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Effects of long-term oral administration of amiodarone on the electromechanical performance of rabbit ventricular muscle

[1] Itsuo Kodama, Ryoko Suzuki, Kaichiro Kamiya, Hirokazu Iwata & Junji Toyama

Department of Circulation, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-01, Japan

1 The effects of long-term administration of oral amiodarone on transmembrane action potential and contraction of ventricular muscle were investigated in rabbits.

2 ECGs of rabbits that received oral amiodarone 50 mg or 100 mg kg^{-1} daily for 4 weeks, showed a significant prolongation of RR, QT and corrected QT (QTc) intervals, whereas PQ and QRS were unaffected. Serum and myocardial tissue amiodarone concentrations were 0.14–0.18 $\mu g\ ml^{-1}$ and 1.47–3.63 $\mu g\ g^{-1}$ wet wt. respectively.

3 Right ventricular papillary muscles isolated from treated rabbits were characterized by a moderate prolongation of action potential duration (APD) compared with controls. A slight decrease of the maximum upstroke velocity ($\dot{V}_{max}$) was also observed at the higher dose. The APD prolongation by chronic amiodarone, unlike acute effects of sotalol, E-4031, Cs^+ and 4-aminopyridine, did not show marked reverse use-dependence.

4 APD and $\dot{V}_{max}$ restitution following slow basic stimuli (0.03 Hz) were unaffected by chronic treatment with amiodarone.

5 Acute application of amiodarone (10 μM) caused a significant decrease in APD and developed tension, as well as a marked use-dependent $\dot{V}_{max}$ inhibition with fast recovery kinetics.

6 These findings suggest that a major and consistent electro-physiological effect of chronic amiodarone is repolarization delay (Class-III action) showing minimal frequency-dependence. However, when amiodarone above a certain concentration is present in the extracellular space, a fast kinetic Class-I action would be added as an acute effect.

Keywords: Amiodarone; ventricular muscle; action potential; $\dot{V}_{max}$; frequency-dependence

Introduction

It is now well established that long-term treatment of patients with oral amiodarone is extremely effective for prophylactic control of most supraventricular and ventricular tachyarrhythmias (Heger *et al.*, 1984; Zipes *et al.*, 1984). Amiodarone has long been referred to as a Class III antiarrhythmic agent, because it prolongs both the action potential duration (APD) and the refractory period of cardiac muscle especially when administered chronically (Vaughan Williams, 1984). Many recent studies, however, have shown that the pharmacological actions of this compound are complex. For instance, it possesses an inhibitory effect on the fast sodium channels as well as on the slow calcium channels (Mason, 1987; Singh *et al.*, 1989). Amiodarone also has non-competitive antisympathetic effects and an action to modulate thyroid function (Singh, 1990). Which action or which combinaiton of actions is fundamental and salutory for its potent antiarrhythmic activity is not known. This question is still a matter of debate, and no unequivocal answer has been presented. In order to obtain further insight into this point, we investigated the electromechanical performance of ventricular muscles isolated from rabbits following long-term oral administration of amiodarone, since this animal (and the dog) are more predictive for cardiac electrophysiology in man than any other species. Our major aims were to determine whether chronic amiodarone exerts use-dependent sodium channel inhibitory actions like local anaesthetic type (Class I) antiarrhythmic drugs, and whether the Class III action of chronic amiodarone depends on stimulation frequency. We also examined the acute effects of amiodarone in some experiments to obtain a better understanding of its total electropharmacological profile.

[1] Author for correspondence.

Methods

Experimental protocol

Japanese white rabbits of either sex weighing 1.8 to 2.2 kg were treated for 4 weeks with oral amiodarone. The dose was 20 mg kg^{-1} daily for 5 rabbits, 50 mg kg^{-1} daily for 8 and 100 mg kg^{-1} daily for 6 rabbits. On the last day of drug treatment, peripheral venous blood sampling was carried out to measure serum amiodarone concentrations. Scalar electrocardiograms (ECGs) of extremity leads (I, II) were also recorded from the conscious rabbits caged in a small dark box. The rabbits were then killed by intravenous administration of pentobarbitone sodium (30 mg kg^{-1}), and the right ventricular papillary muscles removed. Fifteen untreated rabbits of corresponding weight were used as references. The muscles (0.4 to 0.6 mm in diameter and 3 to 4 mm in length) were mounted in a tissue bath (0.5 ml) and superfused at 32°C with Krebs-Ringer solution 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 base of the muscle was fixed, and the tendinous end connected to a force-displacement transducer (Nihon Kohden TB 612T) for isometric tension recording. The resting tension was adjusted to obtain maximal twitch contraction during the equilibration period. The preparation was stimulated through a pair of 1.0 mm platinum wire electrodes placed 1 mm apart on either side of the muscle. By means of this field stimulation technique, the whole muscle was excited simultaneously, and no conduction occurred within the preparation. Unless otherwise stated, pulses used for stimulation were 2 ms in duration and 1.2 times diastolic threshold. Transmembrane potential was recorded through two glass microelectrodes filled with 3 M KCl, one intracellularly and the other extracellularly,

placed close together. The electrodes were each connected by Ag-AgCl wire to a high input-impedance buffer amplifier connected to a differentiated amplifier (Nihon Kohden, MEZ-7101). The maximum upstroke velocity ($\dot{V}_{max}$) of the action potential was obtained by electronic differentiation. Action potential duration (APD) was measured by an electronic device, which produced a ramp voltage corresponding to APD at a given level of membrane potential (Kentish & Boyett, 1983). Single cell impalements of the microelectrodes were maintained throughout each experiment.

A stabilization period of 3 to 4 h under constant stimulation at 1.0 Hz was allowed before data were collected. Frequency-dependent effects were assessed during fixed-rate pacing at cycle lengths of 10 s, 5 s, 2 s, 1 s, 500 ms and 330 ms. Measurements at steady-state were obtained 3 to 5 min after pacing at each cycle length. To study restitution of the action potential configuration, regular basic stimuli at a long cycle length (30 s) were followed by a single test stimuli with various coupling intervals. The intensity of the test stimulus was adjusted to obtain a constant latency from the stimulus artifact to the initiation of the action potential upstroke.

In some experiments, amiodarone (1, 10 μM), Cs (5 mM), 4-aminopyridine (4-AP, 2 mM), E-4031 (N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methane sulphonamide dihydrochloride dihydrate; 0.1 μM) and sotalol (30 μM) were added to the superfusate for 30 to 180 min to examine their acute effects.

