

Effects of MS-551, a new class III antiarrhythmic drug, on action potential and membrane currents in rabbit ventricular myocytes

¹Haruaki Nakaya, Noritsugu Tohse, Youji Takeda & Morio Kanno

Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060, Japan

1 Electrophysiological effects of MS-551, a new class III antiarrhythmic drug, were examined and compared with those of (+)-sotalol in rabbit ventricular cells.

2 In rabbit ventricular muscles stimulated at 1.0 Hz, MS-551 (0.1–10 μM) and (+)-sotalol (3–100 μM) prolonged action potential duration (APD) and effective refractory period without affecting the maximum upstroke velocity of phase 0 depolarization (\dot{V}_{max}). The class III effect of MS-551 was approximately 30 times more potent than that of (+)-sotalol.

3 Class III effects of MS-551 and (+)-sotalol showed reverse use-dependence, i.e., a greater prolongation of APD at a longer cycle length.

4 In rabbit isolated ventricular cells, 3 μM MS-551 and 100 μM sotalol inhibited the delayed rectifier potassium current (I_{K}) which was activated at more positive potentials than -50 mV and saturated around $+20$ mV.

5 MS-551 at a higher concentration of 10 μM decreased the transient outward current (I_{to}) and the inward rectifier potassium current (I_{K1}) although 100 μM sotalol failed to inhibit these currents.

6 MS-551 is a non-specific class III drug which can inhibit three voltage-gated K^+ channels in rabbit ventricular cells.

Keywords: Class III antiarrhythmic drugs; MS-551; (+)-sotalol; delayed rectifier K^+ current; transient outward current; inward rectifier K^+ current

Introduction

Class I antiarrhythmic drugs have been used for a long time in the treatment of life-threatening ventricular tachyarrhythmias. However, most currently used class I drugs may not adequately protect sudden cardiac death resulting from ventricular tachycardia and fibrillation (Friedman, 1984; CAST investigators, 1989). Such results provide further rationale for the continued search for alternative drug therapy to prevent or reduce likelihood of ventricular fibrillation. Recently much attention has been focused on class III antiarrhythmic drugs as the most promising candidates of antifibrillatory activity (Singh *et al.*, 1992). Several experimental studies have demonstrated that class III drugs such as (+)-sotalol (Lynch *et al.*, 1984; 1985), E-4031 (Lynch *et al.*, 1990; Katoh *et al.*, 1990; Chi *et al.*, 1991) and UK-68,798 (Black *et al.*, 1991; Zuanetti & Corr, 1991) prolonged ventricular effective refractory period (ERP) and showed anti-fibrillatory action in dogs with a previous myocardial infarction.

MS-551 is a new class III drug having a pyrimidinedione structure (Figure 1), which is reported to prolong ventricular ERP and exert antifibrillatory action in dogs with myocardial infarction (Kamiya *et al.*, 1992). However, the underlying ionic mechanism(s) responsible for class III effect of MS-551 have not been evaluated. Accordingly, in the present study effects of MS-551 on ionic currents were examined and compared with those of (+)-sotalol, a specific blocker of I_{K} (Komeichi *et al.*, 1990), in rabbit ventricular myocytes. The rabbit ventricular cell may be a suitable preparation for the evaluation of class III effect because a variety of voltage-gated K^+ channels including I_{to} channels (Hiraoka & Kawano, 1989; Giles & Imaizumi, 1988) are known to exist in the

cells as in human cardiac cells (Escande *et al.*, 1987). In addition, it has recently been reported that many class III drugs easily induce torsades de pointes in rabbits (Carlsson *et al.*, 1990). Therefore, in order to minimize the life-threatening proarrhythmias it is of importance to evaluate class III effects in rabbit ventricular cells.

Methods

Action potential study

New Zealand white rabbits weighing 2–4 kg were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} , i.v.) and the hearts were removed rapidly. The heart was immersed in an oxygenated Tyrode solution and small papillary muscles 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 solution equilibrated with 95% O_2 and 5% CO_2 . The composition of the solution was (mM): NaCl 125, KCl 4, NaH_2PO_4 1.8, MgCl_2 0.5, CaCl_2 2.7, NaHCO_3 25 and glucose 5.5. The bath temperature was kept constant at $36.0 \pm 1.0^\circ\text{C}$.

Transmembrane potentials were recorded by standard microelectrode techniques, as previously described (Nakaya *et*

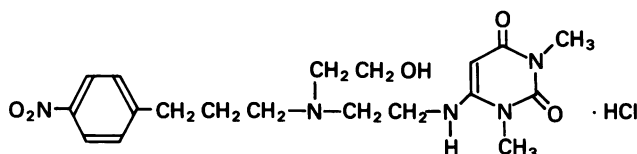


Figure 1 Chemical structure of MS-551.

¹ Author for correspondence at present address: Department of Pharmacology, School of Medicine, Chiba University, Chiba 260, Japan.

al., 1987). In brief, the preparation was electrically stimulated at 1.0 Hz with pulses of 1 ms duration at twice the diastolic threshold with a bipolar electrode. The stimuli were delivered by an electronic stimulator (Nihon Kohden SEN 6100, Tokyo, Japan) through an isolation unit (Nihon Kohden SS-302J). Transmembrane action potentials were recorded with glass microelectrodes filled with 3 M KCl, which had a tip resistance of 10–30 megohms. The microelectrode was coupled to the input stage of a high impedance amplifier with capacitance neutralization (Nihon Kohden MEZ 8201). The V_{\max} of the action potential was measured with an electronic differentiator having a linearity up to 1000 Vs⁻¹. The amplified signals were displayed on a dual beam oscilloscope (Nihon Kohden VC-10), photographed on 35 mm film and recorded on a chart recorder (Watanabe Sokki WR 3101, Tokyo, Japan).

After an equilibration period of 2 h, a stable impalement was obtained and control recordings were made. Effective refractory period (ERP), defined as the longest interval at which the premature stimulation failed to generate an active response, was determined by delivering a single test stimulus at progressively earlier diastolic intervals after a 30-s pulse train with an interstimulus interval of 1000 ms. The preparations were then exposed to a solution containing the lowest concentration of a class III antiarrhythmic drug, and the concentration was increased in a stepwise fashion at intervals of 30 min. Preliminary experiments revealed that a 30 min superfusion period was sufficient for action potential changes to reach a steady state. The recording of the membrane potential and the determination of ERP were repeated at the end of drug superfusion period. Only experiments in which a stable impalement was maintained were used for data analysis.

