Heterogeneous Sympathetic Innervation Influences Local Myocardial Repolarization in Normally Perfused Rabbit Hearts

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Background—Heterogeneity of sympathetic innervation is thought to contribute to the potential for fatal arrhythmia. However, little is known about the effects of heterogeneous innervation on repolarization.

Methods and Results—To assess this relationship, we measured activation recovery intervals (ARIs) from 64 epicardial sites in 11 rabbits studied 2 weeks after regional denervation produced by phenol and 4 sham-operated rabbits. ARIs were compared with the distribution of sympathetic innervation measured from 3D reconstructions of serial autoradiographs of [125I]metaiodobenzylguanidine and 99mTc-sestamibi. ARIs were recorded during baseline sinus rhythm, norepinephrine (NE) infusion (0.1 μg · kg\(^{-1}\) · min\(^{-1}\)), and left stellate ganglion stimulation (SS). NE shortened ARI in 98% of electrodes in the denervated region. The degree of ARI shortening and dispersion increased (P<0.001 and P<0.01, respectively) as denervation became more severe. SS shortened ARI in 30% of electrodes in the denervated area, with increased shortening and dispersion related to increased severity of denervation (P<0.01). SS prolonged ARI in 70% of electrodes in the denervated area, with no correlation with severity of denervation.

Conclusions—The magnitude and dispersion of local repolarization responses are related to the severity of denervation, as well as the type of stimulation: neural (SS) versus humoral (NE). The differences may relate to the concentration of NE released. (Circulation. 2000;101:1060-1066.)

Key Words: myocardium ■ repolarization ■ MIBG ■ nervous system

Repolarization abnormalities are thought to be related to malignant tachyarrhythmias and sudden cardiac death in patients with various cardiac diseases, and reentry is thought to be the most common underlying mechanism. Heterogeneity in the spatial distribution and activity of sympathetic innervation is also thought to contribute to the potential for fatal arrhythmia, possibly through effects on reentry. Recently, several investigations have suggested a relationship between regional neuronal dysfunction and ventricular arrhythmia in humans and animals in the absence of infarction or perfusion abnormalities. Denervation supersensitivity to circulating catecholamines has been implicated in the pathogenesis of these arrhythmias. However, little is known about the effects of the magnitude and spatial distribution of denervation on the distribution of local ventricular repolarization.

The purpose of this study was to evaluate the effects of sympathetic stimulation on myocardial repolarization in regions of mild, moderate, and severe denervation. We applied phenol to the epicardium in rabbit hearts to create regional denervation. Phenol causes necrosis to a depth of 0.25 mm on the epicardium and interrupts both afferent and efferent sympathetic fibers, denervating myocardium downstream from the injured area. Sympathetic innervation was measured from 3D reconstructions of serial autoradiographs of metaiodobenzylguanidine (MIBG) to map sympathetic nerves and sestamibi (MIBI) to map perfusion. Scintigraphic results were compared with activation recovery intervals (ARIs) measured simultaneously at 64 epicardial sites. We tested the hypothesis that various degrees of denervation would result in various local repolarization responses during adrenergic stimulation.

Methods

Surgical Preparation

Healthy New Zealand White rabbits (n=15) weighing 3.5 to 4 kg were used. These rabbits were kept in their cages for 1 week before experiments to allow adaptation. After the administration of ketamine (20 mg/kg) and xylazine (4 mg/kg) IM, a catheter was placed in a marginal ear vein. The rabbits were mechanically ventilated with a mixture of room air and oxygen via an endotracheal tube, and isoflurane (2%) was added during surgery. The surface ECG lead II was monitored throughout the procedure. Under sterile conditions, a thoracotomy was performed in the left fifth intercostal space, and the pericardium was incised. A cotton swab soaked in an 88% aqueous solution of carbolic acid (phenol) (n=11) or saline (n=4) was applied 3 times to the posterolateral region of the left ventricle. The
Thorax was closed, and pleural air was aspirated. The rabbits were allowed to recover and received analgesia and antibiotic therapy. After a mean recovery period of 8 ± 4 days, the rabbits were returned to the laboratory, where they were anesthetized with α-chloralose (80 mg/kg) and urethane (1000 mg/kg). The rabbits were intubated and ventilated on room air and supplemental oxygen. Arterial pressure was monitored continuously by means of a Teflon catheter placed in the femoral artery and connected to a pressure transducer. ECG leads I, II, and aVF were monitored continuously.

