Modulation of Drug Effects by Regional Sympathetic Denervation and Supersensitivity

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Background. Regional sympathetic denervation, such as that produced by a myocardial infarction, causes electrophysiological heterogeneity in the ventricles. The purpose of this study was to test the hypothesis that such denervation could cause drugs to exert heterogeneous myocardial effects.

Methods and Results. Sympathetic stimulation increases the amplitude of cesium chloride–induced early afterdepolarizations (EADs). The amplitude of these induced EADs was used to determine whether drug responses were different in innervated versus denervated areas of the heart. A canine model of sympathetic denervation was created at the cardiac apex by either transmural myocardial infarction (n=19) or phenol application (n=11). Cesium chloride (84 mg/kg) was infused while monophasic action potential recordings were simultaneously obtained from the base and apex of the left ventricle using an epicardial contact electrode. We found that control (innervated) dogs (n=17) showed no difference in the EAD amplitude recorded from the apex compared with the base. In dogs with apical sympathetic denervation, the EAD amplitude was greater at the innervated base during ansae subclaviae stimulation than at the denervated apex (25.8±6.6% at base versus 18.8±6.7% at apex, p<0.001). However, during norepinephrine infusion, the EADs recorded from the denervated apex were greater than those recorded from the innervated base (23.3±7.6% at apex versus 20.6±6.0% at base, p<0.02) due to denervation supersensitivity.

Conclusions. These data show that regional myocardial denervation creates autonomic and electrophysiological heterogeneity and the substrate for heterogeneous drug actions. This drug-induced electrophysiological heterogeneity may be another mechanism for proarrhythmia. (Circulation 1991;84:1709–1714)

Factors that increase the electrical heterogeneity in the ventricles may be important in promoting the development of ventricular arrhythmias. Theoretically, a ventricle that is totally homogeneous electrically would not fibrillate. One such factor responsible for electrical heterogeneity is autonomic heterogeneity, specifically, sympathetic heterogeneity. Sympathetically denervated areas of myocardium exhibit minimal sympathetically induced shortening of refractoriness or sympathetically mediated changes in other electrophysiological parameters but display an enhanced response when exposed to circulating catecholamines as a result of denervation supersensitivity. Such heterogeneous responses can occur after myocardial infarction that produces regional sympathetic denervation of noninfarcted myocardium. This has been shown to occur clinically as well as experimentally. Because sympathetic stimulation can modulate antiarrhythmic drug effects, drugs might exert heterogeneous actions in ventricles that exhibit areas of denervation after infarction. This could be a potential proarrhythmic mechanism that might be eliminated by concomitant β-adrenoceptor blockade.

To test the hypothesis that sympathetic denervation can cause drugs to exert heterogeneous myocardial effects, we investigated the response to cesium chloride in ventricles with regional sympathetic denervation. Cesium causes early afterdepolarizations (EADs) that exhibit a relatively homogeneous waveform and amplitude throughout a normal ventricle, as demonstrated by recordings made with a contact electrode. Ansae subclaviae stimulation and norepinephrine infusion or superfusion increase the am-
plitude of EADs and can promote the development of ventricular tachyarrhythmias predominantly by an α-adrenoceptor agonist mechanism.14,15 Therefore, if the heterogeneity hypothesis is correct, cesium should cause larger-amplitude EADs in innervated than in denervated areas of the ventricle during ansae subclaviae stimulation. In contrast, norepinephrine infusion should reverse this reaction, causing EADs with larger amplitude in the myocardial regions showing denervation supersensitivity. The results of the present study are consistent with this hypothesis.

Methods

Forty-seven mongrel dogs of either sex were included in the study. Thirty dogs underwent surgery for latex injection of a diagonal coronary artery to produce a transmural myocardial infarction (n = 19) or for phenol application (n = 11). Both techniques result in regional sympathetic denervation.1-5 Seventeen dogs served as controls.

