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Effects of sematilide, a novel class III antiarrhythmic agent, on membrane currents in rabbit atrial myocytes

Yasunori Ishii [a,b], Katsuhiko Muraki [a], Atsushi Kurihara [b], Yuji Imaizumi [a,*], Minoru Watanabe [a]

[a] *Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan*
[b] *Laboratory for Pharmacology, Preclinical Development Laboratories, Nippon Hoechst Marion Roussel Ltd., Saitama, Japan*

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Abstract

The effects of sematilide, a novel class III antiarrhythmic agent, on membrane currents were examined in single myocytes isolated from the rabbit left atrium, using the whole-cell voltage clamp technique. Application of 10, 30, 100 and 300 μM sematilide caused a concentration-dependent inhibition of the delayed rectifier K^+ current (IC_{50} approx. 25 μM). The sematilide-sensitive current, which was recorded by means of a triangular voltage command, showed a strong inward rectification and had a peak at about −40 mV, suggesting that sematilide inhibits the rapidly activating delayed rectifier K^+ current. The Ca^{2+}-independent transient K^+ and the inward rectifier K^+ currents were not affected significantly by application of 100 μM sematilide. Moreover, voltage-dependent Na^+ and Ca^{2+} currents were not affected significantly by 100 μM sematilide. These findings indicate that sematilide selectively blocks the rapidly activating delayed rectifier K^+ current in atrial myocytes and provide evidence supporting the usefulness of the drug as a class III antiarrhythmic agent. © 1997 Elsevier Science B.V.

Keywords: Sematilide; Atrium, rabbit; Membrane current; Antiarrhythmic agent

1. Introduction

Sematilide hydrochloride (*N*-[2-(diethylamino)ethyl]-4-[(methylsulfonyl)-amino] benzamide HCl) is currently being evaluated as a new antiarrhythmic agent in clinical trials. It has been reported that sematilide increases action potential duration and effective refractory period without affecting other action potential parameters in rabbit atrium (Argentieri et al., 1991), guinea-pig ventricle (Sawanobori et al., 1994) and atrium (Ishii et al., 1995) and canine ventricle (Argentieri, 1992). Sematilide is, therefore, classified as a class III antiarrhythmic agent (Vaughan Williams, 1984), which preferentially blocks the delayed rectifier K^+ current (I_K) and consequently prolongs action potential duration and the effective refractory period (for reviews, see Hondeghem, 1992; Sanguinetti, 1992). Actually, it has been shown that 30 μM sematilide significantly inhibits I_K in guinea-pig ventricular myocytes (Sawanobori et al., 1994). It is, however, well known that amiodarone, which is classified as a class III antiarrhythmic agent, inhibits voltage-dependent Na^+ (I_{Na}) and Ca^{2+} currents (I_{Ca}) in addition to I_K (Kodama et al., 1992). Moreover, it has also been reported that MS-551, which is a new class III antiarrhythmic agent, affects not only I_K but also Ca^{2+}-independent transient K^+ currents (I_t) and inward rectifier K^+ currents (I_{K1}) (Nakaya et al., 1993). Although sematilide increases action potential duration and effective refractory period in atrial myocytes of the rabbit, it might be possible that the effect is partly due to the inhibition of I_t, which is one of the major repolarizing currents in the action potential in atrial myocytes of the rabbit (Giles and Imaizumi, 1988; Qu et al., 1994) as well as those of humans (Shibata et al., 1989; Fermini et al., 1992). In a previous study, we showed that I_K in the rabbit atrial myocyte is small in comparison with I_t but contributes significantly to the middle and late phases of action potential repolarization at a physiological heart rate (Muraki et al., 1995). The present study was undertaken to record the membrane ionic currents which are affected by sematilide and to elucidate the mechanisms underlying the increase in

* Corresponding author. Fax: (81-52) 836-3432; e-mail: yimaizum@phar.nagoya-cu.ac.jp

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action potential duration and refractory period in the rabbit atrial myocytes.

2. Materials and methods

2.1. Cell-isolation procedure

Rabbit atrial myocytes were enzymatically dissociated as previously described (Giles and Imaizumi, 1988). Briefly, after young male rabbits weighing 1.5 to 2.0 kg were anesthetized, the heart was dissected out and immediately mounted on a Langendorff-perfusion system. The heart was perfused with normal Krebs solution for approximately 5 min, with nominally Ca^{2+}-free Krebs solution for 5–10 min, and then with Ca^{2+}-free Krebs' solution containing 0.03–0.06% collagenase (Yakult, Tokyo, Japan) for 15–30 min. Thereafter, the heart was washed free of enzyme by perfusion with KB solution (Isenberg and Klöckner, 1982) for approximately 5 min. These procedures were carried out at 36°C. Finally, the left atrium was cut off, and agitated gently in the KB solution (see below), using a fire-polished Pasteur pipette to isolate single cells. After the suspension was kept quiescent for 30 min, the supernatant was discarded and Ca^{2+}-free HEPES-buffered solution containing high $MgCl_2$ was added. The tissue was agitated gently, using the Pasteur pipette, for several minutes and 100 μM Ca^{2+} was added to the high Mg^{2+} HEPES-buffered solution. The cell suspension was kept at 10°C and used within 12 h.

