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Electrophysiological effects of SD-3212, a new antiarrhythmic agent with vasodilator action, on guinea-pig ventricular cells

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1 The effects of SD-3212 on transmembrane action potentials were examined in right ventricular papillary muscles and in single ventricular myocytes isolated from guinea-pig hearts.

2 In papillary muscles, SD-3212 $\geqslant 3\,\mu$M caused a significant decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of action potential without affecting resting membrane potential. The inhibition of $\dot{V}_{max}$ was enhanced at higher stimulation frequencies.

3 In the presence of SD-3212, trains of stimuli at rates $\geqslant$0.5 Hz led to a use-dependent inhibition of $\dot{V}_{max}$. The time constant for the recovery of $\dot{V}_{max}$ from the use-dependent block was 1.3 s.

4 Voltage-dependence of $\dot{V}_{max}$ inhibition by SD-3212 was investigated in single myocytes. The curves relating membrane potential and $\dot{V}_{max}$ were shifted by SD-3212 (10 μM) in a hyperpolarizing direction by 6.2 mV.

5 In myocytes treated with SD-3212 (10 μM), the $\dot{V}_{max}$ of test action potentials preceded by conditioning clamp to 0 mV was decreased progressively as the clamp pulse duration was prolonged. $\dot{V}_{max}$ of test action potentials following a long (1 s) 0 mV clamp recovered at a time constant ranging from 1.01 to 1.22 s, being shorter at the more negative potential within a range from -70 to -90 mV.

6 These findings suggest that the primary electrophysiological effect of SD-3212 is a use- and voltage-dependent inhibition of sodium channels. From the onset and offset kinetics of the use-dependent block, SD-3212 is located between fast and intermediate kinetic Class-I drugs. From the state-dependence of sodium channel block, SD-3212 belongs to inactivated channel blockers.

Keywords: SD-3212; ventricular cell; action potential; $\dot{V}_{max}$; use-dependent block

Introduction

SD-3212 ((−)-(s)-2-[5-methoxy-2-[3-[methyl [2-[3,4 (methylenedioxy) phenoxy] ethyl] amino] phenyl]-4-methyl-2H-1,4-benzothiazin-3(4H)-one hydrogen fumarate) is a newly synthesized antiarrhythmic agent with vasodilator action (Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992; Hirasawa *et al.*, 1992). This compound is a stereoisomer of semotiadil fumarate (SD-3211), a recently developed non-dihydropyridine type calcium antagonist (Miyawaki *et al.*, 1990; Fukuchi *et al.*, 1990; Teramoto, 1993). *In vitro* and *in vivo* experiments on rats, guinea-pigs, and dogs, showed that SD-3212 had a potent and long lasting inhibitory or protective action against ventricular tachyarrhythmias induced by chloroform, ouabain, adrenaline, coronary occlusion and reperfusion (Fukuchi *et al.*, 1990; Miyawaki *et al.*, 1991; Nagashima *et al.*, 1992). The antiarrhythmic potency of SD-3212 in these animal models is appreciably higher than that of SD-3211.

As to underlying mechanisms for the antiarrythmic activity of SD-3212, only limited information is available, and much remains to be clarified. The calcium antagonistic action of SD-3212 in vascular smooth muscle was shown to be less potent (approximately one tenth) than that of SD-3211 (Nakayama *et al.*, 1992). In experiments on cardiac muscle, SD-3212 (1–10 μM), unlike SD-3211, decreased the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential in a concentration-dependent manner (Miyawaki *et al.*, 1991). In Langendorff-perfused rabbit hearts, SD-3212 caused an inhibition of atrio-ventricular conduction with comparable prolongation of atrio His bundle (AH) and His bundle-ventricular (HV) intervals (unpublished data). *In vivo* experiments in dogs revealed that SD-3212 prolonged the intraventricular conduction time when the coupling interval of stimulation was relatively short, whereas the parameter was unaffected by SD-3211 (Nagashima *et al.*, 1992). These findings suggest that SD-3212 possesses an inhibitory effect on cardiac sodium channels, and this Class-I action could be responsible for its potent antiarrhythmic activity.

In the present study, we investigated the effects of SD-3212 on the transmembrane action potential in right ventricular papillary muscles as well as in single ventricular myocytes isolated from guinea-pig hearts. The modulation of drug-induced $\dot{V}_{max}$ inhibition by stimulation frequencies or by membrane potential level was studied extensively, in order to compare the characteristics of the sodium channel blocking actions of SD-3212 with other Class-I antiarrhythmic drugs.

