# PROARRHYTHMIC ACTIONS OF CLASS III ANTIARRHYTHMIC DRUGS IN RABBIT SINO-ATRIAL NODAL CELLS

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Received March 3, 1995

*Abstract*: Arrhythmias induced by class III antiarrhythmic drugs (amiodarone, bretylium and sotalol) in spontaneously beating rabbit sino-atrial nodal cells were investigated using voltage-clamp and $Ca^{2+}$-sensitive fluorescent dye (fura-2). These drugs caused a negative chronotropic effect, and amiodarone and sotalol prolonged the action potential duration. Amiodarone (10 $\mu$M) induced arrhythmias in 3 out of 10 cells, and simultaneously elicited a transient inward current. Bretylium (10 $\mu$M) increased the incidence of arrhythmias in 5 out of 11 cells, and sotalol (10 $\mu$M) caused arrhythmias in only one of 10 cells. These drugs inhibited the $Ca^{2+}$ and delayed rectifier $K^+$ and hyperpolarization-activated inward currents. The kinetics for activation and inactivation of these currents were unaffected. After a washout, the regular rhythm was resumed and the transient inward current also disappeared. Change in the cytosolic $Ca^{2+}$ concentration was monitored with fura-2. Amiodarone (10-30 $\mu$M) and bretylium (0.1-10 $\mu$M) significantly elevated the cytosolic $Ca^{2+}$ level, but sotalol, even at 1 mM, did not affect it. These results indicate that the class III antiarrhythmic drugs possess proarrhythmic actions, probably resulting from elevation of cellular $Ca^{2+}$ concentration as well as the prolongation of action potential.

**Index Terms**

class III antiarrhythmic drugs, proarrhythmic action, intracellular $Ca^{2+}$ concentration, calcium overload, transient inward current

## INTRODUCTION

Class III antiarrhythmic drugs possess characteristics for prolongation of action potential duration (APD) without affecting the fast $Na^+$ current ($I_{Na}$), according to a classification of antiarrhythmic drugs[1]. The APD prolongation would be produced mainly by inhibition of delayed rectifier $K^+$ current ($I_K$), and thereby an effective refractory period (ERP) would be expected to increase. However, it has recently been demonstrated that the drugs also cause inhibitory actions on $I_{Na}$ and $Ca^{2+}$ current ($I_{Ca}$)[2][3]. These effects appear to share their antiarrhythmic actions.

By contrast, the class III drugs might induce reentrant arrhythmia due to APD prolongation (a torsades de pointes type ventricular tachycardia)[4]. In addition, the APD prolongation can cause a positive inotropic effect, presumably resulting from the elevation of intracellular $Ca^{2+}$ concentration ($[Ca]_i$)[5]. Amiodarone and bretylium increased the threshold to elicit ventricular

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fibrillation with coronary ligation[6-9]. In the present experiments, therefore, arrhythmias induced by the class III drugs (amiodarone, bretylium and sotalol) were examined in spontaneously-beating rabbit sino-atrial (SA) nodal cells, using two-microelectrode voltage-clamp technique. Also, the $[Ca]_i$ in single SA nodal cells was measured directly using fura-2, a $Ca^{2+}$-sensitive fluorescent dye.

## METHODS

### SA node preparations

Rabbits, weighing 1.5-2.0 kg, were anesthetized with pentobarbital sodium (30 mg/kg, i. v.), and exsanguinated. After removing the right atrium, strips of the SA nodal tissue were made by dissecting the tissue in a direction perpendicular to the crista terminalis, as described previously[3),10)]. The preparations were made smaller by dissections to a final dimension of about 0.2 x 0.2 mm. The preparation was superfused with the bath solution oxygenated with 100% $O_2$ at 36°C, and was left to beat spontaneously.

Single SA nodal cells were dissociated from hearts of rabbit (1.5-2.0 kg)[11),12)]. The SA node region was excised under a dissection microscope and cut into small strips of about 0.5 -1 mm in width. The SA node strips were incubated in $Ca^{2+}$-free bath solution. The spontaneous heart beat ceased. The perfusate was changed to $Ca^{2+}$-free Tyrode solution containing 0.1% collagenase (Type 1, Sigma Chemical Co., St. Louis, MO, USA) for 30-35 min at 36°C. The specimens were then transferred to high $K^+$, low $Cl^-$ solution and stored at 4°C for 1 h. The cell size was in the range of 10-25 $\mu$m in length and 10-15 $\mu$m in width.

### Recordings of action potentials and ionic currents

Two-microelectrode voltage-clamp technique was used[3),10)], which is similar to the method developed initially by Noma & Irisawa[13)]. Conventional glass microelectrodes filled with 3 M KCl were used, and the resistances were 20-30 MΩ. The holding potential was −40 mV. Between applications of the voltage-clamp, the cells invariably showed spontaneous activity. The amplitude of $I_{Ca}$ was determined as the difference between the peak current and the current level measured at 100 msec after the onset of the step, according to the method developed by McDonald and Trautwein[14)]. The magnitudes of $I_K$ and hyperpolarization-activated inward current ($I_f$) were determined by taking the difference between the value of the current at the end of a long clamp-pulse and zero current level. The amplitude of the transient inward current ($I_{ti}$) was determined as the difference between the major inward peak and a line drawn between the beginning and the end of that peak[15),16)]. The values represent mean ±S.E.M.

