Comparative electrophysiologic effects of intravenous amiodarone and desethylamiodarone in dogs: evidence for clinically relevant activity of the metabolite

MARIO TALAJIC, M.D., MICHAEL R. DERROODE, B.SC., AND STANLEY NATTEL, M.D.

ABSTRACT It has been suggested that some of the effects of long-term amiodarone therapy may be due to accumulation of a metabolite, desethylamiodarone. To evaluate the pharmacologic actions of the metabolite, we gave single intravenous doses (10 or 25 mg/kg) of amiodarone or desethylamiodarone to anesthetized dogs. The resulting plasma and myocardial concentrations of both agents were similar to levels achieved with long-term oral amiodarone therapy in man. Amiodarone and desethylamiodarone produced frequency-dependent slowing in ventricular and atrioventricular nodal conduction and increased atrial and ventricular refractory periods. The relative effects of these agents on fast- and slow-channel tissues differed, with amiodarone producing significantly greater prolongation of Wenckebach cycle length and desethylamiodarone producing larger increases in QRS duration, atrial refractory period, and ventricular refractory period. We conclude that desethylamiodarone has substantial electrophysiologic effects at clinically relevant concentrations and has relatively greater effect on fast-channel tissue in vivo than does amiodarone. The accumulation of desethylamiodarone probably accounts for some of the delayed electrophysiologic effects in patients receiving long-term treatment with amiodarone.


AMIODARONE is very effective in the treatment of a wide variety of ventricular and supraventricular arrhythmias.1–4 Enthusiasm for its use is increasing, but its mechanism of action remains unclear. Long-term therapy with amiodarone results in atrioventricular and intraventricular conduction slowing as well as prolongation of atrial, atrioventricular, and ventricular refractoriness.5–10 In contrast, single-dose administration of amiodarone depresses atrioventricular (AV) nodal properties but has little or no effect on atrial and ventricular refractoriness despite the achievement of equivalent plasma and myocardial amiodarone concentrations.9, 10, 17, 18

The different effects of single-dose administration of amiodarone compared with long-term therapy may be due to time-dependent changes in thyroid status,19, 20 delayed biochemical effects,21 or the accumulation of an active metabolite. Desethylamiodarone, a major metabolite of amiodarone, accumulates significantly in the plasma and myocardium of patients on long-term amiodarone therapy.22–24 To date, little is known about desethylamiodarone’s electrophysiologic actions, although it has been found to be more potent than amiodarone in prolonging QRS, QT, and QTc intervals in rats after both short- and long-term administration.25 Preliminary results have been presented showing that intravenous desethylamiodarone suppresses inducible ventricular arrhythmias after experimental myocardial infarction in dogs.26 However, no information concerning the effects of desethylamiodarone on myocardial refactoriness and conduction is available. Since changes in myocardial conduction and refractoriness are typically delayed after oral loading doses of amiodarone, accumulation of the N-desethyl metabolite may be partly responsible for these delayed changes. The purpose of this study was to evaluate the initial electrophysiologic effects of desethylami-
amiodarone relative to amiodarone in vivo in a canine preparation.

Methods

General. Mongrel dogs of either sex were anesthetized with morphine (2 mg/kg sc) and α-chloralose (100 mg/kg iv). Catheters were inserted into both femoral arteries and femoral veins and kept patent with heparinized isotonic saline. Dogs were ventilated via a cuffed endotracheal tube at a rate of 10/min with a tidal volume obtained from a nomogram. Arterial blood gases were measured to ensure adequate oxygenation (SaO$_2$ ≥ 90%) and physiologic pH (7.38 to 7.45). A right thoracotomy was performed and bipolar Teflon-coated stainless steel electrodes were inserted into the right atrial and right ventricular free wall for recording and stimulation. A Statham P23 1D transducer (Statham Medical Instruments, Los Angeles), electrophysiological amplifiers, and a paper recorder (Siemens Mingograph 80 recorder) were used to record blood pressure, electrocardiographic leads II and aVR, stimulus artifacts, and right atrial and ventricular electrograms. Stimulation was applied with 4 msec square-wave pulses at twice diastolic threshold current controlled by a programmable stimulator (Caltronics, Inc.). A previously described protocol was used to produce autonomic blockade. Briefly, β-blockade was achieved by administering a 0.3 mg/kg intravenous bolus of propranolol followed by a maintenance infusion of 0.45 mg/kg/hr. Muscarinic blockade was achieved by surgical division of the cervical vagi followed by a 1 mg bolus of atropine. This regimen has been shown to produce stable β-adrenergic and muscarinic blockade for several hours, with concentrations of propranolol far below those producing local anesthetic effects. The sinus node was then crushed to allow for a wide range of pacing rates.