Methods

Papillary muscle

Guinea-pigs of either sex weighing 200 to 250 g were killed by cervical dislocation under ether anaesthesia and hearts were quickly removed. Papillary muscles, 2 to 3 mm in length and 0.3 to 0.4 mm in diameter were dissected from the right ventricle. The preparation was mounted in a tissue bath and superfused continuously with Krebs-Ringer solution kept at 33°C and gassed with 95% O_2 and 5% CO_2. The composition of the solution was as follows (in mM): NaCl 120.3, KCl 4.0, $CaCl_2$ 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 25.2 and glucose 5.5 (pH 7.4). The preparations were stimulated by a pair of 1.0 mm platinum wire electrodes placed 1.0 mm apart of either side of the muscles. Pulses used for stimulation were 0.5–1.0 ms in duration and 20% higher than the diastolic threshold in intensity unless otherwise specified. Equipment for recording transmembrane potential was the same as described pre-

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viously (Kodama *et al.*, 1985; Toyama *et al.*, 1987). To study the use-dependent effect of SD-3212 on the maximum upstroke velocity ($\dot{V}_{max}$) of action potentials, the preparation was stimulated repetitively at varying rates ranging from 0.1 to 2.0 Hz. Resting periods of 30 s, which were sufficient to ensure full recovery from the rate-dependent decrease in $\dot{V}_{max}$, were interposed between the trains of stimuli. The experimental protocol is able to detect the existence of two types of drug-induced $\dot{V}_{max}$ inhibition, tonic and use-dependent block. The former is defined by the decrease of $\dot{V}_{max}$ of the first action potential preceded by the rest period, and the latter is the decrease of $\dot{V}_{max}$ during the trains (from the value of first action potential to the new steady-state level). The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by applying a single test stimulus at various coupling intervals following a stimulation for 60 s at 1.0 Hz. The intensity of the test stimulus was adjusted to obtain a constant latency from the stimulus artifact to the initiation of action potential upstroke.

Single ventricular myocytes

Single ventricular myocytes were isolated enzymatically from guinea-pig hearts by the same procedure as described in our previous paper (Kodama *et al.*, 1990). A few drops of cell suspension were placed in a recording chamber attached to an inverted microscope. The chamber was perfused at a rate of 2 ml min^{-1} with normal Tyrode solution of the following composition (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHPO_4$ 0.33, HEPES 5.0 and glucose 5.0; pH was adjusted to 7.4 by adding NaOH, and the solution was equilibrated by 100% O_2. The temperature was maintained at 35°C. Following the increase in calcium concentration of the medium to 1.8 mM (normal Tyrode solution), 30 to 40% of myocytes deteriorated into round-shaped cells due to irreversible contracture. The remaining cells were tolerant to calcium; their intact rod-shape was maintained without spontaneous beating, and the experiments were carried out on these myocytes.

For $\dot{V}_{max}$ measurement, the whole-cell clamp technique was used with a patch pipette (2–3 Mohm) containing a solution of (mM): KCl 120.0, NaH_2PO_4 10.0, EGTA 1.0, MgATP 5.0 and HEPES 10.0; the pH was adjusted to 7.2 by adding KOH. Single cells were voltage-clamped by Axoclamp-2A (Axon Instruments, Burlingame, California, U.S.A.) in a single-electrode discontinuous mode (sampling frequency: 8–11 kHz). To elicit action potential, the voltage-clamp was switched to the current-clamp mode by computer-operated TTL signals, during which a square pulse (2 ms) for stimulation was applied. The upstroke velocity ($\dot{V}_{max}$) was sampled at 70 kHz by an on-line analysis system (Anno & Hondeghem, 1990). Details of the clamp pulse protocols are given in the results section.

Drug and data analysis

SD-3212 (Santen Pharmaceutical Co., Osaka, Japan) was dissolved in dimethyl sulphoxide (DMSO) to prepare stock solution at 10 mM, and it was diluted further with Krebs Ringer or Tyrode solution to obtain the final concentration required. Final DMSO concentrations did not exceed 0.3%, and did not significantly affect the results. Values are presented as means ± s.e. or means unless otherwise stated. Data were analysed by *t* test, analysis of variance, Dunnett's test and regression analysis. Differences were considered significant at $P < 0.05$.

Results

Action potentials of papillary muscle

Effects of SD-3212 (1 μM to 10 μM) on the action potential configuration were examined in six papillary muscles constantly stimulated at 0.2 Hz or 1.0 Hz (Figure 1, Table 1).

After exposure to 1 μM SD-3212 for 40 min, action potential duration (APD) at late repolarization (APD_{80}) was slightly prolonged at both 0.2 Hz and 1.0 Hz stimulation. At higher concentrations of SD-3212, APD especially at early repolarization (APD_{30}) tended to be shortened, in a concentration-dependent manner. The latter change was, however, statistically insignificant because of variability among the preparations. $\dot{V}_{max}$ was decreased significantly by SD-3212 ≥ 3 μM only at the higher stimulation frequency