2.2. Solutions and drugs

The standard Krebs solution used for cell isolation was composed of (mM) 117 NaCl, 4.7 KCl, 2.2 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 14 glucose, 25 $NaHCO_3$. A Ca-free Krebs solution was prepared by omitting $CaCl_2$. The solutions were gassed with 95% O_2/5% CO_2. KB solution contained (mM) 20 taurine, 70 KCl, 70 potassium glutamate, 2 KH_2PO_4, 11 glucose, 0.5 EGTA, 10 HEPES, 5 $MgCl_2$. The pH of this solution was adjusted to 7.2 with 10 M KOH. Standard external solution contained (mM) 137 NaCl, 5.9 KCl, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 14 glucose, 10 HEPES. A Ca^{2+}-free, high Mg^{2+} HEPES solution was prepared by omitting $CaCl_2$ and increasing $MgCl_2$ concentration to 10 mM. Tetraethylammonium-HEPES solution to record I_{Na} contained (mM) 50 tetraethylammonium–Cl, 87 NaCl, 5.9 KCl, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 14 glucose, 10 HEPES. The pH of these HEPES solutions was adjusted to 7.2 with 10 M NaOH. The pipette filling solution (internal solution) contained (mM) 100 K-aspartate, 50 KCl, 1 $MgCl_2$, 0.85 $CaCl_2$, 5 EGTA, 5 Na_2ATP, 5 HEPES. The pH was adjusted to 7.2 with 10 M KOH. The pCa of the internal solution was maintained at 7.5 with a Ca^{2+}-EGTA buffer.

The following drugs were used in the present experiments; sematilide (produced by Berlex, USA, and supplied to Nippon Roussel, Tokyo, Japan), E-4031 (a gift from Eisai Pharmaceutical, Japan), $CdCl_2$ (Wako, Tokyo, Japan), $BaCl_2$ (Wako), $CoCl_2 \cdot 6H_2O$ (Wako) and 4-aminopyridine (Tokyokasei, Tokyo, Japan). These drugs were dissolved in distilled water and the concentrations of the stock were 100 mM (sematilide, $CdCl_2$, $BaCl_2$) or 1 M ($CoCl_2$, 4-aminopyridine).

2.3. Electrical recordings

The methods used to record transmembrane currents under whole-cell voltage clamp were similar to those originally developed by Hamill et al. (1981). To make microelectrodes, a borosilicate tube of 1 mm outer diameter was heated and pulled by gravity, using a vertical micropipette puller (PB-7, Narishige Scientific, Tokyo, Japan). The resistance of microelectrodes filled with internal solution was 2–3 MΩ, except for Na^+ current measurement. For the recording of Na^+ current, the resistance of microelectrodes was approximately 0.5–1 MΩ and series resistance was compensated electronically. A single atrial myocyte was voltage-clamped using an amplifier (CEZ-2200, Nihon-Kohden, Tokyo, Japan). When a recording pipette was filled with an aspartate rich solution, the junction potential was −8.9 mV, therefore, membrane potentials were corrected by −9 mV (Clark et al., 1990). To record I_{Na}, 50 mM Na^+ in the standard external solution was replaced with equimolar tetraethylammonium (tetraethylammonium-HEPES) and the solution was kept at 10°C. Experiments were carried out at 36 ± 1.0°C unless mentioned otherwise.

2.4. Data storage and analyses

Membrane currents were monitored on a storage oscilloscope (Kikusui 5020A, Tokyo, Japan) and stored on video-tape, using a PCM system (Sony 501ES, Tokyo, Japan, modified to allow DC recordings). The stored data on video-tape were replayed later and loaded into an IBM-AT computer, using an analog-to-digital conversion board (Data Translation DT2801A, USA) for analysis. Data acquisition (AQ) and analysis (Cellsoft) programs for the IBM-AT computer were developed at the University of Calgary (Calgary, Alberta, Canada). Selected records were printed out by a laser printer (Yokokawa Hewlett Packard, Laser Jet Series IV, Sagamihara, Japan) or plotted with *X–Y* plotter (Roland Digital Group, DXY-1300, Hamamatsu).

2.5. Statistics

Data are expressed as means ± S.E.M. in text and figures. Comparison between two and multiple groups was performed by Student's *t*- and Tukey's tests, respectively.

3. Results

3.1. Effects of sematilide on delayed rectifier K current (I_K)

To record the delayed rectifier K^+ current (I_K) in myocytes from rabbit left atrium, experiments were carried out at 36°C using a pipette solution of pCa 7.5 prepared with Ca^{2+}-EGTA buffer (Tohse, 1990). To inhibit the voltage-dependent Ca^{2+} current (I_{Ca}), 1 mM $CoCl_2$ was added to the external solution. When a cell was depolarized every 5 s from the holding potential of −39 to +1 mV for 200 ms, transient and slowly developing outward currents were activated, as shown in Fig. 1Aa. Repolarization to −39 mV caused a slowly decaying tail current ($I_{K_{tail}}$). The transient outward current was derived from the activation of the Ca^{2+}-independent transient K^+ current (I_t) since (1) the current was sensitive to 4-aminopyridine (see Fig. 3), (2) the inactivation of the current was fast (within 100 ms) and (3) the current was inactivated substantially at the holding potential of −39 mV. As shown in Fig. 1Aa, application of sematilide decreased the amplitude of $I_{K_{tail}}$ in a concentration-dependent manner. Mean data (Fig. 1Ab) shows the concentration-dependent effect of sematilide on $I_{K_{tail}}$. The amplitude of $I_{K_{tail}}$ in the presence of sematilide was normalized with that in the absence and plotted as a percentage of the tail current against the corresponding concentration of sematilide. Addition of 300 μM sematilide almost completely blocked the tail current (4.8 ± 4.8% of the control, $n = 6$). The concentration of sematilide required for a 50% decrease in $I_{K_{tail}}$ was approximately 25 μM. In Fig. 1Ba, the amplitude of $I_{K_{tail}}$ activated at various potentials was measured in the absence and presence of 100 μM sematilide. Mean data were plotted as the current–voltage (I–V) relationship against the test potentials (Fig. 1Bb). The $I_{K_{tail}}$ was observed when the test potentials were positive to −29 mV and reached the maximum at around +21 mV (64.4 ± 7.6, 73.2 ± 8.4 and 72.2 ± 8.3 pA at +11, +21 and +31 mV, respectively). The $I_{K_{tail}}$ was markedly reduced and almost abolished after the application of 100 and 300 μM sematilide, respectively. The inhibition of $I_{K_{tail}}$ by sematilide was not voltage-dependent over a range from +1 to +41 mV (79.6 ± 3.6, 78.4 ± 3.9, 82.1 ± 1.8, 77.6 ± 4.8 and 84.2 ± 2.8% inhibition at +1, +11, +21, +31 and +41 mV, respectively, $P > 0.05$ by Tukey's test).

