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Effects of class III antiarrhythmic drugs on the Na^+-activated K^+ channels in guinea-pig ventricular cells

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1 Class III antiarrhythmic drugs are known to block the outward currents through voltage-gated K^+ channels. However, effects of class III antiarrhythmic drugs on the ligand-gated K^+ channels have not been thoroughly examined. In this study effects of amiodarone and newer class III antiarrhythmic drugs, E-4031 and MS-551, on the Na^+-activated K^+ (K_{Na}) current were examined in inside-out membrane patches and in whole cells isolated from guinea-pig ventricle.

2 The K_{Na} channel current was activated by increasing $[Na^+]_i$ from 0 mM to 30-100 mM with 150 mM $[K^+]_o$ in inside-out membrane patches of ventricular myocytes. The channel current showed a larger slope conductance (210 pS), inward-going rectification and subconductance levels of various amplitudes.

3 E-4031 and MS-551 at high concentrations (300 μM) inhibited the K^+ current by decreasing the open time (flickering block). On the other hand, amiodarone at relatively low concentrations (0.1-10 μM) inhibited the K_{Na} channel current by decreasing the open probability rather than by decreasing the open time. The IC_{50} value of amiodarone for inhibiting the K_{Na} channel current was 1.0 μM.

4 These drugs also inhibited the whole-cell outward current activated by intracellular loading of 50 mM $[Na^+]_i$ and extracellular application of 10 μM ouabain.

5 These results indicate that class III antiarrhythmic drugs inhibit the K_{Na} channel current in cardiac cells. However, there are sharp differences in the effective concentrations and the mode of inhibition between amiodarone and the newer class III antiarrhythmic drugs.

Keywords: Class III antiarrhythmic drugs; Na^+-activated K^+ current; amiodarone; E-4031; MS-551

Introduction

Recently much attention has been focused on class III antiarrhythmic drugs as one of the candidates to reduce the likelihood of sudden cardiac death (Singh *et al.*, 1993; Uprichard & Lucchessi, 1994). These drugs are known to block cardiac K^+ channels and thereby prolonging action potential duration (APD). More than six types of K^+ channels have been described in the heart (Carmeliet, 1993). Of these, most class III antiarrhythmic drugs have been shown to inhibit the outward currents through voltage-gated K^+ channels such as the delayed rectifier K^+ current (I_K), the transient outward current (I_{to}) and the inward rectifier K^+ current (I_{K1}) (Singh *et al.*, 1993; Colatsky *et al.*, 1990, 1994). However, effects of class III antiarrhythmic drugs on the outward current through ligand-gated K^+ channels such as the muscarinic acetylcholine-receptor-operated K^+ current ($I_{K.ACh}$), the ATP-sensitive K^+ current ($I_{K.ATP}$) and the Na^+-activated K^+ current ($I_{K.Na}$) have not been thoroughly examined. Recently we have demonstrated that some class III antiarrhythmic drugs inhibit $I_{K.ACh}$ by blocking the atrial muscarinic receptors while others inhibit the current not only by blocking the muscarinic receptors but also by depressing the function of the K^+ channel itself and/or G proteins (Mori *et al.*, 1995). In addition, class III antiarrhythmic drugs produce little or minimal inhibition of $I_{K.ATP}$ in cardiac cells (Escande *et al.*, 1994; Mori *et al.*, 1994). However, to the best of our knowledge, effects of class III antiarrhythmic drugs on $I_{K.Na}$ have not been evaluated. Therefore, the purpose of the present study was to examine the effects of class III antiarrhythmic drugs, including amiodarone, on $I_{K.Na}$ in isolated ventricular myocytes.

The Na^+-activated K^+ (K_{Na}) channels that are activated by an increase in extracellular Na^+ concentration were first described in cardiac cells by Kameyama *et al.* (1984). However, the pathophysiological significance of the K_{Na} channel is not fully understood although activation of the cardiac K_{Na} channels during digitalis toxicity has been suggested (Luk & Carmeliet, 1990; Carmeliet, 1992). Therefore, the development of potent blockers of the K_{Na} channels may lead to a better understanding of the pathophysiological roles of these channels. The findings presented here indicate that class III antiarrhythmic drugs such as amiodarone, E-4031 and MS-551 inhibit the single channel current of the K_{Na} channels of guinea-pig ventricular cell by different modes and at different concentrations, and that these drugs commonly inhibit the ouabain-induced outward current.

Methods

Cell preparation

Single ventricular cells of the guinea-pig heart were isolated by an enzymatic dissociation method, as described previously (Tohse *et al.*, 1992). Briefly, guinea-pigs weighing 250-350 g were anaesthetized with pentobarbitone sodium. The heart was removed from the open chest guinea-pigs 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 Ca^{2+}-free Tyrode solution and then to a solution containing 0.02% w/v collagenase (Wako, Osako, Japan). After digestion, the heart was perfused with a high K^+, low Cl^- solution (a modified KB solution) (Isenberg & Klockner, 1982; Tohse *et al.*, 1992). 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, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5, and HEPES-NaOH buffer (pH 7.4) 5.0. The composition of the modified

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KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 1.0 and HEPES-KOH buffer (pH 7.4) 10. The ventricular cells were placed in a recording chamber attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES-Tyrode solution.

Single-channel recording

Single K_{Na} channel current was recorded in the inside-out configuration of the patch clamp technique (Hamil *et al.*, 1981). Patch electrodes were fabricated from glass capillaries (o.d. 1.5 mm) by a two-stage puller (Narishige, PB-7, Tokyo, Japan), coated near their tips with silicone and heat-polished. These electrodes had a tip resistance of 5–10 MΩ when filled with the pipette solution.

