1 μM), $I_x$ tail decreased almost to the same extent at all the depolarizing levels tested. This indicates that the inhibitory effects of these drugs on $I_x$ are voltage independent, at least at depolarizing voltage levels ranging between 40 mV and +60 mV. This is consistent with their lack of effects on the activation parameters.

The deactivation time course for $I_x$ could well be fitted with one exponential. Analysis of the tail current after depolarization to +20 mV showed that neither MS-551 nor E-4031 significantly altered the kinetics of $I_x$ deactivation. The time constants before and during the application of 10 μM MS 551 (n = 7) were 480 ± 46 ms and 516 ± 54 ms, respectively. Values in the absence and presence of E-4031 (1 μM) were 461 ± 44 ms and 440 ± 52 ms, respectively (n = 6).

Voltage dependence of the $I_x$ block induced by MS-551 and E-4031 was further evaluated using the envelope tests for the tail current. Envelopes of the $I_x$ tail current were generated by applying depolarizing pulses to +10 mV at durations ranging from 50 to 3000 ms from two different holding potentials of −50 mV or −75 mV. Tail currents elicited by repolarization to −50 mV were evaluated before and during the application of the two drugs. Under control conditions, tail currents increased as the durations of the pulses were prolonged and reached a plateau level at about 2 s at both holding potentials of −50 mV and −75 mV. The time courses of activation could be fitted by a single exponential with time constants of 451 ± 34 ms (n = 6) and 400 ± 54 ms (n = 6), respectively.

Time courses of the block development on $I_x$ by MS-551 (3 μM) are shown in Fig. 6A. They were dependent on the holding potentials. At a holding potential of −50 mV, MS-551 suppressed the tail current to a similar extent (21–34%, n = 6) across all the tested durations of the depolarizing pulses from 50 to 3000 ms. At the holding potential of −75 mV, however, MS-551 inhibited the tail currents in a time-dependent manner. For a 50-ms depolarizing pulse, the tail current was only reduced to 58 ± 9% (n = 6) of the control value. The intensity of the block by MS-551 progressively increased thereafter as the duration of the depolarizing pulses was prolonged. The time course of block development was estimated exponentially with a time constant of 132 ± 36 ms (n = 6).

In the case of E-4031 (0.3 μM), no time or voltage dependence could be observed in the envelope tests from the holding potential of either −50 mV or −75 mV. E-4031 reduced the tail current to a similar extent across all the tested durations of the depolarizing pulses. A typical recording and the time courses of the block development on $I_x$ by E-4031 (0.3 μM) are shown in Fig. 6B. At the holding potential of −50 mV and −75 mV, E-4031 reduced the tail current to 30–40% (n = 6) and 36–46% (n = 6) of each control value, respectively.

Recovery from block was evaluated using a paired-pulse protocol. A conditioning pulse to +10 mV was applied for 1 s to induce a steady-state block. The potential was then clamped at −50 or −75 mV for varying periods and was followed by a 0.2-s test pulse to +10 mV before being returned to the holding potential of −50 mV. The relative amplitude of the tail current in the presence of the drugs was compared with the control to determine recovery from the block at the particular holding potential. The results of MS-551 (3 μM), shown in Fig. 7A, differed for −50 mV and −75 mV. At −75 mV, the recovery was rapid, although incomplete, and steady-state recovery was obtained after 1 s of rest. The time course could be fitted exponentially with a time constant of 577 ± 179 ms (n = 6). At −50 mV, recovery was barely detectable even after 30 s of rest. Fig. 7B shows the results of E-4031 (0.3 μM).
4. Discussion

The present study showed that MS-551 demonstrated more pronounced APD prolongation at higher stimulating frequencies ranging from 0.5 to 3.3 Hz, but lacked significant effects at lower stimulating frequencies, resulting in a bell-shaped frequency response, whereas the APD prolongation by E-4031 attenuated at higher frequencies, showing the reverse frequency-dependence, as has been previously demonstrated by other investigators [16]. In this respect, MS-551 differs notably from other conventional and newer class III agents, such as sotalol [3,4], E-4031 [16], dofetilide [5,6], UK-66914 [7], and almokalant [8]. Theoretically, selective APD prolongation at high frequencies of stimulation offers the advantages of being efficient against tachyarrhythmias with minimum risk of prolonging the action potential at low frequencies and provoking "torsade de pointes" arrhythmia [26].