Experimental protocol. Wenckebach cycle length (WBCL) was determined under control conditions by decreasing atrial pacing cycle length by 10 msec decrements until second-degree atrioventricular block occurred. Atrialventricular conduction time (AVCT), ventricular paced QRS and QT duration, atrial effective refractory period (AERP), and right ventricular effective refractory period (VERP) were measured under steady-state conditions over a wide range of pacing cycle lengths (300 to 1000 msec). Steady state was achieved by pacing either the atrium or ventricle at a given rate for 2 min. After the above control measurements were completed, either amiodarone (10 or 25 mg/kg) or desethylamiodarone (10 or 25 mg/kg) was infused intravenously over 45 min and the experimental protocol was repeated. All doses of drugs were dissolved in 2 ml of 50% ethanol. This vehicle has previously been shown to have no short-term electrophysiologic effects. Electrophysiologic study was begun 30 min after the completion of drug infusion. Blood samples for measurement of plasma drug concentration were drawn 60 min after completion of the drug infusion at the approximate midpoint of the electrophysiologic study. Myocardial samples from the left ventricle were obtained at the completion of the experimental protocol for the subsequent determination of myocardial drug concentrations. Plasma and myocardial concentrations of amiodarone and desethylamiodarone were measured by previously described high-performance liquid chromatographic methods. A total of 28 dogs were studied.

Data analysis. Electrocardiographic recordings were obtained at 250 mm/sec paper speed. AVCT was measured from the stimulus artifact to the onset of the QRS complex. Atrial and ventricular effective refractory periods were defined as the longest S$_1$/S$_2$ interval that failed to capture the atrium or ventricle. In five experiments (two high-dose and one low-dose amiodarone, and one low-dose and one high-dose desethylamiodarone), an epicardial His bundle electrode was used. AH interval was found to be linearly related to AVCT in these experiments. Accordingly, AVCT is used in this article as an index of conduction through the AV node. Group data are presented as mean ± SD. Comparisons between single sets of control and experimental data were made with paired t tests. Multiple comparisons between experimental groups were made by two-way analysis of variance with Scheffe contrasts. Comparisons between two groups of experimental data were made with unpaired t tests. Two-tailed tests were used for all statistical comparisons and a probability of 5% or less was taken to indicate statistical significance.

Results

Hemodynamic response and drug concentrations (Table 1). Reductions in systolic and diastolic blood pressure were observed after administration of both amiodarone and desethylamiodarone. The reductions in systolic pressure approached but did not reach statistical significance. Diastolic blood pressure was significantly reduced only after high-dose amiodarone or desethylamiodarone. Both amiodarone and desethylamiodarone accumulated extensively in ventricular myocardium in a dose-dependent manner (Table 1). Administration of desethylamiodarone resulted in significantly higher myocardial concentrations than amiodarone for equivalent intravenous doses.

Electrophysiologic effects on atrial and ventricular myocardium. Drug-induced changes in electrophysiologic measurements at a basic cycle length of 400 msec are

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Blood pressure and drug concentrations after infusion of amiodarone or desethylamiodarone</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td>Control 9</td>
<td>122 ± 15</td>
</tr>
<tr>
<td>A10</td>
<td>113 ± 15</td>
</tr>
<tr>
<td>Control 6</td>
<td>137 ± 9</td>
</tr>
<tr>
<td>A25</td>
<td>112 ± 36</td>
</tr>
<tr>
<td>Control 7</td>
<td>128 ± 21</td>
</tr>
<tr>
<td>D10</td>
<td>116 ± 19</td>
</tr>
<tr>
<td>Control 6</td>
<td>144 ± 12</td>
</tr>
<tr>
<td>D25</td>
<td>117 ± 22</td>
</tr>
</tbody>
</table>

Doses studied were 10 and 25 mg/kg amiodarone (A10 and A25, respectively) and desethylamiodarone (D10 and D25, respectively). Plasma concentrations were measured 60 min after administration of each dose. Electrophysiologic studies were begun 30 min after drug administration and completed approximately 90 min after drug administration.