To characterize the I–V relationship of the sematilide-sensitive current (I_{sema}) during repolarization in an action

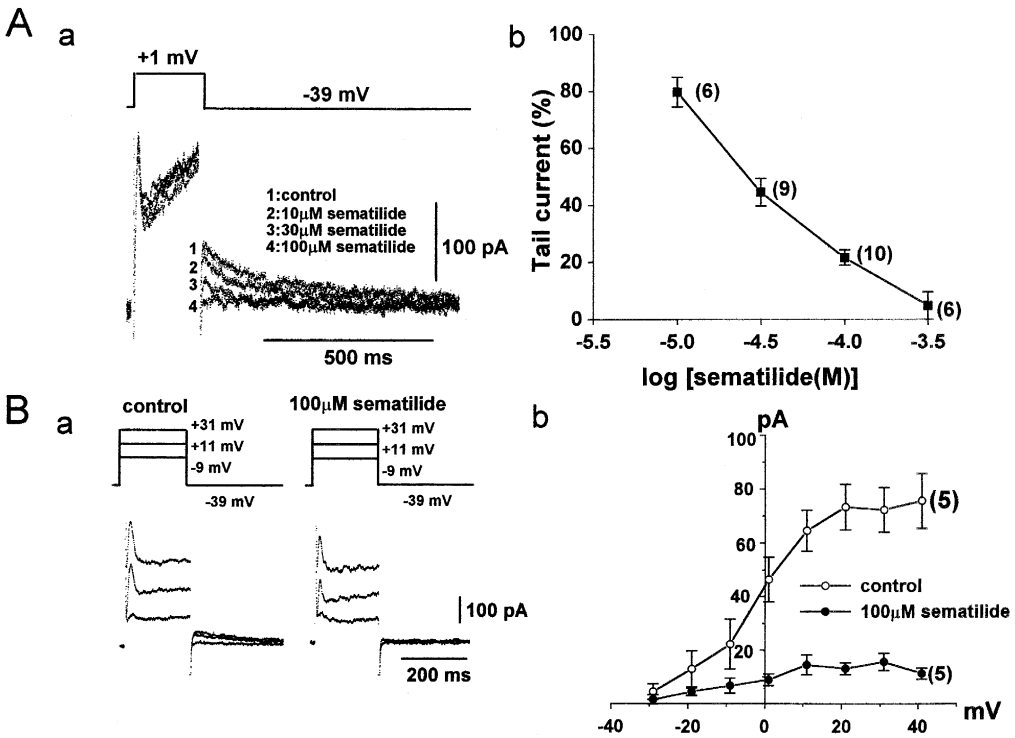

Fig. 1. Effects of sematilide on delayed rectifier K^+ current (I_K) in rabbit atrial myocytes. (A) Concentration-dependent effects of sematilide on I_K. (a) A myocyte was depolarized for 200 ms from the holding potential of −39 to +1 mV. The tail current ($I_{K_{tail}}$) measured at −39 mV was decreased by sematilide in a concentration dependent manner (from '1' to '4'). (b) Data obtained in experiments typically shown in (a) were summarized. Peak amplitude of $I_{K_{tail}}$ in the presence of sematilide was normalized with that in the absence and plotted against the concentration of sematilide. (B) Effects of 100 μM sematilide on the current-voltage (I–V) relationship of $I_{K_{tail}}$. (a) Current traces obtained upon depolarization from −39 to −9, +11 and +31 mV for 200 ms in the absence (left panel) and presence (right panel) of 100 μM sematilide were superimposed. (b) The I–V relationships of $I_{K_{tail}}$ in the absence (open circles) and presence (closed circles) of 100 μM sematilide obtained from five cells. Cells were depolarized for 200 ms from −39 mV to various potentials between −29 and +41 mV in a 10 mV step. Numbers in the parentheses are the number of cells used.

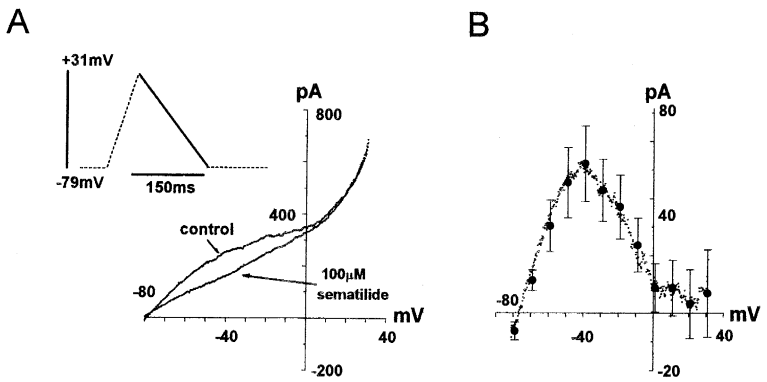

Fig. 2. Sematilide sensitive current (I_{sema}) recorded using a triangular voltage command. (A) $I–V$ relationships of I_{sema} obtained using a triangular voltage command. A cell was depolarized at 0.2 Hz from −79 mV at a rate of 5 V/s and thereafter repolarized to −79 mV at a rate of 0.83 V/s, as shown in the inset. The outward currents recorded during the repolarization in the absence and presence of 100 μM sematilide were plotted against the potential. (B) Summarized $I–V$ relationship of I_{sema}. I_{sema} was obtained by subtracting the current in the presence of 100 μM sematilide from that before sematilide application. Dotted points and closed circles denote the average amplitude of I_{sema} from six cells at each potential. The bars on the closed circles indicate S.E.M. Number of cells used was eight. Note that I_{sema} shows a strong inward rectification.

