

Review

Cellular electropharmacology of amiodarone

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1. Introduction

Antiarrhythmic strategies have changed dramatically during the last decade. The Cardiac Arrhythmia Suppression Trial (CAST) reported the harmful effects of flecainide, encainide and moricizine in patients with ventricular premature beats who had had myocardial infarction [162,163]. Compared with placebo, these three drugs were associated with increased mortality from cardiac death. Numerous meta-analytic studies also provided evidence that drugs that act fundamentally by blocking cardiac sodium channels (Class I agents in Vaughan Williams' classification [171]) may, in general, have the potential to increase mortality in patients with significant structural heart disease even though they may suppress cardiac arrhythmias [2,31,49,99,161,187]. The use of Class I drugs in subsets of patients at risk for sudden death is therefore declining. Along with this trend, the therapeutic role of drugs that have antiarrhythmic actions mainly through a prolongation of action potential duration (APD) and refractoriness (Class III agents) have attracted renewed interest [29,56,86,135,148–151]. Class III agents include relatively old compounds having complex electropharmacological profiles (e.g., bretylium, sotalol and especially amiodarone) and newer compounds that exhibit the sole propensity to lengthen APD and the refractory period (i.e., sotalol, dofetilide, *d*-sotalol, ibutilide, ambasilide, risotilide, almokalant, MK-499, E-4031 and MS-551) [86,147–149].

It was shown in large-scale clinical studies that sotalol is superior to Class I agents in preventing and terminating life-threatening ventricular tachyarrhythmias (ventricular tachycardia and fibrillation, VT/VF) [93,94]. The advan-

tage of sotalol might have been mainly due to the drug's action to prolong APD through the entire heart. This hypothesis has led to research to develop a new series of pure compounds devoid of other associated properties. *d*-Sotalol is one of these new and pure Class III agents, in which the beta-blocking action of the racemic form of sotalol is minimized in order to eliminate its possible undesirable effects on hemodynamics. The long-term clinical effect of *d*-sotalol has been tested in a large-scale double-blind, placebo-controlled study (Survival with Oral *d*-Sotalol; SWORD) in post-infarct patients at risk for high mortality [174]. The trial was initially planned to enrol 6400 patients, but was stopped prematurely when 3119 patients had been enrolled because of the significantly higher total mortality in the drug-treated patients than in the placebo group. The adverse effect of *d*-sotalol on mortality could be due to proarrhythmic reaction, and most other pure Class III compounds might have a similar deleterious effect. These possibilities remain to be tested or confirmed. The outcome of SWORD, however, had a great impact on the development of new antiarrhythmic drugs. A large number of pure Class III compounds under investigation have been discontinued from clinical development [151]. Emphasis is therefore shifting to the compounds with a multifaced pharmacological profile [83,128,151] (with multiple molecular targets in the framework of the Sicilian Gambit [96,160]) or to drugs with more specific targets based on advances in the understanding of ion channels and their molecular physiology and pharmacology.

Many investigators now believe that amiodarone is the most promising drug in the treatment of life-threatening

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supraventricular and ventricular tachyarrhythmias [4,83,103,128,151]. Amiodarone was synthesized in Belgium (Labaz Inc.) originally as an antianginal agent in 1962 during a systematic search for potent coronary vasodilators [143]. The antiarrhythmic effects of amiodarone in experimental animals appeared in 1969 [27]. A great deal of experimental and clinical studies carried out during the subsequent period in the 1970s and 1980s confirmed the extremely potent antiarrhythmic activity of amiodarone, and it has been used to treat patients with a wide variety of tachyarrhythmias, mainly in European countries and in South America [42,52,92,109,143,145,146]. In the United States, clinical use of amiodarone had long been more limited because of its significant extracardiac toxicity involving the liver, lungs, and thyroid. The use of amiodarone was approved in 1985 by the Food and Drug Administration as a 'last resort' drug only for life-threatening ventricular arrhythmias refractory to other agents [92]. It was also approved in Japan in 1992 with a similar indication [68]. The advantage of amiodarone over other antiarrhythmic drugs has been demonstrated in many clinical trials in the 1990s in the treatment of atrial fibrillation and flutter, the treatment of non-sustained ventricular tachycardia in patients with cardiomyopathy and congestive heart failure, the treatment of patients who have recently had a myocardial infarction, and the prevention of recurrent sustained ventricular tachycardia and ventricular fibrillation [4,128,151]. Unlike other antiarrhythmic agents, amiodarone has not been shown to increase mortality in any population studied [128].

Amiodarone has long been referred to as a prototype of Class III antiarrhythmic agents because it was demonstrated in early experimental studies in 1970s that this compound prolongs both APD and the refractory period of cardiac muscle when administered chronically [52,143,188]. Many studies during the past two decades have shown that the pharmacological action of amiodarone is very complex. For instance, it possesses an inhibitory effect on the fast sodium channel as well as on the slow calcium channel [92,109]. Amiodarone also has non-competitive antisympathetic effects, and modulates thyroid function and phospholipid metabolism [71,145,147]. Which action or combination of actions is fundamental and salutary for its potent antiarrhythmic activity is not known.

One reason the pharmacological profile of amiodarone remains so mysterious is the difference in its acute and chronic effects [63,73,145,146]. Clinical electrophysiological studies on amiodarone have revealed that its main acute effect is suppression of conductivity of the atrioventricular (AV) node with minimal effect on the effective refractory periods of the atrial muscle, ventricular muscle, and the bypass tract or the His-Purkinje tissue when the drug is administered intravenously [51,58,98,177]. There is no significant effect on the corrected QT intervals (QTc) in ECGs. QRS duration is prolonged only at fast stimulus frequencies [98]. In contrast, when administered orally for

more than several weeks, amiodarone predictably lengthens repolarization (QTc) and refractoriness in most cardiac tissues with little or no change in QRS duration and a modest increase in atrio-His bundle (AH) or His bundle-ventricular (HV) intervals [38,102,145,177]. There is now substantial evidence that such a difference, also noted in animals, is not explicable in terms of the pharmacokinetics (serum and myocardial tissue concentrations of amiodarone and its active metabolite) [145,146].

In the present article, we review the current knowledge on the electrophysiological effects of amiodarone on the heart based on acute and chronic experimental studies in single channel, single cell, multicellular tissue preparation and whole heart levels, and discuss the molecular and cellular modes of action of this unique antiarrhythmic agent.

2. Acute effects of amiodarone

Amiodarone is a highly lipophilic drug (log P = 5.95 and pK_a = 8.7 at 37°C [28]) and is almost insoluble in water or aqueous buffer solution. In experiments to assess the acute effects of amiodarone, this compound is usually dissolved in crystalloid solutions using several different types of vehicles (Table 1). In previous reports, amiodarone was often dissolved first in 50–100% ethanol, and then added to physiological salt solutions containing plasma or serum albumin (0.1–1%) [57,58,63,73,120,127,182,183]. Vehicles specifically prepared for intravenous injection in clinical use (Polysorbate 80 or Tween 80) were also employed [6,7,11,39,89,113,119,130]. At higher drug concentrations, however, amiodarone does not completely dissolve, and suspension or precipitation occurs. In some studies in which amiodarone was dissolved directly in crystalloid solutions [46,62,111,112], this might have posed a more serious problem, and the concentrations presented in those articles must have been much higher than those at the site of drug action. The vehicles, above a certain concentration, are known to cause their intrinsic effects on the electrophysiological properties of cardiac tissues [164]. Amiodarone penetrates deeply into the lipid matrix of the membrane, and is released from cardiac tissues very slowly when washed out. Consequently, acute effects of amiodarone cannot be reversed during the experiments [131]. These limitations often make it difficult to interpret and compare data obtained in experiments using acute perfusion or superfusion with this drug.

