Rate-Related Electrophysiologic Effects of Long-term Administration of Amiodarone on Canine Ventricular Myocardium In Vivo

Kelley P. Anderson, MD, Richard Walker, Ted Dustman, ME, Robert L. Lux, PhD, Philip R. Ershler, PhD, Robert E. Kates, PhD, Paul M. Urie, MD, PhD

The electrophysiologic effects of amiodarone were examined in 13 dogs that received 30 g amiodarone orally during 3 weeks and compared with 13 control dogs that did not receive amiodarone. Longitudinal and transverse epicardial conduction velocities were estimated with a square array of 64 closely spaced electrodes and a computer-assisted acquisition and analysis system. Amiodarone caused a rate-dependent decrease in conduction velocity with a slightly greater effect in the longitudinal direction of propagation. Rate-related depression of conduction velocity developed rapidly after abrupt shortening of the pacing cycle length; 67% of the change occurred between the first two beats of the rapid train, and little change occurred after the 10th beat. Recovery from use-dependent depression of conduction velocity was exponential with a mean time constant of 447±172 msec in the longitudinal direction and 452±265 msec in the transverse direction. Repolarization intervals, defined as the interval between the activation time and the repolarization time in the unipolar electrograms, correlated highly with refractory period determinations in the absence and presence of amiodarone at each cycle length tested. The increase in repolarization intervals and refractory periods resulting from amiodarone treatment did not vary with cycle length. Amiodarone treatment also resulted in a significant rate-related reduction in systolic blood pressure. The systolic blood pressure in the group that received amiodarone decreased by a mean of 50±23% between steady-state pacing cycle lengths of 1,000 and 200 msec, whereas the corresponding decrease in the control group was 21±32% (p<0.05). Plasma and myocardial amiodarone and desethylamiodarone levels were comparable to those observed clinically. We conclude that long-term amiodarone administration causes rate-dependent reductions in conduction velocity and blood pressure and causes rate-independent increases in repolarization intervals. (Circulation 1989;79:948–958)

Amiodarone is an effective, widely used antiarrhythmic drug. Reduction of conduction velocity may be an important antiarrhythmic effect of amiodarone. Previous investigations showed that amiodarone decreases sodium current, reduces the maximum upstroke velocity of the action potential (Vmax) and prolongs QRS duration, but its effects on myocardial conduction velocity have not been reported. Amiodarone’s effect on sodium current and Vmax are use dependent, but discrepancies exist in the reported rates of development of use-dependent block. Mason et al delivered trains of impulses at a cycle length of 300 msec to guinea pig papillary muscles treated with amiodarone, and they found that 80% of the change in Vmax developed between the first two impulses but that a steady-state Vmax was probably not reached after 19 impulses. In contrast, using a similar stimulation protocol, Varro et al noted that steady-state block was reached after an average of only 4.2 stimuli, and Follmer et al found that steady-state block of sodium current was obtained by the second pulse with an interstimulus interval of 500 msec. Discrepancies also have been reported in the rates of recovery from block, with recovery time constants ranging from 282 to 1,600 msec. These indexes of rate-dependent effects on sodium channel block are of more than theoretical interest because they reflect the heart rates at which conduction velocity depression devel-
ops and are characteristic for a particular antiarhythmic drug.

To complicate matters further, drug-related changes observed at the cellular level may not perfectly mirror events in intact tissue. Time constants of recovery from drug-induced conduction velocity depression observed in vivo tend to be shorter than their in vitro counterparts. Moreover, myocardial conduction is anisotropic, it is faster in the direction parallel to fiber orientation and slower in the direction transverse to fiber axes. Theoretical and experimental evidence exists that the effects of sodium channel blocking agents on conduction velocity are also anisotropic, having proportionally greater depressant effects on longitudinal conduction velocity. The primary purpose of this investigation was to measure the rate-dependent effects of long-term amiodarone administration on longitudinal and transverse conduction velocity in canine left ventricular myocardium in vivo.

Alteration of repolarization is another important property of antiarrhythmic drugs. Although amiodarone is known to prolong action potential duration and refractoriness, little information exists regarding the effects of heart rate on these actions. Therefore, we determined the effects of rate on myocardial repolarization in dogs treated with long-term administration of amiodarone in doses sufficient to cause reduction of conduction velocity.

Because of their potential clinical significance, we also examined the effects of long-term amiodarone administration on normal automaticity, stimulation threshold current, and blood pressure.

Methods

Animal Preparation

Thirteen adult mongrel dogs of either sex (15–30 kg) received amiodarone 2 g/day orally, 5 days a week for 3 weeks. This dose is equivalent to the cumulative per kilogram dose that patients with severe ventricular arrhythmias receive after 6 months of treatment according to a recommended schedule. Thirteen other dogs served as controls. The animals were anesthetized with an intravenous bolus of sodium pentobarbital 30 mg/kg followed by a constant intravenous infusion of 0.03 mg/kg/min. They were then intubated and ventilated with a Harvard respirator (South Natick, Massachusetts). Arterial blood gases were obtained every 30 minutes, and pH was maintained between 7.35 and 7.45 by altering the rate and depth of ventilation. Catheters were inserted into the right femoral artery and both femoral veins for continuous arterial blood pressure monitoring, blood sampling, and drug infusion. Normal saline was continuously infused at 1 ml/kg/hr.