It has been shown that the single channel current of the K_{Na} channel has inward or outward rectification, which is dependent on the K^+ and Na^+ concentrations of the internal and external solutions (Luk & Carmeliet, 1990; Wang *et al.*, 1991). Accordingly, we used two different internal and external (pipette) solutions to record the inward and outward single channel current of the K_{Na} channel. To record the inward current, an internal solution containing: (mM): NaCl 100, KCl 50, $NaHPO_4$ 0.3, $MgCl_2$ 0.5, EGTA 5, ATP-Na_2 1 and HEPES-NaOH buffer (pH 7.4) 5 and an external solution containing (mM): KCl 150, $CaCl_2$ 1.8 and HEPES-NaOH buffer (pH 7.4) 5 were used. To record the outward current of the K_{Na} channel, an internal solution containing (mM): NaCl 30, KCl 120, $NaHPO_4$ 0.3, $MgCl_2$ 0.5, EGTA 5, ATP-Na_2 1 and HEPES-NaOH buffer (pH 7.4) 5 and an external solution containing normal HEPES-Tyrode solution and 100 μM ouabain were used.

The single-channel currents from inside-out patches were recorded by a patch-clamp amplifier (Nihon-Kohden CEZ-2300, Tokyo, Japan) at room temperature (20–25°C) and stored on video tape recorder (Hitachi VT-F20, Tokyo, Japan) through a pulse code modulation system (Instrutech Corp. VR-10B, New York, NY) for later analysis. The frequency response of the recording system was flat up to 37 kHz. The data were filtered at 2 kHz with digital Guassian filter and digitilized at 5 kHz for data analysis with pCLAMP software (Version 5.5, Axon Instruments, Foster City, CA) and an IBM compatible computer (Compaq Prolinea 4/50 with a 200 M Byte hard disc, U.S.A.). Channel openings were identified by an algorithm that used both amplitude and slope information, and measured with an interactive threshold for detecting events which was set at 50% of the expected amplitude. The probability of opening (P_o) of a single channel was defined as the amount of time in which the channel remained in the open state by the total time of the recordings.

Whole cell recording

Whole cell recordings were performed by the same patch-clamp amplifier at 36.0±1.0°C. Glass patch pipettes with a diameter of 1.5 mm were filled with an internal solution containing (in mM): KOH 100, NaCl 30, L-aspartate 100, $MgCl_2$ 1.0, ATP-Na_2 5.0, phosphocreatinine-Na_2 5.0, EGTA 10 and HEPES-KOH buffer (pH 7.4) 5.0. The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8.0 according to the calculation by Fabiato and Fabiato (1979) with the correction of Tsien and Rink (1980). The resistance of the patch pipette filled with the internal solution was 2–3 MΩ. After the formation of giga-ohm-seal, the bath solution was switched from the normal HEPES-Tyrode solution to a nominally Ca^{2+}-free solution in order to block the outward Na^+-Ca^{2+} exchange current. Then, the membrane patch was disrupted by applying more negative pressure to make the whole cell voltage-clamp mode. Voltage command pulses were generated, and data were acquired by the IBM compatible computer and the pCLAMP software. Current signals were digitized with a sampling interval of 2 kHz and stored on the hard disk of the computer.

A ramp pulse protocol was used to record the quasi-steady-state membrane current. The membrane potential was held at −40 mV and depolarized first to +50 mV at a rate of 1.2 $mVms^{-1}$. It was then repolarized or hyperpolarized to −100 mV with a slope of −1.2 $mV\ ms^{-1}$, during which time the change in the membrane current was automatically plotted against the membrane potential. The voltage protocol was repeated at a frequency of once every 20 s. After stabilization of the steady-state current induced by the ramp pulses, cells were exposed to 10 μM ouabain to induce an outward current, i.e., the Na^+-activated K^+ current ($I_{K.Na}$). Then, the effects of class III antiarrhythmic drugs on the ouabain-induced outward current were examined. To calculate the % inhibition of $I_{K.Na}$ by various drugs, the difference between the steady-state current in solution containing 10 μM ouabain and the current level in the absence of ouabain was taken as 100%.

Drugs

The following drugs were used: amiodarone (Taisho Pharmaceutical Co., Omiya, Japan), MS-551 (1,3-dimethyl-6-{2-*N*-(2-hydroxyethyl)-3-(4-nitrophenyl)propylamino]ethylamino}-2,4-(1H,3H)-pyrimidinedione hydrochloride) (Mitsui Pharmaceuticals, Tokyo, Japan), E-4031 (*N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonylphenyl] methanesulphonamide dihydrochloride dihydrate) (Eisai Co., Ltd. Tsukuba, Japan), and ouabain (Wako, Osaka, Japan). Amiodarone was dissolved in absolute ethanol at a concentration of 1 mM, and

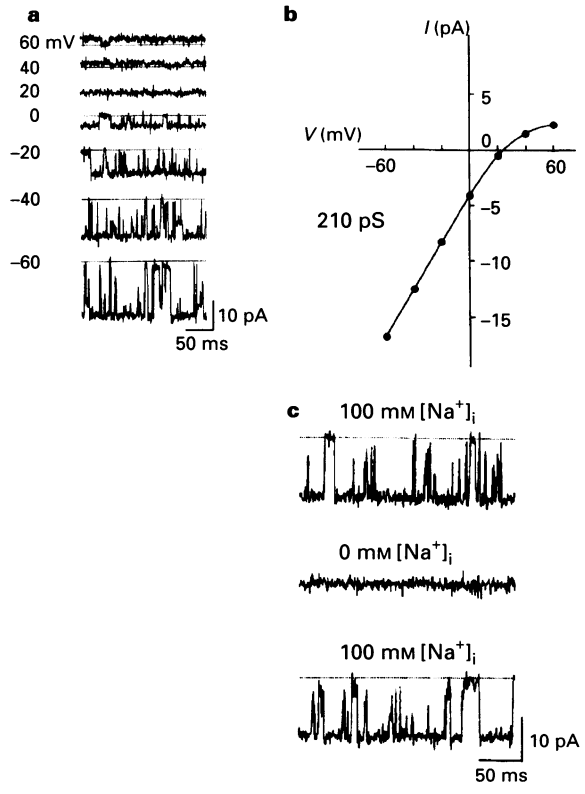

Figure 1 Na^+-activated K^+ channel current recorded in inside-out membrane patch of guinea-pig ventricular cells. (a) Current traces at clamp potentials between +60 and −60 mV with the internal solution of 50 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+. The dotted lines indicate the closed state current level. (b) Current-voltage relationship of the channel current recorded with the internal solution of 50 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+. The slope conductances were measured over the linear part of the *I-V* curve. (c) The channel activity disappeared in Na^+-free internal solution (substituted by $choline^+$).

then added to the bath solution containing bovine serum albumin (1%), as described by Honjo *et al.* (1991). It was confirmed that the solvent of amiodarone did not affect the K_{Na} channel current. The other drugs were dissolved in distilled water.