potential, a triangular wave form which had a repolarization rate similar to that for action potentials from rabbit atrium at 1 Hz was applied as the voltage command pulse in Fig. 2. Depolarization and repolarization rates were 5 V/s and 0.83 V/s, respectively (inset in Fig. 2A). After the current response to the triangular wave form at 0.2 Hz became stable, cells were exposed to 100 μM sematilide. Fig. 2A shows the $I–V$ relationships obtained during the repolarization in the triangular wave form in the absence and presence of 100 μM sematilide. A part of the outward current during repolarization was reduced by sematilide. In Fig. 2B, I_{sema}, obtained by subtracting the current in the presence of sematilide from that in the absence, was averaged from six separate cells and expressed against the repolarizing potential. It is notable that I_{sema} exhibited a marked inward rectification, and reached the maximum at

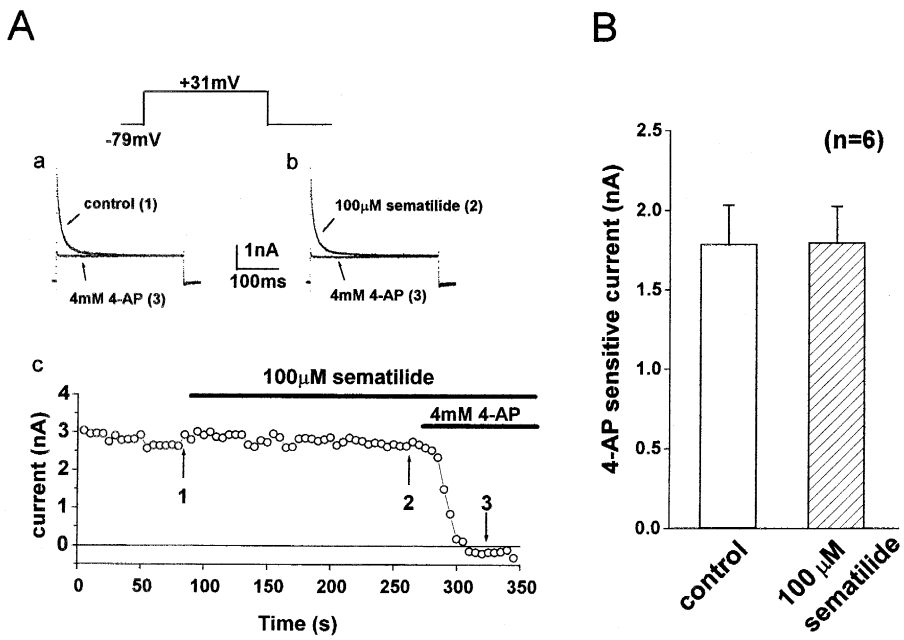

Fig. 3. Effects of sematilide on Ca^{2+}-independent transient outward K^+ current (I_t). Cells were depolarized from −79 to +31 mV for 300 ms every 5 s in the presence of 50 μM $CdCl_2$. (A) Outward currents in the absence and presence of 100 μM sematilide are shown in (a) and (b), respectively. To estimate the total I_t, 4 mM 4-aminopyridine was applied ('3' in panel of (a) and (b)). Panel (c) illustrates the time course of peak amplitude of I_t, which was estimated as the transient component during the depolarizing pulse. Current traces shown in (a) and (b) were obtained at the times indicated by the corresponding numbers in (c). (B) Summarized data describing the peak amplitude of the 4-aminopyridine sensitive current in the absence (open column) and presence (hatched column) of 100 μM sematilide. The 4-aminopyridine sensitive current was not affected by 100 μM sematilide. Number of cell used was six.

around −39 mV. When 3 μM E-4031, a selective blocker of the fast-activating component of I_K, was applied under the same experimental protocol, the outward current over a voltage range of −79 to +11 mV was reduced. The *I–V* relationship of the E-4031-sensitive current has a peak at approximately −39 mV, as shown in the previous work (Muraki et al., 1995), and is very similar to that of I_{sema}. Addition of 300 μM sematilide did not further reduce the outward current ($n = 2$). Conversely, when 100 μM sematilide was first applied and 3 μM E-4031 was added, I_{sema} at −39 mV was 65 ± 5% ($n = 4$) of the total current reduced by sematilide and E-4031. In the presence of 300 μM sematilide, the outward current at −39 mV was not affected by addition of 3 μM E-4031 ($n = 3$).

