The effects of doxorubicin on ventricular tachycardia

HERVE LE MARREC, M.D.,* WALTER SPINELLI, PH.D., AND MICHAEL R. ROSEN, M.D.

ABSTRACT Doxorubicin, in concentrations that have no effect on fast or slow response action potentials, has been shown to suppress ouabain-induced delayed afterdepolarizations. In this study, we used standard microelectrode techniques to determine the effects of doxorubicin on isolated canine Purkinje fibers. We studied automaticity induced at normal and low membrane potentials, conduction in normal and K⁺-depolarized Purkinje fibers, and triggered activity induced by ouabain and by experimental myocardial infarction. Doxorubicin, 50 μM, suppressed the triggered activity and the delayed afterdepolarizations that induced it, but had no effect on the other variables. We then studied the effects of intravenous doxorubicin, 16 to 64 mg/m² body surface area, on ouabain-induced ventricular tachycardia and the ventricular tachycardia that occurs 24 hr after ligation of the left anterior descending coronary artery in the intact dog. There was no effect on the infarct-induced arrhythmia, but concentrations of doxorubicin that had no other effect on the electrocardiogram suppressed those ouabain-induced arrhythmias that appeared to have been triggered. The automatic arrhythmias induced by ouabain were not affected. Both the latter mechanisms were verified in studies of isolated Purkinje fibers that were obtained on completion of the intact animal experiments. These results indicate that agents having high selectivity for specific arrhythmogenic mechanisms can be useful adjuncts in discriminating among the mechanisms responsible for arrhythmias in intact animals. Circulation 74, No. 4, 881–889, 1986.

BECAUSE the characteristic responses to programmed electrical stimulation of such disparate mechanisms as reentry, triggered activity, and automaticity at low levels of membrane potential may at times be overlapping, the use of pacing alone to discriminate among them can be misleading (see references 1 to 4 for detailed discussion). For this reason, we and others have been using electrophysiologic testing and matrixes of drugs in an attempt to identify mechanisms with a greater degree of accuracy in experimental animals and in patients. Although results with this approach have been promising, its use has been limited by the fact that most drugs act via more than one mechanism, thereby rendering discrimination among mechanisms difficult. Here we report results with the anthracycline antibiotic doxorubicin, which has been shown in isolated tissue studies to predominantly modify one mechanism, delayed afterdepolarizations and resultant triggered activity. Although the doxorubicin dosages used clinically for cancer chemotherapy result in significant cardiac and noncardiac toxicity, we will demonstrate that the selectivity of this drug for a specific arrhythmogenic mechanism in isolated tissues and intact animals is such to encourage the search for other agents having comparably high selectivity and lesser toxicity.

Methods

Studies were performed in isolated cardiac fibers and in intact animals.

Isolated tissue preparation. Because a previous study had shown that doxorubicin, 50 μM, depressed delayed afterdepolarizations while not affecting either Na⁺-dependent fast responses or Ca⁺⁺-induced slow responses, our intent in the present isolated tissue studies was to test (1) whether this same concentration suppressed the triggered activity induced by digitalis or myocardial infarction and (2) whether it had any effects on normal or abnormal automaticity or conduction.

Therefore, we tested the ability of doxorubicin to modify conduction and to suppress arrhythmias in each of several preparations. To study conduction we used unbranched Purkinje fiber bundles impaled with microelectrodes and superfused with Tyrode's solution containing [K⁺]₀ = 4, 8, or 10 mM (see below). To study automaticity, we used three preparations: (1)
automaticity at low membrane potentials in subendocardial canine Purkinje fibers 24 hr after ligation of the left anterior descending artery. (2) similar automaticity at low membrane potentials in free-running Purkinje fibers superfused with BaCl2, and (3) automaticity at high membrane potentials in free-running Purkinje fibers superfused with Tyrode's solution containing [K+]0 = 2.7 mM. For studies of triggered activity induced by delayed afterdepolarizations, we used both ouabain-supersurfaced free-running Purkinje fibers and the above-mentioned 24 hr infarct.