Statistics

All values are presented in terms of mean ± s.e. Student's *t* test was used for statistical analysis of the data. A *P* value of less than 0.05 was considered significant. IC_{50} values were obtained by use of Macintosh computer (Apple Computer, Inc., U.S.A.) and Sigma Plot program (Jandel Corporation, CA, U.S.A.).

Results

The single Na^+-activated K^+ channel current was recorded from the inside-out membrane patches. Figure 1a shows typical records at various clamp potentials with the internal solution of 50 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+. The channel current showed subconductance levels of various amplitudes between the closed level and the full-open level at each membrane potential. The amplitudes of the full-open channel current were plotted against the membrane potentials, as shown in Figure 1b. The current-voltage relationship showed inward-going rectification. The mean slope conductances, calculated from the slope of the current-voltage curve at negative potentials, were 210.4 ± 3.6 pS ($n=6$). The reversal potential (+24.5 ± 1.1 mV) was close to the potassium equilibrium potential calculated by using the Nernst equation. The activity of the channel disappeared in a Na^+-free internal solution (substituted by choline$^+$) (Figure 1c). The channel activity reappeared when the bath was perfused with 100 mM Na^+.

Effects of amiodarone on the inward K_{Na} channel current recorded at a holding potential of −40 mV with the internal solution containing 100 mM Na^+ and 50 mM K^+ and the external solution containing 150 mM K^+ were examined. As shown in Figure 2a, amiodarone inhibited the openings of the K_{Na} channel. The inhibitory effect of amiodarone rapidly appeared and reached a steady state within 1 min after the introduction of amiodarone. Amiodarone at a concentration of 1 μM significantly decreased the open probability (P_o) from 0.72 ± 0.04 to 0.36 ± 0.04 without affecting the single channel conductance ($n=7$) (Table 1). The K_{Na} channel activity returned to the control level after 3 min washout of amiodarone.

Effects of amiodarone on the open and closed time distributions were also evaluated. Probability density histograms of the open and closed times measured at −40 mV in the absence and presence of 1 μM amiodarone are shown in Figure 3. The open time histogram revealed a single exponential distribution with a time constant (τ_o) of 5.81 ± 0.80 ms, which was not significantly affected by 1 μM amiodarone ($n=7$) (Table 1). The closed time histogram consisted of two time constants of a fast ($\tau_{c.f}$) and a slow component ($\tau_{c.s}$) (Figure 3). The value of $\tau_{c.s}$ was significantly increased from 4.16 ± 0.26 ms to 10.04 ± 1.86 ms by amiodarone (Table 1).

The outward K_{Na} channel current was also recorded at a holding potential of +40 mV with the internal solution containing 30 mM Na^+ and 120 mM K^+ and the external solution containing 143 mM Na^+, 5.4 mM K^+ and 100 μM ouabain. Amiodarone at a concentration of 1 μM also inhibited the openings of the K_{Na} channel (Figure 2b). The inhibitory effect of amiodarone on the outward K_{Na} channel current also appeared very rapidly and reached a steady state within 1 min. It significantly decreased P_o of the outward K_{Na} channel current from 0.76 ± 0.06 to 0.36 ± 0.02 ($n=3$). Although amiodarone did not affect the values of τ_o and $\tau_{c.f}$ it increased the $\tau_{c.s}$ value