3.2. Other K currents and sematilide

The effects of sematilide on the Ca^{2+}-independent transient K^+ current (I_t) were examined in rabbit atrial myocytes. Atrial myocytes were depolarized from the holding potential of −79 to +31 mV at 0.2 Hz. To inhibit I_{Ca}, 50 μM $CdCl_2$ was added to the bathing solution. As shown in Fig. 3A, I_t was not affected by 100 μM sematilide but was markedly reduced by 4-aminopyridine ('1', '2' vs. '3' in Fig. 3Aa, b and c). In Fig. 3Ac, the amplitude of the transient component of the outward current between the peak and the steady level during the depolarizing pulse was plotted against time. Since sematilide-sensitive I_K was almost absent at +31 mV (< 10 pA; Fig. 2), the transient component was considered as I_t. Fig. 3B shows the mean data describing the peak amplitude of I_t at +31 mV in the absence and presence of 100 μM sematilide. Here, I_t was measured as the 4-aminopyridine sensitive outward current. The average peak amplitude of I_t at +31 mV was 1.78 ± 0.27 nA ($n = 6$) and was not affected by 100 μM sematilide (101 ± 6%, $n = 6$, $P > 0.05$). Even 300 μM sematilide did not affect I_t amplitude (109%, $n = 2$).

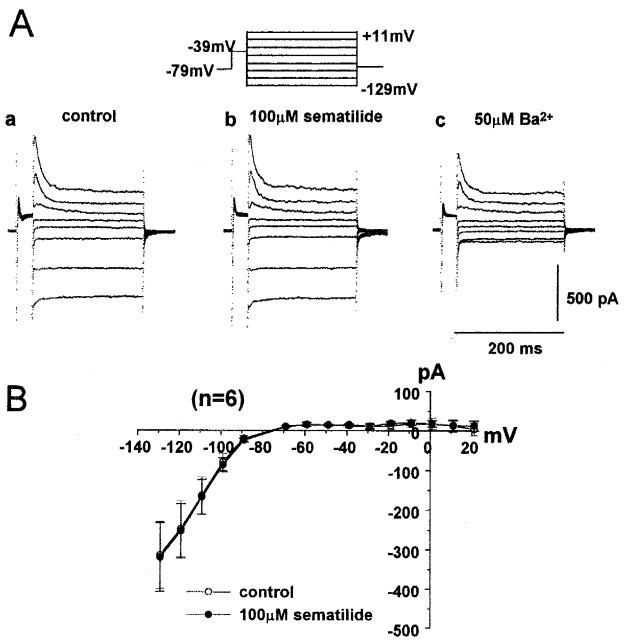

Fig. 4. Effects of sematilide on inward rectifier K^+ current (I_{K1}). (A) Cells were depolarized for 30 ms from −79 to −39 mV to inactivate the voltage-dependent Na^+ current. Thereafter, a test voltage-jump was applied for 200 ms to various potentials between −129 and +11 mV with 20 mV step. Experiments were carried out in the presence of 50 μM $CdCl_2$. (a), (b) and (c) were superimposed current traces in the absence and presence of 100 μM sematilide, and in the presence of 50 μM $BaCl_2$, respectively. (B) Summarized data describing the *I–V* relationship of I_{K1} as the Ba^{2+}-sensitive current. The amplitude of the Ba^{2+}-sensitive current at the end of pulse was measured as peak I_{K1}. Open and closed circles indicate the amplitude of I_{K1} in the absence and presence of 100 μM sematilide, respectively ($n = 6$).

In Fig. 4, the inward rectifier K^+ current (I_{K1}) was recorded from rabbit atrial myocytes. After a cell was depolarized from −79 to −39 mV to inactivate I_{Na}, it was clamped at test potentials between −129 and +11 mV for 200 ms. I_{Ca} was abolished by addition of 50 μM $CdCl_2$ to the bathing solution. Fig. 4Aa, b and c show superimposed current traces recorded at test potentials between −129 and +11 mV with a 20 mV step in the absence and presence of 100 μM sematilide, and after the application of 50 μM $BaCl_2$, respectively. As described above, I_t was observed at potentials positive to −29 mV but decayed within 70–80 ms after the start of a test potential. I_{K1} was estimated as the 50 μM Ba^{2+}-sensitive current and its amplitude was measured at the end of test pulse. The *I–V* relationship of I_{K1} from six separate cells is shown in Fig. 4B and shows that I_{K1} was not affected by 100 μM sematilide ($p > 0.05$ at −99, −109 and −119 mV)

3.3. Voltage-dependent Na^+ and Ca^{2+} currents and sematilide

In Fig. 5A, the effects of sematilide on the voltage-dependent Na^+ current (I_{Na}) were examined. To record I_{Na} accurately, the concentration of extracellular Na^+ was reduced to 87 mM by replacing 50 mM Na^+ by equimolar tetraethylammonium$^+$ and the recording was performed at 10°C. To block I_{Ca}, $CdCl_2$ (50 μM) was added to the bathing solution. When a cell was depolarized from a holding potential of −89 mV to test potentials between −79 and +31 mV, a transient inward current was activated at potentials positive to −49 mV (Fig. 5A). The maximum inward current was recorded at around −30 mV (see Fig. 5Ab; 561.3 ± 54.1 pA at −29 mV, $n = 6$) and was completely inactivated within 20 ms after the start of depolarization. The current was reduced by 10 μM tetrodotoxin to about 5% of the control (not shown). Application of 100 μM sematilide neither reduced the peak amplitude of I_{Na} nor altered the shape of I_{Na} (Fig. 5A). Fig. 5Ab shows the *I–V* relationship between peak I_{Na} and test potentials in the absence and presence of 100 μM sematilide ($n = 6$).

