Scintigraphic and Electrophysiological Evidence of Canine Myocardial Sympathetic Denervation and Reinnervation Produced by Myocardial Infarction or Phenol Application

Joseph D. Minardo, MD, Mahmoud M. Tuli, MD, Bruce H. Mock, PhD,
Ronald E. Weiner, PhD, Harald P. Pride, BS,
Henry N. Wellman, MD, and Douglas P. Zipes, MD

Epicardial phenol application or transmural myocardial infarction in dogs produces sympathetic denervation of myocardium apical to the site of the intervention. Because efferent denervation is probably postganglionic, reinnervation most likely occurs but has not been shown. We investigated whether \(^{123}\)I-labeled metaiodobenzylguanidine (MIBG), a norepinephrine analogue taken up by sympathetic nerve terminals, could provide a scintigraphic image that would detect apical sympathetic denervation and possible reinnervation. Dogs underwent MIBG scintigraphic imaging at various times after phenol application or transmural myocardial infarction. The results of MIBG scintigraphy were correlated with electrophysiological responses obtained during ansae subclaviae and norepinephrine stimulation to establish the presence of neural denervation and reinnervation. Apical defects in the MIBG scan, which were associated with either normal perfusion by thallium or a smaller-sized defect, were found consistently in dogs that had apical sympathetic innervation. MIBG scintigraphic images returned to normal after 14 weeks (mean) at a time when reinnervation was shown to have occurred. Thus, the results of MIBG scintigraphy correlated accurately with the presence of denervation and reinnervation established by neuroelectrophysiological testing. Supersensitive refractory period shortening in response to norepinephrine infusion was present after denervation and persisted for more than 3 weeks after scintigraphic and electrophysiological evidence of reinnervation. Conclusions are that 1) MIBG can be used noninvasively to determine the presence of regional myocardial efferent sympathetic denervation and subsequent reinnervation, 2) reinnervation occurs after phenol application or transmural myocardial infarction, and 3) denervation supersensitivity persists even after reinnervation occurs. (Circulation 1988;78:1008–1019)

There is general agreement that the autonomic nervous system influences the development of some cardiac arrhythmias.\(^1\) The parasympathetic limb is thought to stabilize the electrophysiological milieu, whereas sympathetic stimulation is considered to be arrhythmogenic.\(^2\) However, those studies on the autonomic nervous system for the most part have been descriptive, and little information exists about actual electrophysiological mechanisms by which the autonomic nervous system modulates or precipitates the onset of arrhythmias. Work in our laboratory has focused on the hypothesis that an interplay exists between the autonomic nervous system and the myocardium: autonomic stimulation alters electrophysiological characteristics of myocardial cells, but myocardial injury,
either functional and transient or anatomic and permanent, disrupts autonomic neural transmission. Subsequently, the damaged myocardium can interact with altered autonomic innervation to produce arrhythmias.

The pattern of myocardial sympathetic innervation in dogs has been well described and is based on data from morphological and functional studies. Postganglionic sympathetic fibers course near the epicardium with the coronary arteries, penetrating the myocardium to innervate the endocardium. Disruption of sympathetic fibers by interventions that affect the epicardium such as epicardial phenol application or transmural myocardial infarction results in sympathetic denervation of otherwise normal ventricular myocardium apical to the site of intervention. Compared with the normally innervated basal myocardium, the denervated apical myocardium exhibits a lesser shortening of the effective refractory period in response to anseae subclaviae stimulation, loss of afferent sympathetic reflex responses produced by epicardial application of bradykinin, supersensitive refractory period shortening in response to infused norepinephrine and isoproterenol, a decrease in the myocardial norepinephrine content, and reduced tissue histofluorescence of catecholamine-containing fibers. Also, and most important, the development of denervation supersensitivity of the apical myocardium appears to be arrhythmogenic.

Although these studies have been limited to canine hearts, it is possible that similar denervation after myocardial damage, such as myocardial infarction, occurs in humans. However, it is very difficult, if not impossible, to determine whether sympathetic cardiac innervation in a patient is normal or abnormal.