**Stellate Ganglion Stimulation**

A median thoracotomy was performed, and before any other instrumentation, the left stellate ganglion was dissected and isolated from surrounding connective tissue. Central connections were not cut to avoid alteration of nerve function before MIBG was injected. A shielded platinum Harvard stimulating electrode was placed firmly around the body of the ganglion at the point of exit of the ventral ansae subclaviae. Constant stimulation was obtained through a stimulator (Grass S44, Grass Instruments) at 4 to 10 Hz with pulses of 2-ms duration and adjusted to yield a minimum 10 mm Hg rise in systolic blood pressure that was sustained and constant for ≥ 2 minutes before the start of recording. The pulse amplitude required to achieve this was 6 to 12 V. After a stable electrode position was obtained, the electrode was fixed in place with dental acrylic.

**Norepinephrine Infusion**

After measurement of arterial blood pressure, sinus cycle length, and ARI (see below), norepinephrine (NE) was infused at a rate of 0.1 µg kg⁻¹ min⁻¹. This infusion rate of NE was selected because it is within the range of rates used in previous experimental studies of denervation supersensitivity. After ≥ 10 minutes of NE infusion to allow a steady state to be achieved, arterial blood pressure, sinus cycle length, and ARI were repeated.

**Measurement of Activation Recovery Time**

A 64-electrode recording sock was custom-built for the study. A plaster cast of the rabbit heart was made, which was covered with a nylon mesh stocking. AgCl electrodes were then sutured to the sock, with a 3- to 5-mm interelectrode distance. For short-term experiments, the sock was fitted over the heart without alteration of left ventricular pressure or ST segments from the epicardial ECG. The sock was oriented such that the seam was aligned with the left anterior descending coronary artery. Electrode positions were drawn on a diagram of the heart that contained landmarks such as the anterior descending coronary artery. The sock was not shifted during the course of the experiments, which was verified by the lack of change in the QRS morphology in the epicardial leads. A PC-based mapping system (Pruka) was used for simultaneous recording of up to 64 unipolar epicardial leads from rabbit hearts. The data were obtained from an average of 60 seconds of recordings. Data were stored directly to hard disks and subsequently transferred to a Sun workstation for processing of ARIs. ARIs were calculated from the first temporal derivative of each unipolar electrogram in an automated fashion with custom computer software. The time of maximum negative dV/dt of the QRS was taken as local activation time, and the recovery time was assessed as the time of maximum positive dV/dt during the T wave according to the method of Millar et al. The difference between activation and recovery times gave the ARI. After measurement of arterial blood pressure, sinus cycle length, and ARI during stimulation, the left stellate ganglion was dissected and isolated from surrounding connective tissue. The heart was arrested with hypertonic potassium chloride and removed. The hearts were rinsed in ice-cold saline, immersed in embedding medium (OCT compound), and frozen on crushed dry ice. The frozen hearts were mounted on a cryostat microtome, and serial sections 20 µm thick were cut from apex to base. Every 30th section was mounted on a glass coverslip (average 32 sections per heart), and the coverslips were glued to cardboard for autoradiography. The tissue sections were exposed to a storage phosphor imaging plate (Molecular Dynamics) for 1 hour to allow imaging of ⁹⁹mTc. The imaging plate was then scanned with a Molecular Dynamics Phosphorimager to record the distribution of ⁹⁹mTc-MIBI. The sections were removed for 3 days to allow decay of ⁹⁹mTc, then reapplied to an imaging plate for 24 hours to capture the distribution of [¹²⁵I]MIBG. The plate was scanned to record the distribution of [¹²⁵I]MIBG. Results of a pilot study showed that a mixture of 15 mCi of ⁹⁹mTc-MIBI (half-life 6 hours) and 250 mCi [¹²⁵I]MIBG (half-life 60 days) resulted in autoradiographic images that were > 99% pure for each isotope.

**Computer Processing of Autoradiographs**

The data from each study comprised dual-isotope autoradiographs of MIBI and MIBG. The 2 autoradiographs were compared on a pixel-by-pixel basis by a color method described previously. Color slices were then aligned to create a set of 3D reconstructions of the heart. The 3D maps were used to assign epicardial electrode positions (see below) to either normally innervated or denervated regions. The color scale allowed the severity of denervation to be classified according to the reduction of MIBG relative to MIBI. Denervation was considered to be mild with a 37% reduction of MIBG compared with the normal region, moderate with 65% reduction, and severe with 88% reduction. In addition to the 3D color maps, standardized 2D bull’s-eye maps were generated, which displayed the isotope distributions in the right and left ventricles.