In another set of experiments the rate-dependence of APD-prolonging effects were examined in preparations stimulated at basic cycle lengths of 500, 1000, 2000 and 5000 ms. At each cycle length sufficient time (about 5 min) was allowed for APD to reach a steady state before measurements were made. Relationships between APD and stimulation rate were determined before and after 30-min exposure to class III drugs or 4-aminopyridine.

Patch clamp study

Single ventricular myocytes were obtained from New Zealand white rabbits by enzymatic dispersion, as described previously (Tohse *et al.*, 1990). Briefly, the heart was removed from the open chest rabbits anaesthetized with pentobarbitone sodium, and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal HEPES-Tyrode solution. The perfused medium was then changed to a nominally Ca²⁺-free Tyrode solution and then to a solution containing 0.01% w/v collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with a high K⁺, low Cl⁻ solution (KB solution) (Isenberg & Klockner, 1982). Ventricular tissue was cut into small pieces in the KB solution and the cell suspension was stored in a refrigerator (4°C) for later use. The composition of the normal HEPES-Tyrode solution was (mM): NaCl 143, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 0.33, glucose 5.5 and HEPES-NaOH buffer (pH 7.4), 5.0. The composition of the KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH₂PO₄ 20, MgCl₂ 3, glucose 10, EGTA 1.0 and HEPES-KOH buffer (pH 7.4) 10.

Whole-cell membrane currents were recorded by the patch clamp method (Hamil *et al.*, 1981). Single ventricular cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES-Tyrode solution at a rate of 5 ml min⁻¹. The temperature of the external solution was kept constant at 36.0 ± 1.0°C. Glass patch pipettes with a diameter of 3–4 µm were filled with an internal solution composed of (mM): K-aspartate 110, KCl 20, MgCl₂ 1.0,

ATP-K₂ 5.0, phosphocreatinine-K₂ 5.0, EGTA 0.5–10 and HEPES-KOH buffer (pH 7.4), 5.0. The resistance of the pipette filled with the internal solution was 1.5–2.5 megohms. After the gigaohm-seal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by applying more negative pressure to make the whole cell voltage-clamp mode. The electrode was connected to the input stage of a current-voltage converter with a feedback resistor of 100 megohms. The signals were displayed on a storage oscilloscope (Tektronics OP03, Beaverton, OR, U.S.A.) and were simultaneously fed to a data recording system, consisting of a converter system (SONY PCM-501 ES, Tokyo, Japan) and a video cassette recorder (National VF-F1, Tokyo, Japan) as a back up. The current and voltage signals, filtered at 2 kHz, were also stored on a personal computer (NEC PC98XA, Tokyo, Japan) equipped with a 20 Mbyte hard disc and an AD converter (Canopus Electronics ADX-98, Tokyo, Japan) for later analysis. A liquid junction potential between the internal solution and the bath solution of –8 mV was corrected.

In some experiments the delayed rectifier potassium current (I_K) was isolated from other membrane currents by using Na⁺- and K⁺-free external solution and the Ca²⁺ channel blockers nisoldipine (1 µM), as described previously (Tohse *et al.*, 1987). The Na⁺- and K⁺-free solution was prepared by replacing NaCl and KCl with equimolar choline chloride. By use of this solution, we could block the inward rectifier potassium current (I_{K1}), the calcium current (I_{Ca}), the electrogenic Na⁺/Ca²⁺ exchange current and the electrogenic pump current. In order to record the transient outward current (I_{to}), I_{Ca} was blocked by adding 1.8–2.0 mM Co²⁺ to the external solution. In a part of experiments for I_{to} recording, I_{Na} was incompletely blocked by adding 6–7 µM tetrodotoxin.

Drugs

The compounds used are as follows: MS-551 (Mitsui Pharmaceuticals, Tokyo, Japan), (+)-sotalol (Bristol-Myers Squibb Company, Wallingford, CT, U.S.A.), nisoldipine (Bayer AC, Leverkusen, Germany), tetrodotoxin (Sankyo, Tokyo, Japan) and 4-aminopyridine (Wako, Osaka, Japan). All drugs except for nisoldipine were dissolved in distilled water or suitable buffer solution. Nisoldipine was prepared as a stock solution (10 mM) in absolute ethanol.

Statistics

All values are presented in terms of mean ± s.e. Analysis by Student's *t* test was performed for paired or unpaired observations. *P* value of less than 0.05 was considered significant.

Results

Effects of MS-551 and (+)-sotalol on action potentials and ERP

Figure 2a illustrates representative changes in action potential configuration produced by MS-551 in rabbit papillary muscles stimulated at 1.0 Hz. MS-551 at 0.3–10 µM prolonged APD in a concentration-dependent manner with little effect on V_{\max} . The prolongation of APD induced by a high concentration of MS-551 was more marked at the late repolarization phase. In some preparations exposed to 10 µM MS-551, early afterdepolarizations appeared, as shown in Figure 2b. Similar changes in action potential configuration were also produced by (+)-sotalol although early afterdepolarizations were not evoked by the drug in concentrations examined (up to 100 µM).

Changes in action potential durations and ERP produced by MS-551 and (+)-sotalol are summarized in Table 1. The baseline characteristics of action potentials recorded from 12 preparations stimulated at 1.0 Hz were as follows: resting