Metaiodobenzylguanidine (MIBG) is an analogue of norepinephrine and guanethidine, and it shares the same uptake mechanism at sympathetic nerve terminals. Decreased MIBG uptake after chemical sympathectomy with either reserpine or 6-hydroxydopamine supports the fact that this agent is taken up by myocardial sympathetic nerve terminals. MIBG can be labeled with an iodine radioisotope (I) and used by a standard gamma camera to image organs that have dense adrenergic innervation. Previous work with this agent has shown its usefulness in localizing pheochromocytoma and other catecholamine-secreting tumors. Also, it has been shown to provide high-quality myocardial images in mammalian species including dogs and humans.

The purpose of this study was to 1) investigate whether an MIBG-generated scintigraphic image could be used noninvasively to demonstrate myocardial sympathetic innervation and denervation, 2) verify the MIBG scintigraphic results with neuroelectrophysiological testing, and 3) document the time course of denervation and possible reinnervation in dogs after epicardial phenol application and transmural myocardial infarction.

Materials and Methods

Forty-five healthy, adult mongrel dogs of either sex, weighing 16–28 kg were used for this study. All dogs underwent preoperative MIBG scintigraphy followed by one of four operative procedures and imaging protocols described below, which are designated as groups 1–4.

I-Labeled MIBG Scintigraphy

Nonlabeled MIBG was obtained from The University of Michigan, Ann Arbor, Michigan. Radiolabeling with high purity sodium I was achieved by heating the vented vial to dryness at 155°C for 30 minutes. One ml sterile water for injection was added to the cooled vial, and the heating step was repeated. Radiochemical purity of the I-labeled MIBG averaged greater than 98% by thin-layer chromatography. Specific activity ranged from 4 to 7 mCi/mg.

The dogs were lightly anesthetized with sodium secobarbital (25 mg/kg i.v.) before the imaging procedure. MIBG scintigraphy was accomplished 2–4 hours after the intravenous injection of 2 mCi I-MIBG to allow for significant clearance of the extra-neuronal MIBG uptake and to optimize the cardiac to lung ratio (A). All data were recorded onto a dedicated nuclear medicine minicomputer for display, video formatting, and quantitative analysis. Scintigraphic images were obtained with a commercially available wide field-of-view gamma camera (Elscint, Itasca, Illinois), equipped with a low-energy all-purpose collimator, with a 20% window centered upon the 159-keV photopeak of the I. During planar imaging, the liver and the spleen were shielded for a better dynamic range in cardiac images. Preoperative (baseline) and postoperative images consisted of one million count planar images (anterior, 45° left anterior oblique, and left lateral).

For quantification, a commercially available computer software program originally developed for TI (Washout Analysis, Elscint) was used. A circular region of interest was automatically drawn around the heart and visually adjusted so that the distance between the circumference of the region of interest and the visual edge of the myocardium averaged 4 pixels. After smoothing, bilinear interpolative background subtraction was performed as described by Goris and colleagues and modified by Watson and others. The center of the region of interest was positioned at the center of the left ventricular cavity separately for the baseline and the postoperative images to compensate for changes in the dog positioning during imaging. The com-
puter then generated angular profiles of the myocardial distribution by sampling the myocardial activity along 60 radii spaced at 6° intervals in a clockwise fashion, starting at a specified location on the region of interest for each of the three projections. These angular circumferential profiles thus quantify the segmental myocardial activity as an angular function from the visually located center of the ventricle. Because the amount of radioactivity injected and the time of imaging for each dog were closely standardized, the serial angular circumferential profiles could be displayed together for direct comparison, much in the same way as the stress and redistribution 201Tl circumferential profiles are routinely compared.

Tomographic images of the heart were obtained by means of single-photon emission computed tomography (SPECT). Data were obtained in a 180° or 360° gamma camera rotation about the thorax, (45° right anterior oblique to 45° left posterior oblique for the 180° rotation), stored in a 64 × 64 computer matrix with a Hann filter (cut-off frequency = 0.50) and subsequently reconstructed in short axis (apex to base), horizontal long axis (inferior to superior), and vertical long axis (septum to free wall).

Serial imaging was carried out every 2–4 weeks in a subgroup of dogs to define the time course of reinnervation. All scans were interpreted by a single observer blinded to the type of operative procedure and to the electrophysiological findings. The postoperative scans were compared with the baseline and the preceding postoperative scans. In addition, 50 randomly selected scans (of 110 performed) were independently interpreted by three blinded observers to establish interobserver correlation.