**Electrophysiological-Scintigraphic Correlation**

Before the removal of the sock, a photograph was taken to aid localization of the electrodes. In addition, electrode locations were drawn on a diagram of the heart that contained landmarks such as the left anterior descending coronary artery. Electrode positions were transcribed to the 3D innervation/perfusion maps with the assistance of the diagrams and a CT scan of the sock (see below). Electrode sites were assigned independently of any knowledge of the ARI data.

A CT scan was taken of the electrode sock mounted on a cast of the rabbit heart. CT slices were reconstructed to show the distribution of the electrodes in 3D. These CT images were used to aid in the assignment of electrode positions to the scintigraphic 3D maps. These CT images were also used as a template to generate ARI bull’s-eye maps for qualitative comparison to MIBG/MIBI bull’s-eye images.

**Statistical Analysis**

For each region of myocardium (normal and denervated [mild, moderate, severe]), mean ARI, Δ ARI (ARI at sinus rhythm minus ARI during stimulation), and ARI dispersion (defined as the average of the deviation of ARI at each electrode from the mean ARI) were measured. Comparisons were made by ANOVA. Regression analysis was used to correlate the severity of denervation to mean ARI, Δ ARI, and ARI dispersion.

**Results**

**Scintigraphic Data**

Figure 1 shows representative MIBG and MIBI autoradiographs from a phenol-treated rabbit and a color image combined with MIBI and MIBG. The region of decreased MIBG is clearly visible. A 3D color-coded function map is shown in Figure 2. The area of yellow to green corresponds to the region of denervation. The mean extent of denervation was 44.3 ± 13.0% of the myocardium, with 22.8 ± 7.7% mild, 16.7 ± 6.1% moderate, and 4.8 ± 4.4% severe denervation. The Table summarizes the results of severity and extent of regional MIBG uptake. No perfusion defects were present in any of the hearts. All of the sham rabbits had homogeneous and normal MIBG uptake and perfusion.
Baseline Measurement of ARI
ARI was measured at a total of 642 epicardial sites (mild sites 206, moderate sites 188, severe sites 41, normal sites 207) in the 11 phenol-treated rabbits. Mean ARI was greater in denervated areas than in normally innervated areas during baseline sinus rhythm (Figure 3, A). However, there were no differences in the magnitude of ARI among the 3 levels of denervation.

Effects of NE
NE infusion was successful in all 11 phenol-treated rabbits. NE increased mean blood pressure (72±9 versus 90±10 mm Hg; P<0.001) and did not alter the sinus cycle length (227±35 versus 214±21 ms, P=NS). NE shortened ARI in 98% of the electrodes in the denervated region (122±16 to 99±17 ms, P<0.01) (Figure 3, A). There was no significant shortening in normal areas (110±19 versus 110±17 ms, P=NS). The magnitude of shortening of ARI from sinus rhythm to NE infusion (∆ARI) increased as the degree of severity of denervation increased (Figure 3, B). The dispersion of ARI (defined as the average of the differences between ARI at each electrode and mean ARI for the region) increased in the denervated region, with the largest dispersion of ARI occurring in the severely denervated areas (Figure 4). A total of 248 electrodes were recorded in the 4 sham-operated rabbits during NE infusion. There was no significant change in ARI from baseline (111±17 to 110±19 ms, P=NS).

Effects of SS
SS was successful in 6 of 11 animals. In the remaining 5, either the stellate ganglion was not identified (n=3) or no hemodynamic response was elicited with stimulation (n=2). In the 6 animals with successful SS, mean blood pressure increased (73±11 versus 81±14 mm Hg; P<0.05), with no significant changes in the sinus cycle length (235±28 versus 232±22 ms, P=NS). ARI was measured in a total of 346 epicardial sites in the 6 animals with successful SS (135 normal, 108 mild, 85 moderate, 18 severe). Differences in
ARI between baseline and SS were determined in these 6 animals.