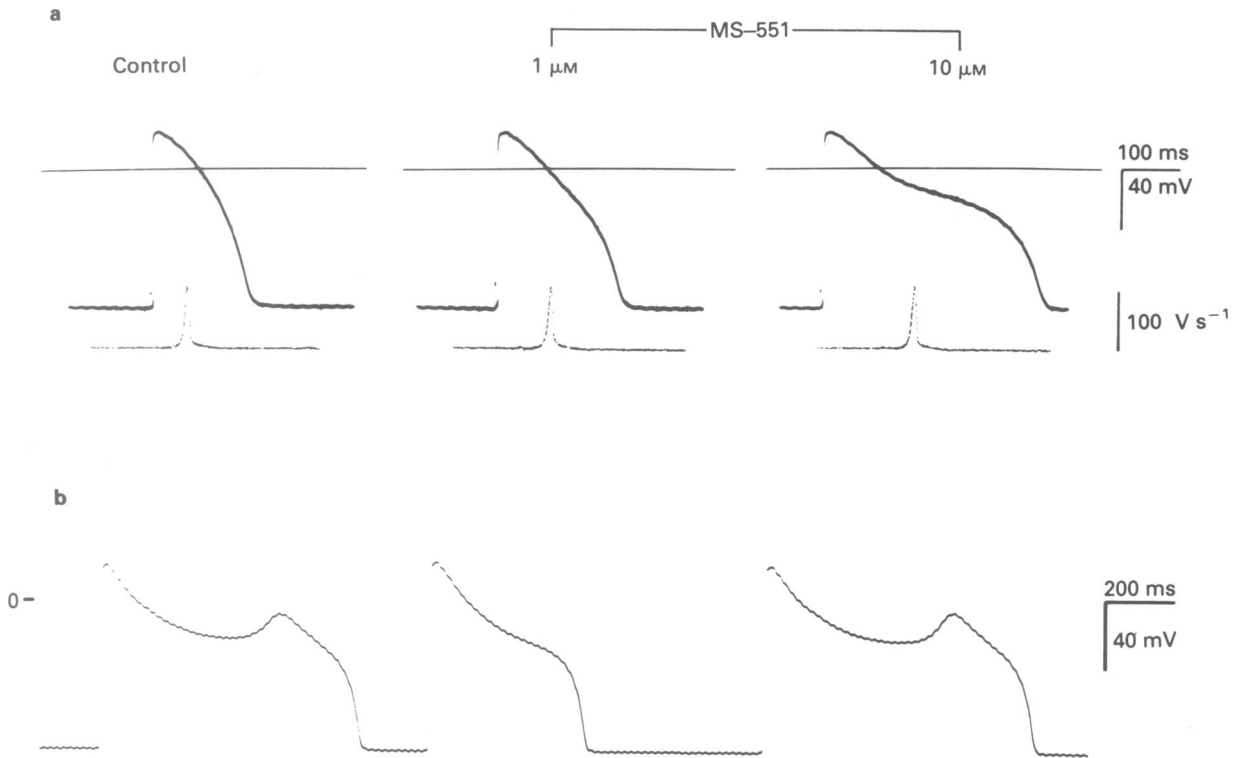


Figure 2 Effect of MS-551 on action potentials in rabbit papillary muscles driven at 1.0 Hz. (a) Concentration-dependent effects of MS-551 on action potentials. The upper, middle, and lower tracings in each record indicate zero potential, the transmembrane potential and dV/dt (on expanded scale), respectively. (b) Early afterdepolarizations induced by $10 \mu\text{M}$ MS-551 in a preparation.

Table 1 Effects of MS-551 and (+)-sotalol on action potential durations and effective refractory period in rabbit papillary muscles stimulated at 1.0 Hz

		APD_{20} (ms)	APD_{50} (ms)	APD_{90} (ms)	ERP (ms)
Control		63.3 ± 4.8	109.2 ± 8.1	147.5 ± 8.6	151.7 ± 9.5
MS-551	$0.1 \mu\text{M}$	61.7 ± 4.8	111.2 ± 9.0	154.2 ± 9.4	158.3 ± 10.5
	$0.3 \mu\text{M}$	61.7 ± 5.3	119.2 ± 11.1	170.8 ± 17.9	$171.7 \pm 15.3^*$
	$1 \mu\text{M}$	65.8 ± 5.5	$148.3 \pm 17.6^*$	$196.2 \pm 18.1^*$	$205.8 \pm 21.6^*$
	$3 \mu\text{M}$	$70.8 \pm 5.1^*$	$211.7 \pm 40.2^*$	$272.5 \pm 42.7^*$	$301.7 \pm 59.1^*$
	$10 \mu\text{M}$	$72.0 \pm 7.2^*$	246.0 ± 61.0	$313.0 \pm 64.5^*$	$321.0 \pm 73.1^*$
Control		61.5 ± 4.1	107.5 ± 5.4	149.2 ± 6.8	148.3 ± 8.9
(+)-Sotalol	$3 \mu\text{M}$	62.0 ± 5.0	110.3 ± 6.3	154.5 ± 9.3	155.0 ± 10.9
	$10 \mu\text{M}$	61.8 ± 5.2	$121.3 \pm 7.8^*$	$172.1 \pm 12.1^*$	$170.8 \pm 14.6^*$
	$30 \mu\text{M}$	62.0 ± 4.6	$153.3 \pm 19.4^*$	$210.5 \pm 24.2^*$	$212.5 \pm 29.3^*$
	$100 \mu\text{M}$	62.8 ± 6.0	180.5 ± 32.0	$248.7 \pm 41.8^*$	249.2 ± 47.4

Values are mean \pm s.e. of 5–6 experiments. APD_{20} , action potential duration at 20% repolarization level; APD_{50} , action potential duration at 50% repolarization level; APD_{90} , action potential duration at 90% repolarization level; ERP, effective refractory period. * $P < 0.05$ versus control value by paired t test.

membrane potential (RMP), -92.8 ± 0.5 mV; action potential amplitude (APA), 119.8 ± 6.4 mV; V_{max} , 158.5 ± 20.2 Vs^{-1} ; APD at 20% repolarization level (APD_{20}), 62.4 ± 3.0 ms; APD at 50% repolarization level (APD_{50}), 108.3 ± 4.7 ms; APD at 90% repolarization level (APD_{90}), 148.3 ± 5.2 ms. There were no significant differences in any of the baseline values of action potential parameters between subgroups. APDs and ERP were increased by MS-551 and (+)-sotalol in a concentration-dependent fashion. The APD- and ERP-prolonging effects of MS-551 were approximately thirty times more potent than those of (+)-sotalol. Neither MS-551 nor (+)-sotalol significantly affected V_{max} in the concentrations examined. MS-551 did not produce any

significant changes in RMP and APA in concentrations of 0.1 – $10 \mu\text{M}$.