2.1. Action potentials

In cardiac cells or tissues whose excitation depends on activation of fast sodium channels, the most consistent change of action potential configuration elicited by acute application of amiodarone is a decrease of the maximum upstroke velocity (V_{max}). Mason et al. [90,91] demon-

Table 1
Acute effects of amiodarone on action potential duration (APD)

| Changes in APD | Tissue | Animal species | Amiodarone concentration | Diluent | References |
|----------------|-------------------------------|------------------|--------------------------|--------------------|------------------------|
| Increase | atrial muscle | frog | 20–200 μM | distilled water | Nélat et al. [111] |
| | atrial muscle | rat | 5.9–29 μM | Polysorbate 80 | Northover et al. [119] |
| | ventricular muscle | guinea pig | 44–88 μM | albumin | Mason et al. [91] |
| | ventricular muscle | guinea pig | 5–10 μM | ethanol + albumin | Pallandi et al. [120] |
| | ventricular muscle | guinea pig | 50 μM | Polysorbate 80 | Maruyama et al. [89] |
| | ventricular muscle | ferret | 20 μM | distilled water | Nélat et al. [111] |
| | ventricular muscle | dog | 1–50 μM | ethanol + serum | Yabek et al. [182,183] |
| | ventricular muscle | dog | 59 μM | Polysorbate 80 | Quinteiro et al. [130] |
| | Purkinje fiber | sheep | 20–200 μM | distilled water | Nélat et al. [111] |
| Decrease | atrial muscle | rabbit | 14.7 μM | distilled water | Kadoya et al. [62] |
| | ventricular muscle | guinea pig | 44–1100 μM | Polysorbate 80 | Aomine et al. [6,7] |
| | ventricular muscle | rabbit | 10 μM | ethanol + albumin | Kodama et al. [73] |
| | ventricular cell ^a | rabbit | 1–10 μM | ethanol + albumin | Kamiya et al. [63] |
| | Purkinje fiber | guinea pig | 44–110 μM | Polysorbate 80 | Aomine et al. [6] |
| | Purkinje fiber | dog | 10–50 μM | ethanol + serum | Yabek et al. [182,183] |
| | Purkinje fiber | dog | 7.3–73 μM | albumin | Varró et al. [169] |
| | Purkinje fiber | dog | 50 μM | albumin | Gallagher et al. [41] |
| | Purkinje fiber | dog | 1.45 μM | blood ^b | Gallagher et al. [41] |
| No effect | SA node | rabbit | 5 μM | ethanol + plasma | Ikeda et al. [58] |
| | AV node | rabbit | 5 μM | ethanol + plasma | Ikeda et al. [58] |
| | atrial muscle | rabbit | 5 μM | ethanol + plasma | Ikeda et al. [58] |
| | atrial muscle | dog | 1–30 μM | ethanol + serum | Yabek et al. [183] |
| | ventricular muscle | guinea pig | 29 μM | albumin | Varró et al. [169] |
| | ventricular muscle | rabbit | 14.7 μM | distilled water | Kadoya et al. [62] |
| | ventricular muscle | dog ^c | 1–50 μM | ethanol + serum | Yabek et al. [182] |

^a Single myocytes isolated enzymatically from ventricle. ^b Blood supplied by cross-perfusion from donor dogs. ^c Newborn dogs.

strated that relatively high concentrations of amiodarone (44–88 μM) depressed V_{max} of guinea pig papillary muscle without affecting the resting membrane potential, and that this V_{max} inhibition was enhanced in a frequency- or use-dependent manner like Class I antiarrhythmic drugs. Similar V_{max} inhibition at high stimulation frequencies with amiodarone (5–50 μM) was reported by other investigators in ventricular muscles as well as in Purkinje fibers isolated from dog, rabbit and guinea pig hearts [6,41,89,120,130,168,182]. Use-dependent V_{max} inhibition by acute amiodarone (1–10 μM) was also shown in single ventricular myocytes isolated from guinea pig and rabbit hearts [57,63].

Honjo et al. [57] investigated the voltage- and time-dependence of V_{max} inhibition by amiodarone in single guinea pig ventricular cells using single pipette whole-cell current-clamp and voltage-clamp techniques. In myocytes treated with 1 μM amiodarone, V_{max} of reference action potential elicited at 0.03 Hz from a holding potential at -82 mV was decreased by 11%, indicating minimal tonic (or resting) block of sodium channel. Application of a single conditioning depolarization to those myocytes resulted in a significant decrease in V_{max} of test action potential. The V_{max} reduction from the reference level was minimal at the shortest (10 ms) depolarization, whereas it was enhanced in a single exponential function as the clamp pulse duration was prolonged. The more positive depolar-

ization was associated with the greater V_{max} inhibition within the range of -70 to -10 mV (Fig. 1). These characteristics of V_{max} inhibition are qualitatively similar

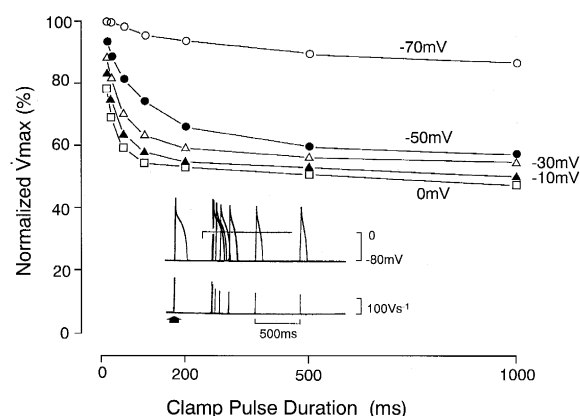


Fig. 1. Influence of conditioning depolarization on V_{max} inhibition by acute amiodarone. Inset shows superimposed action potentials (upper traces) and their differentiated upstroke spikes (lower traces). Action potentials were elicited without clamp pulse as a reference level (left end) or 100 ms after a conditioning clamp to -70 – 0 mV for 10–1000 ms. Each conditioning depolarization was preceded by a 30 s rest period. The records were obtained 20 min after application of amiodarone (1 μM). In the graph, V_{max} of test action potential normalized to the reference level was plotted against the conditioning clamp pulse duration. The time constant (τ_R) for the exponential decay of V_{max} at 0 mV conditioning depolarization was 29 ms. From Ref. [57], with permission.

to lidocaine [72], and consistent with those presented by Mason et al. [91] in guinea pig papillary muscle experiments, where the membrane potential was controlled by the single sucrose gap technique. Amiodarone may therefore block the sodium channel primarily when it is in the inactivated state.

Onset and offset kinetics of the use-dependent V_{\max} inhibition with acute amiodarone are relatively rapid. As to the recovery time constant of V_{\max} from the use-dependent block, Mason et al. [91] and Pallandi and Campbell [120] reported the values of 1.48–1.63 s in guinea pig papillary muscles. These values fall between those reported for the fast kinetic Class I drugs such as lidocaine and mexiletine (200–500 ms) and intermediate kinetic Class I drugs, including quinidine and procainamide (2–12 s) [21]. Varró et al. [168] demonstrated much shorter values in canine Purkinje fibers (289 ms). Our data obtained in experiments using rabbit papillary muscle (452 ms) [73] and in guinea pig single ventricular cells (418 ms) [57] are close to the latter reports, and still comparable to other fast kinetic Class I drugs.

Reports on the effects of acute amiodarone on APD have been very conflicting (Table 1). In atrial muscle, Northover [119] observed an appreciable prolongation of APD at 90% repolarization (APD_{90}) associated with a comparable prolongation of the effective refractory period (ERP). Kadoya et al. [62], however, showed a significant shortening of APD_{50} , whereas Ikeda et al. [58] and Yabek et al. [183] observed no significant changes in APD.

In ventricular muscle, a moderate prolongation of APD (10–23%) with amiodarone (5–58 μM) was reported in several studies using guinea pigs, ferrets and dogs (Table 1). In our experiments in rabbit papillary muscle, however, 10 μM amiodarone caused a significant 12–26% shortening of APD (at -70 mV level) [73]. We also observed a significant shortening of APD in single rabbit ventricular cells [63]. Kadoya et al. [62] observed no significant changes in APD and ERP in ventricular muscle from rabbits. In Purkinje fibers, most of the previous studies using dogs showed a significant shortening of APD (and ERP) with acute amiodarone (Table 1). Gallagher et al. [40] also reported a shortening of APD of dog Purkinje fibers when they were superfused with blood from other dogs treated with oral amiodarone. Néliat et al. [111], however, reported a concentration-dependent prolongation of APD_{90} in sheep Purkinje fibers by 30–60%.