A p < .05 vs corresponding control value; b p < .05; c p < .01 vs ventricular concentration of amiodarone after a corresponding intravenous dose.
summarized in table 2. Both doses of amiodarone produced small increases in QRS duration and QT interval. Desethylamiodarone prolonged the QRS duration in a clearer dose-dependent manner. Changes in QRS duration produced by high-dose amiodarone and desethylamiodarone depended on stimulation cycle length, with decreases in cycle length substantially augmenting drug effect (figure 1). Small, nonsignificant increases in QT interval were seen after administration of 25 mg/kg desethylamiodarone. These increases were accounted for by the increase in QRS duration, with no observable change occurring in the JT interval.

Small, nonsignificant increases in the VERP occurred after both doses of amiodarone and low-dose desethylamiodarone. High-dose desethylamiodarone prolonged the VERP by 22% (p < .05). Low doses of either drug did not significantly change the AERP, but both drugs increased the AERP after infusion of 25 mg/kg. Desethylamiodarone was more potent in altering AERP, producing a 54% increase, significantly greater than the 16% increase seen after 25 mg/kg amiodarone (p < .05). Drug-induced changes in AERP and VERP were not dependent on activation frequency.

Effects on AV nodal properties. In contrast to desethylamiodarone's significant effects on atrial and ventricular myocardial properties, amiodarone's major effect was limited to depression of AV nodal properties (table 2). WBCL was increased in a dose-dependent manner.

**TABLE 2**
Electrophysiologic variables before and after infusion of amiodarone or desethylamiodarone

<table>
<thead>
<tr>
<th></th>
<th>QRS</th>
<th>QT</th>
<th>AERP</th>
<th>VERP</th>
<th>WBCL</th>
<th>AVCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>96 ± 13</td>
<td>274 ± 31</td>
<td>167 ± 19</td>
<td>184 ± 16</td>
<td>216 ± 17</td>
<td>125 ± 17</td>
</tr>
<tr>
<td>A10</td>
<td>100 ± 19</td>
<td>291 ± 37</td>
<td>180 ± 8</td>
<td>202 ± 21</td>
<td>384 ± 62</td>
<td>195 ± 37</td>
</tr>
<tr>
<td>Control</td>
<td>101 ± 13</td>
<td>260 ± 26</td>
<td>153 ± 15</td>
<td>172 ± 13</td>
<td>220 ± 12</td>
<td>124 ± 14</td>
</tr>
<tr>
<td>A25</td>
<td>109 ± 11</td>
<td>265 ± 30</td>
<td>178 ± 4^a</td>
<td>176 ± 14</td>
<td>413 ± 29</td>
<td>199 ± 20</td>
</tr>
<tr>
<td>Control</td>
<td>108 ± 4</td>
<td>262 ± 12</td>
<td>155 ± 9</td>
<td>180 ± 8</td>
<td>225 ± 25</td>
<td>134 ± 7</td>
</tr>
<tr>
<td>D10</td>
<td>121 ± 9</td>
<td>278 ± 11</td>
<td>165 ± 15</td>
<td>188 ± 8</td>
<td>298 ± 51</td>
<td>174 ± 17</td>
</tr>
<tr>
<td>Control</td>
<td>88 ± 8</td>
<td>254 ± 15</td>
<td>138 ± 18</td>
<td>170 ± 14</td>
<td>234 ± 25</td>
<td>139 ± 11</td>
</tr>
<tr>
<td>D25</td>
<td>115 ± 11^a</td>
<td>283 ± 23</td>
<td>213 ± 8^a</td>
<td>208 ± 16^a</td>
<td>374 ± 45^a</td>
<td>213 ± 21^a</td>
</tr>
</tbody>
</table>

All measurements are expressed in milliseconds as mean ± SD. QRS duration, QT interval, and VERP were measured during ventricular pacing with a BCL of 400 msec. AERP and AVCT were measured during atrial pacing at a BCL of 400 msec or at a cycle length just above the WBCL for WBCL ≥ 400 msec. Doses used were 10 and 25 mg/kg amiodarone (A10, A25, respectively) and desethylamiodarone (D10, D25). Results shown are for nine experiments with A10, six with A25, seven with D10, and six with D25.

^p < .05; ^p < .01; ^p < .001 all vs the corresponding control value.

![FIGURE 1. QRS duration as function of basic cycle length.](image-url)
by both amiodarone and desethylamiodarone. However, the increases observed after amiodarone were greater than those after equal doses of desethylamiodarone (78% for amiodarone vs 32% for desethylamiodarone at 10 mg/kg, p < .001; 88% for amiodarone vs 60% for desethylamiodarone at 25 mg/kg, p < .01). AVCT increased to a similar degree after both drugs. Increases in AVCT caused by amiodarone and its metabolite were markedly frequency dependent (figure 2). Frequency-dependent changes in AVCT resulting from these agents were due solely to alterations in AH interval (figure 3).