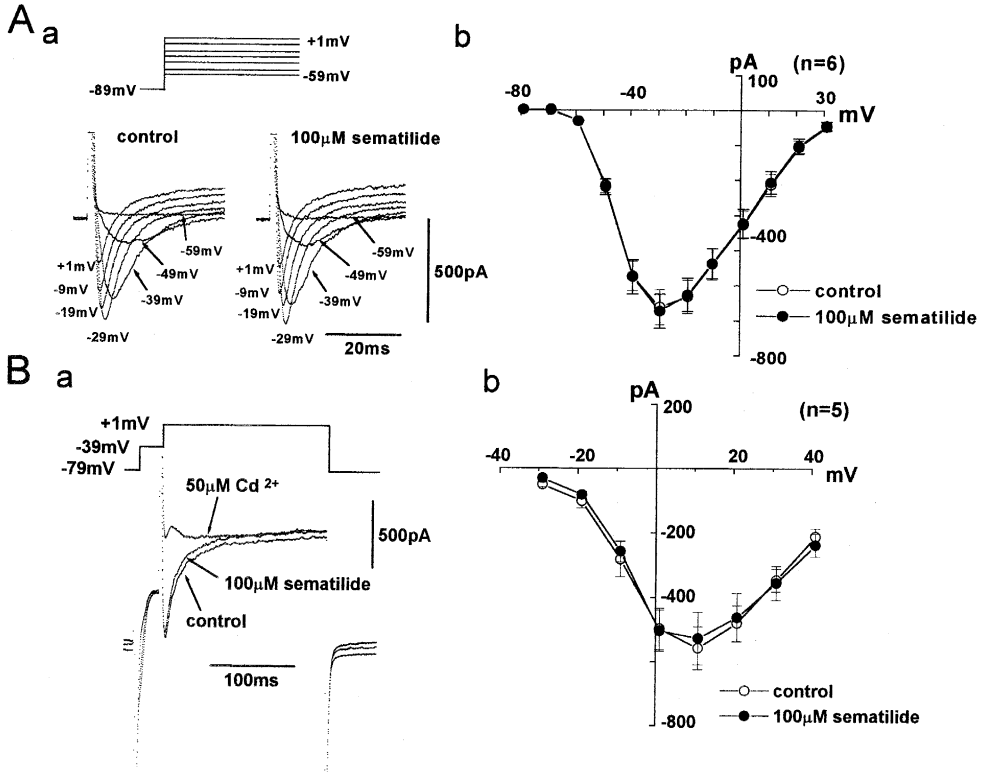

Fig. 5. Effects of sematilide on voltage-dependent Na^+ (I_{Na}) and Ca^{2+} currents (I_{Ca}). (A) Effects of sematilide on I_{Na}. (a) Rabbit atrial myocytes were depolarized from the holding potential of −89 mV to various potentials between −59 and +1 mV with a 10 mV step. The bathing solution contained 87 mM Na^+ and 50 μM $CdCl_2$ and was kept at 10°C. Left and right panels show superimposed current traces in the absence and presence of 100 μM sematilide, respectively. (b) The *I–V* relationship of I_{Na} in the absence (open circles) and presence (closed circles) of 100 μM sematilide. Number of cells used was six. (B) Effects of sematilide on I_{Ca}. (a) I_{Ca} was recorded in the absence and presence of 100 μM sematilide and abolished by application of 50 μM $CdCl_2$. A cell was depolarized for 30 ms from −79 to −39 mV to inactivate I_{Na} and then further depolarized to +1 mV for 200 ms to activate I_{Ca}. Experiments were carried out in the presence of 4 mM 4-aminopyridine. (b) The *I–V* relationship of I_{Ca}, obtained as the Cd^{2+}-sensitive current, in the absence (open circles) and presence (closed circles) of 100 μM sematilide ($n = 5$). I_{Ca} elicited upon depolarization to potentials between −29 and +41 mV in 10 mV steps was plotted against the test potentials.

The effects of sematilide on I_{Ca} were examined in Fig. 5B. A cell was depolarized from −79 to −39 mV for 30 ms to inactivate I_{Na}. Thereafter, I_{Ca} was elicited by depolarization to various test potentials between −29 and +41 mV for 150 ms. To inhibit I_t, 4 mM 4-aminopyridine was added to the external solution. As shown in Fig. 5Ba, I_{Ca} activated at the test potential of +1 mV was not affected by application of 100 μM sematilide but was abolished by 50 μM $CdCl_2$. Fig. 5Bb demonstrates the *I–V* relationship of the peak amplitude of the Cd^{2+}-sensitive inward current. The Cd^{2+} sensitive inward current was activated at potentials positive to −29 mV and reached the maximum at +11 mV (557.9 ± 75.0 pA, $n = 5$). Application of 100 μM sematilide did not affect the *I–V* relationship in the five cells examined. The peak I_{Ca} at +1 mV was not affected significantly by 300 μM sematilide (95.3 ± 2.1% of the control, $n = 4$).

4. Discussion

The present study demonstrated that 10–300 μM sematilide effectively inhibits a delayed rectifier K^+ current (I_K) in rabbit atrial myocytes. Although two types of I_K, rapidly and slowly activating I_K (I_{K_R} and I_{K_S}, respectively), have been identified in cardiac myocytes of several mammals, a large part of I_K in rabbit atrial myocytes is considered to be I_{K_R} (Muraki et al., 1995). Sematilide may preferentially block I_{K_R}, because of the following findings: (i) The half activation voltage of I_K, which was sensitive to 100 μM sematilide, was approximately −5 mV and was obviously negative compared to that of I_{K_S} (+10 to +20 mV) and similar to that of I_{K_R} recorded in guinea-pig ventricle, and guinea-pig and rabbit atrium (Sanguinetti and Jurkiewicz, 1990, 1991; Muraki et al., 1995). (ii) The sematilide-sensitive current obtained by using a triangular command pulse had a marked inward rectification, which is a well-known characteristic of I_{K_R}. In the present study, however, it was not determined whether sematilide inhibited I_{K_S}, since I_{K_S} in the present preparation is a very small current (5–10% of total I_K, Muraki et al., 1995). The residual current in the presence of 100 μM sematilide had a similar *I–V* relationship to I_{K_R} and was abolished by further addition of 3 μM E-4031, which is another class III antiarrhythmic agent and which is selective for I_{K_R} at the concentration used (Sanguinetti and Jurkiewicz, 1990,