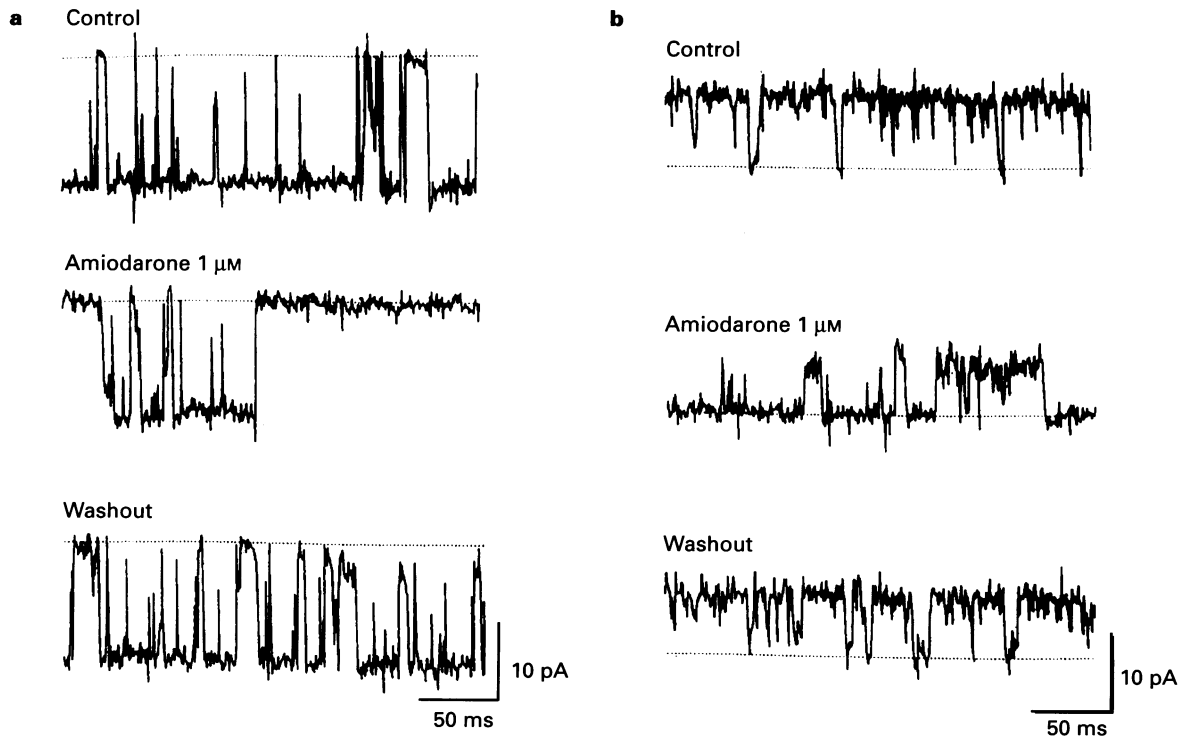

Figure 2 Effects of amiodarone on the Na^+-activated K^+ channel currents. (a) Effect of amiodarone on the inward K_{Na} channel current recorded at a holding potential of −40 mV with the internal solution of 50 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+. (b) Effect of amiodarone on the outward K_{Na} channel current recorded at a holding potential of +40 mV with the internal solution containing 30 mM Na^+ and 120 mM K^+ and the external solution containing 143 mM Na^+, 5.4 mM K^+ and 100 μM ouabain. Note that amiodarone inhibited the inward and outward K_{Na} channel current by decreasing the channel openings.

(Table 1). The inhibitory effect of amiodarone on the outward K_{Na} channel current was also reversible within a few minutes after changing to the drug-free solution.

E-4031 and MS-551, pure class III antiarrhythmic drugs, also inhibited the K_{Na} channel current in their high concentrations. In 8 inside-out membrane patches held at −40 mV, E-4031 at a concentration of 300 μM significantly decreased P_o of the inward K_{Na} channel current from 0.79±0.03 to 0.45±0.04 by decreasing the open time (flickering block) ($n=8$) (Figure 4a and Table 1). As shown in Figure 5, E-4031 markedly decreased the τo value and slightly decreased the τc.s and τc.f values. MS-551 inhibited the inward

Table 1 Effects of class III antiarrhythmic drugs on the open probability, open and closed time constants of the inward and outward K_{Na} channel currents

	Amiodarone		*MS-551*		*E-4031*	
	Control	*1 μM*	*Control*	*300 μM*	*Control*	*300 μM*
Inward K_{Na} channel current						
	($n=7$)		($n=8$)		($n=8$)	
P_o	0.72±0.04	0.36±0.04*	0.77±0.04	0.47±0.02*	0.79±0.03	0.45±0.04*
τ_o (ms)	5.81±0.80	5.60±0.66	6.34±0.90	1.01±0,07*	6.85±0.57	0.80±0.07*
$\tau_{c.f}$ (ms)	0.60±0.03	0.63±0.04	0.60±0.04	0.45±0.03*	0.57±0.02	0.48±0.02*
$\tau_{c.s}$ (ms)	4.16±0.26	10.04±1.86*	5.46±0.61	3.38±0.34*	4.48±0.40	3.18±0.18*
Outward K_{Na} channel current						
	($n=3$)		($n=3$)		($n=3$)	
P_o	0.76±0.06	0.36±0.02*	0.81±0.06	0.38±0.06*	0.72±0.07	0.28±0.03*
τ_o (ms)	7.22±1.76	3.13±0.35	8.47±0.22	0.56±0.04*	6.42±0.21	0.50±0.08*
$\tau_{c.f}$ (ms)	0.48±0.01	0.53±0.04	0.53±0.07	0.59±0.11	0.33±0.08	0.36±0.11
$\tau_{c.s}$ (ms)	3.73±1.02	6.74±1.33	3.85±0.43	2.82±0.52	2.80±1.49	1.95±0.77

Values are mean±s.e. P_o, open probability; τ_o, time constant of open-time distribution; $\tau_{c.f}$, fast component of time constant of closed-time distribution; $\tau_{c.s}$, slow component of time constant of closed-time distribution. *$P<0.05$ versus control value by paired t test.