### Measurement of cellular $Ca^{2+}$ concentration

Aliquots of isolated cells (300 $\mu$l) were incubated with the acetoxy-methyl-ester of fura-2 (fura-2/AM) (Dojin Chemical Co, Kumamoto, Japan) at 30°C with gentle agitation for 30 min in the presence of an initial concentration of 0.3 $\mu$M fura-2/AM[11),12)]. After incubation for 30 min, the cell suspension diluted approximately 1000-fold with the bath solution was used and was served for experiments. The cell was changed from rod-shape to round-shape. Cellular fura-2 was fully $Ca^{2+}$-sensitive at 100 to 500 nM $[Ca]_i$ (measured in populations of cells). The $[Ca]_i$ in cells loaded with fura-2 was monitored as a change in the ratio of the fluorescent

intensity at 340-nm and 380-nm excitations. The fluorescence was quantified with a silicon-intensified target (SIT) camera and a digital imaging system (Hamamatsu Photonics Argus 100, Hamamatsu, Japan).

**Solutins**

The composition of the bath solution was (in mM) : NaCl 137, KCl 2.7, $MgCl_2$ 0.5, $CaCl_2$ 1.8, HEPES [N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid] (Wako Pure Chemical Industries, Ltd., Osaka, Japan) 5, and glucose 5. The pH was adjusted to 7.4 with NaOH. The composition of the high $K^+$, low $Cl^-$ solution was (in mM) : KOH 70, KCl 40, 1-glutamic acid 50, taurine 20, $KH_2PO_4$ 20, $MgCl_2$ 3, glucose 10, EGTA 0.5, and HEPES 10. The pH was adjusted to 7.4 with KOH. The drugs used were amiodarone hydrochloride (Sanofi Recherche, Toulouse, France), bretylium tosylate (American Critical Care, McGraw Park, IL, USA), and (±)-sotalol hydrochloride (Bristol Myers, Brussels, Belgium). Amiodarone and bretylium were dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO, USA), and sotalol was dissolved in distilled water. The stock solution with the concentration of 100 mM was stored at −10°C.

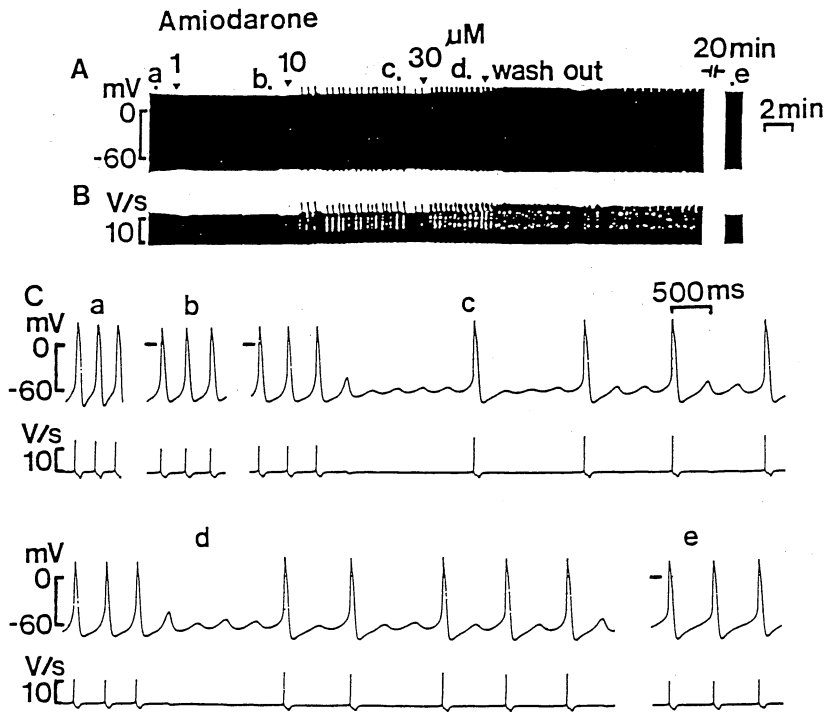


Fig. 1. Modulation of the spontaneously beating sino-atrial nodal action potentials and occurrence of arrhythmia by application of amiodarone. A : Spontaneous action potentials. B : Maximum rate of depolarization. C : Spontaneous action potentials and electrically differentiated action potentials (by fast -speed recording) at various times during the experiments. Short line at the left of the action potentials represents zero mV.

## RESULTS

The class III drugs caused a negative chronotropic effect in a concentration-dependent manner. Amiodarone (100 $\mu$M) decreased the maximum rate of depolarization ($\dot{V}_{max}$), prolonged APD, and hyperpolarized the maximum diastolic potential (MDP), significantly (Fig. 1). Bretylium (10 $\mu$M) decreased $\dot{V}_{max}$. Sotalol at 10 $\mu$M depolarized MDP, and at 100 $\mu$M prolonged APD. These results are summarized in Fig. 2.