Surgical Preparations

In general, the dogs were anesthetized with sodium secobarbital (30 mg/kg i.v.), intubated with a cuffed endotracheal tube, and ventilated with room air by a constant volume ventilator (Model 607, Harvard, South Natick, Massachusetts). By sterile technique, a thoracotomy was performed in the left fifth intercostal space. The ribs and lungs were retracted, and the pericardium was incised with its edges sutured to the wound to support the heart. When the surgical procedure was completed, the chest was closed in layers, and negative pressure was reestablished in the pleural cavity. The dogs were then allowed to recover, and postoperative antibiotics were administered for 5 days to prevent wound infection. Analgesics were given as needed.

Group 1 Dogs: Phenol Application

Nineteen dogs had epicardial phenol (88% carbolic acid) application. Phenol was applied with a cotton-tipped applicator across the left anterior descending coronary artery (LAD) and then along the entire course of a moderate to large-sized diagonal branch of the LAD near the midanterior wall. Three such applications were made 10 minutes apart. Two of these dogs died before postoperative MIBG imaging could be accomplished.

Group 2 Dogs: Latex Injection

Twenty-two dogs underwent latex injections in a diagonal coronary artery. A moderate to large-sized diagonal branch of the LAD was selected near the midanterior wall. Five to seven millimeters of the proximal segment of the diagonal vessel was isolated, and two silk ligatures were placed under the artery. The proximal ligature was tied while the distal ligature was maintained under traction to prevent bleeding due to retrograde arterial flow. The vessel was incised and cannulated with a small plastic catheter. The distal ligature was then used 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 removed, and the distal ligature was tied around the embolized artery. Two of these dogs also died before postoperative MIBG imaging was accomplished. We have shown repeatedly that this isolation and cannulation technique does not interrupt perivascular sympathetic fibers.

Group 3 Dogs: Sham Operation

Two dogs underwent a sham procedure simulating epicardial phenol application. Instead of phenol, three applications of saline were made across the LAD and along a diagonal branch. Similarly, two dogs underwent a sham procedure simulating coronary latex injection. Five to seven millimeters of the proximal segment of the diagonal branch was isolated, but the artery was left intact and neither ligated nor cannulated.

Group 4 Dogs: MIBG and 201Tl Scintigraphy

To determine whether the myocardium apical to the site of phenol application and myocardial infarction produced by coronary latex injection exhibited normal perfusion, four dogs (two dogs with phenol application and two with latex injection) underwent simultaneous imaging with 201Tl at the time of their postoperative MIBG scan. The dogs were positioned in the usual fashion for planar imaging with MIBG. As the third MIBG image was being completed, 2–3 mCi 201Tl was injected intravenously. Ideally, the radionuclide with the lower energy, in this case 201Tl having the 60–80 keV photons of its mercury decay daughter, is used first to avoid the scatter photons that would result from the presence of a higher energy photon such as the 159 keV of 123I. However, in this experimental design, it was necessary to demonstrate that a defect (lesion) was present with 123I-labeled MIBG before 201Tl imaging would be undertaken. Approximately 10 minutes after 201Tl injection, the same planar images were obtained by positioning the gamma camera without moving the dog for direct superimposition of the
images. The \(^{201}\)Tl scans were then interpreted by a single blinded observer.

**Open-chest electrophysiological study.**Thirty-seven of the forty-five dogs underwent an open-chest electrophysiological study. As noted above, four dogs died before postoperative MIBG imaging. Four other dogs died before electrophysiological data could be obtained because of infection (three dogs) or during the operative preparation for the electrophysiological study (one dog). Electrophysiological studies were performed within 25 days (mean, 6 days) after the last MIBG imaging procedure.

For the electrophysiological study, the dogs were anesthetized with sodium secobarbital (30 mg/kg i.v.), intubated, and ventilated with room air by a Harvard respirator. Additional amounts of sodium secobarbital were given as needed to maintain anesthesia during the study. No data were obtained within 15 minutes of anesthetic administration. The right femoral artery was cannulated with a heparinized saline-filled polyethylene catheter that was connected to a Statham (P23Db) pressure transducer (Cleveland, Ohio) and a Honeywell strip chart recorder (1958 CRT Visicorder) (Boulder, Colorado) to monitor blood pressure continuously. The right femoral vein was cannulated with polyethylene tubing for intravenous infusion of normal saline at 10 ml/hr. A midline neck incision was made from which the right and left cervical vagi were isolated, doubly ligated, and transected. A median sternotomy was performed, and the pericardium was opened anteriorly and sutured to the edges of the sternal wound to make a cradle for the heart. The ansae subclaviae were isolated for later transection.