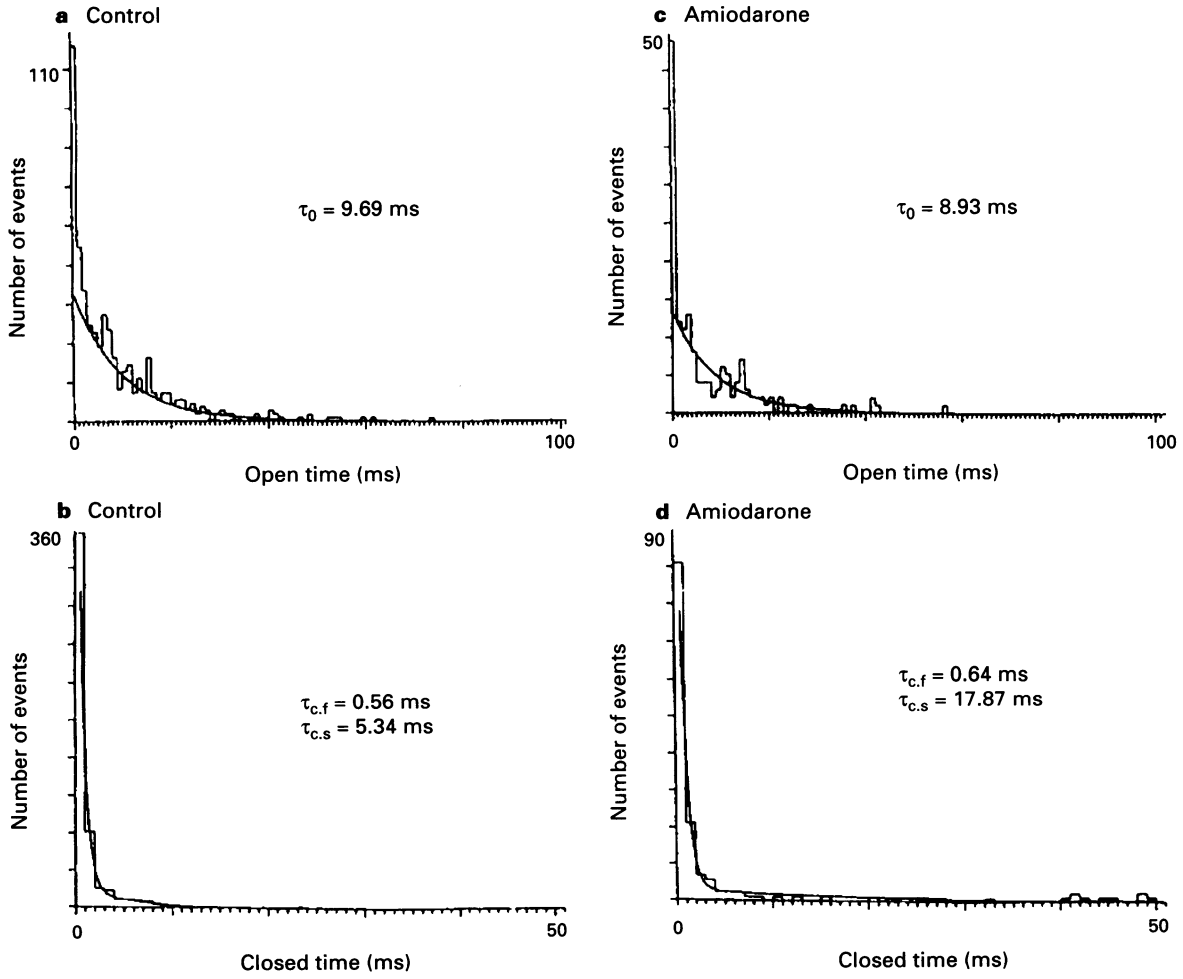

Figure 3 Effects of amiodarone (1 μM c, d) on the open-time (a, c) and closed-time (b, d) histograms of the K_{Na} channel current recorded from an inside-out patch membrane. The open-time histograms were fitted by one exponential curve and the closed-time histograms were fitted by two exponential curves. Note that the τ_o value was hardly affected by amiodarone.

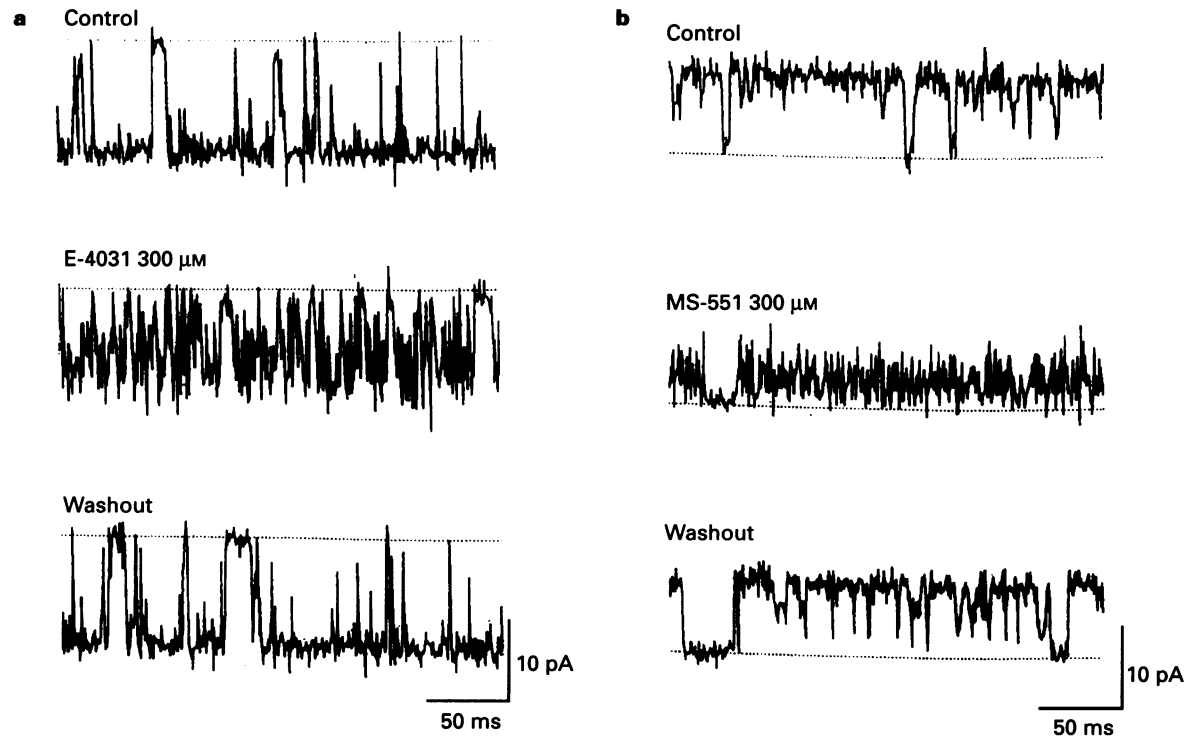

Figure 4 Effects of E-4031 and MS-551 on the Na^+-activated and K^+ channel currents. (a) Effect of E-4031 on the inward K_{Na} channel current recorded at a holding potential of −40 mV with the internal solution of 50 mM K^+ and 100 mM Na^+ and the external solution of 150 mM K^+. (b) Effect of MS-551 on the outward K_{Na} channel current recorded at a holding potential of +40 mV with the internal solution containing 30 mM Na^+ and 120 mM K^+ and the external solution containing 143 mM Na^+ and 5.4 mM K^+, 100 μM ouabain. Note that both E-4031 and MS-551 inhibited the K_{Na} channel current by decreasing the open-time (flickering block).