In some cells, amiodarone (10-30 $\mu$M) induced arrhythmias (Fig. 1 A-C). The incidence of the arrhythmias in summarized in Table 1. During pacemaker potential (a phase 4 depolarization), small depolarizations (not reaching the threshold potential) and irregular oscillatory potentials were elicited. Increasing the concentrations increases the incidence of arrhythmias. When $K^+$ concentration in the bath sulution ($[K]_o$) was reduced from 2.7 to 1.0 mM, amiodarone (30 $\mu$M) newly elicited arrhythmias in three cells. No arrhythmia occurred in these cells by amiodarone alone. Bretylium induced arrhythmias most frequently among the three

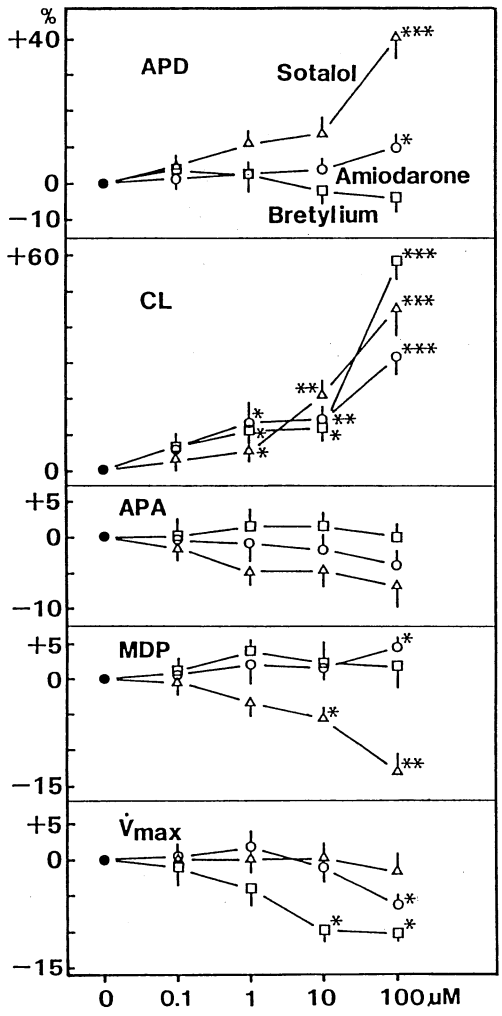


Fig. 2. Summary of changes in the action potential configuration in the class III antiarrhythmic drugs. APD: action potential duration. CL: cycle length. APA: action potential amplitude. MDP: maximum diastolic potential. $\dot{V}_{max}$: maximum rate of depolarization. Symbols are amiodarone (open circles), sotalol (triangles) and bretylium (squares). * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, with respect with control value.

drugs, and the incidence was in 8 out of 11 cells at 1 mM (Table 1). In two SA nodal cells, a sinus arrest occurred after washout of bretylium. The resting potential was approximately −60 mV. Sotalol at 100 μM elicited arrhythmias in only one of 10 cells. After about 30 min-washout of the drugs, regular rhythm was recovered in all the cells.

In voltage-clamp experiments, the class III drugs inhibited the $Ca^{2+}$ current ($I_{Ca}$), the delayed rectifier $K^+$ current ($I_K$), and the hyperpolarization-activated inward current ($I_f$)(Fig. 3). Current-voltage curves are given in Fig. 4. The percentage changes induced by the drugs are summarized in Table 2. Voltages of half-maximum activation and inactivation for $I_{Ca}$ were −37.3±2.5 mV (n=18) and −17.9±2.3 mV (n=17) in control, and these drugs did not affect

Table 1. Incidence of arrhythmia induced by class III antiarrhythmic drugs in spontaneously beating rabbit sino-atrial nodal cells

| | n | 100 nM | 1 μM | 10 μM | 30 μM | 100 μM | 1 mM |
|---|---|---|---|---|---|---|---|
| Amiodarone | 10 | — | 1 | 3 | 5 | — | — |
| Bretylium | 11 | 2 | 2 | 5 | — | 8 | 8 |
| Sotalol | 10 | — | — | 1 | | 1 | 1 |

n : Number of experiments.

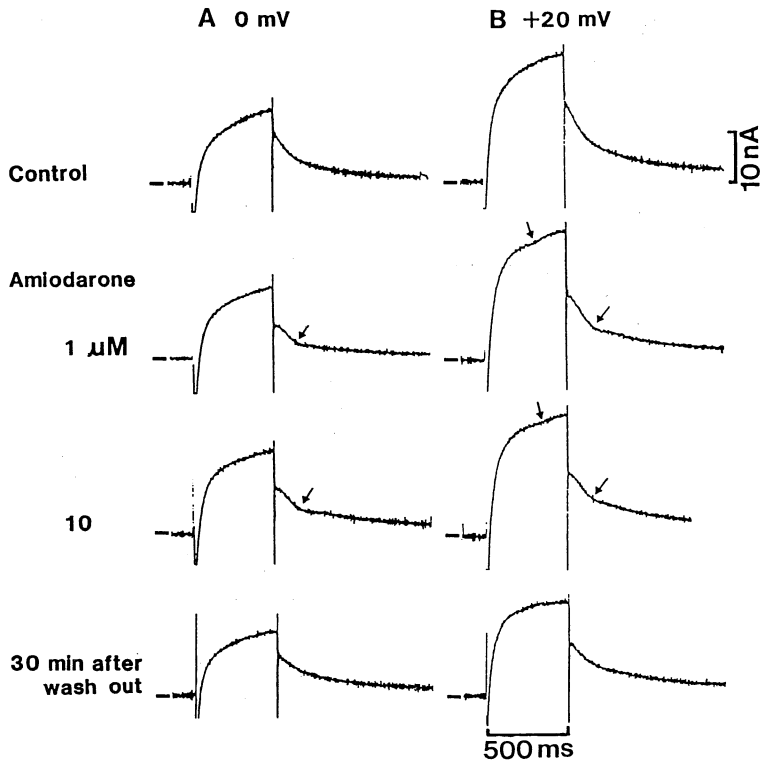


Fig. 3. Effects on the ionic currents and induction of transient inward current in the absence and presence of amiodarone. Test pulses were applied to 0 mV (panel A) and to +20 mV (panel B) from holding potential of −40 mV. Arrows indicate transient inward currents in the presence of amiodarone (1 and 10 μM). Short line at the left of the current recordings represents the zero current level.

them. In addition, voltage of half-maximum activation for $I_K$ was $-13.8\pm1.5$ mV (n=17) in control, and was unaffected in the presence of the drugs.