A single bipolar plunge electrode, constructed with two Teflon-coated stainless steel wires threaded through a 21-gauge needle and bent at the tip to form a hook, was placed near the left ventricular apex to record ventricular activity. The region of the sinus node was crushed with a large clamp applied to the lateral right atrial border. We have shown previously that this procedure does not interrupt neural innervation of the ventricle.\(^6\) Finally, four unipolar stimulating electrodes constructed in a similar manner were inserted into the midmyocardium of the anterior left ventricular wall. Two of these electrodes were placed in normal myocardium near the base of the heart, whereas the other two were placed in normal myocardium apical to the site of phenol application or myocardial infarction produced by latex injection. The site of phenol application was identified by discoloration and superficial scarring. The site of myocardial infarction was determined by visual inspection and by palpation of the indurated area of infarction. A subcutaneous indifferent electrode plate for unipolar pacing was placed in the superior portion of the anterior abdominal wall. After insertion of all electrodes, 30 minutes was allowed for stabilization of the electrode sites. After completion of the electrophysiological studies, the dogs were killed, and the hearts were removed for examination. The hearts were sliced along the long axis at 1-cm intervals and were stained with nitro blue tetrazolium to establish the presence or absence of transmural infarction and to confirm that the electrodes were not placed in infarcted myocardium.

The effective refractory period of the left ventricular myocardium at the two apical and two basilar sites was determined by the extrastimulus technique at a pacing cycle length of 300 msec. Pacing was accomplished with either a Bloom (Philadelphia, Pennsylvania) or custom made (Krannert Medical Engineering, Indianapolis, Indiana) programmable stimulator and an isolated constant-current stimulus source. The late diastolic threshold was measured at each site before each determination of the effective refractory period, and each site was stimulated with unipolar cathodal rectangular pulses of 2 msec duration at twice diastolic threshold. The diastolic threshold remained stable within 0.5 mA throughout the experiment. A train of eight stimuli (S1) was followed by a late premature stimulus (S2) that initially produced a propagated ventricular response. The ventricular response was recorded in the surface electrographic lead II and from the bipolar recording electrode near the left ventricular apex and was displayed at a rapid-sweep speed on a storage oscilloscope triggered from the seventh stimulus of the S1 drive train. The S1-S2 interval was shortened by 1-msec decrements near the effective refractory period until the effective refractory period was reached. The effective refractory period was defined as the longest S1-S2 interval at which the S2 stimulus failed to produce a propagated response on two successive attempts. The S1-S2 interval was then increased by 8–10 msec, and the S1-S2 interval was again decreased by 1-msec decrements until the effective refractory period was again reached. The second measurement of the effective refractory period had to be within 2 msec of the first, or the data were discarded and the procedure for measurement of the effective refractory period repeated.

The effective refractory period was determined at all four test sites after transection of the vagi but with intact, isolated ansae subclaviae. Next, the ansae subclaviae were doubly ligated and transected. Shielded bipolar electrodes were placed on both the left and right ansae subclaviae. At this point, the effective refractory periods were determined again. The ansae subclaviae were then stimulated bilaterally with square-wave pulses at 3 Hz, 3 mA, and 4 msec duration from a Grass S88 stimulator delivered through a constant-current stimulus isolator. Ansae subclaviae stimulation was continued for 4–6 minutes and was uniformly associated with at least a transient increase in mean systemic blood pressure (mean increase, 14 mm Hg). The effective refractory period was then determined at the four test sites while ansae subclaviae stimulation was continued. Ansae subclaviae stimulation was then discontinued, and the blood
pressure was allowed to return to baseline. After approximately 10 minutes with a stable baseline blood pressure, the effective refractory periods were again measured. After recovery, norepinephrine was infused on two different occasions at 0.5 and 1.0 μg/kg/min for 4–6 minutes and was uniformly associated with a large increase in mean systemic blood pressure (mean, 86 to 134 mm Hg after 0.5 μg/kg/min; mean, 86 to 161 mm Hg after 1.0 μg/kg/min norepinephrine i.v.).

While the norepinephrine infusions were continued, the effective refractory periods were again determined at all four test sites. Between infusions, the blood pressure was allowed to return to baseline and remain there for approximately 10 minutes before the next infusion was started.