ARI dispersion significantly increased in severely denervated sites (2 to 13 ms, \(P<0.05\)) (Figure 5, A). Although there were no significant changes in global mean ARI (mean ARI in the entire area) in the denervated regions after SS (125 ± 16 versus 124 ± 9 ms, \(P=NS\)) or in the normal region (111 ± 18 versus 115 ± 17 ms, \(P=NS\)) (Figure 5, B), differential responses within the denervated and normal regions did occur.

In the denervated region, 30% of the electrodes showed a shortening in ARI (132 ± 16 versus 113 ± 19 ms, \(P<0.01\)), whereas 70% showed ARI prolongation (121 ± 18 versus 128 ± 9 ms, \(P<0.05\)). In the sites showing shortening, the magnitude of shortening (Figure 6, A and B) and the dispersion (Figure 7) increased with the severity of denervation. In the sites showing prolongation, there was no correlation with the magnitude of prolongation and severity of denervation (Figure 8, A). However, the degree of prolongation was less in the severely denervated region than in the control region (Figure 8, B). Dispersion tended to decrease with SS (Figure 8, C). In the normal area, 71% of sites showed ARI prolongation (107 ± 16 versus 114 ± 18 ms, \(P<0.01\)), 25% showed shortening (126 ± 16 versus 118 ± 17 ms, \(P<0.01\)), and 4% showed no change (107 ± 11 ms). Baseline ARI was greater in both the denervated and normal sites that showed shortening during SS than in sites that showed prolongation (132 ± 16 versus 115 ± 17 ms, shortening versus prolongation, respectively, \(P<0.01\)).

In the 4 sham-operated rabbits, 228 electrodes were recorded during SS. ARI prolonged in 66% of the electrodes (112 ± 18 to 117 ± 17 ms, \(P<0.01\)), showed no change in 30% (113 ± 16 to 114 ± 18 ms, \(P=NS\)), and shortened in 4% (114 ± 19 to 112 ± 16 ms, \(P=NS\)).

**Bull’s-Eye Maps**

Figure 9 shows bull’s-eye maps of MIBG (left) and of AARI during NE (middle) and SS (right). In the ARI maps,
shortening is color-coded green and prolongation purple. The region of denervation shown in the MIBG map corresponds to the region of shortening in the NE ARI map. The SS map shows predominant prolongation of ARI.

Discussion
The major findings of this study were as follows. (1) ARI was prolonged during the basal state in the denervated region. (2) Consistent shortening of ARI occurred within the denervated region during NE infusion. The magnitude and dispersion of shortening increased as the severity of denervation increased. (3) SS led to a differential response with shortening in 30% of the electrodes in the denervated area, with increased magnitude and dispersion related to severity of denervation. Prolongation of ARI occurred in 70% of the electrodes in the denervated area.

Scintigraphic Imaging of Sympathetic Innervation
MIBG has been studied extensively and has been validated as an imaging marker of regional myocardial sympathetic innervation.13 Scintigraphic imaging in each of the phenol-treated rabbits demonstrated a heterogeneous distribution of MIBG uptake (innervation) and a homogeneous distribution of MIBI uptake (perfusion). Previous studies of MIBG uptake in the phenol model have shown that the reduction in uptake is due to an absence of sympathetic nerve terminals, or denervation.8,14 A gradient in MIBG uptake was found, ranging from a severe reduction, relative to the unaffected area, to a mild reduction. As discussed below, the local repolarization responses were different within the different regions of MIBG uptake.

Activation Recovery Intervals
A primary goal of this study was to test the hypothesis that spatial heterogeneity of innervation would result in heterogeneity of local repolarization. The optimum measure of repolarization is the action potential duration.15 Although recordings obtained via an intracellular microelectrode are the truest measure of action potential duration, recording from multiple simultaneously placed intracellular microelectrodes in an intact beating heart is not feasible and thus cannot be used to estimate spatial dispersion of repolarization. Therefore, another method of estimating action potential duration was used, which has been validated previously by use of simultaneously acquired intracellular recordings. The method uses ARI derived from the first derivative of epicardial unipolar electrograms.10 The ARI has as its advantage the ability to simultaneously record a large number of sites. ARI is a measure related to the action potential duration of cells near
the extracellular electrode. More importantly for our purposes, changes in ARI at a given site during an intervention correlate extremely well with changes in action potential duration measured with microelectrodes.