Simultaneously, a transient inward current ($I_{ti}$) occurred on the activating outward current as well as on the tail current recordings by depolarizing test pulses to +20 mV, as shown in Fig. 3. Holding potential was −40 mV. The amplitudes of $I_{ti}$ reached peak at +20 mV. The peak amplitudes were $4.5\pm1.1$ nA (n=5) at 1 $\mu$M, $5.7\pm0.7$ nA (n=5) at 10 $\mu$M, and $6.3\pm0.5$ nA (n=4) at 30 $\mu$M of amiodarone. At 30 min after washout, the $I_{ti}$ disappeared accompanied with the abolishment of arrhythmias.

Using a single SA nodal cell loaded with fura-2, the $[Ca]_i$ during exposure to class III antiarrhythmic drugs was measured. Since the shape of a single SA nodal cell was variable from rod to round, the localization of cytoplasmic $[Ca]_i$ would not be identified clearly. Amiodarone (30 $\mu$M) gradually increased $[Ca]_i$ level (Fig. 5). The absolute value of $[Ca]_i$ was $130\pm20$ nM (n=4). The $[Ca]_i$ elevation was concentration-dependent, and the maximum values (%) in the presence of the three drugs are summarized in Table 3. After washout of

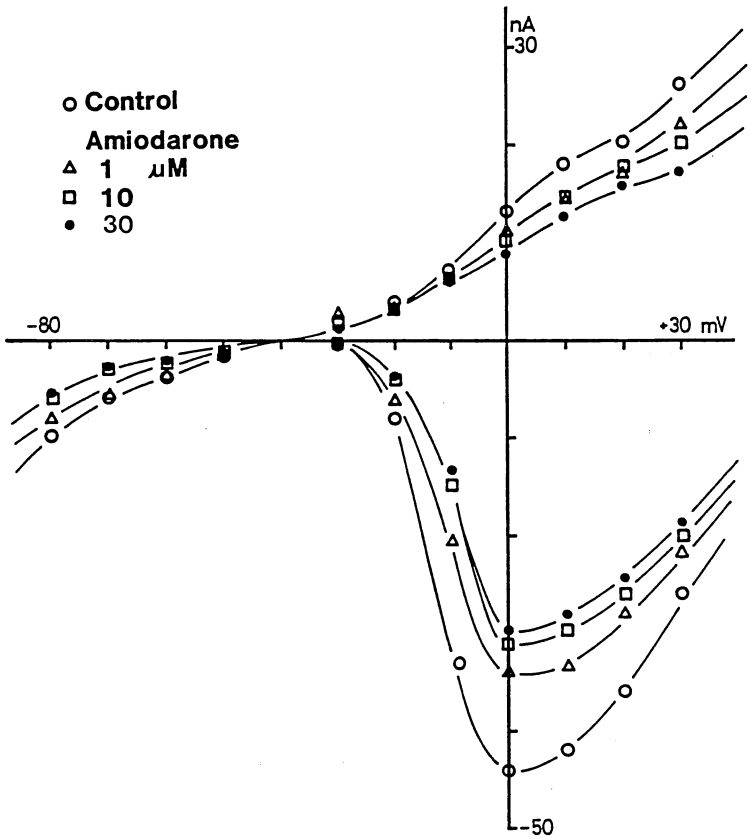


Fig. 4. Current-voltage relationship for the ionic currents in the presence of amiodarone. Amplitudes of transient inward current were plotted along voltage axis. Between applications of voltage-clamp, the SA node preparations showed dysrhythmia. Symbols are control (open circles), 1 $\mu$M (triangles), 10 $\mu$M (squares) and 30 $\mu$M (filled circles) of amiodarone.

Table 2. Inhibitions of ionic currents in rabbit sino-atrial nodal cells

| | n | $I_{Ca}$ | $I_K$ | $I_f$ |
|---|---|---|---|---|
| Amiodarone | | | | |
| 1 $\mu$M | 7 | 22.5±4.1 ** | 18.7±4.2 * | 2.7±2.5 |
| 10 $\mu$M | 8 | 27.3±3.2 ** | 26.3±3.1 ** | 25.0±2.9 *** |
| 30 $\mu$M | 8 | 33.2±4.8 ** | 33.6±5.5 *** | 43.7±3.7 |
| Bretylium | | | | |
| 100 nM | 5 | 10.2±3.7 | 8.7±2.4 | 0.5±0.1 |
| 300 nM | 5 | 16.1±2.9 ** | 21.7±3.1 ** | 2.9±2.6 |
| 1 $\mu$M | 5 | 20.9±5.2 ** | 20.3±4.1 ** | 5.8±4.8 |
| Sotalol | | | | |
| 10 $\mu$M | 5 | 3.8±4.4 | 46.6±4.2 ** | 70.3±5.1 *** |
| 100 $\mu$M | 5 | 6.8±3.8 | 49.9±5.1 ** | 73.4±5.6 *** |
| 1 mM | 5 | 9.8±3.6 | 53.7±3.5 *** | 80.1±5.3 *** |

Values (%) represent mean ± S. E. M. n : number of experiments. $I_{Ca}$ : $Ca^{2+}$ current at 0 mV. $I_K$ : delayed rectifier $K^+$ current at +30 mV. $I_f$ : hyperpolarization-activated inward current at −70 or −80 mV. * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, with respect to control value.

amiodarone and bretylium, further transient enhancement of $[Ca]_i$ (rebound) was observed and then, the $[Ca]_i$ was fallen down. In contrast, sotalol (even at 1 mM) did not induce any significant change in $[Ca]_i$ (by 8.6±4.1%).