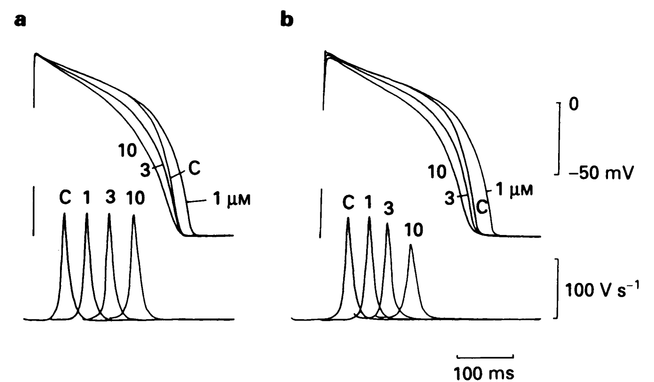

Figure 1 Effects of SD-3212 on transmembrane action potentials of papillary muscles. Upper traces are membrane action potentials, and lower traces are their differentiated upstroke spikes ($\dot{V}_{max}$. $\dot{V}_{max}$ was recorded at a faster sweep velocity. The preparation was constantly stimulated at 0.2 Hz (a) or at 1.0 Hz (b). The records were obtained before (control C) and 30–40 min after application of SD-3212 at 1, 3 and 10 μM.

Table 1 Effects of SD-3212 on action potential characteristics

		RP (mV)	*AMP* (mV)	APD_{30} (ms)	APD_{80} (ms)	$\dot{V}_{max}$ (Vs^{-1})
1.0 Hz	Control (*n* = 5)	−91.4 ± 0.6	125.8 ± 2.0	223 ± 15	289 ± 11	174 ± 13
	SD-3212					
	1 μM	−91.8 ± 0.7	125.5 ± 2.2	239 ± 10	320 ± 8*	176 ± 10
	3 μM	−92.6 ± 0.1	124.8 ± 2.1	225 ± 13	301 ± 15	164 ± 15*
	10 μM	−90.6 ± 1.2	124.4 ± 2.6	208 ± 17	295 ± 16	143 ± 10*
0.2 Hz	Control (*n* = 5)	−92.3 ± 0.6	126.3 ± 1.9	220 ± 13	292 ± 13	178 ± 12
	SDS-3212					
	1 μM	−92.7 ± 0.3	127.5 ± 1.3	237 ± 12*	314 ± 14*	184 ± 8
	3 μM	−92.4 ± 0.4	128.8 ± 1.1	218 ± 13	304 ± 14	183 ± 7
	10 μM	−92.7 ± 0.3	128.3 ± 1.2	199 ± 15	297 ± 17	176 ± 7

Values are means ± s.e.mean of five experiments. The preparations were constantly stimulated at 1.0 Hz or 0.2 Hz. Data were obtained before (control) and 40 min after application of SD-3212 at a given concentration. RP: resting membrane potential; AMP: amplitude of action potential; $\dot{V}_{max}$: maximum upstroke velocity of action potential; APD_{30} and APD_{80}: action potential duration from the upstroke to 30% and 80% repolarization. *Significantly different from control at $P < 0.05$.

(1.0 Hz). Resting potential (RP) was unaffected even at 10 μM. IC_{20} of $\dot{V}_{max}$ inhibition induced by SD-3212 at 1.0 Hz, which was obtained by interpolation on a graph of log molar drug concentration versus response was 9.4 μM.

Use-dependent effects of $\dot{V}_{max}$

The effects of SD-3212 on $\dot{V}_{max}$ were examined with stimulation trains at different rates separated from each other by 30 s rest period. In control preparations, the value of $\dot{V}_{max}$ was almost unchanged with stimulation trains at rates from 0.1 to 2.0 Hz. After treatment with SD-3212, $\dot{V}_{max}$ of the first action potential in each train was slightly decreased, indicating a negligible or minimal tonic block (2.6 ± 1.0% at 3 μM and 4.4 ± 0.9% at 10 μM, $n = 5$). Further decline of $\dot{V}_{max}$ during the repetitive activity (use-dependent block) was observed at rates ≥ 0.5 Hz; the higher the stimulation frequency, the greater the block (Figure 2).

Figure 3 summarizes the percentage decrease of $\dot{V}_{max}$ from the first action potential to the new steady-state level, which was attained at around the 5th to 12th action potential. The use-dependent block of $\dot{V}_{max}$ by SD-3212 was greater at higher stimulation frequencies and at higher drug concentrations.

The beat-to-beat decline of $\dot{V}_{max}$ during stimulation trains at ≥ 0.5 Hz fitted a single exponential curve well (Figure 2), so that the onset rate per action potential (AP^{-1}) at which $\dot{V}_{max}$ fell to the new steady-state level could be calculated in each experiment (Table 2). The onset rate of the use-dependent block by SD-3212 was larger at the higher drug concentration and at the lower stimulation frequency (Table 2).