For the acute effects of amiodarone on cardiac tissues whose excitation depends on activation of slow calcium channels, only limited information is available. In sinoatrial (SA) node preparations of isolated rabbit atria, amiodarone (15–50 μM) was shown to cause significant decreases in the action potential amplitude, the slope of phase 4 depolarization (pacemaker potential) and the rate of spontaneous excitation [46,62,183]. Nattel et al. [107] demonstrated frequency-dependent inhibition of slow action potentials elicited in dog Purkinje fibers exposed to

high extracellular potassium (25 mM) and isoproterenol. In their study, 5 μM amiodarone caused a significant decrease in V_{\max} when the preparations were stimulated at 1.4–2.0 Hz; the higher the stimulation-frequency, the greater the V_{\max} inhibition. In the presence of 5 μM amiodarone, V_{\max} recovery of the slow action potential showed a much slower recovery (with a time constant of 940 ms) than that before the drug application (time constant of 74 ms) [107]. Slow action potentials in isolated guinea pig ventricular muscles exposed to high extracellular potassium (15–24 mM) and isoproterenol were inhibited by acute amiodarone as well [7,89]. Acute application of amiodarone (50–88 μM) was also found to suppress the depolarization-induced spontaneous action potentials (abnormal automaticity) in ventricular muscles [91] and in Purkinje fibers [157].

Based on these experimental studies in vitro, it seems reasonable to describe the major and consistent acute effects of amiodarone as an inhibition of depolarization of action potentials which depends on activation of either fast sodium channels or slow calcium channels, and the inhibition is enhanced in a use- and frequency-dependent manner. On the other hand, acute amiodarone has no consistent effects on the repolarization phase of action potentials. Such characteristics correlated well with the acute effects of amiodarone on conductivity and refractoriness in whole hearts perfused in vitro or in vivo. In isolated hearts from rabbits and guinea pigs, acute perfusion with amiodarone (1–10 μM) resulted in a depression of AV nodal conduction but failed to prolong ventricular repolarization and refractoriness [122,131,153,154].

Intravenous application of amiodarone (1.25–25 mg/kg) to anesthetized dogs resulted in a decrease in sinus rate, a prolongation of effective and functional refractory periods of the AV node, and a frequency-dependent conduction delay in the AV node and in the ventricle (greater inhibition of AV nodal conduction than intraventricular conduction). The QT interval was unaffected by acute amiodarone, and a prolongation of ERP in ventricular muscle was minimal or negligible [30,60,107,158]. Nattel et al. [107] quantified the frequency-dependence of drug effects by studying the response of AV conduction in dogs to changes in coupling intervals. Under control conditions, premature atrial stimulation increased the AV conduction time with a time constant of 70 ms. In the presence of amiodarone, a biexponential relationship between AV conduction time and coupling interval was observed—one component with a time constant similar to control, and the slower component with a time constant of about 1 s. Nanas and Mason [105] demonstrated in anesthetized open-chest dogs that intracoronary amiodarone administration resulted in a rate-dependent decrease in the conduction velocity in the ventricular myocardium with no significant changes in the repolarization intervals of epicardial unipolar electrograms. Rate-dependent intraventricular conduction delay after acute amiodarone application (10 mg/kg, i.v.) was

also shown in human patients under pacing of the right ventricle [97].

2.2. Ionic currents

Acute effects of amiodarone on cardiac ionic currents were first examined by Néliat et al. [112] in frog atrial and ferret ventricular muscles using double sucrose-gap techniques. They showed that amiodarone (20–200 μM) caused a concentration-dependent decrease in the delayed outward current (i_{out}), and that at high concentrations ($> 200 \mu\text{M}$) the fast sodium inward current (i_{Na}) and the slow inward current (i_{si}) were also depressed in association with a significant delay in their recovery kinetics.

Follmer et al. [39] investigated the mode of sodium channel block with amiodarone by measuring i_{Na} of single canine Purkinje cells and cat ventricular myocytes using the suction pipette voltage-clamp technique. They demonstrated that amiodarone (0.1–7.3 μM) produced a substantial tonic block and a marked use-dependent block of i_{Na} . The tonic block was enhanced at less negative holding potential, consistent with a shift in the steady-state availability curve to more negative potential (–16 mV). The use-dependent block was enhanced in conjunction with increase in rate (0.5–5.0 Hz) and duration (2–200 ms) of depolarizing pulses. The recovery of i_{Na} from inactivation was significantly delayed in the presence of amiodarone. They therefore concluded that amiodarone may block both rested and inactivated sodium channels with higher affinity to the inactivated ones (i.e., with a minimal affinity to the activated channels). This is essentially in accord with the findings in experiments using V_{max} measurement [57,91].

Sodium channel block by acute amiodarone was also demonstrated in single channel recording in cultured cardiocytes of neonatal rats [76]. In a cell-attached patch-clamp study, external application of amiodarone (10–20 μM) resulted in a decrease of ensemble-averaged i_{Na} through a reduction of open-probability of the channel. Open time, open-time distribution, unitary current size and the tendency to reopen in unblocked channels were unaffected by amiodarone. The inhibition of i_{Na} was accentuated by increasing the frequency of step depolarization (use-dependent block), and at less negative holding potential (voltage-dependent block). Sheldon et al. [140] reported biochemical evidence for amiodarone binding to a sodium channel receptor. They showed in radioligand assay in rat ventricular myocytes that amiodarone inhibited the binding of [^3H]batorachotoxinin A 20 α -benzoate ([^3H]BTXB: a toxin that binds to the activated state of the sodium channel) with an estimated IC_{50} of 3.6 μM . Scatchard analysis of [^3H]BTXB binding indicated that amiodarone reduced the maximal binding for [^3H]BTXB, suggesting allosteric inhibition by amiodarone. This result is consistent with the idea that amiodarone binds preferentially to the inactivated sodium channels.

Acute effects of amiodarone on L-type calcium current

(i_{Ca}) were studied in single ventricular myocytes from guinea pig or rabbit hearts using the suction pipette whole-cell voltage clamp method [63,113,166,170], and the following results were obtained: (1) amiodarone (1–100 μM) decreased i_{Ca} in a concentration-dependent manner without affecting the time course of i_{Ca} decay; (2) amiodarone (5–16 μM) shifted the steady-state inactivation curve for i_{Ca} in the hyperpolarizing direction by 9–10 mV; (3) amiodarone caused both a tonic and a phasic (use-dependent) reduction of i_{Ca} . Nishimura et al. [113] observed that development of i_{Ca} block with amiodarone (5 μM) at depolarized potentials was voltage-dependent between –20 and 10 mV, and that i_{Ca} recovery from inactivation in the presence of amiodarone (0.2 μM) was fitted by double exponentials, most likely reflecting rapid recovery of the drug-free Ca^{2+} channels and slow recovery of the drug-associated channels with time constants of 44 and 1080 ms, respectively, at –80 mV. Apparent dissociation constants of amiodarone were estimated to be 5.8 μM in the resting state, and 0.36 μM in the inactivated state [113]. These facts suggest that amiodarone blocks i_{Ca} in both the resting and inactivated states, but at therapeutic concentrations (plasma level at 1–3 μM [48,145]) the contribution of the inactivated channel block may be much greater than that of the resting channel block.

Biochemical evidence for the interaction of amiodarone with Ca^{2+} channel receptors has been presented in radioligand binding studies. Nokin et al. [117] observed that amiodarone inhibited [^3H]nitrendipine binding to rat myocardial and guinea pig cerebral cortex membrane particulate in an apparently competitive manner (ID_{50} of 0.98 nmol/mg protein or IC_{50} of $\sim 0.25 \mu\text{M}$). Wagner et al. [175] also showed that amiodarone is a potent inhibitor of binding of both dihydropyridine (DHP) and phenylalkylamine ligands to membrane preparations of rat heart, brain, and both skeletal and smooth muscle, and that the pattern of receptor blockade by amiodarone corresponds to that of verapamil in terms of both competitive inhibition and allosteric interaction. Lubic et al. [84] showed that the binding affinity of amiodarone to DHP receptors correlated well with its pharmacologic potency as a calcium channel blocker in terms of inhibition of myocardial contraction, depolarization-induced aortic contraction and $^{45}\text{Ca}^{2+}$ uptake into cultured cells.

In cardiac cells, there are more than 6 different types of potassium (K^+) channels [22,23,165]. They are composed of voltage-gated channels, including the delayed rectifier K^+ current (i_{K}), the transient outward current (i_{to}) and the inward rectifier K^+ current (i_{K1}), and ligand-gated channels such as the muscarinic acetylcholine receptor-operated K^+ current ($i_{\text{K,ACh}}$), the ATP-sensitive K^+ current ($i_{\text{K,ATP}}$) and the Na^+ -activated K^+ current ($i_{\text{K,Na}}$). Effects of amiodarone on these K^+ currents have not yet been thoroughly investigated.