1991), suggesting that the residual current is due to unblocked I_{K_R} but not to I_{K_S}. In the presence of 300 μM sematilide, however, addition of 3 μM E-4031 did not affect the outward current. Moreover, in the presence of 3 μM E-4031, addition of 300 μM sematilide did not further reduce the outward current. Although the details of sematilide-induced block of I_{K_R} were not examined in the present study, the block was not voltage-dependent. It has been reported that sematilide reduces $I_{K_{tail}}$ voltage independently and preferentially interacts with the channel in the resting state in guinea-pig ventricular myocytes (Sawanobori et al., 1994).

In our previous study using a conventional microelectrode technique, application of sematilide to guinea-pig atrium prolonged action potential duration at 90% repolarization and the effective refractory period in a concentration-dependent manner (Ishii et al., 1995). The concentration of sematilide required for a 50% increase (EC_{50}) in action potential duration at 90% repolarization and in the effective refractory period in guinea-pig atrium was about 10 μM. In addition, both action potential duration at 95% repolarization and the effective refractory period in rabbit atrium were effectively prolonged in the presence of 10–100 μM sematilide (Argentieri et al., 1991). The IC_{50} of sematilide for $I_{K_{tail}}$ inhibition was approximately 25 μM, indicating a reasonable agreement with I_K inhibition and action potential prolongation. In canine cardiac Purkinje fibers, however, it has been reported that 1 μM sematilide causes a substantial increase in action potential duration and effective refractory period (Argentieri, 1992). In guinea-pig ventricle, the IC_{50} of sematilide for $I_{K_{tail}}$ inhibition is approx. 30 μM (Sawanobori et al., 1994), consistent with that in rabbit atrium. The reason for the differences in the potency of sematilide for prolongation of action potential duration in canine Purkinje fibers, guinea-pig atrium and ventricle, and rabbit atrium is not clear, but the contribution of I_K to action potential repolarization may be somewhat different between species and/or portions of the heart. It has also been reported that action potential shape and current density of I_K are quantitatively different even between epi- and endocardial region of canine ventricle (Liu and Antzelevitch, 1995).

It is notable that sematilide had high selectively for I_K over I_t, I_{K1}, I_{Na} and I_{Ca}. The functional importance of I_t offsetting membrane potential in the early phase of repolarization of the action potential has been established in human atrial myocytes (Shibata et al., 1989; Fermini et al., 1992). I_t is also a major early repolarizing current in rabbit atrial myocytes, while the current has a slower recovery time course from inactivation and, thereby, a somewhat different frequency dependence in comparison with that in humans. It has been shown that application of 100 μM E-4031 decreases I_t by approx. 25% in rabbit atrium (Muraki et al., 1995). MS-551, which is another class III antiarrhythmic agent, has also an inhibitory effect on I_t (Nakaya et al., 1993). I_t was, however, not affected by 100 μM sematilide in the present study. A selective increase in the late phase of repolarization in the action potential in the presence of sematilide has been reported in a multi-cellular preparation of rabbit atrium (Argentieri et al., 1991). The present result is consistent with this finding. The late phase of action potential may be strongly regulated by I_{K1}, whereas I_{K1} in rabbit atrial myocyte is small in comparison with that in ventricle (Giles and Imaizumi, 1988). Inhibition of I_{K1} may also contribute to the increase in action potential duration at 90% repolarization, as has been suggested for MS-551 (Nakaya et al., 1993). I_{K1} was, however, not affected by 100 μM sematilide. Correspondingly, it has been reported that the resting membrane potential in cardiac muscles is not affected by sematilide. It can be, therefore, strongly suggested that the increase in action potential duration and effective refractory period is not due to the inhibition of I_t or I_{K1}. Small inhibition of I_{Ca} by 10 μM E-4031 has been reported in rabbit atrio–ventricular myocytes (Verheijck et al., 1995). In the present study, 100 μM sematilide did not affect I_{Ca} which should be mainly the current through L-type channels. In addition, I_{Na} was not affected by 100 μM sematilide, which is consistent with the fact that the maximum upstroke velocity of phase 0 depolarization (dV/dt_{max}) is not affected by sematilide in guinea-pig atrium (Ishii et al., 1995). The effects of sematilide on other membrane currents, such as Ca^{2+}-dependent K^+ current, Ca^{2+}-dependent Cl^- current, c-AMP-dependent Cl^- current, Na^+–Ca^{2+} exchange current, nonselective cationic current, were not examined in the present study. The first two Ca^{2+}-dependent currents have been reported to be the repolarizing currents in the action potential under normal conditions in atrial myocytes (Escande et al., 1987; Wang et al., 1995). It has been reported that the transient outward current which is activated by caffeine in guinea-pig ventricle myocytes is a Ca^{2+}-dependent current and not changed by application of sematilide (Sawanobori et al., 1994). Although these may not be major repolarizing currents in rabbit atrial myocytes under control conditions, further study is required before one can draw the conclusion that I_{K_R} is the only ionic current susceptible to sematilide at therapeutic doses in cardiac myocytes.

The electropharmacological and safety profiles of sematilide have been evaluated in patients with ventricular tachycardia and fibrillation (Sager et al., 1993). Induction of sustained ventricular tachycardia was prevented by sematilide. This effect was associated with an increase in the ventricular effective refractory period. In addition, sematilide showed a tendency to prolong the atrial effective refractory period, suggesting the possible usefulness of sematilide for the treatment of superventricular tachyarrythmias. The effect of sematilide may be due to selective block of I_K.