The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by applying a single test stimulus at various coupling interval following a stimulation train for 60 s at 1.0 Hz. Before the application of the drug, the $\dot{V}_{max}$ of the test action potential recovered almost completely within 100 ms of the diastolic interval (the interval from the end of the last action potential to the beginning of the test action potential). The recovery process was approximated by a single exponential function with a mean time constant of 19 ± 2 ms ($n = 5$). After treatment with SD-3212 (10 μM), much slower $\dot{V}_{max}$ recovery was observed. Representative results are shown in Figure 4, where $\dot{V}_{max}$ of the test action potentials were plotted against the diastolic interval. In the presence of SD-3212, the recovery time course of longer than 100 ms was approximated by a single exponential function. The average time constant (τ_R) was 1.3 ± 0.2 s ($n = 5$).

V_{max} *of single ventricular myocytes*

In single ventricular myocytes, voltage-dependence of $\dot{V}_{max}$ inhibition by SD-3212 was investigated by using three clamp protocols. The baseline characteristics of action potential elicited in the cell at a long interstimulus interval (60 s) were as follows: RP, −80.6 ± 0.3 mV; $\dot{V}_{max}$, 568 ± 17 Vs^{-1}; APD_{80}, 178 ± 20 ms ($n = 8$).

First, the relationship between $\dot{V}_{max}$ and resting membrane potential from which the action potential originates were examined. Following a rest period of 60 s at a holding potential of −80 mV, which is long enough to eliminate the use-dependent depression of $\dot{V}_{max}$ by the drug, the membrane potential was clamped for 10 s to various levels (from −110 mV to −55 mV). At the end of the conditioning clamp, the voltage clamp was released and a stimulus was applied to elicit a test action potential. The decrease of $\dot{V}_{max}$

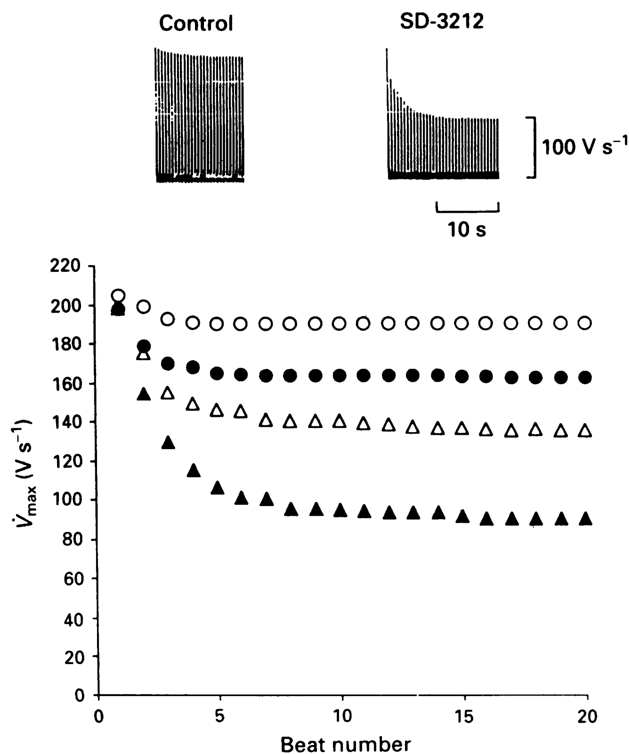

Figure 2 Use-dependent decrease of the maximum upstroke velocity ($\dot{V}_{max}$) of action potential in papillary muscles. Upper panels: differentiated upstroke spikes of action potentials during stimulation trains at 2.0 Hz after 30 s rest. The records were obtained before and 40 min after application of SD-3212 (10 μM). Lower graph: beat-to-beat change in $\dot{V}_{max}$ at the onset of stimulation trains in the presence of SD-3212 (10 μM) Ordinate scale: $\dot{V}_{max}$ Abscissa scale: number of beats (action potentials) from the initiation of stimulation train. Frequencies of stimulation were 0.2 Hz (○), 0.5 Hz (●), 1.0 Hz (△) and 2.0 Hz (▲).

Table 2 Tonic and use-dependent block of $\dot{V}_{max}$ by SD-3212

	n	*Tonic block* (%)	*Onset of use-dependent block* 1.0 Hz (AP^{-1})	2.0 Hz (AP^{-1})
SD-3212				
3 μM	(5)	2.6 ± 1.0	0.18 ± 0.03	0.14 ± 0.02
10 μM	(5)	4.4 ± 0.9	0.22 ± 0.05	0.20 ± 0.05

Values are means ± s.e.mean. n = number of preparations. AP^{-1}: per action potential.