Surgical Procedure

The dogs were anesthetized with sodium pentobarbital, intubated with a cuffed endotracheal tube, and ventilated with room air by a constant-volume ventilator (model 607, Harvard Apparatus, South Natick, Mass.). Using sterile technique, a thoracotomy was performed in the left fifth intercostal space, the ribs and lungs were retracted, and the pericardium was incised. After either phenol application or latex injection (described below), the chest was closed in layers, and negative pressure was reestablished in the pleural cavity. Antibiotics were administered for 5 days after surgery, and analgesics were given as needed.

Phenol Application

Eleven dogs had epicardial phenol (88% carbolic acid) applied. Topical application of phenol selectively interrupts the sympathetic nerves coursing through the subepicardial region without affecting the underlying myocardium.1 Phenol was applied across the mid left anterior descending coronary artery and then along the entire course of a moderate-to-large-size diagonal vessel near the midanterior wall. This was repeated three times at 10-minute intervals.

Latex Injection

Nineteen dogs underwent latex injection into a moderate-to-large-size diagonal branch of the left anterior descending coronary artery to create a transmural myocardial infarction in the midanterior wall. From 5 to 7 mm of the proximal segment of the diagonal vessel were isolated, and two silk ligatures were placed under the artery. The proximal ligature was tied, and the distal ligature was maintained under traction to prevent bleeding. The vessel was incised and cannulated with a small plastic catheter. The distal ligature was then tightened to secure the catheter in the artery, and 0.3–0.7 ml of a rapidly hardening vinyl latex solution (Carolina Biological Supply) was injected into the artery to embolize the vasculature. The catheter was then removed, and the distal ligature was tied around the artery. We have shown repeatedly that a careful surgical dissection does not, in itself, interrupt sympathetic innervation.2,4,5 Latex-induced myocardial infarction is not arrhythmogenic.10,16

Electrophysiological Study

All dogs that were operated on underwent open-chest electrophysiological study 7–14 days after surgery to confirm the presence of sympathetic denervation and supersensitive shortening of the effective refractory period (ERP) to norepinephrine infusion. For electrophysiological study, dogs were anesthetized with sodium pentobarbital, intubated, and ventilated with room air by a Harvard respirator. The right femoral artery was cannulated with a heparinized saline-filled polyethylene catheter to monitor blood pressure continuously. The right femoral vein was cannulated with a polyethylene tube to infuse normal saline and administer cesium chloride or norepinephrine during the study. A midline neck incision exposed the right and left cervical vagi, which were isolated, doubly ligated, and transected. After a median sternotomy, the pericardium was opened and served as a cradle when sutured to the sternal wound. The left and right ansae subclaviae were isolated, doubly ligated, and transected. Shielded bipolar electrodes were placed on both ansae subclaviae for later stimulation. Bipolar plunge electrodes were inserted in the right atrial appendage and right ventricular myocardium to record atrial and ventricular electrograms. Four unipolar stimulating electrodes were placed in the midmyocardium of the anterior left ventricle—two in normal myocardium basal to and two in normal myocardium apical to the site of the phenol application or myocardial infarction. An indifferent electrode plate was placed subcutaneously in the anterior abdominal wall. The region of the sinus node was crushed with a large clamp applied to the lateral right atrial border. This does not influence ventricular innervation.1

The ERP of the left ventricular myocardium at the two apical and two basal sites was determined by the extrastimulus technique at a pacing cycle length of 300 msec. Late diastolic threshold was measured at each site before each determination of the ERP, and unipolar cathodal stimulation was performed with rectangular pulses of 2-msec durations at twice diastolic threshold. A train of eight stimuli (S1) was followed by a late premature stimulus (S2) that initially produced a conducted ventricular response. The S1-S2 interval was shortened by 1-msec decrements near the ERP until the ERP was reached. ERP was defined as the longest S1-S2 interval at which the S2 stimulus failed to produce a response on two successive attempts. The S1-S2 interval was then increased by 10 msec and again decreased by 1-msec decrements until the ERP was reached. The second measurement of the ERP had to be within 2 msec of the first, or the data were discarded and the testing
TABLE 1. Early Afterdepolarization Amplitude During Cesium Infusion