All the data were digitized at a sampling interval of 5 kHz for ECGs, action potentials and contraction curves, or at 20 kHz for the derivative of action potential upstroke, and recorded on a magnetic tape (SONY PC-108M) for off-line computer analysis (NEC 9801-DA). From 16 s consecutive ECG records of each rabbit, mean values of RR, PQ, QT and QRS intervals were calculated through the use of ECG processing software (Softron EP98-1). The mean QT interval was divided by the root mean of the RR interval to provide the corrected QT interval (QTc). Parameters measured in papillary muscles were resting membrane potential (RP), amplitude of action potential (AMP), $\dot{V}_{max}$, APD at − 70 mV, peak developed tension (DT) and time to peak tension (tPT).

Blood samples withdrawn into heparinized tubes were centrifuged at room temperature, and the serum removed and frozen. The ventricle remaining after excision of the papillary muscle was also frozen. Amiodarone and its major active metabolite, desethylamiodarone in the serum and in the ventricular myocardial tissues homogenates were measured by high performance liquid chromatography as modified by Brien *et al.* (1983). The limit of sensitivity for amiodarone and desethylamiodarone was 0.025 μg ml^{-1} of serum and 0.1 μg g^{-1} wet weight of ventricular tissue samples.

Drugs and data analysis

Amiodarone HCl was kindly donated by Taisho Pharmaceutical Co. Ltd. (Tokyo, Japan), and E-4031 by Eisai Pharmaceutical Co. Ltd. (Tokyo, Japan). Sotalol and 4-aminopyridine (4-AP) were purchased from Sigma Chemical Co. E-4031, sotalol and 4-AP were dissolved in deionized water and diluted with superfusate (Krebs-Ringer solution) to achieve the final concentrations required. In experiments to test the acute effects of amiodarone, the compound was dissolved in Krebs-Ringer solution containing ethanol (0.005–0.05 mg 100 ml^{-1}) and bovine serum albumin (0.1–1.0%) as described previously (Honjo *et al.*, 1991). At the concentration used in the present study (1–10 μM), there was no visible precipitation of amiodarone in the superfusate.

Values given are means or means ± s.e. One-way analysis of variance with an *F*-test was applied to evaluate the chronic effects of amiodarone in comparison with control animals. Dunnett's test was used, and differences were considered significant at $P<0.05$. The time course of $\dot{V}_{max}$ recovery was defined using a least square exponential fitting routine.

Results

Electrocardiograms and amiodarone concentration

There were no significant differences in RR, PQ, QT and QRS intervals between untreated conscious control rabbits and rabbits that received amiodarone at 20 mg kg^{-1} daily (data not shown). In rabbits that received amiodarone at 50 mg or 100 mg kg^{-1} daily, RR, QT and QTc were significantly prolonged in comparison with controls (Table 1); PQ and QRS remained unchanged.

Table 2 summarizes serum and myocardial tissue amiodarone concentrations in rabbits which received amiodarone at 50 mg and 100 mg kg^{-1} daily. Amiodarone levels in both serum and myocardium tended to be higher in the 100 mg kg^{-1} daily group than in the 50 mg kg^{-1} daily group. These differences were not however statistically significant because of relatively large variability among the animals. Desethylamiodarone concentrations in 100 mg kg^{-1} daily group were 0.04 ± 0.01 μg ml^{-1} in serum ($n = 6$), and 2.18 ± 0.48 μg g^{-1} wet weight in myocardium ($n = 6$).

Action potential characteristics and contraction in papillary muscles

Steady-state action potentials and contractions during stimulation at 1.0 Hz were compared. There were no significant differences in RP, AMP, $\dot{V}_{max}$, DT and tPT between muscles excised from control animals and those taken from animals treated with 20 mg kg^{-1} daily amiodarone. In muscles from the 50 mg kg^{-1} daily amiodarone group, APD at − 70 mV was significantly prolonged, whereas RP, AMP, $\dot{V}_{max}$, DT and tPT were similar to control (Table 3). In muscles from the 100 mg kg^{-1} daily amiodarone group, APD was further prolonged. A slight decrease in $\dot{V}_{max}$ and DT was also observed, but RP, AMP and tPT were still unchanged (Table 3, Figure 1).

When the cycle length of stimulation to the control preparations was shortened in steps from 10 s (0.1 Hz) to 330 ms (3.0 Hz), APD was initially increased and then decreased resulting in a bell-shaped frequency-response curve (Figure 2). The longest APD was obtained at a cycle length of 2.0 s (0.5 Hz). $\dot{V}_{max}$ was slightly decreased at a cycle length

Table 1 Effects of oral amiodarone administration on electrocardiograms (ECGs) of rabbits

	(n)	*RR* (ms)	*PQ* (ms)	*QRS* (ms)	*QT* (ms)	*QTc*
Control	15	257 ± 8	47 ± 2	40 ± 3	156 ± 6	308 ± 7
Amiodarone						
50 mg kg^{-1} daily	8	315 ± 20**	51 ± 1	43 ± 2	196 ± 9**	353 ± 9**
100 mg kg^{-1} daily	6	300 ± 23*	51 ± 2	45 ± 4	198 ± 11**	363 ± 7**

Values are means ± s.e. *n*: number of rabbits treated or untreated with oral amiodarone for 4 weeks.
Significant difference from control at *$P<0.05$ and at **$P<0.01$.

shorter than 1.0 s (Figure 3a). Since, the $\dot{V}_{max}$ reduction was not accompanied by a decrease in RP, it may reflect insufficient recovery of sodium channels from the slow inactivation (Saikawa & Carmeliet, 1982). DT was increased progressively at the shorter cycle length reflected in a positive staircase of contraction (Figure 3b).

In the preparations treated with amiodarone, 50 mg or 100 mg kg^{-1} daily, APD was prolonged over the entire range of cycle lengths (Figures 1 and 2). Absolute changes in APD from control were larger at the longer cycle length within a range from 330 ms to 2.0 s (Table 4). However, the frequency-dependence of APD prolongation by chronic amiodarone was relatively small, and the bell-shaped cycle length-APD relationship as observed for controls was well preserved (Figure 2). The frequency-response relationship of $\dot{V}_{max}$ and that of DT were also essentially unchanged from control (Figure 3).