The heart was exposed through a median sternotomy and cradled in the pericardium. Care was taken to minimize blood loss. A quadrupolar electrode plaque was sewn to the right ventricle. Formalin was injected into the atrioventricular conduction system by a previously described method to interrupt atrioventricular conduction. An epoxy plaque with 64 flat unipolar silver electrodes (0.6-mm diameter) in an 8 by 8 array with 2-mm interelectrode distance was sewn to the left ventricle near the left anterior descending artery one third of the distance from apex to base. The unipolar electrodes were referenced to a Wilson central terminal. A flat thermistor was placed facing the left ventricular epicardium near the electrode plaque to monitor epicardial temperature. The pericardial cradle was then taken down, and the chest wall apposed and covered.

Shortly after the animal was anesthetized, a baseline rectal temperature was obtained. After the chest was closed, the ventricles were paced at 120 beats/min for 30 minutes while the core temperature returned to baseline with the aid of a thermal blanket. Pacing maneuvers and measurements were then performed. Rectal and cardiac temperatures were maintained within 0.5°C of the baseline rectal temperature throughout the experiment. These experiments conformed to the guiding principles of the American Physiological Society, and the experimental protocol was approved by the University of Utah Internal Animal Care Use Committee.

Analysis System

The input circuitry of the data acquisition and analysis system consisted of 64 differential amplifiers (input impedance greater than 10^12 Ω), each with sample and hold outputs. Electrograms were recorded at a band width of 0.03 to 500 Hz, sampled at a 1 kHz rate, multiplexed into a programmable-gain amplifier by four 16:1 multiplexers, and digitized by a 12-bit analog-to-digital converter. The digital output of the converter was sequentially multiplexed through a saturation detection circuit and into a first in–first out memory that permitted asynchronous buffering between the intermittent bursts of sampled data and the random access memory. The computer (MicroVAX II, Digital Electric, Maynard, Massachusetts) acquired the data through an optically isolated interface and then stored the data on a fixed disk. The data were transferred from the hard disk to high-speed digital magnetic tape for subsequent analysis. A smoothing procedure with parabolic least-squares fit was used to minimize effects of noise.

Conduction Velocity

Unipolar cathodal stimulation was applied through an electrode on the plaque array with the anodal input directed to a needle inserted into the right chest wall. Stimulation was performed with a World Precision Instruments (New Haven, Connecticut) interval generator and a custom constant current source with 2 msec rectangular impulses and current adjusted to twice diastolic capture threshold. Pacing thresholds were measured before each pacing sequence and were determined at the cycle length planned for that maneuver. Thresholds were determined with 0.01-mA precision.
For each experiment, two pairs of electrodes were chosen between which all conduction velocities for that experiment were calculated. The electrodes used to calculate conduction velocities were chosen by examination of the isochrone map of electrical activation. Activation time was defined as the time of the minimum negative derivative of the local potential.18 An activation map with 2-msec isochrones was constructed by the computer program based on the 63 available activation times (Figure 1). Rapid (longitudinal) and slow (transverse) directions of propagation were clearly discernible from the maps. A line was drawn from the pacing site to the outer edge of the map perpendicular to the most widely spaced isochrones (Figure 1). Longitudinal conduction velocity was calculated between two electrode sites collinear with this line by dividing the distance between them by the difference of their activation times. A second line, perpendicular to the first, was drawn through the densely spaced isochrones, and transverse conduction velocity was computed between two electrode sites collinear with the second line. For consistency, the isochrone map used to select electrodes for determining conduction velocities was constructed from data obtained during pacing at a cycle length of 500 msec. However, when isochrone maps from all pacing cycle lengths used in a given experiment were compared, the directions of maximum and minimum propagation did not differ despite rate-related changes in conduction velocities. The mean (±SD) distances between the pair of electrodes used to compute conduction velocity in the longitudinal and transverse directions were 10.3±2.5 and 6.2±1.6 mm, respectively, in the group that received amiodarone, and the distances were 9.8±1.0 and 6.7±1.1 mm, respectively, in the control group. No significant differences were found between the two groups concerning the distances over which conduction velocities were computed. The calculation of conduction velocity as we have defined it presumes that the path of propagation is direct and in the superficial epicardial layer. This cannot be proved without knowledge of activation in three dimensions. However, we carefully reviewed electrode sites for evidence of indirect propagation, which can sometimes be identified by the presence of sudden changes in the density of isochrone lines. This is most likely to occur in the transverse direction where more rapid conduction in a subepicardial layer can “preexcite” distal tissue before it is activated by the wave of excitation in the superficial tissue. Electrode site selections were adjusted to avoid such areas. For this reason, the distances between the electrodes used to compute transverse conduction velocities were shorter than those used for longitudinal conduction velocities.

The rate-dependent effects of amiodarone on conduction velocity were assessed during fixed-rate pacing at cycle lengths of 1,000, 500, 300, 250, and 200 msec. Measurements were obtained after 3 minutes of pacing at each cycle length except 200 msec, which was performed for 1 minute to avoid prolonged hypotension. Development of drug-induced rate-related conduction velocity depression was assessed by abruptly decreasing the pacing cycle length from 1,000 to 300 msec and by measuring the conduction velocities of the first 10 beats and the 600th beat of the shorter cycle length.