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Phenol and myocardial infarction groups</th>
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<tbody>
<tr>
<td></td>
<td>Base (%)</td>
<td>Apex (%)</td>
</tr>
<tr>
<td>Basae</td>
<td>18.1±5.9</td>
<td>17.5±5.1</td>
</tr>
<tr>
<td>Ansae stim</td>
<td>20.9±4.4</td>
<td>21.3±5.5</td>
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<tr>
<td>Norepi</td>
<td>20.5±3.2</td>
<td>21.7±2.9</td>
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</table>

Shown is comparison of early afterdepolarization amplitude at base vs. apex during cesium administration in baseline state, during ansae subclaviae stimulation (ansae stim), and during norepinephrine infusion (norepi). Probability value is for base compared with apex for each condition.

was repeated. The two obtained values were then averaged for each electrode.

The ERP was determined at all four test sites under three conditions: baseline (after vagi and ansae subclaviae transection), during bilateral ansae subclaviae stimulation, and during norepinephrine infusion.

The ansae subclaviae were stimulated bilaterally with square wave pulses of 4-msec duration, 3 mA, and 3 Hz from a Grass 588 stimulator delivered through a constant-current stimulus isolator. Ansae subclaviae stimulation began 4–6 minutes before beginning ERP testing and was continued until testing was completed. It uniformly produced a 10–30 mm Hg increase in mean systemic arterial blood pressure. The dog was then allowed to recover for 30 minutes before beginning the norepinephrine infusion. Norepinephrine was infused at a rate of 0.5 μg/kg for 5–10 minutes to allow a stable increase in blood pressure before measuring ERPs.

Supersensitive shortening of refractoriness in response to norepinephrine was said to occur when greater shortening of the ERP occurred at the apex than at the base during norepinephrine infusion compared with baseline.

**Monophasic Action Potential Recordings**

Monophasic action potential (MAP) recordings were obtained simultaneously from two epicardial sites using epicardial MAP contact electrodes (EP Technologies Inc., Mountainview, Calif.).17,18 Signals were then filtered through a DC amplifier (Kranzett Medical Engineering, Indianapolis, Ind.) before being displayed and recorded on a strip-chart recorder (VR-12, Electronics for Medicine, Pleasantville, N.Y.).

MAP recordings were deemed acceptable if they demonstrated a stable amplitude greater than 10 mV, stable morphology, smooth contour of phase III repolarization, stable phase IV resting membrane potential, and no suggestion of an EAD. EADs were defined as an interruption in the smooth contour of phase III repolarization. EAD amplitude was measured as the difference between the potential at which the interruption of smooth repolarization occurred and the phase IV resting potential, as previously reported.13,15,19 The total MAP amplitude was defined as the potential difference between phase IV and phase II membrane potentials. EAD amplitude was then expressed as a percentage of total MAP amplitude. We have shown previously that the EAD amplitude calculated in this manner correlates closely with the area of the EAD expressed as a percentage of total MAP area.13

**Experimental Protocol**

After completion of ERP testing, one epicardial MAP probe was placed on the anterobasal region of the left ventricle near the site of the two unipolar pacing electrodes, and the other MAP probe was similarly placed on the apical left ventricle. The ventricle was paced at a constant cycle length of 600 msec. MAPs were recorded in the baseline state during cesium injection, during ansae subclaviae stimulation and cesium injection, and during norepinephrine infusion and cesium injection.

Cesium chloride (84 mg/kg) was administered as a bolus over 10 seconds. MAP recordings, surface electrocardiographic lead II, and right atrial and right ventricular electrograms were recorded during each cesium injection and for 60 seconds thereafter. The dog was allowed to recover for 30 minutes, and the cesium bolus was given after 4–6 minutes of bilateral ansae subclaviae stimulation, as described above. After 30 minutes of recovery, norepinephrine was infused for 5–10 minutes, as described above, and cesium administration at the same dose was repeated.