Figure 4 compares the percentage changes of APD by chronic amiodarone with those induced by acute application of 4-AP (2 mM), Cs (5 mM), E-4031 (0.1 μM) and sotalol (30 μM). With the exception of 4-AP, all the drugs tested prolonged APD even at the shortest cycle length. The APD prolongation by E-4031, sotalol, Cs and 4-AP was enhanced progressively at the longer cycle length, giving rise to a marked 'reverse use-dependence' (Hondeghem & Snyder, 1990). Chronic amiodarone differed from the other drugs in that such a marked reverse use-dependence was not seen.

Table 2 Serum and myocardial concentration of amiodarone

	(n)	*Serum* ($\mu g\ ml^{-1}$)	*Myocardial tissue* ($\mu g\ g^{-1}$)
Amiodarone			
50 mg kg^{-1} daily	(8)	0.14 ± 0.05	1.47 ± 0.48
100 mg kg^{-1} daily	(6)	0.18 ± 0.06	3.63 ± 1.20

Values are means ± s.e. *n*: number of rabbits treated with oral amiodarone for 4 weeks.

Restitution of action potential configuration

Restitution of the action potential configuration was examined by applying a single test stimulus following a very slow basic cycle length (30 s) with various coupling intervals. As in controls, $\dot{V}_{max}$ recovered rapidly after full repolarization of the basic action potential in muscles taken from animals given amiodarone, 100 mg kg^{-1} daily. The recovery process was approximated by a single exponential function with a time constnat (τR) of 42 ± 11 ms ($n = 4$) for amiodarone-

Table 3 Effects of chronic oral amiodarone on transmembrane action potential and contraction in rabbit papillary muscle

	(n)	*RP* (*mV*)	*AMP* (*mV*)	$\dot{V}_{max}$ ($V\ s^{-1}$)	*APD* − 70 mV (*ms*)	*DT* (*mg*)	*tPT* (*ms*)
Control	(15)	− 88 ± 1	120 ± 2	209 ± 11	277 ± 7	288 ± 41	169 ± 6
Amiodarone							
50 mg kg^{-1} daily	(8)	− 90 ± 1	121 ± 2	196 ± 6	312 ± 8*	218 ± 32	171 ± 6
100 mg kg^{-1} daily	(6)	− 87 ± 1	119 ± 1	172 ± 8*	333 ± 6*	207 ± 29*	175 ± 8

Values are means ± s.e. *n*: number of rabbits. Oral amiodarone was administered at a dose of 50 mg kg^{-1} daily or 100 mg kg^{-1} daily for 4 weeks. RP: resting membrane potential. AMP: amplitude of action potential. $\dot{V}_{max}$: the maximum upstroke velocity of action potential. APD − 70 mV: action potential duration at − 70 mV. DT: peak developed tension. tPT: time to peak tension. Significantly different from control at $^*P < 0.05$.

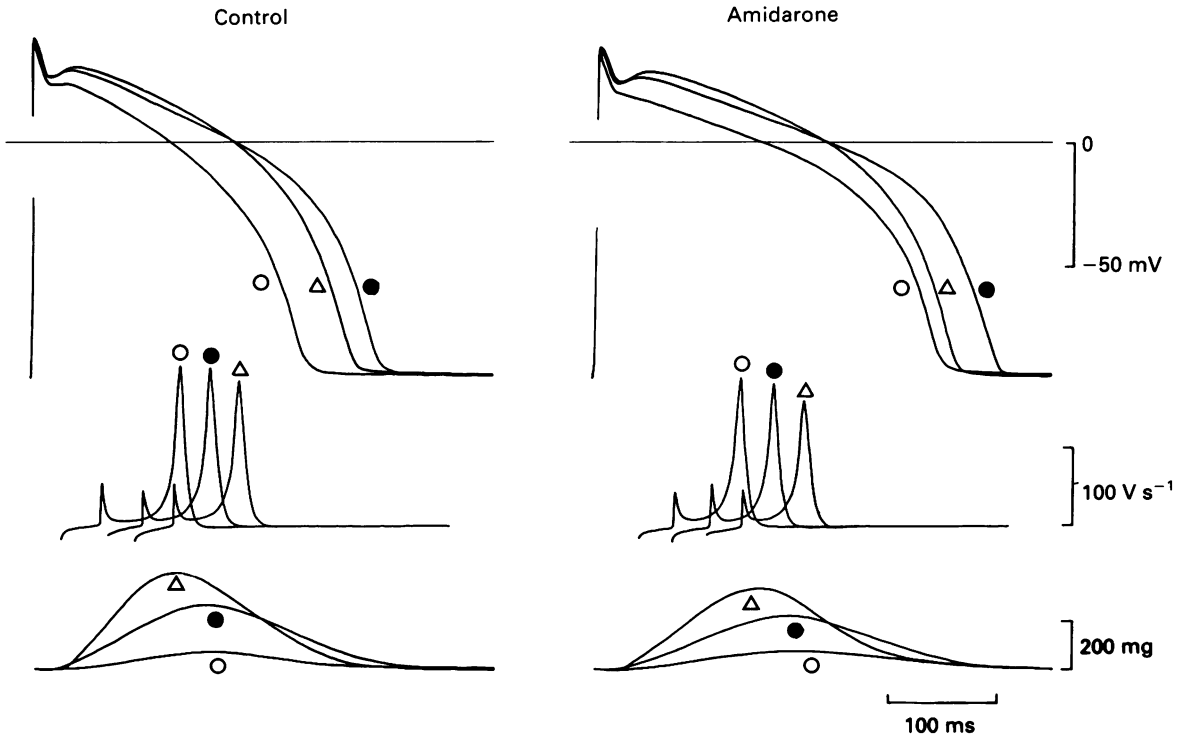

Figure 1 Effects of chronic amiodarone on transmembrane action potential and contraction of rabbit papillary muscles. The preparations from an untreated rabbit (control) and a rabbit treated with amiodarone (100 mg kg^{-1} daily) were stimulated at 0.1 Hz (○), 1.0 Hz (●) and 3.0 Hz (△). The differentiated upstroke spike ($\dot{V}_{max}$) of the action potential (middle traces) was recorded at a faster sweep velocity.