**Statistics**

The changes in the effective refractory periods were analyzed with a three-way repeated measures analysis of variance. The independent grouping factor was the result of the MIBG scan, whereas the repeated measures were between the two electrodes at each location (apex and base) and the location of the electrode (apex vs. base). For the analysis of dogs with apparent reinnervation, a two-way repeated measures analysis of variance was used because the independent grouping factor was no longer necessary.

Because differences exist in the absolute value of the effective refractory period among the dogs, the electrophysiological data were analyzed by comparing the changes in the effective refractory period with each intervention by a two- or three-way repeated measures analysis of variance. The data from both denervation models were grouped together, although similar findings were noted when each model was analyzed individually.

**Results**

**Scintigraphic Findings**

MIBG provided high-quality myocardial scintigraphic images (Figures 1A and 2A). Also, MIBG images clearly demonstrated defects in anteroapical regions that correlated with myocardial sites proven by neuroelectrophysiological testing to be sympathetically denervated after either epicardial phenol application (Figure 1B) or transmural myocardial infarction from coronary latex injection (Figure 2B). Image interpretation was based primarily on the planar images. However, as reinnervation occurred, the planar images sometimes became equivocal, and the final interpretation was made with the SPECT or circumferential activity curves or both. Overall, the concordance of scan interpretation among observers was excellent. The primary observer had complete agreement with the two other observers for 74% of the scans. For the remaining 26% of the scans, the primary observer concurred with one other observer. The primary observer never disagreed with both secondary observers.

**Group 1 Dogs: Phenol Application**

Figure 3 summarizes the scintigraphic results for the 17 dogs with postoperative MIBG imaging after epicardial phenol application. Fifteen of these dogs exhibited an anteroapical defect on their first postoperative MIBG scan. The defects persisted during serial MIBG imaging for up to 17 weeks postoperatively. One of these dogs died before further imaging and before an electrophysiological study could be performed. Eight of these dogs underwent an electrophysiological study and were killed before any scintigraphic evidence of reinnervation. The six remaining dogs underwent serial imaging and exhibited normalization of their scans, consistent with reinnervation, 12–29 weeks postoperatively. Two of these six dogs with normal MIBG scans at 24 and 29 weeks postoperatively had not been imaged for approximately 22 weeks after their first postoperative scan that demonstrated an anteroapical defect. Thus, it is not clear precisely when reinnervation occurred in these dogs. The other four dogs that were followed serially had normal scans after a scan that showed an anteroapical defect.

Two dogs had normal MIBG imaging when their first scans were performed, 8 and 13 weeks postoperatively (Figure 3, left). Because no scans were obtained earlier after phenol application, it is unclear...
Thus, explanation.

FIGURE 4. Diagram summarizing results of dogs undergoing coronary latex injection. See text for details.

whether phenol failed to cause sympathetic denervation in these dogs or whether reinnervation occurred by 8 and 13 weeks postoperatively. A false-negative MIBG scan is excluded by the subsequent electrophysiological findings (see below). The reliability of phenol-induced sympathetic denervation makes reinnervation the most likely explanation. Thus, these data suggest that MIBG scintigraphy detects apical myocardial sympathetic denervation produced by epicardial phenol application. Denervation persists for a varying time period ranging from 8 to 17 weeks postoperatively. The scintigraphic data suggest evidence of reinnervation between 12 and 19 weeks postoperatively and perhaps as early as 8 weeks postoperatively.

**Group 2 Dogs: Latex Injection**

Figure 4 summarizes the scintigraphic results for the twenty dogs studied after latex injection into the diagonal coronary artery. Seventeen of these dogs had an anterioapical defect evident on their first postoperative MIBG scan. The defects persisted during serial MIBG imaging for up to 13 weeks postoperatively. Two of these dogs died before further imaging or an electrophysiological study could be performed. Ten of these dogs underwent an electrophysiological study and were killed before developing scintigraphic evidence of reinnervation. The five remaining dogs underwent serial imaging that demonstrated normalization of their scans 12 to 14 weeks postoperatively. All dogs with postoperative MIBG defects showed morphological evidence of transmural myocardial infarction, based on postmortem staining with nitro blue tetrazolium.