## DISCUSSION

The present experiments in rabbit SA nodal cells showed the following: (1) Class III antiarrhythmic drugs had inhibitory actions on the spontaneous action potentials and ionic currents; (2) they had no effect on activation and inactivation kinetics for the ionic currents; (3) APD prolongation was produced by application of high concentrations; (4) amiodarone and bretylium induced arrhythmias; and (5) both drugs elevated the $[Ca]_i$ level. In general, class III antiarrhythmic drugs exert antiarrhythmic actions by APD prolongation, with no effect on $I_{Na}$ in cardiac muscle cells[1]. For mechanisms of the APD prolongation, some possibilities emerge: (1) decrease in $I_K$ current; (2) increases in the inward currents ($I_{Na}$ and $I_{Ca}$) and delay of their inactivation. In the SA nodal cells, therefore, the APD prolongation would not be due to the effect on inactivation but due to $I_K$ inhibition, since the SA nodal action potential is not dependent on $I_{Na}$.

The drugs possess other multiple actions on the cardiovascular system. Amiodarone depressed $I_{Na}$ and $I_{Ca}$ as well as $I_K$ in ventricular muscle[3),17),18)], consistent with the present results. In addition, amiodarone produces non-competitive inhibitions of $\alpha$- and $\beta$-adrenoceptors[19)], and is able to induce smooth muscle relaxation[20),21)]. On the other hand, bretylium was found to have an indirect action mediated by release of endogeneous catecholamines, and to cause a positive inotropic effect[22)]. Bretylium decreased $I_{Na}$ and $I_K$ in cultured embryonic chick heart cells[23),24)]. Sotalol competitively blocked $\beta$-adrenoceptors in cardiac and vascular smooth muscles[25)]. Both d- and l-sotalol inhibited $I_K$ to the same extent[26)].

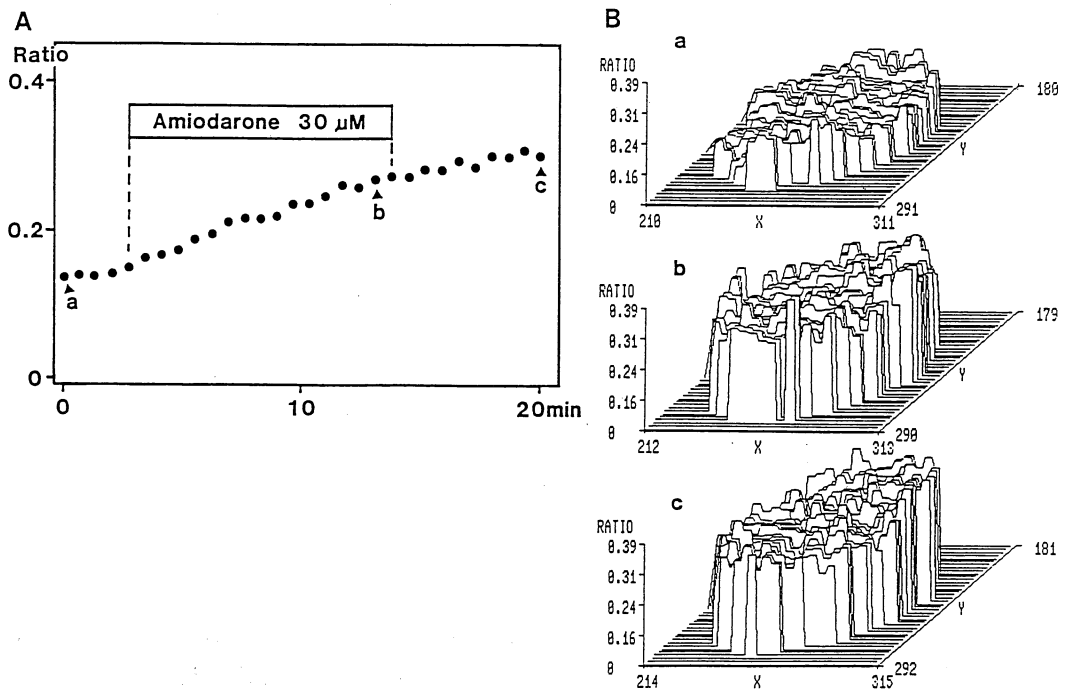


Fig. 5. Elevation of the cytosolic $Ca^{2+}$ concentration of a single SA node cell induced by amiodarone. Ratio of fluorescence intensities of the cytoplasm measured at 340− and at 380-nm excitations is indicated in a fluorescent fura-2 loaded single cell. A : Application of amiodarone (30 μM) increased cellular $Ca^{2+}$ level. B : Stereographs of cellular $Ca^{2+}$ level in a single SA node cell at various times during the experiments as indicated by triangles in A.