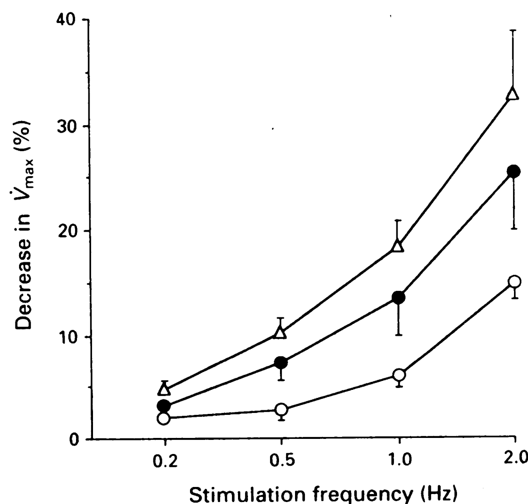

Figure 3 Relation between stimulation frequency and intensity of the use-dependent block. Ordinate scale: % decrease of maximum upstroke velocity ($\dot{V}_{max}$) of action potential from first action potential of stimulation trains to new steady-state level. Abscissa scale: stimulation frequency. Data were obtained 40-60 min after application of SD-3212 at 1 μM (○), 3 μM (●) and at 10 μM (△). Values are presented as means with s.e.means of five preparations.

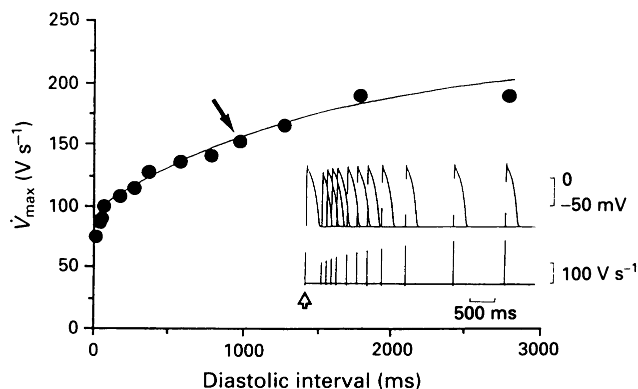

Figure 4 Recovery of $\dot{V}_{max}$ from the use-dependent block by SD-3212. Inset shows superimposed records of action potentials (upper trace) and their differentiated upstroke spikes (lower trace) after application of SD-3212 (10 μM). Following 1.0 Hz stimulation for 60 s, a single test stimulus was applied with various coupling intervals (an open arrow indicates the last conditioning stimulus). The graph shows $\dot{V}_{max}$ recovery in the presence of SD-3212 (10 μM). Ordinate scale: $\dot{V}_{max}$. Abscissa scale: diastolic interval (an interval from the end of the last conditioning action potential to the upstroke of the test action potential). The $\dot{V}_{max}$ recovery after SD-3212 was approximated by a single exponential function at a time constant of 1.0 s (solid arrow) for the data with diastolic intervals of over 100 ms.

by SD-3212 (10 μM) was more pronounced at less negative conditioning membrane potential (Figure 5a). A fraction of $\dot{V}_{max}$ was calculated in each experiment by normalizing the data with the value at −110 mV. The data points fitted the Bolzman equation;

$$y = (1 + \exp(Vm - Vh)k)^{-1} \quad (1)$$

where Vm is the conditioning clamp potential, Vh is the membrane potential showing a half maximal $\dot{V}_{max}$. Vh was changed from −55.2 ± 1.8 mV in control to −61.4 ± 2.1 mV after SD-3212, indicating a hyperpolarizing shift by 6.2 ± 1.1 mV ($n = 4$). The k value was almost identical (0.213 before, and 0.220 after SD-3212).

The second protocol was designed to determine whether the use-dependent $\dot{V}_{max}$ inhibition by SD-3212 is due to the blockade of an activated or inactivated sodium channel (Figure 6). Following a rest period of 60 s, the membrane potential was clamped up from the resting level (holding potential at −80 mV) to 0 mV for 10 to 2,000 ms. At the end of the conditioning depolarization, the membrane potential was clamped back to the holding voltage for 100 ms, which is long enough for a drug-free channel to reactivate fully (Carmeliet & Vereecke, 1979; Ebihara & Johnson, 1980), but short enough so that only partial dissociation of the drug from the blocked channel occurs (Grant *et al.*, 1984). The voltage-clamp was then released, and a stimulus was applied to elicit a test action potential. In untreated control myocytes, such a clamp pulse with a duration of less than 500 ms had no significant effect on the $\dot{V}_{max}$ of the test action potential. However, further prolongation of the clamp pulse duration resulted in a slight but significant $\dot{V}_{max}$ reduction probably due to slow inactivation of sodium channels (Saikawa & Carmeliet, 1982; Clarkson *et al.*, 1984). A clamp pulse of 2,000 ms in duration decreased $\dot{V}_{max}$ by 16.6 ± 1.6% ($n = 4$) from the value of action potential without the conditioning clamp (reference level). Treatment of the myocytes with SD-3212 (10 μM) for 10 to 20 min did not affect RP and $\dot{V}_{max}$ of the reference action potential. In such myocytes, a 0 mV conditioning clamp pulse caused a progressive decrease in $\dot{V}_{max}$ of the test action potential as the clamp pulse duration was prolonged (Figure 6). The decrease in $\dot{V}_{max}$ from the reference value was significant when the clamp pulse duration