Recovery of conduction velocity from rate-dependent depression was examined by determining the conduction velocity of the beat after a test stimulus (S2) delivered with a variable coupling interval to the last beat of a 3-minute pacing train of cycle length 300 msec. Conduction velocity data were normalized and converted to an index of fractional depression by the equation: (θmax - θtest)/θmax, where θmax is the conduction velocity of test stimulus delivered after the longest pause obtainable, and θtest is the conduction velocity of the test impulse. To avoid the possible influence of voltage-dependent effects on conduction velocity resulting from incomplete repolarization, only test intervals 30 msec greater than the effective refractory period were used. Plots of the fractional conduction velocity versus time were fit to

---

**Figure 1.** Activation map of electrical activation sequence from 64-site plaque electrode array from a control animal. Solid circles represent the sites of the 64 electrodes (interelectrode distance 2 mm). Pacing was performed at a cycle length of 500 msec from the site indicated with an asterisk. Isochrones were drawn by the computer program with the method of linear interpolation at 2 msec intervals. The line (L) perpendicular to the widest isochrones was considered the longitudinal path. The two electrode sites (solid arrowheads) along this line were used to compute conduction velocity. A line perpendicular to the first indicates the transverse direction (T). Electrodes from which repolarization intervals and refractory periods were obtained are indicated by the open arrows.
a monoeponential function with a nonlinear least-squares parameter fitting program.\textsuperscript{19}

\textit{Repolarization Intervals and Refractory Periods}

For each experiment, two electrode sites on the plaque were chosen from which refractory periods and repolarization intervals were determined. One site was in the longitudinal direction of propagation, the other in the transverse path (Figure 1). The electrodes were chosen in an area of relatively uniform propagation to avoid acceleration and deceleration effects on repolarization.\textsuperscript{20} The repolarization time was defined as the time of maximum first derivative of the potential of the T wave of the local electrogram as previously described.\textsuperscript{16} This corresponds to the time of maximum rate of change of voltage during phase 3 of the action potential.\textsuperscript{17} The temporal correspondence between repolarization time as measured from electrograms and repolarization measured from action potentials has been shown by simultaneous measurements of intracellular and extracellular potentials.\textsuperscript{17,21} The repolarization interval for an electrode site was defined as the difference between the repolarization time and the activation time obtained at that site. Repolarization intervals and conduction velocities were obtained for the same beat during fixed-rate pacing, that is, after 3 minutes of pacing at cycle lengths of 1,000, 500, 300, and 250 msec and after 1 minute at a cycle length of 200 msec. Refractory period measurements were performed at cycle lengths of 1,000, 500, and 300 msec as follows: Fixed-rate pacing was continued without pause from the original pacing site (S\textsubscript{1}) after the 3-minute data acquisition, whereas single extrastimuli from the second site (S\textsubscript{2}) were delivered after every fourth S\textsubscript{1}. The current of S\textsubscript{2} was set to twice the diastolic stimulation threshold current. To minimize perturbation of the pacing rate, the initial S\textsubscript{1}-S\textsubscript{2} interval was set well within the refractory period and was increased by 1-msec intervals until a propagated response occurred. The S\textsubscript{2} interval was then decreased by 10 msec, and the process was repeated twice. If the disparity of the three S\textsubscript{1}-S\textsubscript{2} intervals that just captured the ventricles was greater than 2 msec (less than 1\% of determinations), then five determinations were obtained. The refractory period of the S\textsubscript{2} site was defined as the mean S\textsubscript{1}-S\textsubscript{2} interval that produced a propagated response minus the activation time at that site.

\textit{Stimulation Threshold Current}

Stimulation threshold current was measured at each cycle length (1,000, 500, 300, 250, and 200 msec). Pacing was initiated at a current of 0.1 mA and then reduced in 0.01 mA intervals until one-to-one capture was lost. The lowest current resulting in one-to-one capture was defined as the stimulation threshold at a given cycle length. This method of stimulation did not result in injury currents or local T wave changes.\textsuperscript{22} The output of the current source was verified by displaying the voltage drop across a calibrated resistor on a Tektronix 7326 oscilloscope (Beaverton, Oregon) and found to be within 5\% of the indicated value.

\textit{Automaticity}

One hour after the production of complete atrioventricular block, external ventricular pacing was terminated, and the native rhythm was allowed to continue for 1 minute. The mean PP and RR intervals of the last 15 seconds of the native rhythm were defined as the sinus and escape cycle lengths, respectively.

\textit{Blood Pressure}

Blood pressures were measured from a large-bore catheter (1.0 mm i.d.) placed in the femoral artery connected to a Gould-Statham P23 transducer (Cleveland, Ohio) with a short (6 in.) stiff arterial tube. Signals from the pressure transducer were amplified by a Gould pressure processor and recorded on a Gould ES1000 electrostatic recorder. Systolic and diastolic pressures were obtained after 3 minutes at each pacing cycle length (1 minute at cycle length of 200 msec). The mean of 10 beats was used for each pressure.

\textit{Histology}

Upon termination of the pacing maneuvers, the animal was killed, and the position of the electrode plaque was marked with sutures. The heart was removed and fixed in a buffered solution of 4\% formaldehyde for at least 1 week. The tissue beneath the electrode plaque was excised, and the edge corresponding to the first column of electrodes was marked with India ink. The tissue was embedded in paraffin, sectioned parallel to the epicardial plane, and stained with hematoxylin and eosin. The angle between the myocardial fiber axes of the most superficial layer and a line perpendicular to the marked edge was estimated. This was compared with the angle between the two electrodes used for calculating longitudinal conduction velocity and a line perpendicular to the first column of electrodes. The mean difference between these two angles was 4.9±3.4° in the group that received amiodarone and was 11.9±10.3° in the control group; the differences were not significant. The small differences between these angles indicates that the method of determining the longitudinal direction of propagation described above reflects histologically determined fiber orientation.