Table 3. Elevation of cellular $Ca^{2+}$ concentration in the presence of class III antiarrhythmic drugs

| | n | maximum | rebound |
|---|---|---|---|
| Amiodarone | | | |
| 1 μM | 6 | 7.6±3.3 | 1.2±0.7 |
| 10 μM | 5 | 95.4±10.0 *** | 11.5±6.3 |
| 30 μM | 6 | 101.8±8.3 *** | 18.6±5.1 ** |
| Bretylium | | | |
| 100 nM | 3 | 68.7±9.2 ** | 8.4±3.5 |
| 1 μM | 4 | 128.3±11.9 *** | 13.6±4.1 * |
| 10 μM | 5 | 487.6±12.0 *** | 16.5±6.2 * |
| Sotalol | | | |
| 1 mM | 5 | 8.6±4.1 | 1.6±0.8 |

Values (%) represent mean ± S. E. M. n : number of experiments. * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, with respect to control value.

## Elevation of $[Ca]_i$

Class III drug-induced APD prolongation may increase $[Ca]_i$. Amiloride (a $K^+$ sparing diuretic drug) also caused profound prolongation of APD in canine ventricular muscle, and simultaneously produced a great positive inotropic effect[5]. Class III drugs have been reported

to cause a positive inotropic effect[27-30]. They suggest that the mechanism for the positive inotropic effect would be due to elevation of $[Ca]_i$, presumably resulting from the APD prolongation, and the prolongation was induced by delayed inactivation of $Na^+$ inward current. However, recent studies have revealed that the class III drugs also inhibited the $I_{Na}$ current, as well as $I_K$[17),18),23),31]. Therefore, it seems unlikely that the positive inotropy might be due to the modulation of $I_{Na}$.

The present experiments actually showed that amiodarone and bretylium elevated $[Ca]_i$ level. Amiodarone did not release $Ca^{2+}$ from intracellular storage sites, but increased intrasynaptosomal free $Ca^{2+}$, resulting in induction of neurotoxicity[32]. The class III drugs did not increase $I_{Ca}$, and prolonged the APD just at high concentrations. Therefore, the drugs would elevate $[Ca]_i$ due not only to the APD prolongation but also to other indirect actions. Less marked APD prolongation might result from stimulation of $Ca^{2+}$-activated $K^+$ current.

**Occurrence of arrhythmias**

Delayed repolarization certainly causes prolongation of ERP[33], but a large APD prolongation may induce arrhythmias. The arrhythmogenicity of class III antiarrhythmic drugs has already been shown in in vivo and in vitro experments, which is a torsades de pointes type ventricular tachycardia[6-8),34]. Disparity of repolarization may predispose to reentrant arrhythmias[4]. It seems that the delayed repolarization might not always behave in a uniform manner and that heterogeneity of refractory periods might be elicited in this situation.

The excess elevation of $[Ca]_i$ level may elicit the triggered activity, and produce arrhythmias. In the arrhythmia-occurring cells, amiodarone elicits $I_{ti}$, which induces the delayed afterdepolarization (DAD) on the action potentials. In general, $I_{ti}$ is exhibited in calcium overloaded cardiac muscle under the conditions of hypoxia, ischemia, and cardiac failure. The $I_{ti}$ (or DAD) is caused by change in permeability of background or leak channels to $Na^+$ or $K^+$, or to both ions, resulting in occurrence of arrhythmias[10),15),35-37]. In the present experiments using fura-2, amiodarone elevated $[Ca]_i$ level. After washout, a sinus arrest was elicited in arrhythmia-occurring SA nodal cells. The induction of sinus arrest was due to further elevation of $[Ca]_i$ during washout. In calcium overloaded cells, $[Ca]_i$ level during washout is enhanced (but transiently), and simultaneously a transient positive inotropic effect is produced[38),39].

Bretylium exerts indirect actions mediated by the release of endogenous catecholamines from nerve endings[22]. It may also elicit arrhythmias, not only due to APD prolongation but also due to elevation of $[Ca]_i$ via $\alpha$-and $\beta$-adrenoceptor stimulations[9]. Recent studies have demonstrated that one mechanism of ventricular tachycardia may be a triggered activity by the early afterdepolarization (EAD)[40-42]. The EAD might probably be produced by decrease in $K^+$ conductance (which may lead to torsades de pointes), and by an inward tail current ($I_{ex}$) during calcium overloading cardiac muscles[10),16]. On switch to low $[K]_o$ solution in the presence of amiodarone, new arrhythmias occurred. This may result from EAD. Hypokalemia may produce instability of the membrane potential[43]. Amiodarone and bretylium hyperpolarized the maximum diastolic potential, suggesting that these drugs might in part have a tendency to produce instability of the membrane potential. Furthermore, it has been known that bretylium possesses the ability to interact directly with the parasympathetic system, and exerts anti-

cholinergic activity in guinea-pig atrium[44]. In the SA nodal cells, however, bretylium did not enhance the pacemaker activity, but prolonged the cycle length.

Sotalol, a $\beta$-adrenergic blocking drug, did not elicit arrhythmia but in only one of 10 cells. The blocking actions could decrease $[Ca]_i$ level, and prevent occurrence of the arrhythmias induced by calcium overload. Thus, sotalol would have a very low incidence of arrhythmia in rabbit SA node preparations.

## CONCLUSION

In the SA node, arrhythmias occurred in the presence of the class III drugs. The drugs caused the APD prolongation, which may elevate $[Ca]_i$, at high concentrations, but not at low concentrations. Amiodarone and bretylium actually elevated $[Ca]_i$. Amiodarone also elicited $I_{ti}$. Since the triggered activity is produced under cellular calcium overload, the class III drugs in rabbit SA nodal cells would elicit arrhythmias mainly due to development of cellular calcium overload, which might result from not only the APD prolongation but also other indirect actions. Under conditions of cardiac failure, the positive inotropic effect induced by class III antiarrhythmic drugs may be a beneficial action. But since these drugs can elevate $[Ca]_i$, administration may develop the cellular calcium overload, and elicit some arrhythmias (i. e. tachycardiac fibrillation).