**Discussion**

We have demonstrated that a single dose of desethylamiodarone has important electrophysiologic effects, including frequency-dependent slowing of intraventricular and AV conduction, as well as prolongation of atrial and ventricular refractoriness. In contrast, amiodarone's most marked effects were due to

\[
\text{FIGURE 2. AVCT as a function of basic cycle length. The right atrium was paced at each cycle length for 2 min and AVCT was measured from the stimulus artifact to the onset of the QRS complex, both under control conditions and after infusion of drug. Values are expressed as percent change of AVCT relative to control ± SD at each cycle length examined. Data shown are from nine experiments with 10 mg/kg amiodarone (A10), six experiments with 25 mg/kg amiodarone (A25), six experiments with 10 mg/kg desethylamiodarone (D10), and five experiments with 25 mg/kg desethylamiodarone (D25). *p < .05; **p < .01; ***p < .001, all vs % change observed at a basic cycle length of 1000 msec.}
\]

\[
\text{FIGURE 3. Analogue records showing changes in AH interval and AVCT as a function of basic cycle length (BCL) after infusion of amiodarone, 25 mg/kg iv. AVCT was defined as the time from the stimulus artifact to the onset of ventricular activation on the surface ECG. As BCL was reduced, the AVCT and AH intervals increased. Over the range of cycle lengths studied, AVCT increased from 152 msec (at BCL 1000 msec) to 196 msec (at BCL 400 msec) and the AH interval increased from 76 msec (at BCL 1000 msec) to 116 msec (at BCL 400 msec). In this experiment, as in four others monitoring the His bundle electrogram, all frequency-dependent changes in AVCT in the presence of amiodarone and its metabolite were caused by changes in AH interval. The horizontal calibration indicates the length of recording corresponding to 100 msec of elapsed time.}
\]
depression of AV nodal properties, with smaller changes seen in intraventricular conduction and refractoriness.

These findings have important potential implications for understanding the mechanism of amiodarone’s long-term effects. Significant serum concentrations of desethylamiodarone are attained within 1 week of high-dose amiodarone therapy and increase thereafter.23 Myocardial concentrations of the metabolite exceed those of the parent drug after long-term therapy,22, 23, 30 achieving concentrations comparable to those produced by high-dose desethylamiodarone in the present study. In view of the observed effects of acutely administered desethylamiodarone, delayed electrophysiologic effects caused by accumulation of this metabolite during long-term amiodarone therapy would be expected. Metabolite accumulation could account for the intraventricular conduction slowing, reflected by QRS widening and HV prolongation, reported in patients receiving long-term therapy with amiodarone6-15, 31 despite the much smaller changes in ventricular conduction seen after a single dose of amiodarone.9, 10, 17 Increases in atrial and ventricular refractoriness occurring after 2 to 4 weeks of amiodarone therapy, with little or no change after intravenous administration,8-15, 31 could also be attributed to accumulation of desethylamiodarone. We found, as have others,9, 10, 17, 18 that a single dose of amiodarone has strong effects on AV nodal conduction. Nonetheless, desethylamiodarone also depressed AV conduction in our dogs, possibly explaining the further AV conduction slowing observed with long-term (compared with short-term) amiodarone therapy.32

We found that both desethylamiodarone and amiodarone caused larger increases in atrial refractoriness than in ventricular refractoriness. Similar differences in tissue sensitivity to amiodarone have been noted in previous experimental33 and clinical studies.8, 9 The mechanism underlying these differences is unknown. Prolongation of the refractory period by amiodarone or desethylamiodarone may occur by several mechanisms, including delayed recovery from inactivation of inward sodium current and prolongation of action potential duration.

Amiodarone and desethylamiodarone depress $V_{\text{max}}$ of cardiac Purkinje and ventricular muscle fibers in a frequency-dependent manner,33-35 presumably because of use-dependent sodium-channel blockade. Ventricular conduction slowing, manifested as QRS prolongation, was frequency dependent after the administration of both amiodarone and desethylamiodarone to our dogs (figure 1). Although the relationship between drug-induced changes in $V_{\text{max}}$ in vitro and ventricular conduction in vivo is as yet unclear, a previous study has shown parallel time dependence for amitriptyline-induced changes in $V_{\text{max}}$ and QRS duration.36 Changes in AVCT (figure 2) and AH interval (figure 3) resulting from amiodarone and its metabolite were also frequency dependent. Calcium-channel blockers cause similar rate-related changes in AV conduction in anesthetized dogs27 with time dependence similar to that of drug-induced changes in slow inward current in vitro. The mechanism of amiodarone-induced changes in AV conduction is unknown, but there is preliminary evidence for a use-dependent slow-channel blocking effect.37 Since our dogs were autonomically blocked, changes in AV conduction could not be attributed to autonomic antagonism or augmentation by amiodarone or desethylamiodarone.