Rate-dependence of APD prolongation produced by MS-551 ($3 \mu\text{M}$) and (+)-sotalol ($100 \mu\text{M}$) were examined and compared with that of APD prolongation produced by 4-aminopyridine in another set of experiments. In drug-free condition, APDs at a stimulation rate of 0.2 Hz were slightly shorter than those at 2.0 Hz (Figure 3a). There was some difference in the rate-dependent effect on APD between these compounds. 4-Aminopyridine at a concentration of 2 mM produced the largest increase in APD_{90} at the longest cycle length and the smallest increase in APD at the shortest cycle length, showing a striking 'reverse use-dependence' (Figure

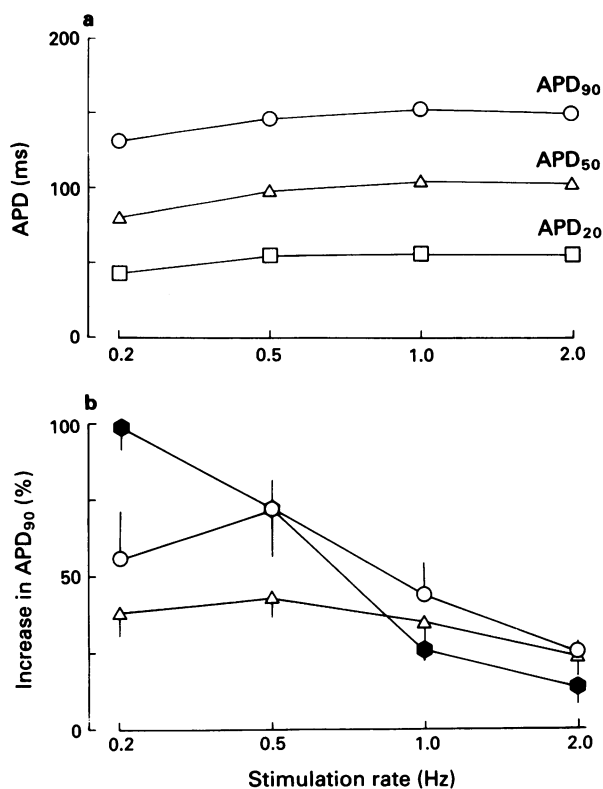


Figure 3 Rate-dependent effects of MS-551, (+)-sotalol and 4-aminopyridine (4-AP) on action potential duration (APD). (a) APD at 90% (APD₉₀, ○), 50% (APD₅₀, △) and 20% repolarization (APD₂₀, □) at various stimulation rates in drug-free condition. Absolute values of APDs are on the ordinate scale and stimulation rates are on the abscissa scale. Values are means of 15 preparations, and s.e. are smaller than the symbols. (b) Percentage increases in APD₉₀ by MS-551 (○), (+)-sotalol (△) and 4-AP (●) at various stimulation rates. Values are expressed as mean \pm s.e. of 5 preparations in each group.

3b). Although the reverse use-dependence of the class III effects of MS-551 and (+)-sotalol was not so marked as that with 4-aminopyridine, these drugs produced the largest increases in APD₉₀ at 0.5 Hz and smaller increases in APD₉₀ at shorter cycle lengths. The reverse use-dependence with MS-551 was more marked than that with (+)-sotalol.

Effects of MS-551 and (+)-sotalol on membrane currents in rabbit ventricular cells

Effects of MS-551 on membrane currents were examined and compared with those of (+)-sotalol in rabbit isolated ventricular myocytes. A representative example of the membrane ion currents of an isolated ventricular myocyte before and after application of 10 μ M MS-551 is shown in Figure 4. Membrane currents were elicited by 300 ms test pulses to various potentials from a holding potential of -38 mV at 0.1 Hz. MS-551 produced a small effect on the calcium current (I_{Ca}) that was induced by depolarizing pulses from the holding potential (Figure 4). MS-551 at concentrations of 3 and 10 μ M slightly decreased an amplitude of I_{Ca} at 2 mV, which was obtained by subtracting the late current from the peak of the initial inward current, by $1.5 \pm 12.7\%$ ($n = 6$, NS) and $26.5 \pm 5.7\%$ ($n = 6$, $P < 0.01$), respectively. On hyperpolarization, the steady state outward and inward currents were recorded and designated as the inward rectifier potassium current (I_{K1}). MS-551 effectively reduced I_{K1} and the slopes of $I-V$ relation at the level of resting potential, as shown in Figure 4. The decreases in I_{K1} at -58 mV by 3 and 10 μ M were $15.2 \pm 6.6\%$ (NS) and $30.1 \pm 10.3\%$ ($P < 0.05$),

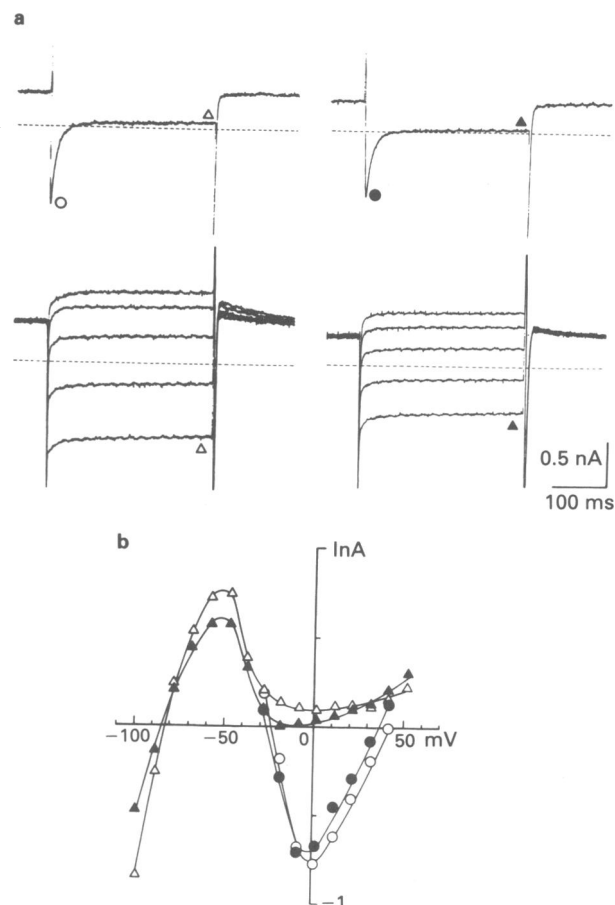


Figure 4 (a) Effects of MS-551 (10 μ M) on membrane currents of a single ventricular cell. Current traces induced by a depolarizing pulse (2 mV) from a holding potential of -38 mV (upper traces) and hyperpolarizing pulses (-58 mV to -98 mV) (middle traces) in the absence (left traces) and presence of 10 μ M MS-551 (right traces). Broken lines indicate 0 current level. Current-voltage relations for the peak current (●, ○) and the current at the end of a 300 ms test pulse (▲, △) in the absence (open symbols) and presence (closed symbols) of MS-551 are shown in (b). Note that MS-551 decreased the steady state outward current at -38 mV to $+12$ mV and the slopes of $I-V$ relation at the level of resting potential.

respectively. In addition, MS-551 decreased the steady state outward current at -38 mV to $+12$ mV, as shown in Figure 4. Similar changes in membrane currents were also elicited by 100 μ M (+)-sotalol. (+)-Sotalol reduced I_{Ca} at 2 mV slightly but significantly ($n = 8$, $26.4 \pm 8.0\%$, $P < 0.05$). The drug insignificantly reduced I_{K1} at -58 mV by $8.7 \pm 12.1\%$.