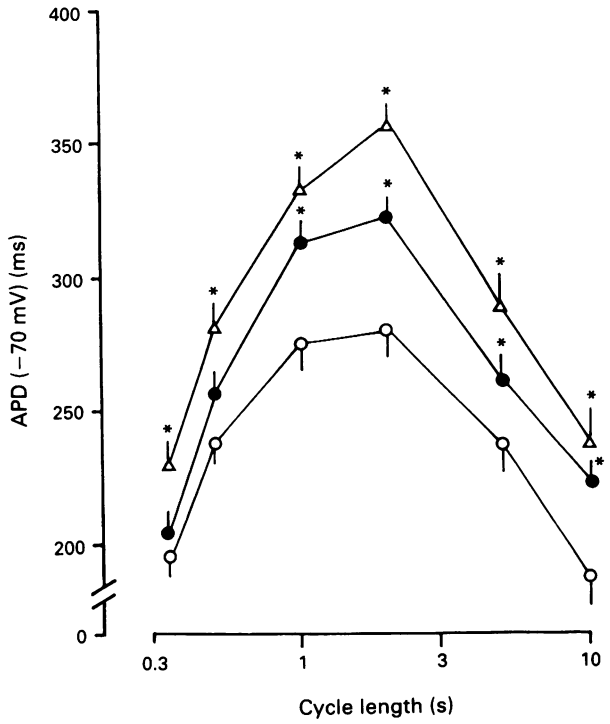

Figure 2 Relationship between action potential duration and stimulation frequency. Ordinate scale: action potential duration (APD) at − 70 mV. Abscissa scale: cycle length of stimulation. Data were obtained from 15 untreated preparations (control, ○), 8 treated with 50 mg kg^{-1} daily amiodarone (●) and 6 with 100 mg kg^{-1} daily amiodarone (△). Values are means; vertical lines show s.e.mean. *The change was statistically significantly different from control at $P<0.05$.

treated preparations and 38 ± 9 ms ($n = 4$) for untreated ones. There was no significant difference between the two values.

Figure 5 shows restitution of APD. In both control and amiodarone-treated muscles, increasing the coupling interval from 200 ms induced a progressive increase in APD reaching a peak at around 300 to 400 ms. Further prolongation of the coupling interval was associated with a gradual shortening of APD toward the level of basic action potential. Percentage decrease of APD in association with a prolongation of coupling interval from 400 ms to 10 s was 46 ± 5% in control preparations ($n = 4$). Comparable values (41 ± 6%, $n = 4$) were obtained in amiodarone-treated muscles.

Acute effects of amiodarone

In the above-mentioned experiments, papillary muscles were superfused with drug-free Krebs-Ringer solution for longer than 3 h. In other words, the data were obtained, unlike *in vivo* experiments or clinical cases, in the presence of negligible extracellular levels of amiodarone. We, therefore, tested the additional acute effects of amiodarone in four preparations which had been pretreated with oral amiodarone, 100 mg kg^{-1} daily for 4 weeks.

Application of 1 μM amiodarone to the superfusate for 120 to 180 min caused no significant changes in action potential configuration or in contractility (data not shown). Application of amiodarone at 10 μM resulted in a significant shortening in APD and a decrease in DT at all the stimulation

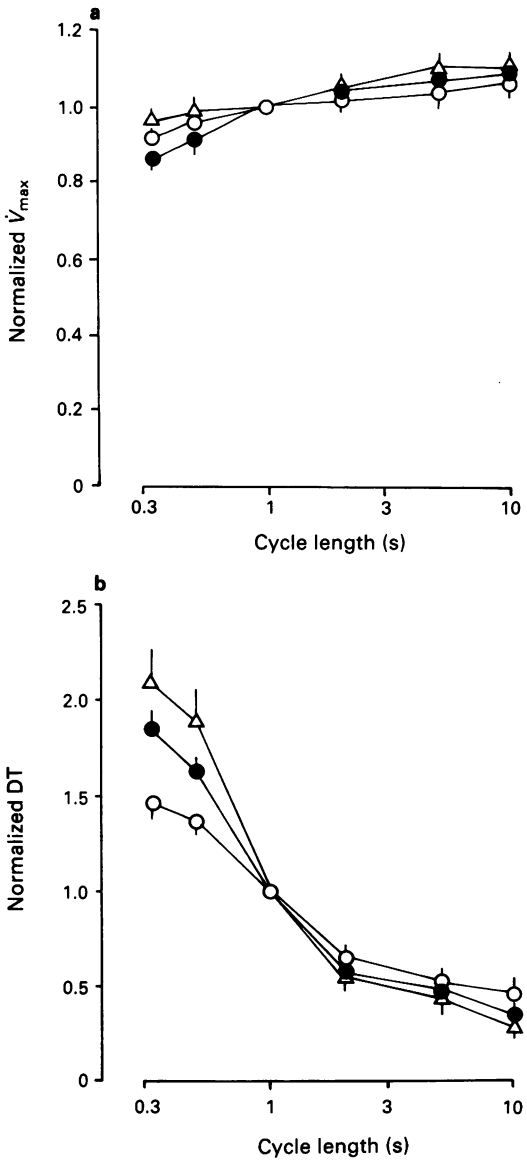

Figure 3 Frequency-response relationship of the maximum upstroke velocity ($\dot{V}_{max}$) and the peak developed tension (DT). Ordinate scale: $\dot{V}_{max}$ (a) and DT (b) normalized by the values at 1.0 Hz stimulation. Abscissa scales: cycle length of stimulation. Data were obtained from 15 untreated preparations (control, ○), 8 treated with 50 mg kg^{-1} daily amiodarone (●), and 6 with 100 mg kg^{-1} daily amiodarone (△). Values are means; vertical lines show s.e.mean.

Table 4 Prolongation of action potential duration in rabbit papillary muscles by chronic treatment with amiodarone

		Cycle length (s)					
	(n)	0.33	0.5	1.0	2.0	5.0	10.0
Amiodarone							
50 mg kg^{-1} daily	(8)	+ 11	+ 19	+ 38	+ 43	+ 24	+ 36
100 mg kg^{-1} daily	(6)	+ 34	+ 43	+ 58	+ 75	+ 52	+ 50

Values are absolute differences (ms) in means of action potential duration (APD) at − 70 mV between amiodarone-treated groups and control ($n = 15$). Data were obtained from the same experiments as in Figure 2.