EAD and MAP measurements were made 30 seconds after cesium injection from measurements of four consecutive ventricular paced complexes.

**Statistics**

Data are given as mean±SD. Statistical comparisons were performed using paired t tests.

**Results**

**Control Group**

The 17 control dogs showed no difference in EAD amplitude at the left ventricular base compared with at the apex after the ansae subclaviae were transected (baseline), during ansae subclaviae stimulation, or during infusion of norepinephrine (Table 1). Compared with values obtained just after ansae subclaviae transection, ansae subclaviae stimulation increased EAD amplitude significantly at the apex but not quite significantly at the base (Table 2). Similarly, norepinephrine infusion compared with baseline significantly
increased EAD amplitude at the apex but not quite at the base.

Phenol and Myocardial Infarction Groups

Effective refractory period testing. There were no significant differences in ERP between each of the two basal sites or apical sites during baseline, ansae subclaviae stimulation, or norepinephrine infusion. Therefore, the ERP measurements from the two electrodes were averaged for each region.

After ansae subclaviae transection, there was no difference between the ERPs at the base compared with the apex in the phenol or myocardial infarction groups (Table 3). However, with ansae subclaviae stimulation, ERPs shortened more at the innervated base than at the denervated apex in both the phenol and myocardial infarction groups. During norepinephrine infusion, the ERPs shortened more at the denervated apex than at the innervated base in both the phenol and myocardial infarction groups. Thus, a supersensitive response of the ERPs was demonstrated during norepinephrine infusion.

Early afterdepolarization measurements. There was no statistically significant difference between the phenol and myocardial infarction groups for cesium-induced EAD amplitude at each site or test condition. Therefore, the phenol and myocardial infarction EAD data were pooled.

After transection of the ansae subclaviae, cesium-induced EAD amplitude was slightly larger at the base than at the apex (Table 1). The EAD amplitude was still greater at the base than at the apex during ansae subclaviae stimulation. However, during norepinephrine infusion, the EAD amplitude was greater at the apex than at the base. Figure 1 shows analog recordings from a dog in the myocardial infarction group.

Seventeen dogs in the phenol and myocardial infarction groups demonstrated supersensitive shortening of ERPs to norepinephrine infusion. That is, norepinephrine-induced ERP shortening was greater at the denervated apex than at the innervated base.

In these dogs, the difference in EAD amplitude between the apex and the base was even more marked (19.3±5.1% at base versus 29.4±6.6% at apex, p<0.001). The remaining dogs, which did not demonstrate supersensitivity, showed no difference in EAD amplitude between the base and apex (21.4±4.4% at base versus 20.6±5.1% at apex, p=0.67) (Table 4). This indicates that a similar supersensitive response occurs with EADs as with ERPs in regions of sympathetic denervation during infusion of norepinephrine.

Discussion

New Findings

The present study demonstrates that compared with innervated areas, the amplitude of cesium-induced EADs, shown previously to be modulated by sympathetic stimulation,13-15 is reduced in sympathetically denervated left ventricular epicardium during ansae subclaviae stimulation. In addition, because the denervated segments exhibit denervation supersensitivity, norepinephrine infusion provokes a larger EAD amplitude in the denervated apex than in the innervated base.

Denervation Supersensitivity

These data show that the actions of drugs on myocardial electrophysiological properties can be altered depending on the sympathetic state of different regions within the same heart. When cesium, a drug that can generate EADs, is administered intravenously to a normal canine heart, EADs of equal amplitude are recorded from the right and left ventricular endocardium and epicardium.13 The data in this report show that no difference exists between the amplitudes of the cesium-induced EADs recorded epicardially in the left ventricular apex or in the base in the control dogs.