To study myocardial infarction we used mongrel dogs of both sexes that weighed about 20 kg each and were anesthetized with 30 mg/kg iv pentobarbital Na. The dogs were intubated and artificially ventilated and under sterile conditions the left anterior descending artery of each was isolated and ligated just distal to the first septal perforator (see Harris's). The thorax was then closed and the animals were permitted to recover from surgery.

Twenty-four hours later the dogs all demonstrated sustained ventricular tachycardia on the electrocardiogram. They again were anesthetized with pentobarbital, and the hearts were excised and placed in ice-cold Tyrode's solution containing (mM): NaCl, 131; KCl, 4; NaHCO3, 18; NaH2PO4, 1.8; MgCl2, 0.5; CaCl2, 2.7; and dextrose, 5.5. The Tyrode's solution was equilibrated with 95% O2, 5% CO2. A subendocardial segment of the anteroseptal infarct measuring approximately 2 cm × 2 cm × 2 mm in depth was placed in a Lucite tissue bath perfused with Tyrode's solution warmed to 38° C and equilibrated with 95% O2–5% CO2. The bath was connected to ground via a 3M KCl-Ag-AgCl bridge.10 Bath pH was approximately 7.3 and the flow rate of the Tyrode's solution was about 12 ml/min. Bath volume was changed three times per minute. The preparations were impaled with 3M KCl-filled glass capillary electrodes having tips resistances of 10MΩ to 25MΩ. The signal was channeled through amplifiers having high input impedance and capacity neutralization to oscilloscopes and a strip-chart recorder.10

Because infarcted tissues gradually hyperpolarize while in the tissue bath,11 control measurements were made within 15 min of placing the infarcted tissue in Tyrode's solution. The spontaneously beating preparations were mapped initially with microelectrodes and bipolar silver wire electrodes to identify the earliest site of impulse initiation. A pacemaker fiber then was impaled with a microelectrode and the pacing and drug superfusion protocols were carried out.12

Our studies of conduction were performed in unbranched Purkinje fiber bundles driven at a cycle length of 800 msec. Square-wave pulses 0.5 to 1.5 msec in duration and having an amplitude about 1.5 times threshold were delivered to one end of the preparation via bipolar, Teflon-coated silver wire electrodes (see Rosen et al.13). The superfusate [K+] in individual experiments was maintained at 4, 8, or 10 mM to permit us to consider the effects of doxorubicin on conduction in normal or partially depolarized fibers. The preparations were impaled with two microelectrodes. The interelectrode distance was at least 2 mm, and the proximal electrode was at least 2 mm distal to the stimulating electrode. The action potential upstrokes and electronically differentiated maximum upstroke velocity (Vmax) of phase 010 were displayed at a rapid sweep speed on an oscilloscope and photographed. Conduction time was measured between the midpoints of the upstrokes of the action potentials and checked against the interval between the peaks of the Vmax. After superfusion with doxorubicin, the measurements were repeated. Five to 10 consecutive cycles were measured for each determination of conduction time. We did not measure conduction velocity since the preparations were resting slack in the floor of the tissue bath, a situation that does not permit the accurate determination of fiber length and velocity.13

Other isolated tissue studies of automatic preparations were performed on free-running Purkinje fiber bundles excised from the left and right ventricles and superfused with Tyrode's solution as described above. To study normal automaticity (i.e., that at high membrane potentials) we changed superfusate [K+] to 2.7 mM. To study abnormal automaticity we maintained [K+] at 4 mM and added 0.25 mM BaCl2 to the superfusate.

To study digitalis-induced triggered activity, we initially drove free-running Purkinje fibers at a basic cycle length of 500 msec during superfusion with Tyrode's having [K+] of 4 mM. Within 30 min of the onset of exposure to 2 × 10−7 M ouabain, stable triggered activity occurred. We then discontinued the ouabain because we were aware from previous studies that the rhythm associated with digitalis toxicity would persist for about 60 min in the absence of further exposure to the drug.14

**Doxorubicin protocols in isolated tissues.** In studies of normal automaticity ([K+]0 = 2.7 mM) drive was maintained throughout the protocol at a basic cycle length of 1000 msec. Periodically during control, and at 15 min intervals after the onset of doxorubicin superfusion, drive was discontinued to permit the observation of automaticity. We then overdrove the rhythm at basic cycle lengths of 600, 500, and 400 msec before returning to drive at a basic cycle length of 1000 msec.