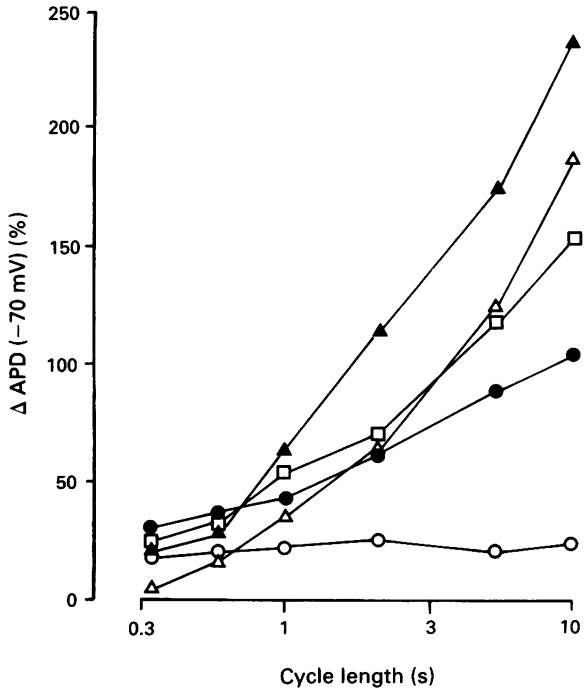

Figure 4 Frequency-dependence of APD prolongation by chronic amiodarone and by acute application of Cs, 4-aminopyridine (4-AP), E-4031 or sotalol in rabbit papillary muscles. Ordinate scale: percentage prolongation of action potential duration (APD) at −70 mV compared with control. Abscissa scale: cycle length of stimulation. Values are means of 6 preparations treated with 100 mg kg^{-1} daily amiodarone (○), and 4 treated with acute application of Cs 5 mM (▲), 4-AP 2 mM (△), E-4031 0.1 μM (●) or sotalol 30 μM (□).

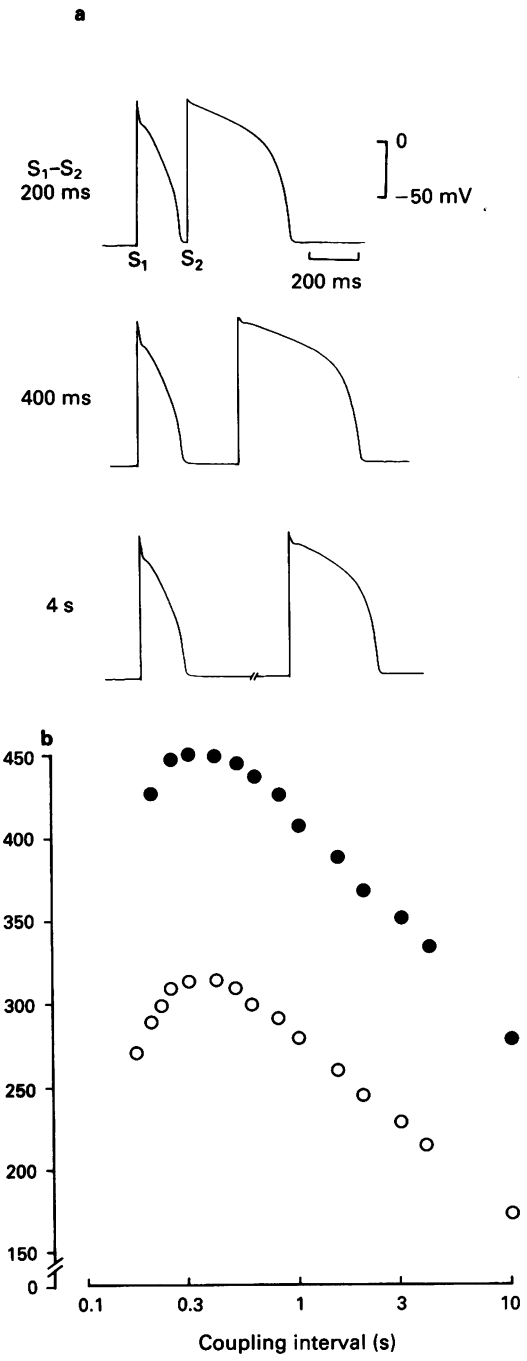

Figure 5 Restitution of action potential duration (APD) of rabbit papillary muscle. Panel (a) shows action potentials elicited by a slow basic stimulus (S1−S1 = 30 s) followed by a test stimulus (S2) with a coupling interval of 200 ms, 400 ms and 4 s. The records were obtained from a preparation treated with amiodarone (100 mg kg^{-1} daily). In (b) are shown restitution curves of APD at −70 mV in the amiodarone-treated muscle (●) and in control muscle (○).

frequencies used (0.1 to 3.0 Hz) (Figure 6). $\dot{V}_{max}$ was also decreased significantly at a frequency higher than 1.0 Hz.The higher the stimulation frequency, the greater the $\dot{V}_{max}$ reduction. Thus, the $\dot{V}_{max}$ inhibition by acute amiodarone was 'use-dependent'.

The recovery of $\dot{V}_{max}$ from use-dependent block was studied by applying a single test stimulus at various coupling intervals following a simulation train for 60 s at 1.0 Hz. Before the addition of amiodarone to the superfusate, $\dot{V}_{max}$ of the test action potential recovered almost completely within 100 ms of full repolarization. After addition of 10 μM amiodarone, a much slower recovery of $\dot{V}_{max}$ was observed (Figure 7). The recovery time course of $\dot{V}_{max}$ with a diastolic interval longer than 100 ms was approximated by a single exponential function with a mean time constant of 452 ± 23 ms ($n = 4$).

Similar APD shortening and use-dependent $\dot{V}_{max}$ inhibition were obtained when 10 μM amiodarone was added to untreated (control) papillary muscle preparations (data not shown).

Discussion

The present study showed that long-term oral administration of amiodarone (50 mg and 100 mg kg^{-1} daily for 4 weeks) caused a significant prolongation of action potential duration (APD) of rabbit papillary muscles. The muscles treated by the higher dose of amiodarone (100 mg kg^{-1} daily) also showed a slight decrease in $\dot{V}_{max}$ and peak developed tension (DT); the changes were independent of stimulation frequencies. The APD prolongation by chronic amiodarone was quite different from that induced by acute application of sotalol, E-4031, Cs and 4-AP in terms of their frequency-dependence; the former was enhanced only minimally, whereas the latter was greatly enhanced at the lower stimulation frequency within a range from 0.1 to 3.0 Hz.