## REFERENCES

1) **Singh, B. N.** and **Vaughan Williams, E. M.**: A third class of antiarrhythmic action. Effects on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ 1999 and AH 3474. Br. J. Pharmacol. **39**: 675-687, 1970.

2) **Aomine, M.**: Inhibition of tetrodotoxin-sensitive plateau sodium current by amiodarone in guinea pig cardiac muscles. Gen. Pharmacol. **20**: 653-658, 1989.

3) **Satoh, H.**: Class III antiarrhythmic drugs (amiodarone, bretylium and sotalol) on action potentials and membrane currents in rabbit sino-atrial node preparations. Naunyn-Schmiedeberg's Arch. Pharmacol. **344**: 674-681, 1991.

4) **Bexton, R. S.** and **Camm, A. J.**: Drugs with a class III antiarrhythmic action. Pharmacol. Ther. **17**: 315-335, 1982.

5) **Satoh, H.** and **Hashimoto, K.**: An electrophysiological study of amiloride on sino-atrial node cells and ventricular muscle of rabbit and dog. Naunyn-Schmiedeberg's Arch.Pharmacol. **333**: 83-90, 1986.

6) **McComb, J. M.**, **Logan, K. R.**, **Khan, M. M.**, **Geddes, J. S.** and **Adgey, A. A. J.**: Amiodarone-induced ventricular fibrillation. Eur. J. Cardiol. **11**: 381-385, 1980.

7) **Sclarovsky, S.**, **Lewin, R. F.**, **Kracoff, O.**, **Stransberg, B.**, **Arditti, A.** and **Agmon, J.**: Amiodarone-induced polymorphous ventricular tachycardia. Am. Heart J. **105**: 6-12, 1983.

8) **Santinelli, V.**, **Chiariello, M.**, **Santinelli, C.** and **Condorelli, M.**: Ventricular tachyarrhythmias complicating amiodarone therapy in the presence of hypokalemia. Am. J. Cardiol. **53**: 1462-1463, 1984.

9) **Gough, W. B.** and **El-Sherif, N.**: The differential response of normal and ischemic Purkinje fibres to clofilium, sotalol and bretylium. Cardiovasc. Res. **23**: 554-559, 1989.

10) **Satoh, H.**, **Tsuchida, K.** and **Hashimoto, K.**: Electrophysiological actions of A 23187 and X-537 A in spontaneously beating and in voltage-clamped rabbit sino-atrial node preparations. Naunyn-Schmiedeber-

g's Arch. Pharmacol. **339**: 320-326, 1989.

11) **Satoh, H.**: Caffeine depression in spontaneous activity in rabbit sino-atrial node cells. Gen. Pharmacol. **24**: 555-563, 1983.

12) **Satoh, H.**: Modulation of the automaticity by histamine and cimetidine in rabbit sino-atrial node cells. Gen. Pharmacol. **24**: 1213-1222, 1993.

13) **Noma, A.** and **Irisawa, H.**: A time-and voltage-dependent potassium current in the rabbit sinoatrial node cell. Pflügers Arch. **366**: 251-258, 1976.

14) **McDonald, T. F.** and **Trautwein, W.**: Membrane currents in cat myocardium: separation of inward and outward components. J. Physiol. (Lond.) **274**: 193-216, 1978.

15) **Hasegawa, J., Satoh, H.** and **Vassalle, M.**: Induction of the oscillatory current by low concentrations of caffeine in sheep cardiac Purkinje fibres. Naunyn-Schmiedeberg's Arch. Pharmacol. **335**: 310-320, 1987.

16) **Satoh, H., Hasegawa, J.** and **Vassalle, M.**: On the characteristics of the inward tail current induced by calcium overload. J. Mol. Cell. Cardiol. **21**: 5-20, 1989.

17) **Kohlhardt, M.** and **Fichtner, H.**: Block of single cardiac $Na^+$ channels by antiarrhythmic drugs: the effect of amiodarone, propafenone and diprafenone. J. Membr. Biol. **102**: 105-119, 1988.

18) **Follmer, C. H., Aomine, M., Yeh, J. Z.** and **Singer, D. H.**: Amiodarone-induced block of sodium current in isolated cardiac cells. J. Pharmacol. Exp. Ther. **243**: 187-194, 1987.

19) **Charlier, R.**: Cardiac actions in the dog of a new antagonist of adrenergic excitation which does not produce competitive blockade of adrenoceptors. Br. J. Pharmacol. **39**: 668-674, 1970.

20) **Remme, W. J., Van Hoogenhuyze, D. C. A., Krauss, X. H., Hoffman, A., Kruyssen, D. A. C. M.** and **Storm, C. J.**: Acute hemodynamic and antiischemic effects of intravenous amiodarone. Am. J. Cardiol. **55**: 639-644, 1985.

21) **Schwartz, A., Shen, E., Morady, F., Gillespie, K., Scheiman, K.** and **Chatterjee, K.**: Hemodynamic effects of intravenous amiodarone in patients with depressed left ventricular function and recurrent ventricular tachycardia. Am. Heart J. **106**: 848-855, 1983.