\textit{Serum and Myocardial Amiodarone Concentration}

In the animals that received amiodarone, a sample of blood was withdrawn into a heparinized tube shortly after insertion of the arterial catheter. The sample was centrifuged at 10° C, and the serum was removed and frozen. After termination of the experiment, a cubic centimeter of left ventricular myocardial tissue was excised and frozen. Amiodarone was measured in plasma and myocardial tissue homogenates by a modification of a previously
reported high-pressure liquid chromatographic procedure. To aliquots of plasma (0.5 ml) 0.5 µg internal standard (L8040) and 2 ml phosphate buffer (pH 5.4) were added. This was then extracted twice with 5 ml hexane. The aqueous phase was discarded, and the pooled organic phases were evaporated to dryness at 35°C under nitrogen. The residue was then redissolved in 0.1 ml methanol, and an aliquot (20–50 µl) was injected onto the column. The 2 ml aliquot resulting from tissue homogenation was evaporated to dryness, and the residue was redissolved in 2 ml phosphate buffer (pH 5.4) and extracted in the same manner as described for plasma. Based on peak height comparisons with nonextracted standards, amiodarone and desethylamiodarone were extracted better than 85% from plasma and myocardium.

Statistical Analysis

Two-tailed Student’s t tests for independent samples were used to compare values between the treated and untreated animals. Paired t tests were used for within-groups comparisons. Analysis of variance was used to assess the differences between treated and untreated groups when repeated measures at different cycle lengths were used. Analysis of covariance was used to determine the significance of the difference between refractory periods and repolarization intervals. Data are mean ± SD.

Results

Rate-Dependent Effects on Conduction Velocity

The effects of fixed-cycle length pacing were compared in 13 dogs that received amiodarone and in 13 controls that did not receive amiodarone (Figure 2). Longitudinal and transverse conduction velocities did not vary significantly with cycle length in the control dogs. In contrast, animals that received amiodarone showed a significant rate-dependent reduction of both longitudinal and transverse conduction velocities. The mean longitudinal conduction velocity during a cycle length of 1,000 msec in the dogs that received amiodarone (0.61 ± 0.02 m/sec) was significantly lower than the value obtained in control animals (0.68 ± 0.09 m/sec, p = 0.02), indicating some drug-induced slowing of longitudinal conduction at the longest cycle length. The percent decreases in longitudinal and transverse conduction velocities are shown in Figure 3. The percent change of conduction velocity was consistently greater in the longitudinal direction, but the differences were not statistically significant (p > 0.1 by ANOVA).

Development of Conduction Velocity Depression

In six control animals, conduction velocity did not vary for the first 10 beats when the pacing cycle length was abruptly decreased from 1,000 to 300 msec but decreased slightly, 5–8%, by 600 beats. In six animals treated with amiodarone, the longitudinal conduction velocity decreased sharply during the first 10 beats of the rapid train, and 67% of the eventual change occurred between the first and second beats of the rapid train, 27% between the second and 10th beats, and 6% between the 10th and 600th beats (Figure 4). The time course of transverse conduction velocity depression paralleled that of longitudinal conduction velocity.
Recovery of Conduction Velocity

Recovery of conduction velocity from rate-dependent depression was examined in 10 dogs that received amiodarone and in eight controls. In control animals, depression of conduction velocity did not occur except at test intervals close to the refractory period (Figure 5, top). In dogs that received amiodarone, conduction velocity was depressed at short test intervals and recovered progressively at longer test intervals (Figure 5, bottom). The mean time constant ($\tau$) for recovery of conduction velocity in the animals treated with amiodarone was $447 \pm 172$ msec in the longitudinal direction and $452 \pm 265$ msec in the transverse direction. These values were not significantly different from each other.

Repolarization Interval and Refractory Period

The correlations between repolarization intervals and refractory periods were very high ($r=0.99$) for both controls and animals treated with amiodarone (Figure 6). The slopes of the regression lines for the two groups of animals were close to unity. The regression line for the group that received amiodarone was shifted slightly, but significantly, to the right of the control line ($p<0.001$). This shift indicates a slightly longer refractory period for a given repolarization interval in the animals treated with amiodarone. This effect is also reflected in the ratio of refractory period to repolarization interval, which was $0.986 \pm 0.027$ in the control group and $1.015 \pm 0.026$ in the amiodarone group ($p<0.0001$).

Rate-Dependent Effects on Repolarization Interval

Repolarization intervals increased with increasing pacing cycle lengths in the control animals (Figure 7). The relation between repolarization interval and cycle length of the animals that received amiodarone paralleled that of the controls, but repolarization intervals remained significantly greater compared with controls at all cycle lengths. On average, the group that received amiodarone showed an increase in repolarization of 17 msec over the control group.

Stimulation Threshold Current

No significant relation was found between pacing cycle length and stimulation threshold current in the control group or in the group that received amiodarone. The mean stimulation threshold in the controls was $0.027 \pm 0.011$ mA and was $0.024 \pm 0.007$ in the group treated with amiodarone ($p>0.1$).

Automaticity

After interruption of atrioventricular conduction, all animals developed a stable escape rhythm with uniform QRS complexes. The sinus cycle length was greater in the group that received amiodarone ($524 \pm 79$ msec) than in the control group ($334 \pm 35$ msec, $p<0.0001$). The cycle length of ventricular escape rhythm was longer in the treated group ($1,626 \pm 463$ msec) than in the control group ($1,168 \pm 318$ msec, $p=0.02$).

Blood Pressure

The animals that received amiodarone showed a significantly greater rate-dependent effect on both systolic and diastolic blood pressures compared with control animals (Figure 8).