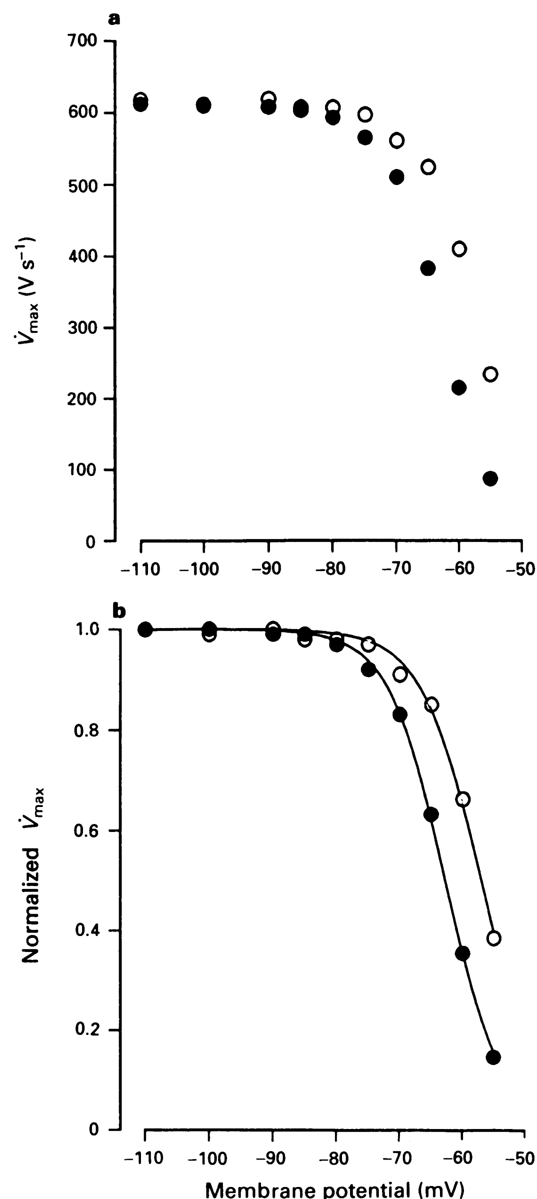

Figure 5 Voltage-dependence of $\dot{V}_{max}$ inhibition by SD-3212 in single ventricular myocytes. Ordinate scale: $\dot{V}_{max}$ of test action potential (a) and its fraction normalized by the value at the conditioning membrane potential of −110 mV (b). Abscissa scales: conditioning membrane potential from which a test action potential was elicited. Following a rest period of 60 s at a holding potential of −80 mV, the membrane potential was clamped for 10 s to various conditioning level (from −110 mV to −55 mV). At the end of the conditioning clamp, the voltage-clamp was released, and a test action potential was elicited to measure $\dot{V}_{max}$. Data were obtained before (○) and 20 min after application of SD-3212 at 10 μM (●).

was longer that 50 ms, and reached 81.2 ± 2.8% ($n = 4$) at 2,000 ms. The change in $\dot{V}_{max}$ with each experiment was well fitted by single exponential function with a time constant of 332 ± 4 ms ($n = 4$). We also tested the effects of very short (2–5 ms) conditioning 0 mV prepulses in the presence of 10 μM SD-3212. They caused no significant decrease in $\dot{V}_{max}$ of test action potentials.

In the third protocol, the recovery process of $\dot{V}_{max}$ following a long (1 s) conditioning clamp to 0 mV was examined by introducing a test action potential with various coupling intervals during which the membrane potential was clamped back to −70 to −90 mV.

A representative experiment is shown in Figure 7. Under drug-free control conditions, the $\dot{V}_{max}$ of the test action

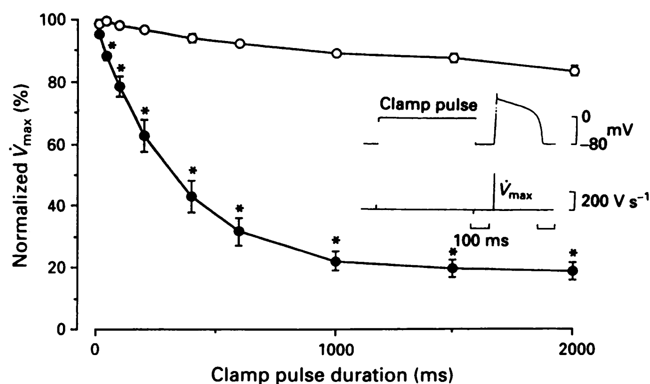

Figure 6 Effects of single 0 mV conditioning clamp pulse on the $\dot{V}_{max}$ inhibition induced by SD-3212. Ordinate scale: $\dot{V}_{max}$ of test action potential normalized by the value of action potential without clamp pulse (referenced at the tonic block subtracted value). Abscissa scale: duration of the conditioning 0 mV clamp. Values are presented as means ± s.e.mean of four cells. Data were obtained before (control, ○) and 20 min after application of SD-3212 at 10 μM (●). *Significantly different from control at $P<0.05$.