22) **Markis, J. E.** and **Koch-Weser, J.**: Characteristics and mechanism of inotropic and chronotropic actions of bretylium tosylate. J. Pharmacol. Exp. Ther. **178**: 94-102, 1971.

23) **Bkaily, G., Payet, M. D., Benabderrazik, M., Sauve, R., Renaud, J-F., Bacaner, M.** and **Sperelakis, N.**: Intracellular bretylium blocks $Na^+$ and $K^+$ currents in heart cell. Eur. J. Pharmacol. **151**: 389-397, 1988.

24) **Bacaner, M. B., Clay, J.R., Shrier, A.** and **Brochu, R. M.**: Potassium channel blockade: a mechanism for suppressing ventricular fibrillation. Proc. Natl. Acad. Sci. USA **83**: 2223-2227, 1986.

25) **Bergamaschi, M., Shanks, R. G., Caravaggi, A. M.** and **Mandelli, V.**: A comparison of the cardiovascular actions of four adrenergic $\beta$-receptor blocking agents in resting conscious dogs. Am. Heart J. **82**: 338-351, 1971.

26) **Carmeliet, E.**: Electrophysiologic and voltage clamp analysis of the effects of sotalol on isolated cardiac muscle and Purkinje fibres. J. Pharmacol. Exp. Ther. **232**: 817-827, 1985.

27) **Millar, J. S.** and **Vaughan Williams, E. M.**: Differential actions on rabbit nodal, atrial Purkinje cell and ventricular potentials of melperone, a bradycardic agent delaying repolarization: effect of hypoxia. Br. J. Pharmacol. **75**: 109-122, 1982.

28) **Amsterdam, E. A., Spann, J. F., Mason, D. T.** and **Zelis, R. F.**: Characterization of the positive inotropic effects of bretylium tosylate: a unique property of an antiarrhythmic agent. Am. J. Cardiol. **25**: 81, 1970.

29) **Parmley, W. W.**: Inotropic and antiarrhythmic effects of sotalol. *in* Advances in Beta-Adrenergic blocking Therapy (Suart, A. G., ed.). Vol. 3, Sotalol. Excerpta Medica, p11-19, 1975.

30) **Platou, E. S., Refsum, H.** and **Hotvedt. R.**: Class III antiarrhythmic action linked with positive inotropy: Antiarrhythmic, electrophysiological, and hemodynamic effects of the sea-anemone polypeptide ATX II in

the dog heart in situ. J. Cardiovasc. Pharmacol. 8 : 459-465, 1986.

31) **Culling, W., Penny, W. J.** and **Sheridan, D. J.** : Effects of sotalol on arrhythmias and electrophysiology during myocardial ischemia and reperfusion. Cardiovasc. Res. **18** : 397-404, 1984.

32) **Kodavanti, P. R. S., Pentyala, S. N., Yallapragada, P. R.** and **Desaiah, D.** : Amiodarone and desethylamiodarone increase intrasynaptosomal free calcium through receptor mediated channel. Naunyn-Schmiedeberg's Arch. Pharmacol. **345** : 213-221, 1992.

33) **Bennet, D. H.** : Acute prolongation of myocardial refractoriness by sotalol. Br. Heart J. **4** : 521-526, 1982.

34) **Moro, C., Romero, M.** and **Peiretti, M. A. C.** : Amiodarone and hypokalemia. A dangerous combination. Int. J. Cardiol. **13** : 365-368, 1986.

35) **Kass, R. S., Lederer, W. J., Tsien, R. W.** and **Weingart, R.** : Role of calcium ions in transient inward current and aftercontractions induced by strophanthidines in cardiac Purkinje fibers. J. Physiol. (Lond.) **281** : 187-208, 1978.

36) **Eisner, D. A.** and **Lederer, W. J.** : Inotropic and arrhythmogenic effects of potassium-depleted solutions on mammalian cardiac muscle. J. Physiol. (Lond.) **294** : 255-277, 1979.

37) **Lederer, W. J.** and **Tsien, R.** : Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. J. Physiol. (Lond.) **263** : 73-100, 1976.

38) **Satoh, H.** and **Vassalle, M.** : Reversal of caffeine-induced calcium overload in cardiac Purkinje fibres. J. Pharmacol. Exp. Ther. **234** : 172-179, 1985.

39) **Satoh, H.** and **Vassalle, M.** : Role of calcium in caffeine-norepinephrine interactions in cardiac Purkinje fibres. Am. J. Physiol. **257** : H 226-H 237, 1989.

40) **Roden, D. M.** and **Hoffman, B. F.** : Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibres. Circ. Res. **56** : 857-867, 1985.

41) **Damiano, B. P.** and **Rosen, M. R.** : Effects of pacing on triggered activity induced by early afterdepolarizations. Circulation **69** : 1013-1025, 1984.

42) **El-Sherif, N., Zeiler, R. H., Craelius, W., Gough, W. B.** and **Henkin, R.** : QTU prolongation and polymorphic ventricular tachyarrhythmias due to bradycardia-dependent early afterdepolarizations. Afterdepolarizations and ventricular arrhythmias. Circ. Res. **63** : 286-305, 1988.

43) **Satoh, H.** : Arrhythmogenic actions of class III antiarrhythmic drugs in spontaneously beating sino-atrial node preparations. Gen. Pharmacol. **24** : 1435-1442, 1993.

44) **Schreiber, G., Friedman, M.** and **Sokolovsky, M.** : Interaction of bretylium tosylate with rat cardiac muscarinic receptors. Possible pharmacological relevance to antiarrhythmic action. Circ. Res. **55** : 653-659, 1984.