Plasma and Myocardial Concentrations

The mean plasma amiodarone and desethylamiodarone levels were $3.8 \pm 3.2$ and $0.38 \pm 0.28$ $\mu$g/ml, respectively. The mean myocardial levels of amiodarone and desethylamiodarone were $104.0 \pm 78.3$. 
FIGURE 5. Plots of conduction velocities in the longitudinal and transverse directions were obtained for a beat delivered after a variable test interval after pacing at a cycle length of 300 msec. Top panel: In a control animal, longitudinal and transverse conduction velocities did not change with test interval. Bottom panel: In an animal treated with amiodarone, conduction velocities were depressed at short test intervals. Data were fit to an exponential function. Time constants of recovery (τ) were 464 msec for longitudinal conduction velocity and 487 msec for transverse conduction velocity.

and 66.0±42.6 μg/g, respectively. A correlation existed between plasma and myocardial concentrations of amiodarone (r=0.84, p<0.01), between plasma and myocardial desethylamiodarone concentrations (r=0.95, p<0.01), between plasma amiodarone and desethylamiodarone levels (r=0.80, p<0.01), and between myocardial amiodarone and desethylamiodarone levels (r=0.91, p<0.01). Because of the strong intercorrelation of drug levels, variables that correlated with one concentration variable tended to correlate with all.

Myocardial amiodarone concentration correlated with the percent decrease in the steady-state longitudinal conduction velocity when the pacing cycle length was changed from 1,000 to 200 msec (r=0.83, p<0.01). Myocardial amiodarone concentration also correlated with the drop in systolic blood pressure when the stimulation cycle length was changed

from 1,000 to 200 msec (r=0.74, p<0.05). No significant correlations were found between drug levels and recovery time constants, repolarization intervals, sinus cycle lengths, or escape cycle lengths.

**Discussion**

**Rate-Dependent Effects on Conduction Velocity**

Drug-induced depression of conduction velocity may be an important antiarrhythmic mechanism as
well as a potential cause of toxicity. Our findings indicate that amiodarone has the desirable characteristic of minimal conduction velocity depression at typical resting heart rates and significant depression at rates typical of tachyarrhythmias. Amiodarone did not reduce transverse conduction velocity at a pacing cycle length of 1,000 msec but caused significant reduction in the longitudinal direction. We could not determine whether or not the effect of amiodarone on longitudinal conduction velocity at this cycle length was a tonic or use-dependent effect. Previous investigations showed rate-dependent changes in variables that are related to conduction velocity. Most studies have shown little or no depression of \( V_{\text{max}} \) by amiodarone at stimulation cycle lengths of 1,000 msec but the effects of amiodarone 

![Plot of repolarization intervals versus pacing cycle lengths for 13 animals treated with amiodarone and 13 control animals. Repolarization intervals of the group treated with amiodarone are significantly greater than those of control animals (\( p<0.0001 \)), but the slopes of the curves are not significantly different. Error bars indicate SEM.](http://circ.ahajournals.org/content/113/11/e335.F7.large.png)

**Figure 7.** Plot of repolarization intervals versus pacing cycle lengths for 13 animals treated with amiodarone and 13 control animals. Repolarization intervals of the group treated with amiodarone are significantly greater than those of control animals (\( p<0.0001 \)), but the slopes of the curves are not significantly different. Error bars indicate SEM.

been observed for drugs blocking open sodium channels is not inconsistent with the theory of Spach et al., who hypothesize that longitudinal propagation increases the time that sodium channels are open compared with transverse propagation, thus permitting greater open sodium channel blockade.

**Development of Conduction Velocity Depression**

Because the onset of tachyarrhythmias is usually abrupt, the rapidity with which use-dependent block develops may be related to antiarrhythmic efficacy. This property was assessed by shortening the pacing cycle length from 1,000 to 300 msec. Under the influence of amiodarone, conduction velocity decreased 16% after 3 minutes of pacing at a cycle length of 300 msec. Most of this decrease (67%) occurred between the first and second beats, and 94% of the change occurred by 10 beats. These findings are comparable with measurements by Mason et al. of \( V_{\text{max}} \) in papillary muscles of guinea pigs treated with short-term and long-term administration of amiodarone in which stimulation at a cycle length of 300 msec was initiated after a

![Bar graphs of rate-related effects of amiodarone on blood pressure. Top panel: Systolic blood pressure versus pacing cycle length in animals treated with amiodarone and in control animals. Bottom panel: Diastolic blood pressure versus pacing cycle length. Animals treated with amiodarone developed significantly greater reductions in systolic (\( p=0.0002 \) by ANOVA) and diastolic (\( p<0.0001 \)) blood pressures with respect to pacing cycle length compared with control animals. Error bars indicate SEM.](http://circ.ahajournals.org/content/113/11/e335.F8.large.png)

**Figure 8.** Bar graphs of rate-related effects of amiodarone on blood pressure. Top panel: Systolic blood pressure versus pacing cycle length in animals treated with amiodarone and in control animals. Bottom panel: Diastolic blood pressure versus pacing cycle length. Animals treated with amiodarone developed significantly greater reductions in systolic (\( p=0.0002 \) by ANOVA) and diastolic (\( p<0.0001 \)) blood pressures with respect to pacing cycle length compared with control animals. Error bars indicate SEM.
20-second rest period. In this study, 80% of the 
reduction of $V_{\text{max}}$ occurred between the first two 
beats of the train, and 13% occurred between the 
second and 16th beats, although steady state was 
probably not reached by the end of the 19-beat 
train. Varro et al detected more rapid onset 
of block (steady state in 4.2 beats) in canine Purkinje 
fibers and guinea pig ventricular fibers during short-
term perfusion with amiodarone. Even more rapid 
onset of sodium channel block (steady state in 1-2 
beats) has been shown after short-term application 
of amiodarone in isolated canine Purkinje fibers and 
feline ventricular myocytes with the suction pipette 
voltage clamp technique. The large differences in 
the experimental preparations may account for the 
differences in the number of impulses required to 
reach steady state. However, it is agreed that most 
of the drug effect occurs between the first and 
second beats of the rapid train and that little further 
change occurs after the tenth beat.