Desethylamiodarone alters fast-channel properties such as ventricular conduction and atrial and ventricular refractoriness to a greater extent than did amiodarone in this study. In contrast, amiodarone was more potent in increasing WBCL, an index of slow-channel properties. Evidence for similar selectivity of action of amiodarone and its metabolite has been provided by pharmacodynamic studies in rats25 and clinical studies in man.11 The mechanism for these differences is unknown. Greater myocardial accumulation of the metabolite than the parent compound, shown in this study as well as in previous investigations,23-25 may account for its greater effects on fast-channel tissue. The potencies of amiodarone and its metabolite in depressing $V_{\text{max}}$ of fast channel tissue in vitro appear to be approximately equal.37 No comparative evaluations of effects on slow-channel tissue in vitro are available.

One potentially important effect of amiodarone that we did not observe after short-term administration of either the parent drug or its metabolite was QT prolongation. The lack of change in QT interval was not due to inadequate plasma or myocardial drug concentration, since the concentrations of amiodarone and its metabolite in our dogs were similar to concentrations measured in the plasma and hearts of patients on long-term amiodarone therapy.22, 23, 30 Unchanged QT intervals after short-term intravenous amiodarone administration have also been reported by other investigators.17, 18 Although QT prolongation has been correlated with clinical efficacy14 and myocardial concentrations of amiodarone,38 it is unclear to what extent changes in repolarization are directly responsible for amiodarone’s antiarrhythmic actions. A recent clinical study demonstrated that despite reversal of amiodarone’s effects on the QT interval by triiodothyronine, no
change in antiarrhythmic effects was seen.\textsuperscript{39} Ventricular action potentials are more prolonged in animals receiving long-term amiodarone treatment than in preparations acutely superinfused with amiodarone or desethylamiodarone in vitro.\textsuperscript{33-35} Delays in repolarization probably contribute to the increase in myocardial refractoriness after long-term amiodarone therapy, both directly by delaying voltage-dependent sodium-channel recovery and indirectly by enhancing state-related sodium-channel blockade.\textsuperscript{34} The mechanism of delayed repolarization is unknown but may involve changes in thyroid metabolism.\textsuperscript{19, 20}

We produced autonomic blockade in our dogs to create a more stable preparation. Alterations in autonomic tone caused by variations in the level of anesthesia, reflex responses to drug-induced vasodilation, and release of local neurotransmitters by pacing stimuli can cause important electrophysiologic changes and mask direct drug effects. We cannot comment on the degree to which the expression of drug effects that we observed would be altered by an intact autonomic nervous system. The effects of intravenous amiodarone in our studies, however, are similar to those noted by others after single-dose intravenous administration to autonomically intact dogs.\textsuperscript{9, 10, 17, 18} Furthermore, it is uncertain whether the effects of long-term exposure to desethylamiodarone (as with oral amiodarone therapy) would be similar to those occurring after one intravenous dose. A study of the effects of long-term oral desethylamiodarone would be useful in this regard.

We have shown that desethylamiodarone has potentially important electrophysiologic effects, which differ in some ways from those of the parent compound. These observations suggest that accumulation of the desethyl metabolite contributes to the electrophysiologic changes seen during long-term amiodarone administration. The roles of desethylamiodarone in mediating the antiarrhythmic effects of long-term oral amiodarone therapy, and as an antiarrhythmic agent in its own right, remain to be established.

We thank Steven Nuara, Carol Matthews, and Melanie Davies for their technical assistance and Maria Materniak for typing the manuscript. We also wish to express our gratitude to Ayerst Pharmaceuticals Canada, Ltd., and Sanofi Corp. for providing purified amiodarone and desethylamiodarone for this study.

References


Comparative electrophysiologic effects of intravenous amiodarone and desethylamiodarone in dogs: evidence for clinically relevant activity of the metabolite.
M Talajic, M R DeRoode and S Nattel

Circulation. 1987;75:265-271
doi: 10.1161/01.CIR.75.1.265

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/75/1/265

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/