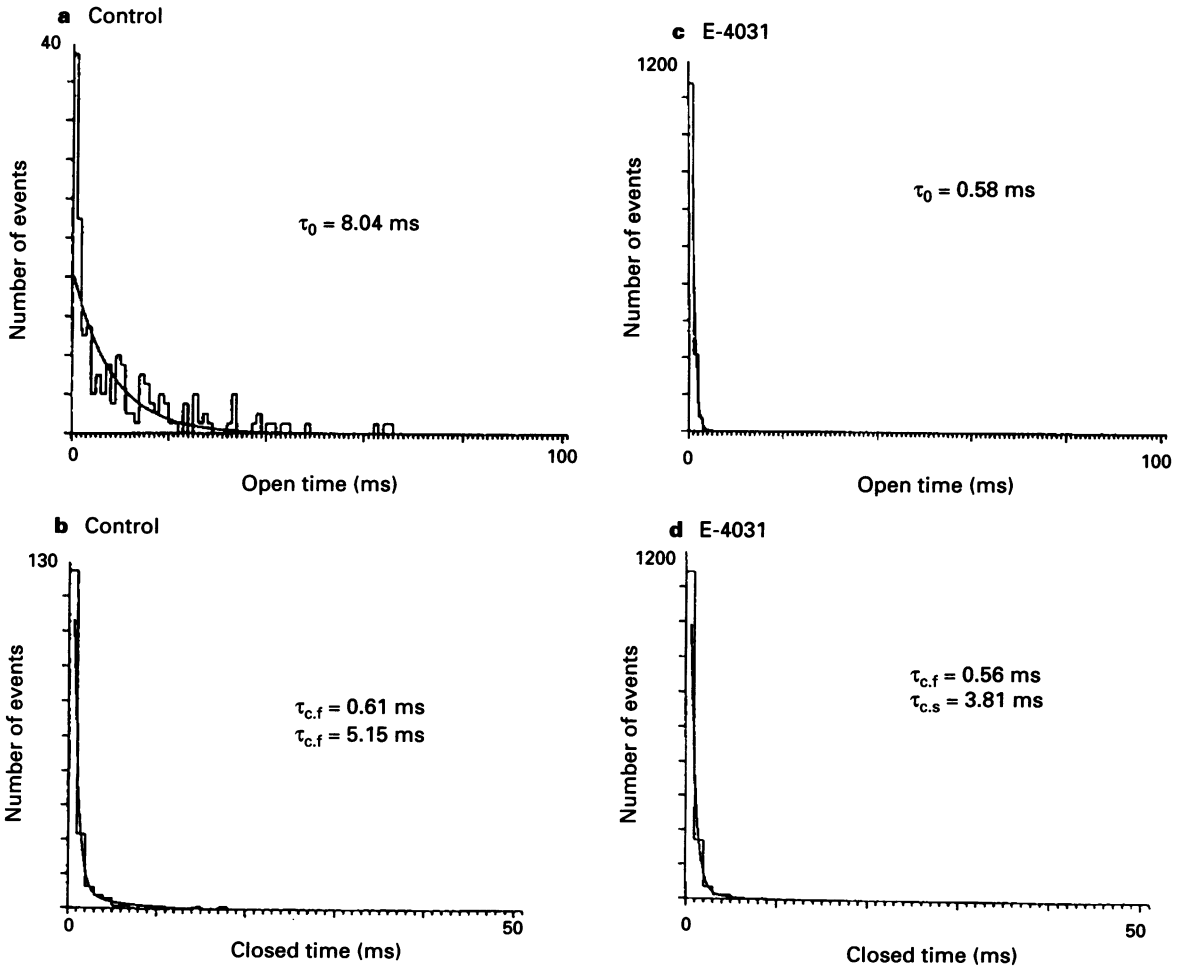

Figure 5 Effects of E-4031 (300 μM c, d) on the open-time (a, c) and closed-time (b, d) histograms of the K_{Na} channel current recorded from an inside-out patch membrane. Note that the value of τ_o was markedly decreased by E-4031.

K_{Na} channel current in a similar fashion. MS-551 at a concentration of 300 μM significantly decreased the values of P_o and τ_o ($n=8$) (Table 1).

These drugs also produced a flickering block of the outward K_{Na} channel current. As shown in Figure 4b, MS-551 markedly decreased the open time, resulting in an apparent reduction of the single channel conductance. Both E-4031 and MS-551 decreased the P_o and τ_o values of the outward K_{Na} channel current (Table 1). The inhibitory effects of these newer class III antiarrhythmic drugs on the inward and outward K_{Na} channel currents also reached a steady-state within 1 min and disappeared within a few minutes after changing to a drug-free solution.

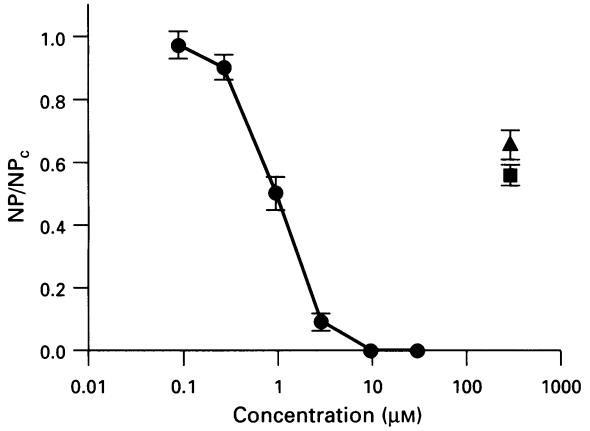

Figure 6 Effects of amiodarone (●), E-4031 (■) and MS-551 (▲) on relative open probability (NP/NP_C) of the inward K_{Na} channel current at −40 mV. Values are expressed as mean of 4–7 experiments; vertical lines show s.e.mean.

Changes in relative open probability (NP/NP_c) of the inward K_{Na}, channel current at −40 mV after amiodarone (0.1–10 μM), E-4031 (300 μM) and MS-551 (300 μM) are summarized in Figure 6. Amiodarone inhibited the K_{Na} channel current in a concentration-dependent manner, and the IC_{50} value was 1.0 μM. However, E-4031 and MS-551 at the relatively high concentration of 300 μM only decreased open probability by approximately half.