Recovery From Conduction Velocity Depression

The time constant of recovery from use-
dependent conduction velocity depression due to 
amiodarone was about 450 msec in our preparation. 
The fact that this value was independent of propa-
gation direction suggests that the kinetics of drug 
unbinding was isotropic. A recovery time constant 
of 1,600 msec was obtained in sucrose gap prepara-
tions of guinea pig papillary muscles in which $V_{\text{max}}$ 
was used as an index of sodium current. The same 
time constant was observed in preparations perfu-
sed with amiodarone and from animals treated 
with long-term administration. However, shorter 
time constants (285 msec) were observed in another 
study of guinea pig papillary muscles and in canine 
Purkinje fibers. The discrepancy between these two 
studies could be attributed, in part, to differences in 
resting potentials (80-85 mV in the former study, 
>90 mV in the latter) because lower resting poten-
tials will prolong recovery. The most precise 
determinations of recovery time constants are prob-
able obtained from direct measurements of sodium 
current because the magnitude of change is greater. 
Follmer et al performed such measurements in 
isolated canine Purkinje cells and feline ventricular 
myocytes. The relevant time constant of recovery 
from amiodarone-induced sodium channel block for 
both preparations was 1,430 msec. However, these 
measurements were obtained at 20° C, which prob-
ably results in an overestimate of two to four times 
the recovery time constant at 37° C. For instance, 
the recovery time constant of lidocaine at 36° C is 
180 msec, whereas at 24° C it is 400-750 msec.

Effects on Repolarization and Refractoriness

Analytic derivations and experimental evidence 
support the temporal coincidence of the rapid phase 
of repolarization of the action potential and the 
maximum first derivative of the extracellular poten-
tial of the local T wave. This relation has been 
found to hold for acute ischemia, a range of heart 
rates and local temperature changes. A high correla-
tion has been shown between repolarization interval 
and refractory period in the presence and absence of 
sympathetic stimulation. In the present study, we 
showed that the high correlation between repolariza-
tion intervals and refractory periods is retained in 
the presence of amiodarone. Thus, rate-related changes 
in repolarization intervals precisely paralleled those 
of refractory periods at the cycle lengths tested.

Previous studies documented that long-term 
administration of amiodarone markedly prolongs 
action potential duration and refractory periods. 
However, the effect of cycle length on these vari-
ables has been less well studied. Amiodarone 
increased repolarization interval during steady-
state pacing at rates that span the range of typical 
resting and tachycardiac heart rates.

Stimulation Threshold Current

Amiodarone had no effect on stimulation thresh-
old, which indicates that it should not increase the 
energy requirements of artificial pacemakers. 
Although sodium current blockade can decrease 
excitability, the resolution of our measurements 
(0.01 mA, ±5%) may not have been sufficient to 
detect a use-dependent change in threshold current. 
Another possibility is that amiodarone affects dif-
ferent determinants of excitability in opposite direc-
tions. Such an effect has been shown for enca
caine. One previous study found that amiodarone 
no effect on pacing thresholds in dogs.

Amiodarone slightly, but significantly, increased 
the duration of refractory period with respect to 
repolarization interval, which reflects a decrease in 
another aspect of excitability. Two previous inves-
tigations did not detect a significant increase in the 
ratio of effective refractory period to action poten-
tial duration but a trend was noted in one of the 
studies. The small magnitude of the effect and the 
larger numbers studied in our investigation may 
account for the discrepancy.

Automaticity

Long-term amiodarone administration signifi-
cantly increased the sinus cycle length in the anes-
thesitized open-chest canine preparation by 
57%. Depression of sinus node automaticity has been 
observed in numerous investigations. We also 
observed a 40% increase in the escape rhythm after 
complete atrioventricular block, a previously unre-
ported observation.

Blood Pressure

We observed a significant decrease in systolic 
blood pressure with increasing stimulation rates in 
the animals that received amiodarone, an effect not 
 presente in the control group except at the fastest 
pacing rates. Although the hemodynamic effects of 
amiodarone have been studied, its rate-dependent 
hemodynamic properties have not, to our knowledge,
been examined previously. Our experiments were not designed to determine the mechanism of amiodarone’s effects on blood pressure, but several known effects could affect blood pressure. Amiodarone is a vasodilator,1 and the treated animals had lower systolic and diastolic blood pressures at slow pacing rates, although the differences were not significant. Amiodarone also inhibits adrenergic stimulation.1 This could blunt normal adrenergic influences that maintain blood pressure at faster heart rates. Amiodarone causes use-dependent block of calcium channels in cardiac tissue,35,36 which could cause use-dependent decreases in contractility.37 The fact that mean systolic blood pressure in the animals treated with amiodarone was slightly lower than in controls at a cycle length of 1,000 msec, but dropped significantly with shorter cycle lengths, is consistent with the reported time constant of recovery from amiodarone-induced block of calcium currents of 1,000 msec.36 Amiodarone also blocks sodium channels in a use-dependent fashion. In theory, resultant decreases in intracellular sodium ion activity could increase loss of intracellular calcium ions by the sodium-calcium exchange mechanism, thereby reducing contractility.38 Whatever the mechanism, rate-dependent hemodynamic effects would be largely undesirable in the clinical setting. The greatest changes were observed at the most rapid pacing rates. Therefore, amiodarone therapy could increase hemodynamic deterioration at a given rate of tachycardia. Moreover, decreases in blood pressure were noted even in the physiologic range of heart rates, that is, from 60 to 120 beats/min. This could result in clinically significant deterioration in a patient with severely compromised heart function.