There is a general agreement between previous investigators that chronic treatment of mammals with amiodarone for several weeks causes moderate prolongation of APD throughout the entire heart (Mason, 1987; Singh *et al.*, 1989). However, only limited information is available as to the frequency-dependence of this Class III action. Anderson *et al.* (1989) demonstrated in dogs *in vivo* that repolarization intervals and refractory periods of epicardial ventricular muscle assessed by surface electrograms were prolonged to a similar extent over stimulation frequencies ranging from 1 to

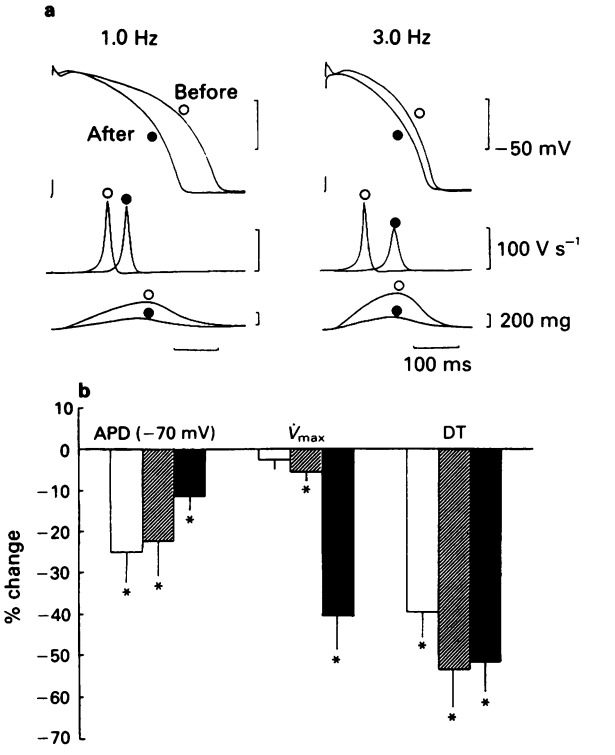

Figure 6 Effects of acute amiodarone on transmembrane action potential and force of contraction of papillary muscles. In (a) are shown superimposed records of membrane action potential (top traces), their differentiated upstroke spikes (middle traces) and isometric tension curves (bottom traces) before (○) and 120 min after (●) application of 10 μM amiodarone. The preparation was stimulated at 1.0 Hz (left) and at 3.0 Hz (right). (b) The change in action potential duration (APD) at − 70 mV, the maximum upstroke velocity ($\dot{V}_{max}$) and peak developed tension (DT) at three different stimulation frequencies; 0.1 Hz (open columns), 1.0 Hz (hatched columns) and at 3.0 Hz (solid columns). Values are presented as % change from control (means with s.e.mean (vertical bars), $n = 4$). *Significantly different from control at $P < 0.05$.

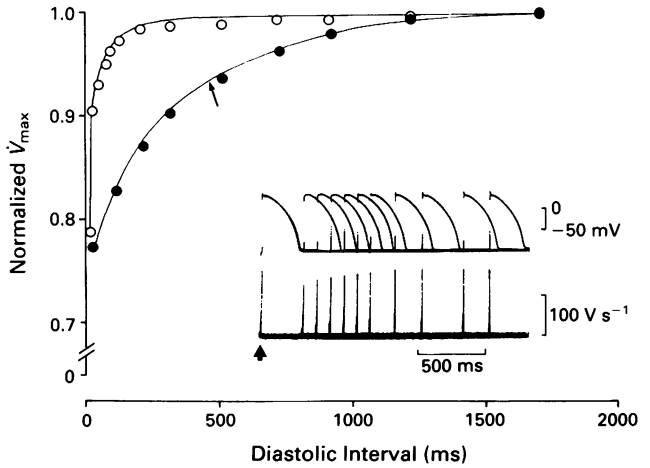

Figure 7 Recovery of $\dot{V}_{max}$ from the use-dependent block by acute amiodarone. Inset shows superimposed records of action potentials (upper trace) and their differentiated upstroke spikes (lower trace) after acute application of amiodarone (10 μM). Following 1.0 Hz stimulation for 60 s, a single test stimulus was applied with various coupling intervals. (A thick arrow indicates the last conditioning stimulus). The graph shows $\dot{V}_{max}$ recovery before (○) and after (●) acute application of amiodarone (10 μM). Ordinate scale: normalized $\dot{V}_{max}$ of test action potential with a reference to the value after full recovery. 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 amiodarone was approximated by a single exponential function at a time constant of 460 ms (thin arrow) for the data with diastolic intervals of over 100 ms.

5 Hz. Hondeghem & Snyder (1990) have suggested in their recent review that such a frequency-independent Class III action of amiodarone (unlike other agents causing greater Class III action at the lower stimulation frequency) might be important for its more antiarrhythmic and less proarrhythmic activities. Our results also revealed much less 'reverse use-dependence' of APD prolongation by chronic amiodarone than that by other Class III agents.

Biphasic restitution curves of APD in control preparations (Figure 5) were similar to those reported by previous investigators using multicellular ventricular tissue preparations (Gibbs & Johnson, 1961; Kukushkin *et al.*, 1983) or single ventricular myocytes (Hiraoka & Kawano, 1987) isolated from rabbit hearts. Based on membrane current analysis during voltage-clamp experiments, Hiraoka & Kawano (1987, 1989) suggested that APD prolongation with progressively longer coupling intervals up to 0.3 s may reflect a relatively fast recovery of high threshold (L-type) inward calcium current (I_{Ca}) from inactivation, whereas APD shortening with further prolongation of coupling intervals is the result of very slow recovery of the transient outward current (I_{to}) from inactivation. Since the APD restitution curves of untreated and amiodarone-treated preparations were virtually identical, the effects of chronic amiodarone on the kinetics of I_{Ca} and I_{to} would be minimal or negligible. Nevertheless, extensive voltage-clamp studies on single ventricular cells isolated from amiodarone-treated rabbits are required to substantiate this assumption.