Balser et al. [11] examined the effects of amiodarone on time-dependent potassium currents of guinea pig ventricu-

lar myocytes using the whole-cell configuration of the patch-clamp technique. The net time-dependent outward current recorded under the condition where non-potassium currents had been eliminated contained two components: a rapidly activating La^{3+} -sensitive current, and a slowly activating, La^{3+} -resistant delayed rectifier current. According to the definition by Sanguinetti and Jurkiewicz [134], the former and the latter may correspond to the rapidly activating component of the delayed rectifier current (i_{Kr}) and the slowly activating component of the current (i_{Ks}). Quinidine (10, 50 μM) reduced both components, whereas amiodarone (10 μM) reduced only the slowly activating (La^{3+} -resistant) component. Amplitude of the La^{3+} -resistant current after treatment with 10 μM amiodarone decreased to approximately half of control [11].

Kamiya et al. [63] examined the acute effects of amiodarone on the delayed rectifier potassium current (i_{K}) in the whole-cell voltage clamp of rabbit ventricular myocytes. i_{K} was recorded in Na^{+} - and K^{+} -free external solution containing nisoldipine (3 μM) to isolate the current. Three-second depolarizing pulses were applied from the holding potential (HP) at -50 mV at 30-s intervals. $i_{\text{K,tail}}$ was measured on repolarization back to HP. Amiodarone (1–10 μM) decreased i_{K} in a concentration-dependent manner. The amplitude of $i_{\text{K,tail}}$ following 0 mV depolarization decreased, on average by 22% at 1 μM and by 51% at 10 μM amiodarone. Varró et al. [170] also showed a significant decrease in i_{K} by acute amiodarone (1–5 μM) in rabbit ventricular cells. It was demonstrated by Carmeliet [24] that the decrease in i_{K} by amiodarone (1 μM) in rabbit ventricular cells is enhanced in a use-dependent manner. The major component of i_{K} in rabbit ventricular cells is i_{Kr} [135]. These results may therefore indicate a substantial inhibition of i_{Kr} by acute amiodarone.

Kamiya et al. [63] measured 4-aminopyridine (4AP)-sensitive transient outward current (i_{to}) in rabbit ventricular cells with an external solution containing 2 mM CoCl_2 and 10 μM TTX to block i_{Ca} and i_{Na} , respectively. i_{to} , elicited by depolarizing pulses of 300 ms (0.1 Hz) from HP at -80 mV, was unaffected by 10 μM amiodarone. Similarly, Varró et al. [170] observed no significant effects of acute amiodarone (5 μM) on i_{to} in rabbit ventricular cells. In a recent report by Guo et al. [47], however, acute application of amiodarone (> 1 μM) to cultured newborn rat ventricular cells was shown to cause a concentration-dependent decrease of i_{to} ($\text{IC}_{50} = 4.9$ μM).

The effects of amiodarone on i_{K1} were examined by Sato et al. [137] in isolated guinea pig ventricular cells. In their whole-cell voltage clamp experiments, in which inward Ca^{2+} and Na^{+} currents were blocked by 100 μM CdCl_2 and 10 μM TTX in the external solution, amiodarone (10–20 μM) reduced i_{K1} in both the inward (14% at -120 mV) and outward (12% at -50 mV) directions. These changes were relatively small, but statistically significant. In inside-out or cell-attached patch-clamp experiments by the same group [137], amiodarone

(5 μM) reduced single i_{K1} channel open probability by increasing interburst intervals. In experiments using rabbit ventricular cells, however, Varró et al. [170] showed that the steady-state current level at the end of 400 ms long voltage pulses to -120 to 0 mV, which reflects i_{K1} , was unaffected by acute amiodarone (5 μM).

Acute amiodarone also inhibits the ligand-gated K^{+} channel currents [85,100,176]. Na^{+} -activated K^{+} (K_{Na}) channel current was recorded in inside-out membrane patches and in whole cells isolated from guinea pig ventricles [85,100]. In the experiments by Mori et al. [100], amiodarone (0.1–10 μM) inhibited the single K_{Na} channel current, which had been activated by increasing $[\text{Na}^{+}]_{\text{i}}$, in a concentration-dependent manner ($\text{IC}_{50} \sim 1$ μM) by decreasing the open probability of the channel [100]. Amiodarone (10 μM) was also shown to inhibit whole-cell outward current activated by intracellular Na^{+} loading and extracellular application of ouabain [100]. It has been suggested that the K_{Na} channel in cardiac cells is activated during repetitive excitation at fast rates, digitalis intoxication or acute ischemia [23]. Amiodarone action to inhibit K_{Na} channel current could be involved in its antiarrhythmic potential under such pathological conditions through prevention of excessive APD shortening.

The effects of amiodarone on the acetylcholine-sensitive muscarinic K^{+} (K_{ACh}) channel were examined by Watanabe et al. [176] using the patch clamp technique. In whole-cell voltage clamp of guinea pig atrial cells, amiodarone inhibited $i_{\text{K,ACh}}$, which had been activated by extracellular application of carbachol (1 μM), adenosine (10 μM) or by intracellular loading of GTP γS (100 μM), with similar potency ($\text{IC}_{50} \sim 2$ μM). In experiments to record single K_{ACh} channel current using a pipette solution containing carbachol (1 μM) or atropine (10 μM) plus theophylline (100 μM) in a cell-attached configuration, amiodarone (3 μM) significantly decreased the open probability of the K_{ACh} channel without affecting the amplitude of unitary current [176]. From these findings, the same authors suggested that amiodarone may directly depress the function of the K_{ACh} channel itself and/or GTP binding proteins. In support of this idea, amiodarone (1–10 μM) was shown to reverse the muscarinic-receptor- and adenosine-receptor-mediated shortening of APD of guinea pig atrial myocytes in a concentration-dependent manner [176]. Such a potent action of acute amiodarone to inhibit $i_{\text{K,ACh}}$ may contribute to the clinical usefulness of this drug in termination and prevention of atrial fibrillation [18,55,115].

From the findings described above, the acute effects of amiodarone on cardiac ionic currents can be summarized as follows. Amiodarone inhibits both inward and outward currents. The inhibition of inward Na^{+} and Ca^{2+} currents is enhanced in a use- and voltage-dependent manner. Suppression of excitability and conductivity by amiodarone would, therefore, be greater in cardiac tissues stimulated at higher frequency, and in those with less negative resting

(or diastolic) membrane potentials. Both voltage- and ligand-gated K^+ channel currents are included in the outward currents. i_K (probably both i_{Kr} and i_{Ks}), $i_{K,ACh}$ and $i_{K,Na}$ were shown to be suppressed by amiodarone at relatively low concentrations corresponding to its therapeutic plasma level. i_{K1} was also inhibited at higher concentrations of amiodarone. The inhibitory action of amiodarone on i_{to} is still unclear. Divergent effects of acute amiodarone on APD as described in the previous section can be explained at least in part by different ionic currents responsible for the repolarization of action potential in different animal species, different cardiac tissues, or different experimental conditions [22,23,165]. APD would be shortened if the inhibitory action of amiodarone on the inward currents is greater than on the outward currents, and vice versa in the opposite case.

Such multifaceted effects of acute amiodarone on ionic currents are comparable to those of bepridil, which has been shown to inhibit the inward Ca^{2+} and Na^+ currents in a voltage- and use-dependent manner [50,74,110,184] as well as outward K^+ currents including i_K , i_{to} , i_{K1} and $i_{K,ACh}$ [15,50]. Bepridil has divergent effects on APD; a mild to moderate prolongation was reported in some studies [65,69,74,180], but a shortening in others [5,114]. In the experiments using ventricular muscle from guinea pigs or rabbits, bepridil-induced APD prolongation was attenuated when the drug concentration was increased to a level causing significant decreases in V_{max} and contractile force [65,74].

3. Chronic effects of amiodarone

During the long-term treatment of animals or humans with amiodarone, not only the parent drug, but also its active metabolite, desethylamiodarone (DEA), accumulates extensively in both the plasma and the myocardium [106,145,146]. In this section, we first describe the chronic effects of amiodarone on action potentials and ionic currents, and then discuss the contribution of DEA to those effects of amiodarone.