**Plasma and Myocardial Drug Levels**

The doses of amiodarone we administered were similar to those that patients with severe ventricular arrhythmias receive during 6 or more months.14 The plasma and myocardial amiodarone and desethylamiodarone levels detected in our study were in the same range as those found in patients on long-term amiodarone therapy.38-41 However, the mean plasma amiodarone level in our study was about twice the mean level typically found in humans, and the mean desethylamiodarone level was about half.39-42 Plasma amiodarone and desethylamiodarone levels correlated with myocardial levels and with each other. This probably reflects relative equilibrium between the compartments and between the parent drug and its metabolite.

Previous investigations have supported42,43 and rejected26 a relation between drug levels and electrophysiologic effects. We found a modest correlation between drug levels and rate-dependent effects on conduction velocity and the rate-related reduction in blood pressure. However, no significant correlation was found between drug levels and repolarization intervals and sinus or escape cycle lengths even though amiodarone significantly prolonged these variables. The lack of correlation with these variables could be due to the large variation between individual animals.43

**Limitations**

We estimated conduction velocity between two electrodes on the epicardial surface of the left ventricle. The path of propagation between the two electrodes was assumed to be direct, which is an assumption that can be verified only by examining activation in three dimensions. On the other hand, complete knowledge of activation in this small volume would require the insertion of multiple electrodes that could cause injury and disturb activation. Our methods provided detailed two-dimensional knowledge of activation over a small (1.6 cm²) area of myocardium with minimal likelihood of perturbing cellular function or structure. Determination of longitudinal and transverse paths of propagation and the choices of electrode sites were guided by the electrical activation sequence throughout the area. The estimates of longitudinal propagation direction correlated closely with histologic determination of myocardial fiber orientation. Although conduction times rather than conduction velocities could be used to avoid specification of the path of propagation, this alternative would not eliminate the necessity that the path be unchanged between maneuvers and would not change the interpretation of the experimental results. Our control values for longitudinal and transverse conduction velocities were similar to those reported previously.10,13,44

Our experimental model permitted examination of a variety of variables relevant to the use of antiarrhythmic drugs. The unconscious state, the anesthetic used, the thoracotomy, and numerous unknown variables could have affected our results, but a more important limitation is that our model does not reproduce the myocardial disturbances that accompany clinical arrhythmias. On the other hand, the fact that our measurements were obtained in the intact heart strengthens the assumption that similar phenomena occur in the clinical setting.

**Acknowledgments**

We acknowledge the invaluable assistance of Robert Krall and David Moddrelle in collection of the experimental data, Karen Allen for preparation of the manuscript, Ronald Moddrelle, for statistical advice, and Jay W. Mason, MD, for his critique of the manuscript.