In this study, the baseline ARI was longer in the areas demonstrating reduced MIBG uptake than in areas demonstrating normal MIBG. Calkins et al reported similar results in patients showing prolongation of effective refractory period at the sites of denervation detected by [11C]hydroxyephedrine and positron emission tomography. The mechanism by which prolongation of baseline ARI occurs in denervated areas could not be determined in our study.

The changes in ARI during NE infusion were different in normally innervated and denervated areas. Ninety-eight percent of the electrodes in the denervated area showed shortening of ARI, consistent with denervation supersensitivity. ARI shortening increased as the severity of denervation increased. In addition, the dispersion of ARI responses increased within the most severely denervated area. The close correlation between regional denervation and regional ARI responses was confirmed by comparison of computer-generated MIBG and ARI polar maps. These results suggest that supersensitive repolarization responses depend not only on the presence or absence of denervation but also on the relative severity of denervation. The mechanisms responsible for postsynaptic denervation supersensitivity are not completely defined. Whereas denervation supersensitivity correlated with an increase in β-adrenergic receptor density and adenylyl cyclase activity in the totally denervated heart, there were no differences in β-adrenergic receptor density, affinity, or adenylyl cyclase activity in regionally denervated hearts. Warner et al suggest that an alteration distal to the β-adrenergic receptor potentiates adrenergic signal transduction in regionally denervated hearts. Our results showing that the supersensitivity response increases as the degree of denervation increases imply that the loss of presynaptic uptake is an important determinant of the response.

SS led to differential responses in the denervated myocardium. Approximately 30% of the electrodes showed ARI shortening, and 70% showed ARI prolongation. Sites that showed ARI shortening had a longer baseline ARI than sites that showed prolongation. This finding may indicate differences in regional myocyte properties. Previous studies have shown that the effects of SS differ substantially within hearts. Opthof et al found both a shortening and a prolongation of ventricular refractoriness in the same heart during SS. Mechanisms to explain these differential responses are not well understood; however, various concentrations of NE were released, and differential α- and β-receptor responses are thought to play a key role. In our study, sites that shortened ARI with SS showed a response qualitatively similar to the ARI responses due to NE infusion. There was an increasing degree of shortening as the severity of denervation increased. This raises the possibility that a sufficient amount of NE was released from adjacent partially denervated areas to evoke a supersensitivity response. Sham-operated rabbits showed ARI responses very similar to those of the normally innervated regions in the phenol-treated rabbits.

Arrhythmogenesis

Although heterogeneous innervation may play a role in increased arrhythmogenesis, particularly during sympathetic activation, this conclusion cannot be supported by our data, because no arrhythmias were induced. Our aim was to study the effects of regional denervation on local ventricular repolarization. For this reason, we chose a model of “pure denervation” without the confounding (but often necessary) additive substrate for arrhythmia: myocardial scar. Our results clearly show increased dispersion of repolarization that related not only to the presence or absence of denervation but also to the severity of denervation. In a recent study by Zabel et al, QT dispersion as measured from the baseline 12-lead ECG failed to predict mortality or arrhythmic events in a series of post–myocardial infarction patients. The lack of predictive power in the study by Zabel et al is probably a result of the limited resolution of regional changes in repolarization obtainable from a conventional 12-lead ECG. Other studies that used higher-resolution isopotential body surface mapping of activation and recovery have shown predictive power to identify patients with ventricular arrhythmias. The study by Zabel et al did show that left ventricular
ejection fraction and heart rate variability were independent predictors of arrhythmia. Myocardial sympathetic nerves are activated in patients with left ventricular dysfunction. This activation is associated with a decrease in heart rate variability. These findings are consistent with the concept that autonomic influences are important contributors to arrhythmogenesis. Our results show that alterations in the spatial distribution of local repolarization are related to the spatial distribution of sympathetic innervation and are further affected by dynamic changes in sympathetic tone.

Conclusions
The magnitude and dispersion of repolarization responses are related to the severity of denervation as well as the type of stimulation: neural (SS) versus humoral (NE). The differences may relate to the concentration of NE released. Our results show that the dispersion of repolarization is significantly influenced by regional changes in sympathetic innervation and dynamic changes in autonomic tone. For clinical studies, the presence or absence of denervated but viable myocardium, the severity of denervation, the underlying level of sympathetic tone, and the subsequent influences on local ventricular repolarization may be important and interactive determinants of arrhythmogenesis.

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