3.1. Action potentials

Investigators of data generally agree that the major effect of chronic amiodarone is a prolongation of APD of cardiac tissues. This was first demonstrated by Singh and Vaughan Williams [142] in atrial and ventricular muscles from rabbits: after treatment of the rabbits with intraperitoneal application of amiodarone (20 mg/kg/day) for 6 weeks, APD_{90} of atrial and ventricular muscles was increased by 34 and 30%, respectively, whereas the resting membrane potential and amplitude of action potential were unaffected. Subsequent studies have shown that treatment of rabbits with amiodarone for several weeks results in moderate APD prolongation not only in atrial and ventricu-

lar muscles but also in SA nodes and AV nodes [58,62,70,172]. A similar APD prolongation by chronic amiodarone was also reported in ventricular muscles from guinea pigs [6,91,169], rats [78], and dogs [81,130,181] as well as in Purkinje fibers from dogs [41]. Such APD prolongation was associated with a comparable prolongation of ERP.

There is considerable controversy regarding the Class I action of chronic amiodarone. Mason et al. [91] showed a marked use-dependent V_{max} inhibition in guinea pig papillary muscle after chronic treatment with amiodarone, which was comparable to the acute effects of the drug. In support of this finding, a rate-dependent decrease in conduction velocity in association with a decrease in V_{max} was demonstrated in epicardial ventricular muscles from dogs treated with chronic amiodarone [3,130]. The use-dependent slowing of conduction in the His-Purkinje system after chronic treatment with amiodarone has also been demonstrated in dogs [36] as well as in human patients [25,141]. In contrast, Singh and his colleagues [58,70,142,172] showed minimal or no significant change in V_{max} in atrial and ventricular muscles from rabbits after long-term amiodarone treatment. Gallagher et al. [41] reported that Purkinje fibers obtained from dogs after long-term oral amiodarone treatment did not show use-dependent V_{max} inhibition despite significant APD prolongation.

We studied the chronic effects of amiodarone in a series of experiments using rabbits [59,63,73]. The animals were treated for 4 weeks with oral amiodarone at doses ranging from 20 to 100 mg/kg daily. On the last day of drug treatment, peripheral blood was sampled to measure serum amiodarone and the ECGs of extremity leads were recorded. The rabbits were then sacrificed to obtain the whole hearts for Langendorff perfusion, right ventricular papillary muscles for superfusion, or single ventricular cells through enzyme digestion. Untreated rabbits of corresponding weight were used as controls. ECGs were not affected at a daily dose of 20 mg/kg amiodarone. In rabbits that received 50 or 100 mg/kg amiodarone per day, the RR, QT and QTc intervals were significantly prolonged in comparison with controls. PQ and QRS remained unchanged [59,73]. Serum and myocardial tissue amiodarone concentrations in rabbits that received 50 or 100 mg/kg of amiodarone per day were 0.14–0.18 $\mu\text{g}/\text{ml}$ and 1.47–3.63 $\mu\text{g}/\text{g}$ wet weight, respectively [59,73].

Papillary muscles isolated from amiodarone-treated rabbits showed longer APD than controls. Under constant stimulation at 1.0 Hz, APD at -70 mV repolarization in the muscles treated with 50 or 100 mg/kg amiodarone was prolonged significantly by 13 and 20%, respectively ($n=6-8$). At the higher dose, V_{max} was slightly but significantly decreased ($\sim 18\%$), whereas resting membrane potential and amplitude of action potential were unaffected [73]. Moderate APD prolongation was also observed in single ventricular cells [63]. APD_{90} in the cells treated with 100 mg/kg amiodarone was significantly

longer than that in control cells by 42% on average ($n = 18$), whereas other action potential parameters including V_{\max} were unchanged [63]. The papillary muscles and ventricular cells treated with chronic amiodarone, like control muscles and cells, did not exhibit use-dependent V_{\max} inhibition when trains of stimulation (0.5–3.0 Hz) were applied following a long rest period [63,73].

In control rabbit papillary muscles, APD was the longest at a stimulation frequency of 0.5 Hz, shortening at both higher and lower frequencies, giving rise to a bell-shaped frequency–APD relationship. In muscles treated with daily doses of amiodarone (50 or 100 mg/kg), APD was prolonged over the entire range of frequencies from 0.1 to 3.0 Hz. Percentage prolongation of APD at each frequency was more or less similar, and the bell-shaped relationship observed in control muscles was well preserved [73]. APD prolongation in ventricular myocytes from rabbits treated with chronic amiodarone was also frequency-independent within a range of 0.1–3.3 Hz [63]. We compared the frequency-dependence of APD prolongation in rabbit papillary muscles between chronic treatment with amiodarone and acute application of 4 compounds having Class III action: sotalol (30 μM), sotalol (30 μM), E-4031 (0.3 μM) and MS-551 (3 μM). APD prolongation by these 4 drugs was, unlike chronic amiodarone, enhanced progressively at lower stimulation frequencies, exhibiting a marked ‘reverse’ frequency dependence [73,75]. Regarding the frequency-dependence of APD prolongation by chronic amiodarone, only limited information is available. Anderson et al. [3] demonstrated in dogs 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 5 Hz. Our data on rabbit ventricular muscles and cells [63,73] are consistent with their results.

In Langendorff-perfused rabbit hearts, we investigated the chronic effects of amiodarone on ventricular repolarization in comparison with the acute effects of 3 Class III antiarrhythmic drugs (sotalol, E-4031 and MS-551) [59]. Forty to 50 electrograms were recorded through modified bipolar electrodes from the anterior to the lateral epicardial surface of the ventricles under His-bundle pacing (1.0 Hz). In control hearts, epicardial activation proceeded from the apex to the base. The interval from the initial sharp negative deflection of the QRS complex to the apex of the T-wave (Q-aT) in the electrograms, which reflects APD at the recording site, was longest at the apex and shortest at the base. Therefore, repolarization proceeded from the base to the apex. In hearts treated with oral amiodarone (100 mg/kg, 4 weeks), Q-aT was uniformly prolonged by 14–16% throughout the mapped area, whereas the activation sequence was unaffected, and a normal Q-aT gradient was well preserved from the apex to the base. The spatial inhomogeneity of ventricular repolarization was not enhanced by drug treatment. In contrast, acute application of sotalol (30 μM), E-4031 (0.1 μM), or MS-551 (1 μM)

caused much greater Q-aT prolongation at the apex than at the base, resulting in a marked enhancement of the spatial inhomogeneity of ventricular repolarization [59].

3.2. Ionic currents

Kamiya et al. [63] investigated changes in ionic currents responsible for the APD prolongation induced by chronic amiodarone in single ventricular cells isolated from rabbits untreated (control, $n = 6$ –16) and treated ($n = 6$ –9) with daily oral doses of amiodarone (100 mg/kg for 4 weeks). L-type calcium current (i_{Ca}), delayed rectifier potassium current (i_{K}), and 4AP-sensitive transient outward current (i_{to}) were recorded using a whole-cell voltage-clamp technique. In ventricular cells from amiodarone-treated rabbits, the current density of i_{Ca} was significantly less (by 13–43%) than control, but its voltage-dependence was unchanged (Fig. 2A). The current densities of $i_{\text{K, tail}}$ and i_{to} were also decreased significantly from control (by 43–62 and 23–44%, respectively) without any appreciable changes in their voltage-dependence (Fig. 2B,C). Recently, Varró et al. [170] reported that chronic treatment of rabbits with amiodarone (50 mg/kg/day, i.p. for 3–4 weeks) resulted in significant decreases in the current density of i_{K} and i_{to} in ventricular cells without affecting i_{Ca} and i_{K1} densities.