The molecular weight of doxorubicin HCl is 580. In our initial studies of normal automaticity at high membrane potentials, 50, 100, and 200 μM doxorubicin were superfused for 30 min each. Based on results of our initial experiments with normal automaticity (see results), the 200 mM concentration was not used in subsequent experiments.

In infarcted and BaCl2-supersurfaced preparations before superfusion with doxorubicin and at 15 min intervals thereafter the following pacing protocol was performed: fibers were driven for 1 min at basic cycle lengths of 600, 500, and 400 msec. Pacing was then discontinued and the first postspacing interval and the characteristics of the rhythm in the absence of pacing were observed.

Previously described methods10, 12, 15 were used to calibrate the equipment and to make the following measurements: maximum diastolic potential, activation voltage, action potential amplitude, Vmax of phase 0, action potential duration to 50% and full repolarization (ADP50 and ADP95, respectively), and conduction time. We also measured the cycle lengths of automatic rhythms and of triggered activity.

**Studies of intact animals.** To study myocardial infarction, we used conscious dogs in which the left anterior descending coronary artery had been ligated 24 hr previously. Those animals to be studied in the conscious state were subjected to the following additional interventions during the surgery that produced the infarct. Biomer plaque electrodes,12 each containing two bipolar pairs, were sewn to the heart (one to the left atrial appendage, and one to the lateral left ventricle). Two electrocardiographic recording plaques were sewn subcutaneously in the right and left thorax. In addition, a polyethylene cannula was inserted into the left atrium through the appendage, and was held in place with a purse-string suture. The electrodes and atrial infusion cannula were exteriorized intrascapularly.

To study ouabain-induced ventricular tachycardia, dogs were instrumented as described above, but infarcts were not induced. They were permitted to recover from surgery, and 7 to 10 days later, were brought to the laboratory for study in the conscious state. Cardiac pacing was effected with a WPI stimulator, and electrocardiograms and electromograms were recorded with a strip-chart recorder and previously described techniques.12

At the start of each study, all hearts were paced via the atrial and ventricular plaques by the following protocol. Initially a drive cycle length 5% shorter than that of the spontaneous rhythm was used, followed by cycle lengths of 300, 250, and
200 msec for 1 min each. None of these dogs had a pacing-induced arrhythmia. The dogs in the ouabain protocol then were given 40 μg/kg iv ouabain as a bolus. Twenty minutes later an additional 10 μg/kg bolus was given and it was repeated every 10 min until a sustained ventricular tachycardia occurred. The hearts were then paced as described above.

Doxorubicin was administered as follows: We calculated body surface area using a nomogram and then injected 16 mg/m² of the drug through the atrial cannula. The duration of the injection was 3 min. We repeated this dose at 30 min intervals until a total of 64 mg/m² had been given. Pacing was repeated 20 to 25 min after each injection. In each of the animals we measured the sinus or tachycardia cycle length. For those in sinus rhythm we measured the PR interval, QRS duration, and QT interval as well, and calculated the corrected QT (QTc) interval. For animals with tachycardia we also tabulated the percentage of beats that were ventricular and supraventricular in origin.

The dogs with myocardial infarction-induced ventricular tachycardia underwent pacing before and after administration of doxorubicin, as described above for the digitalis-toxic dogs.

**Analysis of data.** Results are expressed as means ± SE. The effects of pacing and the actions of doxorubicin both in vivo and in vitro were expressed as curves relating recovery cycle length (interval to the first spontaneous beat after cessation of overdrive) to overdrive pacing cycle length. We analyzed these curves with a nested analysis of variance.

**Results**

**Studies in isolated tissue**

**Effects of doxorubicin on the normal transmembrane potential, normal automaticity, and conduction.** We studied nine free-running Purkinje fibers driven at a basic cycle length of 1000 msec and superfused with [K⁺]₀ = 2.7 mM. As shown in table 1, 50 μM doxorubicin had no effect on the transmembrane potential during drive at a basic cycle length of 1000 msec or on automaticity. Doxorubicin, 100 μM, prolonged repolarization, and the 200 μM dose decreased amplitude and Vₘₐₓ. Even at the highest concentration, automaticity was unchanged (figure 1). Because this experiment and our previous experience indicated that doxorubicin concentrations greater than 100 μM had different effects on the transmembrane potential, we limited the concentrations of doxorubicin in the remainder of our studies to 50 and 100 μM.