Effects of MS-551 and (+)-sotalol on the transient outward current (I_{to}) were examined in isolated ventricular myocytes. I_{to} was recorded by applying depolarizing pulses from a holding potential of -68 mV in Co^{2+} -containing solution with or without tetrodotoxin. In these conditions, we could record I_{to} which was sensitive to 2 mM 4-aminopyridine. Although MS-551 at a concentration of 3 μ M hardly affected I_{to} , the drug at a higher concentration of 10 μ M inhibited the current, as shown in Figure 5a. MS-551 decreased the peak current with little influence on the steady state current at the end of the 300 ms pulse. The decrease in the amplitude of I_{to} at $+12$ mV, which was designed as the difference between a peak current and the current at the end of the depolarizing pulse, after the exposure to 3 and 10 μ M MS-551 were $5.7 \pm 8.3\%$ ($n = 5$, NS) and $30.8 \pm 9.1\%$ ($n = 5$, $P < 0.05$), respectively. In contrast with the high concentration of MS-551, (+)-sotalol at a concentration of 100 μ M hardly affected I_{to} (Figure 5b). The decrease in the amplitude of I_{to} at $+12$ mV after 100 μ M (+)-sotalol was

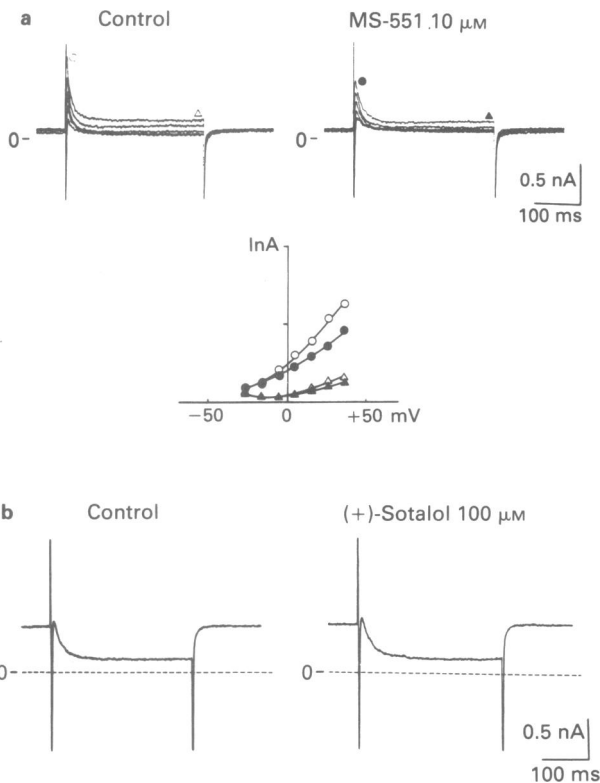


Figure 5 Effects of MS-551 (a) and (+)-sotalol (b) on the transient outward current (I_{to}). (a) I_{to} was elicited by depolarizing pulses of 300 ms duration to various potentials from a holding potential of -68 mV in Co^{2+} (2 mM)- and tetrodotoxin (7 μ M)-containing solution. MS-551 (10 μ M) decreased peak currents (circles) with little effect on the steady state currents (triangles). $I-V$ relationships before (open symbols) and after MS-551 (closed symbols) are also shown. (b) I_{to} was elicited by a depolarizing pulse to $+12$ mV from a holding potential of -68 mV in Co^{2+} (2 mM)-containing solution. I_{to} was hardly affected by (+)-sotalol.

$-5.3 \pm 9.9\%$ ($n = 4$, NS). Thus, MS-551 inhibited I_{to} especially at its high concentration but (+)-sotalol failed to inhibit the current up to the concentration of 100 μ M.

The delayed rectifier potassium current (I_K) was recorded in Na^+ -free, K^+ -free and nisoldipine (1 μ M)-containing external solution, in which I_{Ca} , I_{K1} , the electrogenic Na^+/Ca^{2+} exchange current and the electrogenic pump current were blocked. The membrane potential was held at -68 mV and clamped to more positive potentials for 3 s and then repolarized to the holding potential. The isolated outward current showed the properties of delayed rectification and the sigmoidal time course in its activation process, as shown in Figure 6. The amplitude of the outward current increased progressively in response to progressively larger depolarizing test pulses and so did that of the outward tail current upon subsequent repolarization to -68 mV. The outward tail current almost reached a saturation level around $+20$ mV. MS-551 decreased I_K at all potentials, as shown in Figure 6. The amplitude of the tail current of I_K defined as the difference between the peak current of the tail and the holding current, after the test potential to $+2$ mV was decreased by $29.9 \pm 10.1\%$ ($n = 5$, $P < 0.05$) and $39.8 \pm 16.4\%$ ($n = 3$) after the application of 3 μ M and 10 μ M MS-551, respectively. (+)-Sotalol also decreased I_K in the ventricular cells. The decrease in the amplitude of the tail current of I_K after the test potential of 2 mV was 36.4 and 24.3% after 100 μ M sotalol in two cells.

It has been reported that the class Ia drug, quinidine, delayed the activation of I_K in guinea-pig ventricular cells (Roden *et al.*, 1988). Therefore, the effect of MS-551 on the time course of I_K activation was examined by generating

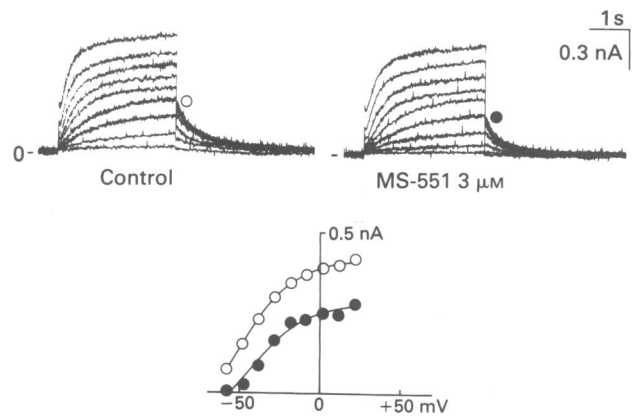


Figure 6 Effect of MS-551 on the isolated I_K . Current traces of I_K were recorded by using depolarizing pulses to nine different potentials with 10 mV steps between -58 mV to $+22$ mV before (left) and after 3 μ M MS-551 (right) in a Na^+ -free, K^+ -free and nisoldipine-containing solution. The holding potential was -68 mV and the duration of the pulse was 3 s. Current-voltage relations of the tail amplitude in the absence (\circ) and presence (\bullet) of MS-551 are illustrated below.