Guo et al. [47] examined chronic effects of amiodarone on potassium outward currents in cultured ventricular cells from neonatal rats. Outward currents were elicited by 300 ms depolarization from a holding potential at -60 mV in the external solution including 10 μM TTX and 3 μM nisoldipine. The currents were composed of 4AP-sensitive transient components and 4AP-insensitive sustained components. The former and the latter were regarded as i_{to} and i_{sus} , respectively. In the myocytes cultured in a serum-supplemented medium containing 0.12 nM triiodothyronine (T_3), long (72 h) exposure to 1 μM amiodarone, which has no acute effects on i_{to} and i_{sus} , resulted in a significant decrease in the current density of both i_{to} and i_{sus} without affecting their voltage-dependence; the current densities of peak i_{to} and i_{sus} were decreased on average by 21 and 24%, respectively, compared with the controls [47]. This study also showed in current-clamp experiments in Tyrode bath solution that APD_{75} of the cultured cells was prolonged significantly (by 32% on average) after 72 h exposure to 1 μM amiodarone.

Bosch et al. [19] reported in an abstract that treatment of guinea pigs with amiodarone (80 mg/kg/day, i.p. for 7 days) resulted in significant decreases in current density of i_{Kr} , i_{Ks} , and i_{K1} in ventricular myocytes by 61, 45, and 44%, respectively. To our knowledge, no other published information is available on the chronic effects of amiodarone on ionic currents of cardiac cells. At present, therefore, APD prolongation induced by chronic treatment with amiodarone may be most likely explained by a de-

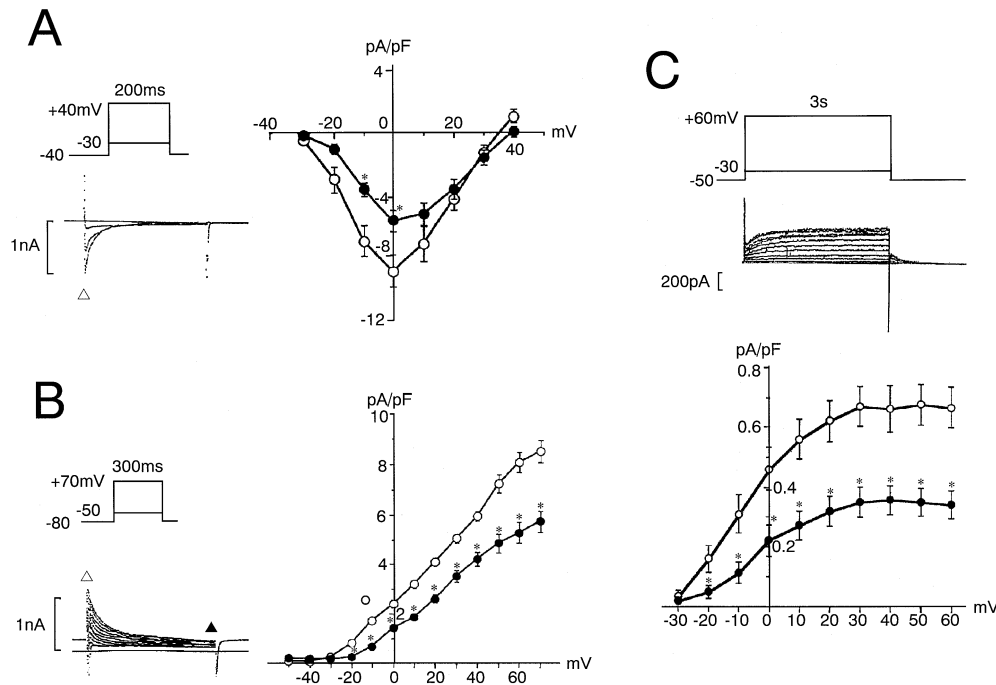


Fig. 2. L-type calcium current (i_{Ca}), transient outward current (i_{To}) and delayed rectifier potassium current (i_K) of ventricular cells isolated from rabbits untreated (control) or treated with amiodarone (100 mg/kg p.o. 4 weeks). (A) In experiments to record i_{Ca} , K^+ in the external and pipette solutions was replaced with Cs^+ , and depolarizing pulses for 200 ms were applied at 0.1 Hz from a holding potential at -40 mV. (B) In experiments to measure i_{To} , external solution containing 2 mM $CoCl_2$ and 10 μ M TTX was used to block i_{Ca} and i_{Na} , respectively, and depolarizing pulses for 300 ms were applied at 0.1 Hz from a holding potential at -80 mV. (C) i_K was recorded in Na^+ - and K^+ -free external solution containing 3 μ M nisoldipine to isolate the current. Depolarizing pulses for 3 s were applied from the holding potential at -50 mV at 30 s intervals. $i_{K,tail}$ was measured on the repolarization back to the holding potential. Midpoint voltage of activation for i_K is shifted to the right probably due to the special conditions of K^+ -free and Na^+ -free medium. The current amplitude was divided by cell capacitance in each experiment to obtain the current density (pA/pF). Averaged values (mean \pm s.e.) of the current density for i_{Ca} , i_{To} and $i_{K,tail}$ were plotted in the graphs of the current-voltage relationship. Open circles indicate control: $n = 16$ for i_{Ca} (A), 16 for i_{To} (B), and 6 for $i_{K,tail}$ (C). Closed circles indicate the data obtained from the cells treated with chronic amiodarone: $n = 8$ for i_{Ca} (A), 9 for i_{To} (B), and 6 for $i_{K,tail}$ (C). * Significantly different from control at $P < 0.05$. From Ref. [63], with permission.

crease of current density for i_K and i_{To} . The inhibition of i_{Ca} current density is supposed to counteract the excessive APD prolongation.

The reduction in ionic current density following chronic treatment with amiodarone might be due to a modulation of gene expression of ion channels. Kamiya et al. [64] investigated the expression of Shaker-related potassium channel genes in rats in order to obtain insight on this point. Male Wistar rats aged 6 weeks were treated for 4 weeks with oral amiodarone at a daily dose of 100 mg/kg. The rats were then sacrificed to obtain blood samples and the ventricles. Total RNA was extracted by the standard AGPC method, and Kv1.5 mRNA levels were measured by Northern blot analysis using densitometry of the autoradiogram. The mRNA for Kv1.5 was detected as a single band (3.4 kb) in both the control ($n = 7$) and amiodarone-treated groups ($n = 7$). The amount of Kv1.5 mRNA in the amiodarone-treated rats was significantly less than the control (by 41% on average), suggesting that gene expression of the Kv1.5 channel in the ventricle is modulated (down-regulated) by long-term treatment with amiodarone. A down-regulation of the transcriptional activity of the

Kv1.5 gene in rat atrial and ventricular muscles following amiodarone treatment was also reported by Peele et al. in an abstract [123].

Recent molecular biological studies have shown that more than several distinct voltage-gated potassium channel genes are expressed in rat cardiac tissues [13,132]. They include 4 Shaker-related (Kv subfamily) genes: Kv1.1, Kv1.2, Kv1.4 and Kv1.5. The structural and functional relations of these channels cloned to the native potassium channels remain unclear. Such channel gene products can form heteromeric potassium channels (hetero-tetramers) that exhibit current kinetics and pharmacologic properties distinct from the original homomeric channels (homo-tetramers). Association of accessory β -subunits with the pore-forming α -subunits may also influence the channel properties, leading to a large diversity of the native voltage-gated potassium channels [13,33]. From the results presented by Kamiya et al. [64] and by Peele et al. [123], it seems intriguing to speculate that chronic amiodarone administration can modulate the gene expression level for potassium channels. Nevertheless, other genes corresponding to a variety of key currents, such as HERG, minK, KvLQT1 or

Kv4.2 [13,33], have not been shown to be affected by chronic amiodarone. Further experimental studies are therefore needed to substantiate this speculation.

3.3. Desethylamiodarone

It has been shown in pharmacokinetic studies that the amount of desethylamiodarone (DEA) accumulated in the myocardium after several weeks of amiodarone treatment is comparable to, or greater than, that of the parent drug [12,108,172]. There is a species difference in the extent of myocardial accumulation of DEA [145,146]. In isolated cardiac tissues, DEA was shown to have acute effects on action potential configuration qualitatively and quantitatively similar to those of amiodarone [107,120,183]. In ventricular muscles and Purkinje fibers from dogs or guinea pigs, DEA (1–57 μM), like amiodarone, caused a marked use-dependent V_{max} inhibition with similar onset and offset kinetics. Acute *in vitro* application of DEA resulted in little or no prolongation of APD in ventricular muscle, and a shortening of APD in Purkinje fibers [120,130,183]. An appreciable decrease in spontaneous rate of rabbit SA node tissues was also reported [183]. *In vivo* animal experiments, however, have shown some differences between the two compounds. A single intravenous dose of DEA was 2.6 times as potent as amiodarone in increasing the QRS duration in rats [106], and was also more potent than the parent compound in producing rate-dependent QRS prolongation in dogs [158]. In contrast, DEA applied intravenously was less potent than amiodarone in frequency-dependent inhibition of AV nodal conduction [107,158]. In radioligand binding studies, both amiodarone and DEA were shown to inhibit [^3H]BTXB binding to sodium channels in ventricular myocytes with a similar potency [140], but the inhibitory action of DEA against binding of [^3H]nitrendipine or (–)[^3H]desmethoxyverapamil to membranes of heart was less than one-tenth as potent as amiodarone [175]. Inhibitory actions of the metabolite on i_{Na} and i_{Ca} have not yet been studied in voltage-clamp experiments in comparison with the parent drug. Thus the chronic effects of amiodarone may be modulated substantially by accumulation of DEA in the heart, but the metabolite does not account for the main chronic effects on the repolarization of action potential.