There is considerable controversy regarding the Class I action of chronic amiodarone. Mason *et al.* (1984) showed a marked use-dependent $\dot{V}_{max}$ inhibition in guinea-pig papillary muscles after chronic treatment with amiodarone, similar to the acute effects of the drug. In support of this finding, Anderson *et al.* (1989) demonstrated a rate-dependent decrease in conduction velocity in the epicardium of dogs after chronic treatment with amiodarone. Epstein *et al.* (1991) also showed use-dependent slowing of conduction in the His-Purkinje system in dogs after chronic amiodarone treatment. The use-dependent His-ventricular conduction delay has also been demonstrated in patients receiving long-term oral amiodarone therapy (Shenasa *et al.*, 1984; Cascio *et al.*, 1988).

In contrast, Singh and his colleagues (Singh & Vaughan Williams, 1970; Ikeda *et al.*, 1984; Venkatesh *et al.*, 1986; Kato *et al.*, 1988) showed minimal or no significant change in $\dot{V}_{max}$ in atrial and ventricular muscles from rabbits after long-term amiodarone treatment. Gallagher *et al.* (1989) also showed in their blood cross-perfusion experiments that Purkinje fibres obtained from dogs after long-term amiodarone treatment did not show use-dependent $\dot{V}_{max}$ inhibition even at higher stimulation frequencies. Our data showing no use-dependent $\dot{V}_{max}$ inhibition and rapid restitution of $\dot{V}_{max}$ (τR 42 ms) are in accordance with the latter group.

These discrepancies concerning Class I actions of chronic amiodarone could be attributed to differences in experimental and clinical conditions. For instance, serum and myocardial concentrations of amiodarone found in experiments conducted by Anderson *et al.* (1989) were several times higher than those found by others (Ikeda *et al.*, 1984; Kato *et al.*, 1988; Gallagher *et al.*, 1989) including us. Species and tissue differences in response to the chronic effects of amiodarone (and its active metabolite) might also play a part.

Acute treatment with 10 μM amiodarone had quite different effects on papillary muscles than chronic treatment. Acute administration caused a significant shortening of APD and a substantial decrease of DT. A marked use-dependent $\dot{V}_{max}$ reduction was also observed. Since the first description by Mason *et al.* (1983), many experimental studies have confirmed the local anaesthetic (Class I) actions of acute amiodarone (Varro *et al.*, 1985; Yabek *et al.*, 1985; 1986; Pallandi & Campbell, 1987; Follmer *et al.*, 1987; Kohlhardt & Fichtner, 1988; Honjo *et al.*, 1991). According to the data presented by Mason *et al.* (1983, 1984) and by Honjo *et al.*

(1991), acute amiodarone may block sodium channels by binding mainly during the inactivated state. There is some discrepancy as to the recovery time constant from use-dependent block by acute amiodarone. Mason *et al.* (1984) and Pallandi & Campbell (1987) reported values of 1.48 to 1.63 s, whilst Varro *et al.* (1985) and Honjo *et al.* (1991) obtained a much shorter time constant (from 282 to 460 ms). The recovery time constant found in the present study (452 ms) is in agreement with the latter reports.

Reports of the effects of acute amiodarone on APD have been conflicting. Some studies have shown minimal to moderate APD prolongation, whilst others have shown an appreciable APD shortening (Singh *et al.*, 1989; Gallagher *et al.*, 1989). This may be explained at least in part by different ionic currents responsible for the repolarization of action potential in different animal species and in different cardiac tissues. In voltage-clamp experiments, acute application of amiodarone has been shown to inhibit not only the fast inward sodium current (I_{Na}), the slow inward calcium current (I_{Ca}) (Nishimura *et al.*, 1989) but also outward potassium currents including the delayed rectifier current (I_K) (Colatsky *et al.*, 1990; Balser *et al.*, 1991). In rabbit ventricular muscle, the action of amiodarone on the inward currents might be greater than that on the outward currents.

The above discussion indicates that the major electrophysiological effect of chronic amiodarone on the ventricle appears to be repolarization delay (Class III action) showing minimal frequency-dependence. Relatively high concentrations of amiodarone in the extracellular space cause additional use-dependent sodium channel inhibition (Class I action). Acute amiodarone may also induce calcium channel inhibition (Class IV action) leading to a negative inotropic effect. In patients receiving long-term oral amiodarone, the serum drug level covers a wide range (0.06 to 4.5 $\mu g\,ml^{-1}$ corresponding to approximately to 0.1 to 7.6 μM) (Harris *et al.*, 1983; Raeder *et al.*, 1985). Accordingly, in individual cases of long-term amiodarone treatment, Class I, II and IV actions may contribute to the antiarrhythmic efficacy of this agent.

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References

ANDERSON, K.P., WALKER, R., DUSTMAN, T., LUX, R.L., ERSHLER, P.R., KATES, R.E. & URIE, P.M. (1988). Rate-related electrophysiologic effects of long-term administration of admiodarone on canine ventricular myocardium in vivo. *Circulation*, **79**, 948–958.

BALSER, J.R., BENNETT, P.B., HONDEGHEM, L.M. & RODEN, D.M. (1991). Suppression of time-dependent outward current in guinea pig ventricular myocytes. Actions of quinidine and amiodarone. *Circ. Res.*, **69**, 519–529.

BRIEN, J.F., JIMMO, S. & ARMSTRONG, P.W. (1983). Rapid high-performance liquid chromatographic analysis of amiodarone and N-desethylamiodarone in serum. *Can. J. Physiol. Pharmacol.*, **61**, 245–248.

CASCIO, W.E., WOELFEL, A., KNISLEY, S.B., BUCHANAN, J.W.Jr., FOSTER, J.R. & GETTES, L.S. (1988). Use dependence of amiodarone during the sinus tachycardia of exercise in coronary artery disease. *Am. J. Cardiol.*, **61**, 1042–1045.

COLATSKY, T.J., FOLLMER, C.H & STARMER, C.F. (1990). Channel specificity in antiarrhythmic drug action. Mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. *Circulation*, **82**, 2235–2242.

EPSTEIN, L.M., SCHEINMAN, M.M., CHIN, M.C. & KATZUNG, B.G. (1991). The use-dependent effects of acute and chronic amiodarone administration on His-Purkinje conduction and the interaction of beta-adrenergic stimulation. *J. Cardiovasc. Electrophysiol.*, **2**, 156–167.

FOLLMER, C.H., AOMINE, M., YEH, J.Z. & SINGER, D.H. (1987). Amiodarone-induced block of sodium current in isolated cardiac cells. *J. Pharmacol. Exp. Ther.*, **243**, 187–194.