<table>
<thead>
<tr>
<th>Table 2. Effect of Sympathetic Modulation on Early Afterdepolarization Amplitude in Control Group</th>
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<tbody>
<tr>
<td>Base</td>
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<tr>
<td>Baseline</td>
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<td>Ansae stim</td>
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<td>Baseline</td>
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<td>Norepi</td>
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Shown is comparison of ansae subclaviae stimulation (Ansae stim) vs. baseline and norepinephrine infusion (Norepi) vs. baseline at each site in the control dogs. Probability value is for intervention compared with baseline at each site.

*p<0.07. †p<0.21. ‡p<0.04. §p<0.007.

<table>
<thead>
<tr>
<th>Table 3. Effective Refractory Periods</th>
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<tbody>
<tr>
<td><strong>Phenol group</strong></td>
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<tr>
<td>Base (msec)</td>
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<td>-----</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>Ansae stim</td>
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<td>Norepi</td>
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</table>

Shown are effective refractory periods in baseline state, during ansae subclaviae stimulation (Ansae stim), and during norepinephrine infusion (Norepi). Cesium was not administered during this portion of the study.

Probability value is for base compared with apex for each condition.
When a portion of the myocardium is sympathetically denervated by either myocardial infarction or regional chemical ablation by phenol, an area containing normal but electrophysiologically different myocardium is created apical to the lesion. Thus, instead of cesium-induced EADs that are of equal amplitude in the innervated and denervated regions, sympathetic stimulation created regional inhomogeneity by affecting only the innervated area. EAD amplitude increased in the innervated area during ansae subclaviae stimulation but did not increase in the denervated region.

It is known that sympathetically denervated myocardium demonstrates a supersensitive response during norepinephrine infusion. For example, norepinephrine elicits greater ERP shortening in the denervated area compared with innervated myocardium. In addition to confirming supersensitive shortening of refractoriness in response to norepinephrine, the present study demonstrates that a similar phenomenon occurs with cesium-induced EADs. During norepinephrine infusion, EAD amplitude became larger in the denervated, supersensitive region than in the innervated region, a reversal of what occurred during neural stimulation.

### Study Importance

These findings have several important implications. Using 125I-radiolabeled metiodobenzylguanidine, a guanethidine analogue taken up by sympathetic nerve terminals to obtain a sympathetic nervous scintigram of the left ventricle, we have shown that some patients after myocardial infarction develop areas of the left ventricle that are probably sympathetically denervated. It is likely, although not yet established, that these areas exhibit denervation supersensitivity to circulating catecholamines. If this

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**TABLE 4. Effect of Supersensitivity on Early Afterdepolarization Amplitude in Phenol and Myocardial Infarction Groups**

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Apex</th>
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<tbody>
<tr>
<td>Supersensitivity present</td>
<td>19.3±5.1</td>
<td>24.4±6.6*</td>
</tr>
<tr>
<td>Supersensitivity absent</td>
<td>21.4±4.4</td>
<td>20.6±5.1†</td>
</tr>
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*p<0.001.  †p=0.67. Probability value is for base compared with apex in each group.
is true, then cardioactive drugs whose actions can be modified by sympathetic stimulation have the potential of exerting heterogeneous effects on the human left ventricle that is regionally denervated. Instead of exerting relatively homogeneous effects and predictable responses at "therapeutic" concentrations, drugs like digitalis and quinidine might promote delayed and early afterdepolarizations, respectively, in denervated regions when stimulated by circulating catecholamines. Similarly, regional effects on refractoriness and conduction will be heterogeneous. These changes can be arrhythmogenic and might represent another mechanism for proarrhythmia.

Acknowledgments

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References

10. Inoue H, Zipes DP: Results of sympathetic denervation in the canine heart: Supersensitivity that may be arrhythmogenic. Circulation 1987;75:877–887

Key Words • triggered activity • nervous system, autonomic • proarrhythmia • arrhythmogenesis