The large discrepancy among investigators regarding the chronic effects amiodarone on V_{max} of action potential and conduction in fast-sodium-channel-dependent cardiac tissues could be attributed to different accumulations of amiodarone and DEA in the hearts. In the experiments reporting a marked use- or frequency-dependent inhibition of V_{max} and conduction velocity, myocardial concentrations of amiodarone and DEA were relatively high: average values were 55–104 $\mu\text{g/g}$ for amiodarone and 56–66 $\mu\text{g/g}$ for DEA [3,130]. Anderson et al. [3] demonstrated a fairly good correlation between the frequency-dependent decrease in the conduction velocity along the fiber orienta-

tion and the myocardial amiodarone concentration (but no significant correlation between the prolongation of repolarization intervals and the myocardial drug levels). On the other hand, myocardial levels of the parent drug and the metabolite were considerably lower in the reports showing minimal or negligible Class I action of chronic amiodarone; average values were 4–15 $\mu\text{g/g}$ for amiodarone and 1–3 $\mu\text{g/g}$ for DEA [41,58,70,73,172]. This might reflect different pharmacokinetics of amiodarone in different animal species [145,146]. High concentrations of amiodarone and DEA accumulated in the cardiac tissue would have their direct (or acute) inhibitory action on the fast sodium channel, resulting in a use-dependent decrease in V_{max} and conduction velocity. Nevertheless, further experimental studies are required to confirm this possibility.

4. Amiodarone and thyroid hormones

4.1. Hypothyroid-like actions of amiodarone

One molecule of amiodarone contains two iodine atoms comprising 37% of its total molecular weight, and it shares some structural similarities with thyroid hormones [71,144]. The major electrophysiologic changes of the heart induced by chronic treatment with amiodarone (a prolongation of the action potential duration and the refractory period of all cardiac tissues) resemble those induced by hypothyroidism [17,71,139,142,144–146]. Many other cardiac effects of chronic amiodarone are also similar to those seen in hypothyroidism: bradycardia, reduced myocardial oxygen consumption, reduced myocardial β -adrenergic receptor density, prolonged systolic time interval, decreased cardiac Ca^{2+} -ATPase activity, decreased $\text{Na}^{+}/\text{K}^{+}$ -ATPase activity, and altered expression of myosin isoforms [8,16,20,32,66,104,116,122,144,178,185,186]. Singh and Vaughan Williams [142] found that administration of iodine alone to rabbits in doses equivalent to those contained in the effective dose of amiodarone had no significant effects on action potential configuration, but concomitant administration of thyroxine (T_4) with amiodarone prevented the development of repolarization changes. Patterson et al. [122] showed that concomitant application of triiodothyronine (T_3) reversed most of the chronic effects of amiodarone on conduction and repolarization of rabbit hearts. Talajic et al. [159] demonstrated in guinea pigs that intact thyroid function is a prerequisite for Class III and sinus node effects of chronic amiodarone. It has therefore been hypothesized that one of the mechanisms of the chronic action of amiodarone could be the induction of a local hypothyroid-like condition in the heart [71,145]. Some investigators, however, have questioned this assumption because hypothyroidism does not mimic all chronic effects of amiodarone on the hearts [19,78,126]. In recent experiments by Bosh et al. [19], hypothyroidism produced in guinea pigs resulted in a prominent decrease in i_{Ks} density

in ventricular cells, but, unlike chronic amiodarone, it did not affect i_{Kr} and i_{K1} densities.

4.2. Modulation of thyroid hormone action

Three mechanisms have been proposed to explain the hypothyroid-like effects of chronic amiodarone (Fig. 3). The first is an inhibition of the peripheral conversion from T_4 to T_3 . This was suggested from an observation that long-term administration of amiodarone causes a modest decrease in plasma levels of T_3 (an active metabolite of T_4) accompanied by a significant increase in serum T_4 and, in particular, reverse T_3 (rT_3 , an inactive metabolite of T_4) [1,20,53,61,101,124,129,144,152,173]. The intracellularly located enzyme, thyroxine 5'-deiodinase, converts T_4 to T_3 by outer-ring deiodination. This process takes place mainly in the liver and kidney, and is predominantly responsible for the production of active thyroid hormone in the blood. Inhibition of this enzyme by amiodarone has been demonstrated in liver and myocardial tissue homogenates from rats treated in vivo [10,26,43,124,125,152] and in isolated rat hepatocytes [1]. DEA, like amiodarone, was also shown to inhibit hepatic (Type I) 5'-deiodinase [43,133,138]. An inhibition of pituitary (Type II) 5'-deiodinase activity was demonstrated as well in rats treated with amiodarone or DEA, leading to an increase of TSH secretion from the anterior pituitary gland [14,71,133,136,144]. The production of rT_3 by inner-ring deiodination of T_4 at the 5-position (by Type III 5-deiodinase) is unaffected by amiodarone [66]. Iopanoic acid, a potent inhibitor of the intracellular conversion of T_4 to T_3 , causes similar changes in serum thyroid hormones as observed during long-term amiodarone administration, but it has failed to duplicate sinus bradycardia [82,152], reduction of cardiac β -adrenergic receptor density [125], changes in electrocardiograms [155], and antiarrhythmic activity [95]. Therefore, the inhibition of peripheral conversion of T_4 to T_3 by amiodarone or DEA cannot account for the major electrophysiological

profile of chronic amiodarone, although it may influence the thyroid hormone-dependent metabolism of the heart.

The second possible mechanism responsible for the hypothyroid-like effects of chronic amiodarone is an inhibition of transport of iodothyronines through the cell membrane [67,71,77]. Schröder-van der Elst et al. [138] showed in experiments using rats that entrance of plasma-derived T_3 is compromised in the heart after 3 weeks of oral administration of amiodarone or DEA, resulting in a marked decrease of myocardial T_3 concentration. It was also reported by Gøtzsche and Ørskov [44,45] that myocardial tissue T_3 in pigs and rats treated with oral amiodarone (4–6 weeks) decreased below the detection level despite only moderately decreased serum T_3 . These facts may suggest a substantial inhibition of thyroid hormone entry into cardiac cells.

The third, and most intriguing, mechanism is a direct inhibition of T_3 nuclear binding by amiodarone and/or DEA. Franklyn et al. [40] demonstrated a concentration-dependent blocking effect of amiodarone (10–1000 μ M) on T_3 binding to isolated nuclei prepared from rat anterior pituitary tissue. Norman and Lavin [118] also reported a competitive antagonism of amiodarone for T_3 binding to nuclear T_3 receptors (TR) of rat liver, brain and pituitary tumor (GC) cells (EC_{50} at 3 μ M for liver-derived receptor). In contrast, Sogol et al. [152] and Eil et al. [35] were unable to demonstrate inhibition of T_3 binding by amiodarone to rat hepatic nuclei and to human skin fibroblasts in culture, respectively. Latham et al. [79] showed that DEA, but not amiodarone, bound with substantial affinities (K_d 8.6–35.0 μ M) to nuclear TR in extracts derived from human lymphocytes, bovine atrium, ventricle and rat liver. In experiments using nuclear preparations from myocardial tissue extracts of pig and rat hearts, Gøtzsche and Ørskov [44,45] also observed that DEA had a much higher potency than amiodarone in displacing T_3 from its receptor. These discrepancies among investigators could be attributed in part to the different tissues investigated, and in part to the different methods employed. Low solubility of amiodarone in the nuclear preparation under assay conditions, and intrinsic effects of a vehicle (Tween 80) on T_3 binding to TR have been considered to make data interpretation more difficult [9,118,179].