Action potential duration in cardiac tissues varies with the frequency of stimulation [27]. In rabbit ventricular myocytes, at least three kinds of potassium currents (I_{to}, I_{K} and I_{Kr}) are responsible for the repolarization. Of these three currents, I_{to} is the determinant one in the frequency-related regulation of APD. This current contributes to the remarkable shortening in APD at slow rates due to its slow reactivating kinetics [22]. Conversely, an inhibition of I_{to} may result in the prominent prolongation of APD at lower stimulating frequency. In our study, neither MS-551 nor E-4031 induced inhibition of I_{to} and I_{Kr} at the same concentrations employed in action potential study. Rather, these agents induced a significant inhibition of I_{K}. Prolongation of APD by MS-551 and E-4031 is therefore considered to be due to the inhibition of I_{K}. MS-551 at the concentration of 10 μM was previously reported by Nakaya et al. to be a nonselective blocker of I_{to}, I_{Kr} and I_{Ca} [15]. However, our results demonstrated that at the same concentration, MS-551 blocked I_{K} selectively, and it appeared as a non-selective blocker only at the much higher concentration of 100 μM.

Recent studies by Sanguinetti and Jurkiewicz have shown the existence of two components of delayed outward current named I_{Kr} and I_{Ks} in guinea-pig ventricular myocytes, with differential sensitivity to block by class III antiarrhythmic agents [28]. In human atrial myocytes, the delayed outward current was even reported to be composed of three components, the classical rapid and slow ones, as described in guinea-pig myocytes, and an "ultra-rapid" one named I_{Kurt} which was sensitive to 4-aminopyridine [29,30]. In human ventricular myocytes, however, the delayed outward current was demonstrated to be small or not discernible [31,32]. Unlike that found in guinea-pig myocytes, the existence of only one component (I_{Kr}) of the delayed outward current was reported in rabbit atrial and ventricular myocytes [9,12]. The deactivation time course of atrial I_{K} tail current was reported to be of one exponential [12]. In our study, the I_{K} deactivation time course could be fitted with one exponential with a fast time constant, consistent with the above report.

To explain the difference in frequency-dependent effects on APD by MS-551 and E-4031, block development and its recovery on I_{K} were analyzed. The envelope tests for the tail current showed that the block by MS-551 on the tail currents was much stronger at a holding potential of −50 mV than at −75 mV after shorter depolarizing
pulses. This indicates that MS-551 may have already blocked the $I_K$ channel at the depolarized holding potential of $-50 \text{ mV}$ before the application of the depolarization pulses. From $-75 \text{ mV}$ holding potential, depolarization for several hundred ms was required for MS-551 to reach a steady-state block. This means that the block during a single action potential (usually about 150 ms in duration in rabbit ventricular myocytes) is substantial but not complete, and that the block will show frequency-dependent change. When the membrane potential was returned to the resting potential level at $-75 \text{ mV}$, recovery from the block by MS-551 was observed, and several hundred ms were required to reach the steady-state level. This indicated that an accumulation of the $I_K$ block would occur, if any additional depolarizing pulses were to be delivered before the recovery from the block could be complete. In addition, increased stimulation frequencies maintain the myocardial cell at depolarized potentials longer, thereby causing much accumulation of the $I_K$ block. Therefore, this voltage-dependent inhibition of $I_K$ by MS-551 may well explain its selective APD prolongation at higher stimulation frequencies and the disappearance of its effect after longer rest.