**References**

properties of isolated cardiac muscle. J Cardiovasc Pharma- 
col 1986;8:197–207
5. Varro A, Nakaya Y, Elharrar V, Surawicz B: Use- 
dependent effects of amiodarone on Vmax in cardiac Purk-
je and ventricular muscle fibers. Eur J Pharmacol 1985; 
112:419–422
6. Cobbe SM, Manley BS: Cellular electrophysiology of amio-
darone in cardiac ischemia. Br J Clin Pract 1986; (symp-
 supp)44:104–108
7. Talajic M, DeRoode MR, Nattel S: Comparative electroph-
ysiologic effects of intravenous amiodarone and desethy-
amiodarone in dogs: Evidence for clinically relevant ac-
dependence of amiodarone during the sinus tachycardia of 
9. Morady F, DiCarlo LA Jr, Krol RB, Baerman JM, de Buitleir 
M: Acute and chronic effects of amiodarone on ventricular 
refractoriness, intraventricular conduction and ventricular 
RL, Roden DM: Frequency- and orientation-dependent 
effects of mexiletine and quinidine on conduction in the 
intact dog heart. Circulation 1987;75:1065–1073
11. Spach MS, Miller WT, Geselowitz DB, Barr RC, Kootsey 
JM, Johnson EA: The discontinuous nature of propagation 
in normal canine cardiac muscle: Evidence for recurrent 
discontinuities of intracellular resistance that affect mem-
12. Spach MS, Dolber PC, Heidlage JF, Kootsey JM, Johnson 
EA: Propagating depolarization in anisotropic human and 
canine cardiac muscle: Apparent directional differences in 
13. Kadish AH, Spear JF, Levine JH, Moore EN: The effects of 
propranolol on conduction in anisotropic canine ventricu-
lar myocardium. Circulation 1986;74:616–625
14. Siddoway LA, McAllister CB, Wilkinson GR, Roden DM, 
Woosley RL: Amiodarone dosing: A proposal based on its 
pharmacokinetics. Am Heart J 1983;106:951–956
15. Steiner C, Kovalik ATW: A simple technique for produc-
tion of chronic complete heart block in dogs. J Appl Physiol 1968; 
25:613–632
16. Millar CK, Kralios FA, Lux RL: Correlation between refrac-
tory periods and activation-recovery intervals from electro-
grams: Effects of rate and adrenergic interventions. Circu-
lation 1985;72:1372–1379
17. Haws CW, Lux RL: Correlation between activation in 
transmembrane 
action potential durations and activation-recovery 
intervals from electrograms: Effects of interventions which 
after repolarization time. Circulation (in press)
18. Spach MS, Dolber PC: Relationing extracellular potentials 
and their derivatives to anisotropic propagation at a microsco-
19. Minsky: Nonlinear Parameter Estimation. Salt Lake City, 
20. Burgess MJ, Steinhaus BM, Spitzer DW, Ersheiler PR: Non-
linear epicardial activation and repolarization speci-
21. Wyatt RF: Comparison of estimates of activation and recov-
ery times from bipolar and unipolar electrograms to in vivo 
New York, McGraw-Hill, 1960, p 212
23. Laks RJ, Connolly ST, Kates RE: Myocardial disposition of 
amiodarone in the dog. J Pharmacol Exp Ther 1983; 
224:603–608
24. Hondegem LM: Antiarrhythmic agents: Modulated recep-
on the electrophysiologic properties of isolated neonatal 
and adult cardiac fibers. J Am Coll Cardiol 1985;5:1109–1115
Singh BN: Electrophysiologic effects of desethylamio-
darone, an active metabolite of amiodarone: Comparison 
with amiodarone during chronic administration in rabbits. 
Am Heart J 1988;115:351–359
27. Shensa M, Denker S, Mahmud R, Lehmann M, Estrada A, 
Akhtar M: Effects of amiodarone on conduction and refrac-
toriness of the His-Purkinje system in the human heart. J Am 
Coll Cardiol 1984;4:105–110
modulated receptor mechanism of action of sodium and 
calcium channel-blocking drugs. Annu Rev Pharmacol Toxic-
ol 1984;24:387–423
29. Cohen CJ, Bean BP, Tsien RW: Maximal upstroke velocity 
as an index of available sodium conductance. Circ Res 1984; 
54:636–651
30. Clarkson CW, Hondegem LM: Evidence for a specific 
receptor site for lidocaine, quinidine, and disopyramide 
associated with cardiac sodium channels in guinea pig ventricular 
31. Sanchez-Chapula J, Tsuda Y, Josephson IR: Voltage- and 
use-dependent effects of lidocaine on sodium current in rat 
32. Arnsdorf MF: Cable properties and conduction of the 
action potential, in Sperelakis N (ed): Physiology and Pathophys-
33. Arnsdorf MF, Schmidt GA, Sawicki GJ: Effects of encainide 
on the determinants of cardiac excitability in sheep Purkinje 
34. Huang SK, Hedberg PS, Marcus FI: Effects of antiarrhyth-
mic drugs on the cardiac pacing threshold and the endocar-
dial R wave amplitude in the conscious dog. PACE 1986; 
9:651–669
35. Nishimura M, Follmer CH, Cigan AL, Yeh JZ, Singer DH: 
Amiodarone blocks Ca++ current in guinea pig ventricular 
36. Nattel S, Talajic M, Quantz M, DeRoode M: Frequency-
dependent effects of amiodarone on atrioventricular nodal 
function and slow-channel action potentials: Evidence for 
calcium channel-blocking activity. Circulation 1987; 
76:442–449
37. Chappell SP, Henderson AH, Lewis MJ: Frequency-
dependent depression of myocardial contractility by slow 
calcium channel blocking drugs. Eur J Pharmacol 1985; 
110:129–132
38. Fozzard HA, Wasserstrom JA: Voltage dependence of intra-
cellular sodium and control of contraction, in Zipes DP, 
Jalife J (eds): Cardiac Electrophysiology and Arrhythmias. 
Orlando, Fla, Grune & Stratton, 1981, pp 51–57
39. Baerman JM, Annesley T, DiCarlo LA Jr, Foley MK, 
Nicklas JM, Crevey BJ, Morady F: Interrelationships 
between serum levels of amiodarone, desethylamiodarone, 
reverse T3 and the QT interval during long-term amiodarone 
40. Holt DW, Tucker GT, Jackson PR, Storey GCA: Amio-
darone pharmacokinetics. Am Heart J 1985;106:840–847
41. Adams PC, Holt DW, Storey GCA, Morley AR, Callaghan J, 
Campbell RWF: Amiodarone and its desethyl metabolite: 
Tissue distribution and morphologic changes during long-
42. Latini R, Tognoni G, Kates RE: Clinical pharmacokinetics 
43. Connolly SJ, Latini R, Kates RE: Pharmacodynamics of 
intravenous amiodarone in the dog. J Cardiovasc Pharmacol 
1984;6:531–535
44. Kuo C, Atarashi H, Reddy CP, Surawicz B: Dispersion of 
ventricular repolarization and arrhythmia: Study of two 
consecutive ventricular premature complexes. Circulation 
1985;72:370–376

KEY WORDS: conduction velocity, repolarization, refractoriness, 
automatcity
Rate-related electrophysiologic effects of long-term administration of amiodarone on canine ventricular myocardium in vivo.

K P Anderson, R Walker, T Dustman, R L Lux, P R Ershler, R E Kates and P M Urie

Circulation. 1989;79:948-958
doi: 10.1161/01.CIR.79.4.948
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 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/79/4/948

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/