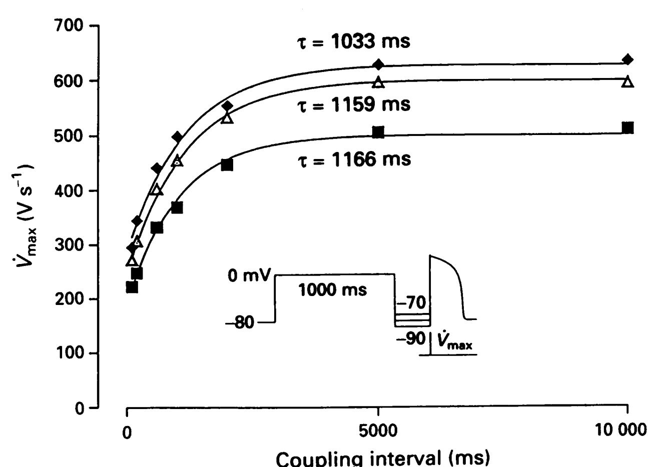

Figure 7 Recovery of $\dot{V}_{max}$ in a single ventricular myocyte treated with SD-3212 (10 μM). Following a long (1 s) conditioning clamp to 0 mV, the membrane potential was clamped back to −70 (■), −80 (Δ) or −90 (◆) mV, and a test action potential was elicited with various coupling intervals. Ordinate scale; $\dot{V}_{max}$ of test action potential. Abscissa scale: coupling interval from the end of 0 mV clamp to the upstroke of test action potentials. The time course of $\dot{V}_{max}$ recovery of test action potentials with a coupling intervals > 100 ms was approximated by a single exponential function with a time constant ranging from 1033 ms to 1166 ms.

potential recovered almost completely (95%) within 100 ms. The recovery process was approximated by a single exponential function with a mean time constant of 29 ± 3 ms at −70 mV, 25 ± 2 ms at −80 mV, and 22 ± 2 ms at −90 mV ($n = 4$). In the presence of SD-3212 (10 μM), the recovery time course of $\dot{V}_{max}$ with a coupling interval longer than 100 ms was approximated by a single exponential function with a time constant (τ_R) ranging from 1.03 s to 1.16 s. The average τ_R in four experiments showed a modest voltage-dependence; being shorter at the more negative potential: 1.14 ± 0.04 s at −70 mV, 1.22 ± 0.04 s at −80 mV and 1.01 ± 0.05 s at −90 mV.

Discussion

The present experiments on guinea-pig papillary muscles have revealed that SD-3212 ≥ 1 μM has a biphasic action on the repolarization of the action potential; low concentrations caused a moderate prolongation of APD, whereas this effect was reversed as the concentration was increased. At concentrations ≥ 3 μM, SD-3212 also caused a significant decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential without affecting the resting membrane potential (RP). The effect on APD showing 'reverse dose-dependence' like quinidine or disopyramide (Wyse *et al.*, 1993) was not affected by stimulation frequencies, whereas the inhibition of $\dot{V}_{max}$ was observed only at higher stimulation frequencies (≥ 0.5 Hz). The biphasic effect on APD is probably due to a balance of the drug action on the outward potassium currents responsible for repolarization and that on the inward sodium and/or calcium currents flowing during the plateau phase of the action potential. Extensive voltage-clamp studies to measure individual ionic current will be required to clarify the mechanism, but this is out of the scope of the present study.

The decrease in $\dot{V}_{max}$ by SD-3212 without any change in RP reflects an inhibitory effect of the drug on the fast sodium inward current (I_{Na}), since the entire papillary muscle was excited simultaneously and there was no conduction within the preparation. Furthermore, a similar $\dot{V}_{max}$ inhibition was observed in single ventricular myocytes. The probable convex-shaped non-linear relationship between $\dot{V}_{max}$ and peak sodium inward current (I_{Na}) in cardiac cells (Cohen *et al.*, 1984; Sheets *et al.*, 1988) might introduce variable errors in estimating the precise amount of sodium channel block. Nevertheless, such a limitation does not invalidate $\dot{V}_{max}$ as a qualitative index, because voltage-clamp techniques currently available for I_{Na} measurement require more artificial experimental conditions (low temperature and low extracellular sodium concentrations) than those for $\dot{V}_{max}$ measurement.

The inhibition of $\dot{V}_{max}$ by SD-3212 was enhanced at the higher stimulation frequency. Thus, in normally polarized papillary muscles, SD-3212 at concentrations ranging from 1 to 10 μM caused negligible or minimal tonic $\dot{V}_{max}$ inhibition despite their marked use-dependent $\dot{V}_{max}$ inhibition. Such frequency-dependent $\dot{V}_{max}$ inhibition can be interpreted within the framework of the 'modulated receptor' hypothesis (Hondeghem & Katzung, 1977) or 'guarded receptor' hypothesis (Starmer *et al.*, 1984) to explain the interaction between local anaesthetic-type (Class-I) antiarrhythmic drugs and cardiac sodium channels. According to these hypotheses, the reduction of I_{Na} is due to the accumulation of drug-associated non-conducting channels (blocked channels). SD-3212, like most other Class-I antiarrhythmic drugs, may bind to the sodium channel receptor mainly during activated and/or inactivated states. This characteristic would lead to an accumulation of blocked channels during stimulation trains above certain rates.