GALLAGHER, J.D., BIANCHI,J. & GESSMAN, L.J. (1989). A comparison of the electrophysiologic effects of acute and chronic amiodarone administration on canine Purkinje fibers. *J. Cardiovasc. Pharmacol.*, **13**, 723–729.

GIBBS, C.L. & JOHNSON, E.A. (1961). Effect of changes in frequency of stimulation upon rabbit ventricular muscle action potential. *Circ. Res.*, **9**, 165–170.

HARRIS, L., MCKENNA, W.J., ROWLAND, E., HOLT, D.W., STOREY, G.C.A., & KRIKLER, D.M. (1983). Side effects of long-term amiodarone therapy. *Circulation*, **67**, 45–51.

HEGER, J.J., PRYSTOWSKY, E.N., MILES, W.M. & ZIPES, D.P. (1984). Clinical use and pharmacology of amiodarone. *Med. Clin. North Amer.*, **68**, 1339–1366.

HIRAOKA, M. & KAWANO, S. (1987). Mechanism of increased amplitude and duration of the plateau with sudden shortening of diastolic intervals in rabbit ventricular cells. *Circ. Res.*, **60**, 14–26.

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 danger of reverse use dependence. *Circulation*, **81**, 686–690.

HONJO, H., KODAMA, I., KAMIYA, K. & TOYAMA, J. (1991). Block of cardiac sodium channels by amiodarone studied by using $\dot{V}max$ of action potential in single ventricular myocytes. *Br. J. Pharmacol.*, **102**, 651–656.

IKEDA, N., NADEMANEE, K., KANNAN, R. & SINGH, B.N. (1984). Electrophysiologic effects of amiodarone: Experimental and clinical observation relative to serum and tissue drug concentrations. *Am. Heart J.*, **108**, 890–898.

KATO, R., VENKATESH, N., KAMIYA, K., YABECK, S., KANNAN, R. & SINGH, B.N. (1988). Electrophysiologic effects of desethylamiodarone, an active metabolite of amiodarone: Comparison with amiodarone during chronic administration in rabbits. *Am. Heart J.*, **115**, 351–359.

KENTISH, J.C. & BOYETT, M.R. (1983). A simple electronic circuit for monitoring changes in the duration of the action potential. *Pflügers Arch.*, **398**, 233–235.

KOHLHARDT, M. & FICHTNER, H. (1988). Block of single cardiac Na^+ channels by antiarrhythmic drugs: The effect of amiodarone, propafenone and diprafenone. *J. Memb. Biol.*, **102**, 105–119.

KUKUSHKIN, N.I., GUINULLIN, R.Z. & SOSUNOV, E.A. (1983). Transient outward current and rate-dependence of action potential duration in rabbit ventricular muscle. *Pflügers Arch.*, **399**, 87–92.

MASON, J.W. (1987). Amiodarone. *N. Engl. J. Med.*, **316**, 455–466.

MASON, J.W., HONDEGHEM, L.M. & KATZUNG, B.G. (1983). Amiodarone blocks inactivated cardiac sodium channels. *Pflügers Arch.*, **396**, 79–81.

MASON, J.W., HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Block of inactivated sodium channels and of depolarization-induced automaticity in guinea pig papillary muscle by amiodarone. *Circ. Res.*, **55**, 277–285.

NISHIMURA, M., FOLLMER, C.H. & SINGER, D.H. (1989). Amiodarone blocks calcium current in single guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.*, **252**, 650–659.

PALLANDI, R.T. & CAMPBELL, T.J. (1987). Resting and rate-dependent depression of $\dot{V}max$ of guinea-pig ventricular action potentials by amiodarone and desethylamiodarone. *Br. J. Pharmacol.*, **92**, 97–103.

RAEDER, E.A., PORDRID, P.J & LOWN, B. (1985). Side effects and complications of amiodarone therapy. *Am. Heart J.*, **109**, 975–983.

SAIKAWA, T. & CARMELIET, E. (1982). Slow recovery of the maximum rate of rise ($\dot{V}max$) of the action potential in sheep Purkinje fibers. *Pflügers Arch.*, **394**, 90–93.

SHENASA, M., DENKER, S., MAHMUD, R., LEHMANN, M., ESTRADA, A. & AKHTAR, M. (1984). Effect of amiodarone on conduction and refractoriness of the His-Purkinje system in the human heart. *J.A.C.C.*, **4**, 105–110.

SINGH, B.N. (1990). Amiodarone: Electropharmacologic properties. In *Antiarrhythmic Drugs*. ed. Vaughan Williams, E.M. pp. 335–364. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer-Verlag.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1970). The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. *Br. J. Pharmacol.*, **39,** 657–667.

SINGH, B.N., VENKATESH, N., NADEMANEE, K., JOSEPHSON, M.A. & KANNAN, R. (1989). The historical development, cellular electrophysiology and pharmacology of amiodarone. *Prog. Cardiovas. Dis.*, **31,** 249–280.

VARRO, A., NAKAYA, Y., ELHARRAR, V. & SURAWICZ, B. (1985). Use-dependent effects of amiodarone on $\dot{V}$max in cardiac Purkinje and ventricular muscle fibers. *Eur. J. Pharmacol.*, **112,** 419–422.

VAUGHAN WILLIAMS, E.M. (1984). A classification of antiarrhythmic action reassessed after a decade of new drugs. *J. Clin. Pharmacol.*, **24,** 129–147.

VENKATESH, N., SOMANI, P., BERSOHN, M., PHARI, R., KATO, R. & SINGH, B.N. (1986). Electropharmacology of amiodarone: Absence of relationship to serum, myocardial, and cardiac sarcolemmal drug concentration. *Am. Heart J.*, **112,** 916–922.

YABEK, S., KATO, R. & SINGH, B. (1985). Acute effects of amiodarone on the electrophysiologic properties of isolated neonatal and adult cardiac fibers. *J.A.C.C.*, **5,** 1109–1115.

YABEK, S., KATO, R. & SINGH, B. (1986). Effects of amiodarone and its metabolite, desethylamiodarone, on the electrophysiologic properties of isolated cardiac muscle. *J. Cardiovasc. Pharmacol.*, **8,** 197–207.

ZIPES, D.P., PRYSTOWSKY, E.N. & HEGER, J.J. (1984). Amiodarone: Electrophysiologic actions, pharmacokinetics and clinical effects. *J.A.C.C.*, **3,** 1059–1072.

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