envelopes of current tails in the present study. An example of control I_K tails following depolarizing pulses of variable duration to -8 mV is shown in Figure 7a. The same envelope of current tails experiment was conducted after the application of 3 μ M MS-551 (Figure 7b). The I_K tail amplitudes as a function of activation pulse duration are shown in Figure 7c. The reduction of I_K after shorter pulses (50 – 300 ms) was greater than that after longer pulses (2000 – 7000 ms). The effect of MS-551 on I_K tails was greater after shorter depolarizing pulses. In other words, MS-551 delayed the activation of I_K . Similar results were also observed in another preparation.

Discussion

The present electrophysiological study has demonstrated that MS-551 is a potent class III antiarrhythmic agent in rabbit ventricular muscles. The class III effect, i.e., APD- and ERP-prolonging effect, of MS-551 was approximately 30 times more potent than that of (+)-sotalol on a molar basis. MS-551 as well as (+)-sotalol prolonged APD, especially at late repolarization phase. In addition, MS-551 induced early afterdepolarizations in isolated rabbit ventricular muscles. Such early afterdepolarizations may potentially lead to torsades de pointes *in vitro*, as observed with other class III drugs such as clofilium, sematilide, UK-68,798, LY97119 and amperozide (Carlsson *et al.*, 1990).

It is well-established that at least three kinds of potassium outward currents, i.e., I_K , I_{to} and I_{K1} , are important for repolarization in cardiac cells. It has been reported that I_{to} is involved in the rate-dependent changes in APD (Hiraoka & Kawano, 1989) and regional variations in action potential configuration (Fedida & Giles, 1991) in the rabbit ventricle. In addition, it was demonstrated that I_{to} is present in human atrial cells and plays a role in the repolarization of the action potential (Escande *et al.*, 1987; Shibata *et al.*, 1989). In terms of I_{K1} , it has been recently reported that I_{K1} is mainly responsible for final repolarization in rabbit ventricular cells (Shimoni *et al.*, 1992). In guinea-pig ventricular cells, basic characteristics of I_K were extensively studied (Matsuura *et al.*, 1987; Tohse, 1990) and inhibitory action of class III drugs on I_K was demonstrated (Arena & Kass, 1988; Komeichi *et al.*, 1990; Sanguinetti & Jurkiewicz, 1990; Gwilt *et al.*, 1991). As far as we know, however, I_K in rabbit ventricular cells has not been characterized. In the present study I_K was isolated from other currents with a Na^+ -free, K^+ -free and nisoldi-

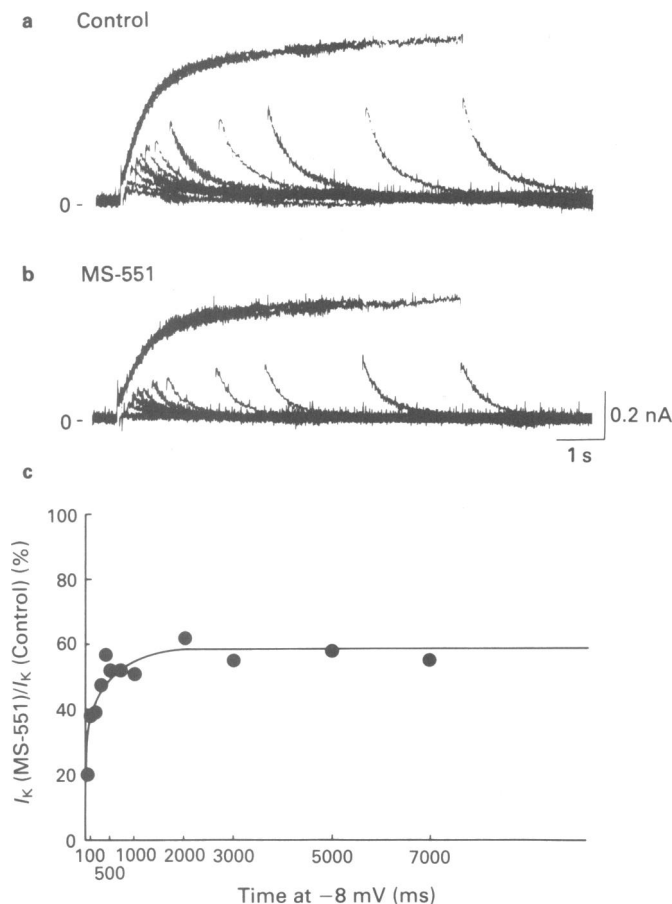


Figure 7 Envelopes of current tails before (a) and after exposure to 3 μM MS-551 (b). Holding potential was -68 mV and depolarizing pulses to -8 mV of various durations (50; 100; 200; 300; 400; 500; 700; 1000; 2000; 3000; 5000; and 7000 ms) were given. All twelve traces during the activating and deactivating sequences are shown superimposed. I_K tail amplitude (MS-551/Control as a %) as a function of activating pulse duration is shown in (c). Note that MS-551 delays I_K activation.

pine-containing solution. I_K of rabbit ventricular cells was activated at potentials more positive than -50 mV and saturated around $+20$ mV. These properties of rabbit ventricular I_K are clearly different from those of guinea-pig ventricular I_K , which is activated at potentials more positive than -30 mV and saturated around $+50$ mV (Komeichi *et al.*, 1990; Tohse, 1990). Such I_K , observed in rabbit ventricular myocytes, appeared to be in part similar to the I_K of rabbit sinoatrial and atrioventricular nodes (Shibasaki, 1987).