**Table 1**

<table>
<thead>
<tr>
<th>MDP (mV)</th>
<th>Amplitude (mV)</th>
<th>Vₘₐₓ (V/sec)</th>
<th>APD₅₀ (msec)</th>
<th>APD₆₀ (msec)</th>
<th>Automatic rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-95 ± 1.5</td>
<td>135 ± 1.6</td>
<td>569 ± 40</td>
<td>224 ± 15</td>
<td>341 ± 7</td>
</tr>
<tr>
<td>Doxorubicin, 50 μM</td>
<td>-96 ± 1.3</td>
<td>136 ± 2.2</td>
<td>580 ± 43</td>
<td>235 ± 17</td>
<td>353 ± 12</td>
</tr>
<tr>
<td>Doxorubicin, 100 μM</td>
<td>-95 ± 1.7</td>
<td>131 ± 7.5</td>
<td>534 ± 33</td>
<td>257 ± 25</td>
<td>392 ± 12</td>
</tr>
<tr>
<td>Doxorubicin, 200 μM</td>
<td>-94 ± 1.3</td>
<td>131 ± 1.4*</td>
<td>484 ± 30*</td>
<td>256 ± 24</td>
<td>430 ± 13*</td>
</tr>
</tbody>
</table>

MDP = maximum diastolic potential.
*p < .05 vs control; *p < .01 vs control.
TABLE 2
Effects of doxorubicin on barium-induced abnormal automaticity (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>MDP (mV)</th>
<th>Activation voltage (mV)</th>
<th>Amplitude (mV)</th>
<th>APD(_{100}) (msec)</th>
<th>Auto CL (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−62 ± 2.0</td>
<td>−50 ± 1.8</td>
<td>68 ± 2.8</td>
<td>399 ± 23</td>
<td>767 ± 48</td>
</tr>
<tr>
<td>Doxorubicin, 50 μM</td>
<td>−65 ± 1.5</td>
<td>−54 ± 2.1</td>
<td>71 ± 3.0</td>
<td>431 ± 23</td>
<td>742 ± 40</td>
</tr>
<tr>
<td>Doxorubicin, 100 μM</td>
<td>−64 ± 1.5</td>
<td>−55 ± 2.3</td>
<td>76 ± 3.8(^\ast)</td>
<td>432 ± 20</td>
<td>729 ± 47</td>
</tr>
</tbody>
</table>

MDP = maximum diastolic potential; CL = cycle length.
\(^\ast\)p < .05 vs control.

1.5, and −77 ± 1.9 mV; the \(V_{\text{max}}\) values were 604 ± 32, 433 ± 20, and 247 ± 44 V/sec. Neither concentration of doxorubicin modified the transmembrane potential or \(V_{\text{max}}\). Conduction time was 3.1 ± 1.0 msec at \([K^+]_o = 4 \text{ mM}\), 3.5 ± 1.2 msec at \([K^+]_o = 8 \text{ mM}\), and 5.9 ± 1.5 msec at \([K^+]_o = 10 \text{ mM}\). With 100 μM doxorubicin these values were 3.7 ± 1.2, 3.5 ± 1.1, and 6.1 ± 1.6 msec, respectively (all p > .05). Hence, at a drive cycle length of 800 msec, there was no evidence of voltage-dependent effects of doxorubicin on \(V_{\text{max}}\) or conduction.

**Effects of doxorubicin on abnormal automaticity.** We superfused seven Purkinje fiber bundles with BaCl\(_2\) (table 2). Doxorubicin, 50 μM, had no effect on the variables studied, and 100 μM increased action potential amplitude. The results of all experiments on overdrive pacing of BaCl\(_2\)-induced automaticity are summarized in figure 2. After 50 μM doxorubicin the curves relating recovery cycle length to pacing cycle length were virtually superimposable. Even after 100 μM doxorubicin the curves did not differ significantly, indicating the absence of overdrive suppression.