Since the cloning of genes encoding TR, at least 4 TR isoforms have been identified: TR- α 1, TR- β 1, TR- β 2 and TR- α 2 [54,80,156]. Recent studies have suggested that the T_3 binding inhibitory effects of amiodarone or DEA for each TR isoform may be different in potency and mode of antagonism [9,34,167]. Such TR isoform-dependent effects might account for the tissue selectivity of the drug action, although the issue remains to be investigated.

4.3. T_3 antagonism

Chronic effects of amiodarone could be mediated by T_3 antagonism at a cellular or subcellular level [118,121]. Guo

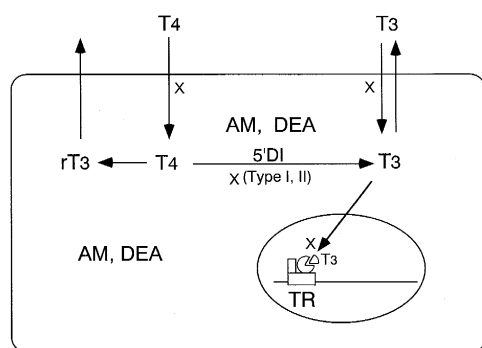


Fig. 3. Schematic illustration of the effects of amiodarone and desethylamiodarone on the metabolism and nuclear binding of thyroid hormones. Amiodarone (AM) and desethylamiodarone (DEA) inhibit intracellular conversion from thyroxine (T_4) to triiodothyronine (T_3) via 5'-deiodination (5'DI) without affecting intracellular conversion from T_4 to reverse T_3 (rT_3). AM and/or DEA may also inhibit cellular uptake of T_4 and T_3 as well as T_3 binding to the nuclear receptors (TR).

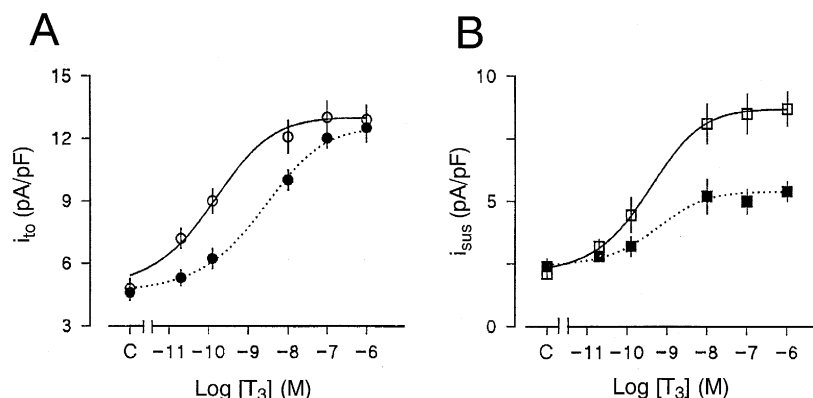


Fig. 4. Effects of 72-h exposure to T_3 with or without amiodarone (1 μM) on 4AP-sensitive transient outward current (i_{to}) and 4AP-insensitive sustained K^+ outward current (i_{sus}) in cultured newborn rat ventricular cells. Ordinates show the current density (pA/pF) of i_{to} (A) and i_{sus} (B) calculated from current amplitude and cell capacitance. Abscissae indicate T_3 concentrations in the medium. Values are means \pm s.e. ($n = 5-7$). Open circles indicate the data obtained in the absence of amiodarone (control), and closed circles in the presence of amiodarone. Amiodarone inhibited the T_3 concentration-dependent increase in the current density of both i_{to} and i_{sus} . The inhibition was competitive for i_{to} , but non-competitive for i_{sus} . From Ref. [47], with permission.

et al. [47] investigated the effects of amiodarone on repolarizing outward K^+ currents in cultured newborn rat ventricular cells using the whole-cell patch clamp technique. In a serum-supplemented medium containing 0.12 nM T_3 , a short (30-min) application of 1 μM amiodarone to cultured myocytes had no effect on the 4-aminopyridine (4AP)-sensitive transient outward current (i_{to}) and the 4AP-insensitive sustained K^+ outward current (i_{sus}), but a long period (72 h) application resulted in significant decreases in the current density of both i_{to} and i_{sus} . In a serum-free medium without T_3 , however, a similar 72-h exposure to 1 μM amiodarone had no effect on either i_{to} or i_{sus} . Single application of T_3 to the culture medium for 72 h resulted in a concentration-dependent enhancement of the current density of both i_{to} and i_{sus} . Concomitant application of 1 μM amiodarone and T_3 caused an inhibition of the T_3 action on i_{to} and i_{sus} in competitive and non-competitive manners, respectively (Fig. 4). These results suggest that long-term treatment with amiodarone may antagonize T_3 and thereby counteract its hormonal effects on potassium channels.

5. Summary and conclusions

The complex profile of amiodarone actions on the electrophysiological properties of cardiac cells reviewed in this article may be summarized as follows. As acute effects, amiodarone inhibits both inward and outward currents. The inhibition of inward Na^+ and Ca^{2+} currents is enhanced in a use- and voltage-dependent manner, resulting in suppression of excitability and conductivity in both i_{Na} - and i_{Ca} -dependent cardiac tissues. The inhibition is greater in the tissues stimulated at higher frequencies, and in those with less negative resting (or diastolic) membrane potentials. As outward currents, i_K (i_{Kf} and i_{Ks}), $i_{K, \text{ACh}}$ and $i_{K, \text{Na}}$ are inhibited by acute amiodarone. i_{K1} could also

be inhibited at high concentrations of amiodarone. Acute effects of amiodarone on i_{to} remain unclear. Previous reports on the acute effects of amiodarone on APD are conflicting, presumably because different ionic currents are responsible for the repolarization of action potential in different animal species, cardiac tissues and experimental conditions. APD would be shortened if the inhibitory action of amiodarone on the inward current is greater than on the outward current, and vice versa in the opposite case.

The major and consistent chronic effect of amiodarone is a moderate APD prolongation with minimal frequency-dependence. This prolongation is most likely due to a decrease in the current density of i_K and i_{to} . Chronic effects of amiodarone are modulated by tissue accumulation of amiodarone and DEA. Variable suppression of excitability and conductivity of the heart by chronic amiodarone might reflect direct acute effects of the parent drug and/or its active metabolite (DEA) retained at the sites of action. Chronic amiodarone was shown to cause a down-regulation of Kv1.5 mRNA in rat hearts, suggesting a drug-induced modulation of potassium channel gene expression.

Electrophysiological changes in the heart induced by chronic amiodarone resemble those induced by hypothyroidism. Three mechanisms have been proposed to explain this hypothyroid-like action of amiodarone. Amiodarone and/or DEA may inhibit peripheral conversion from T_4 to T_3 , cellular uptake of T_4 and T_3 , and T_3 binding to nuclear receptors (TR). The second and third mechanisms are considered to be more important than the first. Amiodarone or DEA could antagonize T_3 action on the heart at a cellular or subcellular level.

Two distinct characteristics in the cellular electropharmacology of amiodarone are different from those of other antiarrhythmic drugs. First, it acts on many different types of molecular targets including Na^+ , Ca^{2+} , and K^+ channels as well as adrenoceptors. Second, it may cause antiar-

rhythmic remodeling of cardiac cells, probably through a modulation of gene expression of ion channels and other functional proteins. We hypothesize that this remodeling is mediated most likely by cellular or subcellular T_3 antagonism. Nevertheless, much remains to be studied as to the acute and especially chronic effects of amiodarone on ionic currents, transporters, receptors and other molecules in cardiac cells. The role of the cardiac hypothyroid state in the genesis of antiarrhythmic activity is still a matter of considerable controversy among investigators. Recently, two amiodarone analogues (SR 33589 and ATI-2001) showing a potent acute antiarrhythmic activity in animal models, have been developed [37,87,88,131]. These new compounds are not known to exhibit chronic antiarrhythmic potential or cardiac hypothyroidism activity. Unraveling these issues will be required to understand the exact molecular and cellular mode of action of amiodarone and to find a new direction for the development of the ideal antiarrhythmic drugs of the future.

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