In the present study 3 μM MS-551 and 100 μM sotalol commonly inhibited the I_K in rabbit ventricular cells. The inhibition of I_K might be involved in the decrease in the steady-state outward current between -40 mV and -20 mV and the prolongation of APD at a late repolarization phase. In this context, Sanguinetti & Jurkiewicz (1990) reported that E-4031, a potent class III drug, and sotalol inhibited a rapid component of I_K (I_{Kr}) with small effect on a slow component (I_{Ks}) in guinea-pig ventricular cells. They showed that I_{Kr} was rapidly activated at -30 mV to 0 mV and was effectively inhibited by class III drugs. However, in their study I_{Kr} was not completely separated from I_{K1} . Recently it has been

reported that E-4031 can also block I_{K1} channels in guinea-pig ventricular cells (Koumi *et al.*, 1991). Therefore, I_{K1} was blocked by using K^+ -free solution and effect of MS-551 on the envelopes of current tails of I_K was examined in rabbit ventricular cells. The envelope test revealed that MS-551 delayed the activation of I_K . Similar delay in the I_K activation was also observed with the class Ia drug, quinidine, in guinea-pig ventricular cells (Roden *et al.*, 1988). These findings might be interpreted as showing that binding of MS-551 to I_K channels is state- and/or voltage-dependent.

Sotalol at a concentration of 100 μM failed to inhibit I_{to} and I_{K1} . Although MS-551 at a concentration of 3 μM did not significantly inhibit I_{to} and I_{K1} , the drug at a concentration of 10 μM suppressed these currents. Therefore, MS-551 appears to nonspecifically inhibit potassium currents at high concentrations. It has been reported that tedisamil, a class III drug, blocks not only I_K but also I_{to} in rat and guinea-pig isolated ventricular myocytes (Dukes *et al.*, 1990). We have also reported that N-acetylprocainamide decreased both I_K and I_{K1} in guinea-pig ventricular myocytes (Komeichi *et al.*, 1990).

In the present study both MS-551 and (+)-sotalol had little effect on V_{max} of action potentials. Therefore, these drugs appear to be of little inhibitory action on the sodium channels. In isolated ventricular myocytes I_{Ca} was slightly but significantly decreased by 10 μM MS-551 and 100 μM (+)-sotalol. Therefore, in high concentrations these drugs may slightly inhibit I_{Ca} .

It is known that many class III drugs except for amiodarone exhibit reverse use-dependent effects on APD, i.e., greater prolongation of APD at a longer cycle length (Hondegheem & Snyder, 1990). The present study revealed that MS-551 and (+)-sotalol showed reverse use-dependence although it was not so marked as that produced by a specific I_{to} blocker, 4-aminopyridine. The reverse use-dependent class III effect may be undesirable, because these drugs may limit their efficacy during tachyarrhythmias and cause torsades de pointes at a slow heart rate. Recently it has been also reported that α -adrenoceptor stimulation causes QT prolongation and torsades de pointes potentially resulting from early afterdepolarization in animals receiving K^+ channel blockers (Carlsson *et al.*, 1990; Ben-David & Zipes, 1990). Although the underlying ionic mechanism(s) responsible for the α -adrenoceptor-mediated QT prolongation and early afterdepolarizations are not well defined, the α -adrenoceptor-mediated inhibition of I_{to} (Apkon & Nerbonne, 1988; Fedida *et al.*, 1989; Tohse *et al.*, 1990) and I_{K1} (Fedida *et al.*, 1991) may play an important role. In addition, hypokalaemia is known to prolong QT, which may be attributable to the inhibition of I_{K1} channels by extracellular hypokalaemia (Kameyama *et al.*, 1983). Therefore, class III drugs including MS-551 should be administered with a great caution to the patients with sinus bradycardia, hypokalaemia, and potentially high sympathetic tone.

In conclusion, MS-551 is a potent class III antiarrhythmic drug which prolongs APD reverse use-dependently. The class III effect of the drug may be ascribed to the inhibitory action on three voltage-gated K^+ channels in rabbit ventricular cells.

The authors wish to thank Ms Y. Shishido, Mr M. Tamagawa and I. Sakurada for their excellent technical assistance. We also thank Ms R. Yamazaki, Y. Yonezawa and I. Sakashita for secretarial work. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, and Culture of Japan.