Abnormal automaticity was also studied in six tissue samples from dogs with subendocardial infarcts. Here, control maximum diastolic potential was −68 ± 1.7 mV; activation voltage, −59 ± 2.0 mV; action potential amplitude, 77 ± 1.9 mV; APD\(_{100}\), 423 ± 13 msec; and automatic cycle length, 730 ± 33 msec. There were no significant effects of 50 or 100 μM doxorubicin on the transmembrane potentials. The automatic cycle lengths were 763 ± 55 and 823 ± 56 msec, respectively, after 50 and 100 μM doxorubicin (p > .05). The effects of doxorubicin on the response of the infarcted tissue to overdrive pacing are shown in figure 3. Note that pacing at a basic cycle length of 400 msec induced significant overdrive suppression during control and after 50 and 100 μM doxorubicin. However, the control curve and the doxorubicin curves did not differ significantly from one another (p > .05).

**Effects of doxorubicin on triggered activity.** We studied five Purkinje fibers in which ouabain 2 × 10\(^{-7}\)M induced delayed afterdepolarizations and triggered activity. The transmembrane potential characteristics during the occurrence of triggered activity (at a cycle length of 632 ± 88 msec) was maximum diastolic potential −84 ± 1.6 mV, activation voltage −73 ± 3.0 mV, action potential amplitude 102 ± 6.5 mV, \(V_{\text{max}}\) 350 ± 80 V/sec, and APD\(_{100}\) 282 ± 12 msec. On exposure to 50 μM doxorubicin for 25 min, triggered activity had ceased completely in all five fibers. Al-
though no changes were seen in maximum diastolic potential, activation voltage, or amplitude, V_{\text{max}} was reduced to 282 ± 63 V/sec and APD_{100} increased to 366 ± 30 msec (both p < .05). Results of a representative experiment are shown in figure 4. Note that after the cessation of triggered activity, small delayed afterdepolarizations persisted.

In two other experiments, ouabain induced an increase in automaticity (i.e., abnormal automaticity) rather than triggered activity. As described above for automaticity, 50 μM doxorubicin had no effect here. Finally, two preparations from dogs with 24 hr infarcts showed triggered activity rather than automatic rhythms. In both, the triggered activity (and the delayed afterdepolarizations) was suppressed by doxorubicin.

Studies in intact animals. We first studied three control dogs, to which we gave doxorubicin in a total dose of 64 mg/m² according to the protocol in the Methods section. As shown in table 3, the third bolus of doxorubicin induced increases in sinus cycle length and the QT and QTc intervals that did not attain statistical significance.

We studied ventricular tachycardia occurring 24 hr after myocardial infarction in five dogs. The control cycle length of ventricular tachycardia was 390 ± 24 msec. The cycle lengths after the first through fourth doses of doxorubicin were 380 ± 17, 386 ± 17, 384 ± 24, and 378 ± 33 msec, respectively. None of these cycle lengths was significantly different from control. Moreover, no effect of doxorubicin was seen on the response to overdrive pacing (figure 5).

We studied the effects of doxorubicin on ouabain-

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**TABLE 3**

<table>
<thead>
<tr>
<th>Sinus cycle length</th>
<th>Control</th>
<th>590 ± 50</th>
<th>100 ± 6</th>
<th>56 ± 2</th>
<th>190 ± 6</th>
<th>270 ± 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First bolus</td>
<td>593 ± 57</td>
<td>103 ± 9</td>
<td>56 ± 4</td>
<td>203 ± 3</td>
<td>266 ± 12</td>
<td></td>
</tr>
<tr>
<td>Second bolus</td>
<td>593 ± 72</td>
<td>107 ± 7</td>
<td>57 ± 3</td>
<td>210 ± 6</td>
<td>276 ± 12</td>
<td></td>
</tr>
<tr>
<td>Third bolus</td>
<td>658 ± 117</td>
<td>110 ± 6</td>
<td>57 ± 3</td>
<td>220 ± 6</td>
<td>300 ± 45</td>
<td></td>
</tr>
<tr>
<td>Fourth bolus</td>
<td>623 ± 95</td>
<td>107 ± 7</td>
<td>58 ± 3</td>
<td>243 ± 3</td>
<td>313 ± 23</td>
<td></td>
</tr>
</tbody>
</table>

All values are in msec. p > .05 for all; n = 3.
induced ventricular tachycardia in eight dogs. The ventricular tachycardia cycle length was 327 ± 18 msec. In four dogs, sinus rhythm recurred 10 to 20 min after the first dose of doxorubicin. In two of these dogs, overdrive pacing for 1 min triggered a few ventricular premature depolarizations (see example in figure 6). We continued the administration of doxorubicin to a total dose of 64 mg/m² in the four dogs, and saw no significant change in sinus cycle length, which had been 620 ± 22 msec at the time the arrhythmia ceased.