In another series of experiments, we examined the effects of the class III antiarrhythmic drugs on the Na^+-activated K^+ current at the whole cell level. The ventricular cells dialyzed with an internal solution containing 50 mM Na^+ were exposed to ouabain. Initially the quasi-steady-state current was downward shifted, probably due to partial blockade of the Na^+-K^+ pump current. Then, a marked outward current with pronounced outward rectification at positive potentials was induced, concomitantly with a further decrease in the reversal potential (Figure 7). The steady-state zero current level was close to the theoretical potassium equilibrium potential. Addition of 10 μM amiodarone or 300 μM E-4031 inhibited the

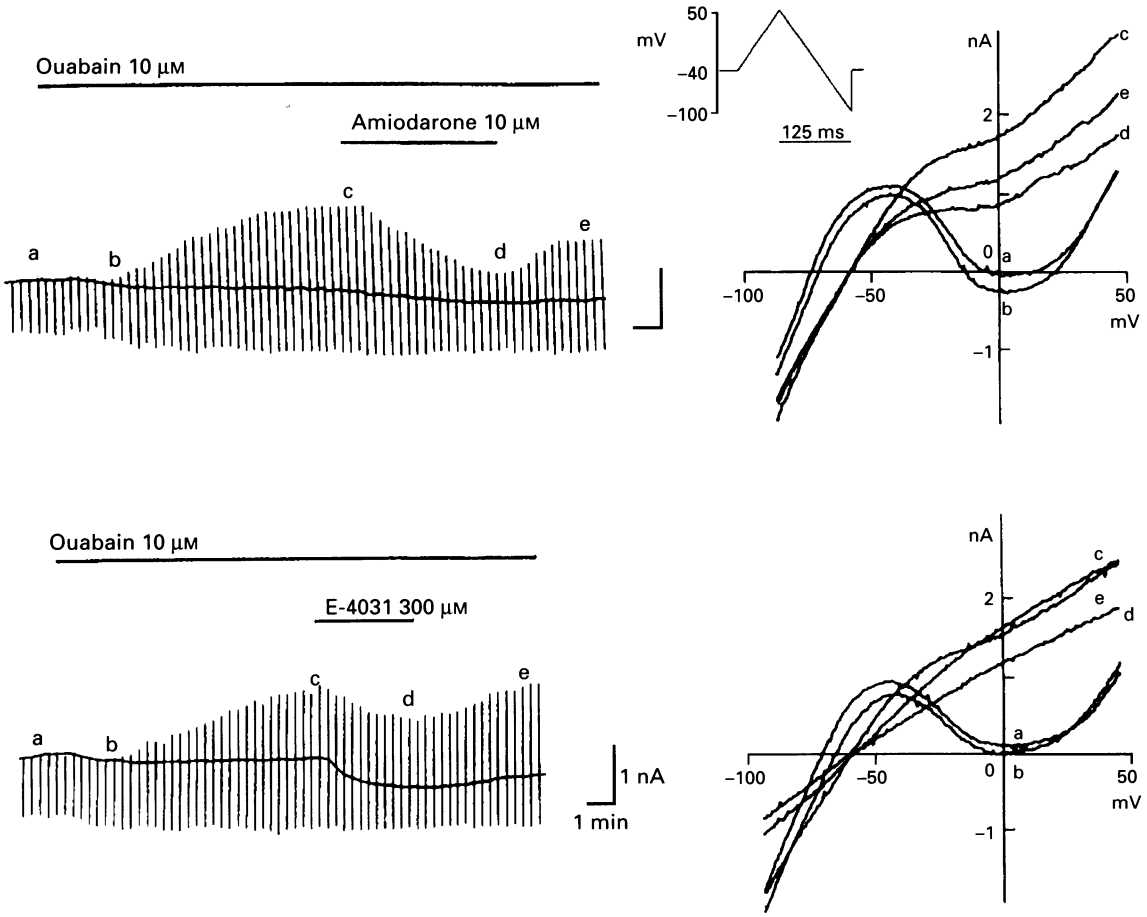

Figure 7 Effects of amiodarone (upper panels) and E-4031 (lower panels) on the ouabain-induced changes of quasi-steady-state membrane current. The ventricular cells dialyzed with a solution containing 50 mM Na^+ were exposed to 10 μM ouabain. Chart recordings of the membrane current are shown on the left. The vertical deflections indicate changes induced by the ramp pulses depicted in upper inset. The negative limb of ramp pulse delivered every 20 s was used to plot the *I-V* curves. The times for superfusion of ouabain and the class III antiarrhythmic drugs are indicated above the records. The quasi-steady-state membrane currents at various time points are illustrated on the right. Letters (a–e) indicate the corresponding ramps on the chart recordings. Note that both amiodarone and E-4031 inhibited the ouabain-induced outward current and the inhibition was partially reversed upon washout.

ouabain-induced outward current. MS-551 at a concentration of 300 μM also inhibited the ouabain-induced current. At the 0 mV level, amiodarone (10 μM), E-4031 (300 μM) and MS-551 (300 μM) decreased the ouabain-induced current by $59.9 \pm 4.6\%$ ($n=5$), $34.7 \pm 10.5\%$ ($n=6$) and $27.5 \pm 5.8\%$ ($n=7$), respectively. The inhibition of the ouabain-induced current by these class III antiarrhythmic drugs appeared gradually and reached a steady-state within 5 min. The inhibitory effect of amiodarone on $I_{K.Na}$ was partially reversible upon washout of the drug. The inhibition of $I_{K.Na}$ by E-4031 and MS-551 almost completely disappeared within 5 min after washout of the drugs. Thus, class III antiarrhythmic drugs also inhibit $I_{K.Na}$ at the whole-cell level.

Discussion

In 1984 Kameyama and coworkers demonstrated K^+ channels that can be activated by application of Na^+ to the cytoplasmic face of inside-out patches from guinea-pig ventricular myocytes. These channels have a very large unitary conductance of 207 pS with 150 mM $[K^+]_o$ and 49 mM $[K^+]_i$ and require more than 20 mM Na^+ to become active. Since intracellular Na^+ activity was shown to be less than 10 mM in normal cardiac cells (Lee & Fozzard, 1975; Ellis, 1977), the K_{Na} channels were considered not to play a significant role in heart cells. Hence the K_{Na} channels have not been extensively studied. In the present study we recorded the K_{Na} channel currents from inside-out membrane patches excised from guinea-pig ventricular cells by exposing them to various concentrations of Na^+ (30–100 mM) with or without ouabain. The single channel current showed a large conductance (210 pS) with several subconductance levels. The open and closed time distributions showed one and two time constants, respectively. These electrophysiological properties of the K^+ channels are consistent with previous data (Kameyama *et al.*, 1984; Wang *et al.*, 1991).