In conclusion, the present results indicate that 100 μM sematilide has no effect on I_t, I_{K1}, I_{Na} and I_{Ca} but effectively blocks I_{K_R}. The inhibition leads to prolongation

of the action potential duration and effective refractory period in atrial muscle. These characteristics indicate that sematilide has clinical potential as a Class III antiarrhythmic drug.

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References

Argentieri, T., 1992. Sematilide. Cardiovasc. Drug. Rev. 10, 182–198.

Argentieri, T.M., Carroll, M.S., Sullivan, M.E., 1991. Cellular electrophysiological effects of the Class III antiarrhythmic agents sematilide and clofilium on rabbit atrial tissues. J. Cardiovasc. Pharmacol. 18, 167–174.

Clark, R.B., Nakajima, T., Giles, W.R., Kanai, K., Momose, Y., Szabo, G., 1990. Two distinct types of inwardly rectifying K^+ channels in bull-frog atrial myocytes. J. Physiol. 424, 229–251.

Escande, D., Coulombe, A., Faivre, J., Deroubaix, E., Coraboeuf, E., 1987. Two types of transient outward currents in adult human atrial cells. Am. J. Physiol. 252, H142–H148.

Fermini, B., Wang, Z., Duan, D., Nattel, S., 1992. Differences in rate dependence of transient outward current in rabbit and human atrium. Am. J. Physiol. 263, H1747–H1754.

Giles, W.R., Imaizumi, Y., 1988. Comparison of potassium currents in rabbit atrial and ventricular cells. J. Physiol. 405, 123–145.

Hamill, O.P., Marty, A., Neher, E., Sackman, B., Sigworth, J., 1981. Improved patch-clamp techniques for high resolution current recording from cell free membrane patches. Pflügers Arch. 391, 85–100.

Hondeghem, L.M., 1992. Development of class III antiarrhythmic agents. J. Cardiovasc. Pharmacol. 20, S17–S22.

Isenberg, G., Klöckner, U., 1982. Calcium tolerant ventricular myocytes prepared by preincubation in a 'KB medium'. Pflüg. Arch. 395, 6–18.

Ishii, Y., Muraki, K., Kurihara, A., Imaizumi, Y., Watanabe, M., 1995. Effects of sematilide, a novel class III antiarrhythmic agent, on action potential in guinea-pig atrium. Jpn. J. Pharmacol. 68, 175–182.

Kodama, I., Suzuki, R., Kamiya, K., Iwata, H., Toyama, J., 1992. Effects of long-term oral administration of amiodarone on the electromechanical performance of rabbit ventricular muscle. Br. J. Pharmacol. 107, 502–509.

Liu, D.W., Antzelevitch, C., 1995. Characteristics of the delayed rectifier current (I_{Kr} and I_{Ks}) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. Circ. Res. 76, 351–365.

Muraki, K., Imaizumi, Y., Watanabe, M., Habuchi, Y., Giles, W.R., 1995. Delayed rectifier K^+ current in rabbit atrial myocytes. Am. J. Physiol. 269, H524–H532.

Nakaya, H., Tohse, N., Takeda, Y., Kanno, M., 1993. Effects of MS-551, a new Class-III antiarrhythmic drug, on action potential and membrane currents in rabbit ventricular myocytes. Br. J. Pharmacol. 109, 157–163.

Qu, A., Yeung-Lai-Wah, J.A., Xiao, J., Kerr, C.R., 1994. Regional differences in rabbit atrial repolarization: importance of transient outward current. Am. J. Physiol. 266, H643–649.

Sager, P.T., Nademanee, K., Antimisiaris, M., Pacifico, A., Pruitt, C., Godfrey, R., Singh, B.N., 1993. Antiarrhythmic effects of selective prolongation of refractoriness. Electrophysiologic actions of sematilide HCl in humans. Circulation 88, 1072–1082.

Sanguinetti, M.C., 1992. Modulation of potassium channels by antiarrhythmic and antihypertensive drugs. Hypertension 19, 228–236.

Sanguinetti, M.C., Jurkiewicz, N.K., 1990. Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class III antiarrhythmic agents. J. Gen. Physiol. 96, 195–215.

Sanguinetti, M.C., Jurkiewicz, N.K., 1991. Delayed rectifier outward K^+ current is composed of two currents in guinea pig atrial cells. Am. J. Physiol. 260, H393–H399.

Sawanobori, T., Adania, H., Namiki, T., Hiraoka, M., 1994. Rate-dependent effects of sematilide on action potential duration in isolated guinea pig ventricular myocytes. J. Pharmacol. Exp. Ther. 271, 302–310.

Shibata, E.F., Drury, T., Refsum, H., Aldrete, V., Giles, W., 1989. Contributions of a transient outward current to repolarization in human atrium. Am. J. Physiol. 257, H1773–H1781.

Tohse, N., 1990. Calcium-sensitive delayed rectifier potassium current in guinea pig ventricular cells. Am. J. Physiol. 258, H1200–H1207.

Vaughan Williams, E.M., 1984. A classification of antiarrhythmic actions reassessed after decade of new drugs. J. Clin. Pharmacol. 24, 129–147.

Verheijck, E.E., van Ginneken, A.C., Bourier, J., Bouman, L.N., 1995. Effects of delayed rectifier current blockade by E-4031 on impulse generation in single sinoatrial nodal myocytes of the rabbit. Circ. Res. 76, 607–615.

Wang, Z., Fermini, B., Feng, J., Nattel, S., 1995. Role of chloride currents in repolarizing rabbit atrial myocytes. Am. J. Physiol. 268, H1992–H2002.