In two of the eight dogs the first bolus of doxorubicin resulted in a change from sustained ventricular tachycardia to a rhythm in which 10% of the beats per minute were sinus and 90% were ectopic. The second and third bolus doses of doxorubicin, respectively, reduced the percentage of ventricular premature beats to 16% and 6%. The mean sinus cycle length in these dogs was 610 msec.

In the final two dogs, doxorubicin in a dose of up to 64 mg/m² had no effect on the arrhythmia. Although the control cycle lengths of the ventricular tachycardias in these two dogs did not differ from those of the other ouabain-treated animals, the following differences were noted: the six dogs whose tachycardias were rapidly suppressed by doxorubicin all demonstrated a decrease in the cycle length of ventricular tachycardia in response to overdrive pacing before administration of doxorubicin (figure 7). In contrast, the two dogs that were unresponsive to doxorubicin (one of whose records is shown in figure 8) demonstrated no overdrive enhancement, but rather had slight suppression of arrhythmia in response to pacing. Purkinje fibers were excised from the hearts of these two animals and studied in the tissue bath. As demonstrated in figure 8, the rhythms were automatic. In contrast, excised fibers from the hearts of animals in which there was suppression of arrhythmia induced by doxorubicin demonstrated no triggered or automatic activity.

Discussion

We have demonstrated that doxorubicin suppresses ventricular tachycardias induced by toxic doses of digitalis (in six of eight animals) but does not suppress those occurring 24 hr after myocardial infarction. This result is consistent with our expectations for these experiments. We state this for the following reasons: First, our prior work and the microelectrode experiments performed in the present study demonstrated
that low concentrations of doxorubicin depress delayed afterdepolarizations and resultant triggered activity, whether induced by digitalis or subsequent to myocardial infarction. Second, the same concentrations of doxorubicin had no effect on automaticity occurring in the normal range of membrane potentials (presumably, the result of the \( i_\text{p} \) pacemaker current) or on automaticity at low membrane potentials resulting from infarction or exposure to \( \text{BaCl}_2 \). Third, these same concentrations of doxorubicin affected neither the normal transmembrane action potential nor that dependent on the slow inward current, nor did they affect conduction in normal or depolarized fibers.

These factors in combination suggested that appropriate concentrations of doxorubicin in plasma should suppress arrhythmias resulting from delayed afterdepolarizations and triggered activity, but not those from other causes.

The fact that doxorubicin had no effect on the ventricular tachycardia occurring 24 hr after myocardial infarction is consistent with the previous observation that most of these arrhythmias result from automaticity at low membrane potentials. Although it has been suggested that triggered activity might be the cause of such arrhythmias, recent studies have suggested that infarct-induced triggered activity is seen only in special instances; for example, in microelectrode studies of 24 hr infarcts at low temperature (36°C rather than the 39°C usually seen in the normal dog), in instances in which hyperpolarization of the membrane is occurring, or in instances in which the infarct (or the tissue sample studied) is small. In the intact animal after myocardial infarction, a response to pacing consistent with triggered activity (i.e., a decrease in the escape interval and/or an increase in ventricular tachycardia rate after overdrive pacing) usually is not seen at 24 hr (except in the presence of small infarcts) and is more frequent at 48 to 96 hr, at which time the infarct has begun to heal, and the membrane to hyperpolarize. It was because of the above information that we did not expect doxorubicin to suppress ventricular tachycardia occurring 24 hr after infarction.