In terms of pharmacological blockers of K_{Na} channels, Luk and Carmeliet (1990) found that R56865 (*N*-{1-[4-(4-fluorophenoxy) butyl]-4-piperidinyl}-*N*-methyl-benzothiazolamine) inhibited the K_{Na} channel current. The drug was also shown to inhibit the cardiac Na^+ current (Himmel *et al.*, 1990; Wilhelm *et al.*, 1991), the delayed rectifier K^+ current (I_K) and the transient outward current (I_{to}) (Leyssens & Carmeliet, 1991). However, effects of class III antiarrhythmic drugs on the K_{Na} channel have not been examined. In the present study we selected two novel class III antiarrhythmic drugs, E-4031 and MS-551, which selectively or non-selectively block the outward current through voltage-gated K^+ channels (Sanguinetti & Jurkiewicz, 1990; Nakaya *et al.*, 1993) and amiodarone which exerts class I, II and IV actions in addition to its class III action (Singh, 1994). E-4031 and MS-551 inhibited the K_{Na} channel current by decreasing the open time. However, the concentration required to decrease the NP_o of the K_{Na} channel current by approximately half were much higher than those to inhibit I_K (Sanguinetti & Jurkiewicz, 1990; Nakaya *et al.*, 1993). Furthermore, in experimental animals the effective plasma concentrations of E-4031 and MS-551 were less than 1 μM and 10 μM, respectively (Katoh *et al.*, 1990; Friedrichs *et al.*, 1995; Hashimoto *et al.*, 1995). Therefore, it would be expected that these new class III antiarrhythmic drugs would not block the K_{Na} channels *in vivo*. In contrast, amiodarone could completely block the K_{Na} channels at therapeutic concentrations by decreasing the open probability.

There were no differences in the mode of the K_{Na} channel blockade between the inward and outward channel current. E-4031 and MS-551 produced flickering block of both the inward and outward K_{Na} channel current by decreasing the open time. It has been suggested that the flickering conductance of one channel implies that single drug molecules enter and leave the pore stochastically (Neher & Steinbach, 1978). However, the primary structure of the K_{Na} channel has not been determined and further studies are needed to clarify the interaction of drugs with the cardiac K_{Na} channels.

The physiological and pathophysiological significance of the K_{Na} channels are not fully understood. It has been found that in stimulated and unstimulated cardiac preparations intracellular Na^+ activity, measured by ion-selective electrodes, is less than 10 mM (Lee & Fozzard, 1975; Ellis 1977; Nakaya *et al.*, 1990), which appears to be insufficient for the activation of the K_{Na} channels in inside-out membrane patches. However, the subsarcolemmal sodium accumulation may be restricted to a narrow space at the inner side of the membrane, so-called fuzzy space (Carmeliet, 1992). During repetitive electrical activity the subsarcolemmal Na^+ accumulation resulting from Na^+ influx through the Na^+ channels and Na^+-Ca^{2+} exchange system may activate the K_{Na} channels. Wendt-Gallitelli *et al.* (1993) have recently demonstrated the sodium microheterogeneity by using electron probe microanalysis. They showed that in stimulated cardiac cells sodium concentrations within 20 nm of the inner side of the sarcolemma reached about 40 mM. They also recorded the K_{Na} channel activity from guinea-pig ventricular cells during paired-pulse stimulation. Therefore, the K_{Na} channels may be activated under repetitive stimulation and amiodarone may readily inhibit the K_{Na} channel current. In addition, it has been suggested that the K_{Na} channel current is activated during digitalis toxicity, leading to action potential shortening (Luk & Carmeliet, 1990). In the present study amiodarone inhibited the ouabain-induced outward current. It has been found that acute administration of amiodarone provides significant protection against ouabain-induced ventricular arrhythmias in anaesthetized guinea-pigs (Singh & Vaughan Williams, 1970). The inhibitory action of amiodarone on the K_{Na} channel might be partly involved in the antiarrhythmic effect of digitalis toxicity. The activation of K_{Na} channels has been also suggested during acute myocardial ischaemia since R56865, a K_{Na} channel blocker, retards the early extracellular K^+ accumulation (Mitani & Shattock, 1992). The K_{Na} channels have been observed not only in cardiac cells but also in several populations of neurones (Dryer, 1994). The K_{Na} channels are thought to contribute to the maintenance of the resting membrane potential and to control neuronal membrane excitability. Amiodarone could be used to identify the physiological significance of the K_{Na} channel in cardiac as well as neuronal tissues. However, amiodarone is known to affect various ion channels (Singh, 1994) and significant progress on understanding the physiological function of the K_{Na} channels may require the development of a more specific blocker.

Class III antiarrhythmic drugs are assumed to prevent sudden cardiac death resulting from ventricular fibrillation (Singh *et al.*, 1992). Since amiodarone and (±) sotalol are class III antiarrhythmic drugs having several actions other than K^+ channel blockade, there has been an increasing impetus to develop newer and more specific K^+ channel blockers. However, newly-developed class III antiarrhythmic drugs cause torsades de pointes more frequently than amiodarone (Leatham *et al.*, 1993). Therefore, it is important to clarify the differences between amiodarone and the newer class III antiarrhythmic drugs. An ideal antifibrillatory drug should increase action potential duration (APD) and effective refractory period (ERP) as the cycle length of venticular arrhythmias is shortened (Hondeghem & Snyder, 1990). However, most new class III compounds show a more prominent APD-prolonging effect at slow heart rate (so-called reverse use-dependence) (Tande *et al.*, 1990; Nakaya *et al.*, 1993; Hiraoka *et al.*, 1994). In contrast, it has been found that amiodarone shows little reverse use-dependence during chronic administration (Anderson *et al.*, 1989). The inhibitory action of amiodarone on the K_{Na} channels that were activated during repetitive stimulation might be partly involved in the more favourable APD prolongation.

In summary, amiodarone and the newer class III antiarrhythmic drugs E-4031 and MS-551 inhibit the K_{Na} channel

current in guinea-pig ventricular cells. Amiodarone blocks the K_{Na} channel by inhibiting the openings in therapeutic concentrations whereas MS-551 and E-4031 produce flickering block in supratherapeutic concentrations. Further studies are needed to clarify the significance of the K_{Na} channel blockade by amiodarone *in vivo*.

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