The use-dependent block of $\dot{V}_{max}$ by SD-3212 was observed during stimulation trains at rates ≥ 0.5 Hz. The onset kinetics of use-dependent block was rapid (0.20 AP^{-1} at 10 μM, 2.0 Hz), and approximately 80 to 90% of the use-dependent block was attained within ten beats. The offset kinetics of use-dependent block by SD-3212 was also rapid; the average time constant of $\dot{V}_{max}$ recovery (τ_R) was 1.34 s. The τ_R value is longer than those described for fast kinetic Class-I drug such as lignocaine, mexiletine and tocainide (200–500 ms) (Campbell 1980; 1983 a,b), but still shorter than those for intermediate kinetic Class-I drugs such as quinidine, procainamide and aprindine (2.3–6.8 s) (Campbell 1980; 1983a,b; Kodama *et al.*, 1990). These observations may suggest that binding and dissociation kinetics of SD-3212 molecules to the cardiac sodium channels are quite rapid.

The relationship between $\dot{V}_{max}$ and membrane potential was examined in single ventricular cells following a rest period of 60 s (Figure 5). Under such experimental conditions, a decrease in $\dot{V}_{max}$ may reflect only the tonic block of sodium channels. The present data showed that the decrease in $\dot{V}_{max}$ by SD-3212 was more pronounced at less negative membrane potentials. This effect could be due to a high affinity of this drug for inactivated sodium channels.

It has been shown in our previous studies (Kodama *et al.*, 1987; 1990) that Class-I antiarrhythmic drugs can be subdivided into two groups in terms of their sodium channel blocking phase during the conditioning clamp pulse to 0 mV; one 'transient' and one 'maintained'. The former group of drugs (quinidine and disopyramide) may block the sodium channel mainly during its activated state, corresponding to the upstroke phase of the action potential, while the latter group (lignocaine, mexiletine, tocainide and aprindine) may do so predominantly during the inactivated state which correspond to the plateau phase of the action potential. In the present single cell experiments with SD-3212, we tested such a 'state-dependence' of sodium channel block by using a similar conditioning 0 mV clamp protocol. In the presence of SD-3212 (10 μM), the $\dot{V}_{max}$ of the test action potential, which was elicited 100 ms after the termination of 0 mV clamp, decreased progressively as the preceding clamp pulse duration was prolonged. The decay of $\dot{V}_{max}$ was well fitted to a single exponential function with a time constant of 332 ms. These findings may indicate that SD-3212 blocks the sodium channel primarily when it is in the inactivated state. Time constants for the onset of the inactivated channel block by SD-3212 are comparable to those for aprindine, but appreciably longer than lignocaine and mexiletine (Kodama *et al.*, 1990). Nevertheless, we cannot rule out a possibility that SD-3212 at higher concentrations blocks the sodium channel not only during the inactivated state but also during the activated state.

In the myocytes treated with SD-3212 (10 μM), the $\dot{V}_{max}$ recovery of test action potential following a long (1 s) 0 mV clamp was expressed by a single exponential function for the data points with coupling intervals > 100 ms. This slow component of recovery may reflect the dissociation of the drug molecules (unbinding) from the sodium channels. Time constants of the $\dot{V}_{max}$ recovery in single myocytes, which are comparable to those of $\dot{V}_{max}$ recovery from the use-dependent block in papillary muscles (Figure 4), showed an appreciable voltage-dependence, being shorter at the more negative potential within the range from −70 to −90 mV. This voltage-dependence can be interpreted by its higher affinity for the inactivated state of sodium channels than the resting one. Under such conditions, hyperpolarization would enhance drug dissociation from channel receptors by increasing the resting fraction of drug-associated channels at the expense of their inactivated fraction (Hondeghem & Katzung, 1984; Grant *et al.*, 1984).

The *in vitro* concentration of SD-3212 used in the present study to decrease $\dot{V}_{max}$ is in a range of effective i.v. dose in dog experiments of arrhythmias (Nagashima *et al.*, 1992; Hirasawa *et al.*, 1992). Accordingly, it seems reasonable to conclude that the primary electrophysiological effect of this compound is a use- and voltage-dependent inhibition of fast sodium channels (Class-I action). From the onset and offset kinetics of the use-dependent $\dot{V}_{max}$ inhibition, SD-3212 is located between the fast and intermediate kinetic drugs. From the state-dependence of sodium channel block, SD-3212 belongs to inactivated channel blockers.

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