Alternatively, we did anticipate that doxorubicin would suppress most of the digitalis-induced ventricular tachycardias, for the following reasons: Although...
some early studies had suggested an abnormal automatic mechanism to be the cause of digitalis-induced ventricular tachycardia in the dog, work in the last 15 years has indicated that both at the cellular level\textsuperscript{22-27} and in intact animals\textsuperscript{28, 29} triggered activity induced by delayed afterdepolarizations is the more prevalent cause. It also has been demonstrated that the ionic mechanism responsible for the delayed afterdepolarizations (accumulation of intracellular Ca\textsuperscript{2+} leading to the oscillatory current, \(i_{\text{h}}\)) is separate and distinct from both the normal pacemaker current at high membrane potentials and that occurring in depolarized membranes.\textsuperscript{31}

The observation that doxorubicin did not suppress all ventricular tachycardias induced by digitalis suggested that those that persisted were the result of a mechanism other than triggered activity, presumably automaticity. The demonstration of automatic firing in isolated tissues obtained from the hearts with digitalis toxicity that had not responded to doxorubicin in vivo supports this interpretation, and provides further evidence for both the specificity of doxorubicin and the multiplicity of mechanisms responsible for digitalis-induced tachycardias.\textsuperscript{32}

It is to be stressed that the concentrations of doxorubicin we used appear not to have affected conduction. This observation was made in our isolated tissue studies, in which conduction time was unaltered by doxorubicin over a wide range of membrane potentials. It also appears that the concentrations attained in the intact animals did not modify conduction, since control dogs given doxorubicin intravenously showed no change in the PR interval or in QRS duration (table 3).

The mechanisms responsible for the actions of doxorubicin are not certain. Prior studies have suggested that doxorubicin depresses the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange mechanism in canine sarcolemmal vesicles.\textsuperscript{33} If such a change resulted in a lesser accumulation of [Ca\textsuperscript{2+}], after the development of digitalis toxicity, this could provide an explanation for the suppression of delayed afterdepolarizations and triggered activity. However, there is no direct evidence that doxorubicin acts in this fashion in the presence of digitalis toxicity. Moreover, there is some disagreement over the consistency of doxorubicin’s effects on the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange.\textsuperscript{34, 35} Finally, even if it modified the exchange, it is uncertain that such an action would, in fact, reduce [Ca\textsuperscript{2+}], in sum, although an explanation has been suggested that might explain doxorubicin’s effects here, more evidence is needed before we have a firm understanding of the mechanism.

Another question to be considered is the relationship of the doses of doxorubicin we administered to the intact animals to the concentrations used in the isolated tissue studies. We previously have shown\textsuperscript{7} and have verified here that the 50 \(\mu\)M concentration in the tissue bath has no effect on repolarization. However, higher concentrations both prolong repolarization and, at times, induce early afterdepolarizations. This would suggest that if we intended to consider a highly specific effect of doxorubicin, the appropriate concentrations to use in the intact animal would be those that did not modify repolarization, as measured in the QT interval. As shown in table 3, only the fourth dose of doxorubicin significantly prolonged repolarization. Since only the first one or two doses were needed to suppress digitalis-induced ventricular tachycardias it is reasonable to conclude that the dose given to the intact animal was sufficiently small to leave repolarization unaltered and to exert a specific effect on the triggered activity.

Our isolated tissue studies also showed early afterdepolarizations occurring during washout of the 200 \(\mu\)M concentration (figure 1). This suggests that in some instances we might have expected early afterdepolarization-induced arrhythmias in the intact animals. However, we saw no signs of increased ectopic activity in any of our experiments.

In conclusion, we have found that a drug having a specific effect on an arrhythmogenic mechanism in isolated tissues can be used to discriminate among arrhythmogenic mechanisms in the intact animal. Although doxorubicin cannot be considered clinically for such use because of its toxic effects on both the heart and other organ systems, this observation should encourage the identification of other specific but less toxic drugs that might then be used in the diagnosis of mechanism and the treatment of arrhythmias. Moreover, it should be stressed that the effects of doxorubicin seen here are unrelated to its actions on cardiac proteins that may be assumed to induce longstanding toxicity and cardiomyopathy.\textsuperscript{8, 36} Rather, the effects seen here, whether resulting from an action on the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange or some other mechanism, are both dose dependent and, as we have shown previously,\textsuperscript{7} completely reversible.

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