References

- APKON, M. & NERBONNE, J.M. (1988). α_1 -Adrenergic agonists selectively suppress voltage-dependent K^+ currents in rat ventricular myocytes. *Proc. Natl. Acad. Sci. USA*, **85**, 8756–8760.
- ARENA, J.P. & KASS, R.S. (1988). Block of heart potassium channels by clofilium and its tertiary analogs: relationship between drug structure and type of channel blocked. *Mol. Pharmacol.*, **34**, 60–66.
- BEN-DAVID, J. & ZIPES, D.P. (1990). α -Adrenoceptor stimulation and blockade modulates cecium-induced early afterdepolarizations and ventricular tachyarrhythmias in dogs. *Circulation*, **82**, 225–233.
- BLACK, S.C., CHI, L., MU, D.X. & LUCCHESI, B.R. (1991). The antifibrillatory actions of UK-68,798, a class III antiarrhythmic agent. *J. Pharmacol. Exp. Ther.*, **258**, 416–423.
- CARLSSON, L., ALMGREN, O. & DUKER, G. (1990). QTU-prolongation and torsades de pointes induced by putative class III antiarrhythmic agents in the rabbit: etiology and interventions. *J. Cardiovasc. Pharmacol.*, **16**, 276–285.
- CAST INVESTIGATORS (1989). Preliminary report: effects of encainide and flecainide on mortality in randomized trial of arrhythmia suppression after myocardial infarction. *N. Eng. J. Med.*, **321**, 406–412.
- CHI, L., MU, D.A. & LUCCHESI, B.R. (1991). Electrophysiology and antiarrhythmic actions of E-4031 in the experimental animal model of sudden coronary death. *J. Cardiovasc. Pharmacol.*, **17**, 285–295.
- DUKES, I.D., CLEEMANN, L. & MORAD, M. (1990). Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glia cells. *J. Pharmacol. Exp. Ther.*, **254**, 560–569.
- ESCANDE, D., COULOMBE, A., FAIVRE, J.F., DEROUBAIX, E. & CORABOEUF, E. (1987). Two types of transient outward currents in adult human atrial cells. *Am. J. Physiol.*, **252**, H142–H148.
- FEDIDA, D., BRAUN, A.P. & GILES, W.R. (1991). α_1 -Adrenoceptors reduce background K^+ current in rabbit ventricular myocytes. *J. Physiol.*, **441**, 673–684.
- FEDIDA, D. & GILES, W.R. (1991). Regional variations in action potentials and transient outward current in myocytes isolated from rabbit left ventricle. *J. Physiol.*, **442**, 191–209.
- FEDIDA, D., SHIMONI, Y. & GILES, W.R. (1989). A novel effect of norepinephrine on cardiac cells is mediated by α_1 -adrenoceptors. *Am. J. Physiol.*, **256**, H1500–H1504.
- FRIEDMAN, L.M. (1984). Can chronic antidysrhythmic treatment prevent cardiac death? *Eur. Heart. J.*, **5** (Suppl. B), 99–101.
- GILES, W.R. & IMAIZUMI, Y. (1988). Comparison of potassium currents in rabbit atrial and ventricular cells. *J. Physiol.*, **405**, 123–145.
- GWILT, M., ARROWSMITH, J.E., BLACKBURN, R.A., BURGESS, R.A., CROSS, P.E., DALRYMPLE, H.W. & HIGGINS, A.J. (1991). UK-68,798: a novel, potent and highly selective class III antiarrhythmic agent which blocks potassium channels in cardiac cells. *J. Pharmacol. Exp. Ther.*, **256**, 318–324.
- HAMIL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **395**, 85–100.
- HIRAOKA, M. & KAWANO, S. (1989). Calcium-sensitive and insensitive transient outward current in rabbit ventricular myocytes. *J. Physiol.*, **410**, 187–212.
- HONDEGHEM, L.M. & SNYDER, D.J. (1990). Class III antiarrhythmic agents have a lot of potential but a long way to go: reduced effectiveness and dangers of reverse use dependence. *Circulation*, **81**, 686–690.
- ISENBERG, G. & KLOCKNER, R. (1982). Calcium-tolerant ventricular myocytes prepared by preincubation in a 'KB medium'. *Pflügers Arch.*, **395**, 6–18.
- KAMEYAMA, M., KIYOSUE, T. & SOEJIMA, M. (1983). Single channel analysis of the inward rectifier K current in rabbit ventricular cells. *Jpn. J. Physiol.*, **33**, 1039–1056.
- KAMIYA, J., ISHI, M. & KATAKAMI, T. (1992). Antiarrhythmic effects of MS-551, a new class III antiarrhythmic agent, on canine models of ventricular arrhythmia. *Jpn. J. Pharmacol.*, **58**, 107–115.
- KATOH, H., OGAWA, S., FURUNO, I., SATO, Y., YOH, S., SAEKI, K. & NAKAMURA, Y. (1990). Electrophysiologic effects of E-4031, a new class III antiarrhythmic agent, on re-entrant ventricular arrhythmias in a canine 7-day-old myocardial infarction model. *J. Pharmacol. Exp. Ther.*, **253**, 1077–1082.
- KOMEICHI, K., TOHSE, N., NAKAYA, H., SHIMIZU, M., ZHU, M.Y. & KANNO, M. (1990). Effects of N-acetylprocainamide and sotalol on ion currents in isolated guinea-pig ventricular myocytes. *Eur. J. Pharmacol.*, **187**, 313–322.
- KOUMI, S., SATO, R., HISATOME, I., NAGASAWA, K. & HAYAKAWA, H. (1991). E-4031 blocks the inward-rectifier K^+ channel through the hydrophobic pathway. *Circulation*, **84** (Suppl. II), 508.
- LYNCH, J.J., WILBER, D.J., MONTGOMERY, D.G., HSIEH, T.M., PATTERSON, E. & LUCCHESI, B.R. (1984). Antiarrhythmic and antifibrillatory actions of the levo- and dextrorotatory isomers of sotalol. *J. Cardiovasc. Pharmacol.*, **6**, 1132–1141.
- LYNCH, J.J., COSKEY, L.A., MONTGOMERY, D.G. & LUCCHESI, B.R. (1985). Prevention of ventricular fibrillation by dextrorotatory sotalol in a conscious canine model of sudden coronary death. *Am. Heart. J.*, **109**, 949–958.
- LYNCH Jr, J.J., HEANEY, L.A., WALLACE, A.A., GEHRET, J.R., SELNICK, H.G. & STEIN, R.B. (1990). Suppression of lethal ischemic ventricular arrhythmias by the Class III agent E4031 in a canine model of myocardial infarction. *J. Cardiovasc. Pharmacol.*, **15**, 764–775.
- MATSUURA, H., EHARA, T. & IMOTO, Y. (1987). An analysis of the delayed outward current in single ventricular cells of the guinea pig. *Pflügers Arch.*, **410**, 596–603.
- NAKAYA, H., TOHSE, N. & KANNO, M. (1987). Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. *Am. J. Physiol.*, **253**, H1089–H1097.
- RODEN, D.M., BENNET, P.B., SNYDER, D.J., BALSER, J.R. & HONDEGHEM, L.C. (1988). Quinidine delays I_K activation in guinea pig ventricular myocytes. *Circ. Res.*, **62**, 1055–1058.
- 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.
- SHIBASAKI, T. (1987). Conductance and kinetics of delayed rectifier potassium channels in nodal cells of the rabbit heart. *J. Physiol.*, **387**, 227–250.
- SHIBATA, E.F., DURTY, 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.
- SHIMONI, Y., CLARK, R.B. & GILES, W.R. (1992). Role of an inwardly rectifying potassium current in rabbit ventricular action potential. *J. Physiol.*, **448**, 709–727.
- SINGH, B.N., SARNA, J.S.M., ZHANG, Z.H. & TANAKA, C. (1992). Controlling cardiac arrhythmias by lengthening repolarization: rationale from experimental findings and clinical considerations. In *QT Prolongation and Ventricular Arrhythmias*. ed. Hashiba, K., Moss, A.J. & Schwartz, P.J. pp. 187–209. New York: The New York Academy of Science.
- TOHSE, N. (1990). Calcium-sensitive delayed rectifier potassium current in guinea-pig ventricular cells. *Am. J. Physiol.*, **258**, H1200–H1207.
- TOHSE, N., KAMEYAMA, M. & IRISAWA, H. (1987). Intracellular Ca^{2+} and protein kinase C modulate K^+ current in guinea pig heart cells. *Am. J. Physiol.*, **253**, H1321–H1324.
- TOHSE, N., NAKAYA, H., HATTORI, Y., ENDOU, M. & KANNO, M. (1990). Inhibitory effect mediated by α_1 -adrenoceptors on transient outward current in isolated rat ventricular cells. *Pflügers Arch.*, **415**, 575–581.
- ZUANETTI, G. & CORR, P.B. (1991). Antiarrhythmic efficacy of a new class III agent, UK-68,798, during chronic myocardial infarction: evaluation using three-dimensional mapping. *J. Pharmacol. Exp. Ther.*, **256**, 325–334.

(Received November 16, 1992
Revised January 12, 1993
Accepted January 13, 1993)