In rabbit atrial and ventricular myocytes, open-state block on $I_K$ has been investigated in detail for various antiarrhythmic agents. Carmeliet has shown that the blocking actions on $I_K$ of dofetilide and almokalant, the class III antiarrhythmic agents, were dependent on test potentials [9,10]. The degree of block increased with increasing depolarizations, and the activation curves were shifted in the hyperpolarizing direction [9,10], whereas propafenone, a class Ic antiarrhythmic agent, blocked $I_K$ to the same extent at all tested depolarizing levels, without altering the kinetics of activation [12]. The effect of MS-551 on the activation curve much resembled that of propafenone. In addition, the recovery from block by dofetilide and almokalant occurred at $-50 \text{ mV}$ of holding potential with a very slow rate and was absent at $-75 \text{ mV}$ [9,10]. The recovery from the block on $I_K$ by MS-551, on the other hand, was very rapid at $-75 \text{ mV}$ and was absent at $-50 \text{ mV}$ of holding potential. Thus, the effect on $I_K$ by MS-551 was assumed to depend on the membrane potentials; that is, block develops at more depolarized levels, while recovery occurs at more hyperpolarized levels. Considering the channel state dependent affinity of this drug, one possible explanation can be made by assuming the existence of some "intermediate state" of the $I_K$ channel between the rest and open states. MS-551 may bind to the intermediate state channel and unbind from the rest state channel. Further study is required to clarify this point.

The development of the block on $I_K$ by E-4031 exhibited no time or voltage dependence. This suggests that E-4031 may have already blocked the $I_K$ channel at the respective holding potentials when the channel was in the closed state or that the block by E-4031 occurs rapidly (during the first depolarizing pulse). A recent study by Ohler and Ravens supports the latter possibility in showing that the full APD-prolonging effects of E-4031 were not present after quiescence but instead required repetitive stimulation to develop [33]. This phenomenon suggests preferential drug interaction with the open channel. It should also be noted that no recovery from the block could be induced at $-75 \text{ mV}$ of the holding potential, indicating that it may be a tonic (steady state) block. This may explain the lack of recovery from APD prolongation after rest. Our observations are consistent with those reported by Ohler and Ravens in that the APD-prolonging effect of E-4031 persisted in the first action potential after 30 min of rest [33]. This suggests that E-4031 may be trapped within the resting channel [33].

The lack of significant APD prolongation effects of E-4031 at higher frequencies could not be explained by the present findings. As has been pointed out by previous investigators [10,34], action potential duration is a complex phenomenon resulting from the interplay between a number of inward and outward ionic currents. All of these currents change with frequency. At high stimulation frequencies, the total amount of current flowing during repolarization may become much greater than at low frequencies, whereas it is speculated that the relative contribution of $I_K$ to repolarization becomes much less important. Thus, the decreased efficacy of E-4031 at higher stimulation frequencies might be explained by a concomitant increase of other membrane currents.

Block of the activated $I_K$ channel and fast recovery from block are two main requirements for ideal class III antiarrhythmic agents. A drug possessing such characteristics would have a frequency-dependent prolonging effect on APD, and thus would be very effective in the treatment of tachyarrhythmias. Unfortunately, although many class III antiarrhythmic agents have proved to be open or activated channel blockers, none of them shows a frequency-dependent APD prolonging effect due to the slow kinetics of recovery from block. For example, dofetilide and almokalant were demonstrated to be open channel blockers with slow kinetics of recovery (time constant of 13.9 s for almokalant and in the order of minutes for dofetilide) [9,10]; previous reports demonstrated that their effects on APD were reverse frequency-dependent [5,6,8]. Our study has for the first time demonstrated that MS-551 possesses specific frequency-dependent characteristics in APD prolongation, owing to its blocking properties at depolarized levels and the fast kinetics of recovery at hyperpolarized levels. If this property of MS-551 proves beneficial in on-going clinical trials, the next generation of delayed rectifier blockers should possess the ability to prolong APD in a frequency-dependent manner.

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References