Three dogs had normal MIBG scans after coronary artery latex injection (Figure 4, left). Two of these dogs underwent their first scan within 2 weeks of surgery, and at the time of killing, 5 days after the MIBG scan, transmural myocardial infarction was absent by nitro blue tetrazolium staining. The third dog with no defect was not scanned until 14 weeks postoperatively. Transmural myocardial infarction was present at autopsy in this dog. As with the phenol data, the normal image in this dog could represent the failure of the transmural myocardial infarction to produce apical sympathetic denervation or initial denervation followed by reinnervation 14 weeks later. A false-negative MIBG scan was excluded by electrophysiological study (see below). Because transmural myocardial infarction reliably produces sympathetic denervation, including the fact that at 14 weeks after transmural infarction all dogs followed serially demonstrated reinnervation, it was believed that reinnervation was the most plausible explanation.

**RESULTS: LATEX**

FIGURE 4. Diagram summarizing results of dogs undergoing coronary latex injection. See text for details.
Thus, these data demonstrate that experimental transmural myocardial infarction produces sympathetic denervation of the apical myocardium that can be detected by MIBG scintigraphy. Denervation was not detected by MIBG imaging in dogs with nontransmural myocardial infarction. Finally, these scintigraphic data provide the first evidence of sympathetic reinnervation 12–14 weeks after transmural myocardial infarction.

**Group 3 Dogs: Sham Operation**

The four dogs undergoing the sham procedures had MIBG scans 2–3 weeks postoperatively followed by electrophysiological studies. All four of these dogs had normal postoperative MIBG scintigraphy.

**Group 4 Dogs: MIBG and 201TI Scintigraphy**

Two dogs treated with phenol and two dogs receiving latex injection underwent simultaneous MIBG and 201TI imaging 5 weeks after phenol application and 7–9 weeks after latex injection. Epicardial application of phenol produces only a superficial line of necrosis that would not be expected to alter the thallium image. As can be seen in Figure 1, after a normal preoperative MIBG scan (Figure 1A), an anteropapical defect is apparent on the postoperative MIBG scan (Figure 1B), whereas the thallium image (Figure 1C) is normal. In contrast, transmural myocardial infarction altered the images obtained simultaneously with both MIBG (Figures 2A and 2B) and thallium (Figure 2C). The image defect is clearly more extensive in the MIBG scan, most likely because MIBG uptake is absent or reduced in both the actual area of infarction (due to the presence of nonviable tissue and absence of blood flow) and the normal denervated myocardium apical to the infarcted area. 201TI uptake is reduced only in the infarcted area. Thus, these findings with simultaneous MIBG and 201TI imaging in both models are consistent with myocardial sympathetic denervation in normally perfused myocardium apical to epicardial phenol application and transmural myocardial infarction.

**Reinnervation**

Serial postoperative imaging with MIBG showed clear evidence of reinnervation in both denervation models (groups 1 and 2). Figure 5A is a preoperative left lateral MIBG image showing homogeneous uptake. This dog had epicardial phenol application. Repeat MIBG imaging 12 weeks postoperatively showed an anteropapical defect indicated by the arrows in Figure 5B. At 19 weeks postoperatively, MIBG imaging revealed essentially complete resolution of the defect with homogeneous MIBG uptake, which is consistent with reinnervation (Figure 5C). The computer analysis of circumferential myocardial activity for all three images is shown in Figure 5D. Zero degrees represents the starting point for a clockwise circumferential analysis and is located at the “2 o’clock” position on the left lateral image (corresponding to the midpoint of the mitral valve anulus). Thus, 180° represents the apex. The red curve was derived from the preoperative image (Figure 5A) and is essentially horizontal. In con-

---

**Figure 5.** Scintigraphic images of phenol application. Left lateral metaiodobenzylguanidine (MIBG) images obtained at control preoperative study (Panel A), 12 weeks after epicardial phenol application (Panel B), and 19 weeks postoperatively (Panel C). Control and the 19-weeks images show homogeneous uptake of MIBG, whereas the scan 12 weeks after phenol application shows a defect (arrows). Angular circumferential profiles of myocardial activity obtained preoperatively (red), 12 weeks postoperatively (yellow), and 19 weeks postoperatively (blue) (Panel D).

**Figure 6.** Scintigraphic images of latex injection. Left lateral preoperative metaiodobenzylguanidine (MIBG) image showing homogeneous uptake (Panel A). MIBG images obtained 7 weeks after latex injection showing anteropapical defect (Panel B) (arrow) and 14 weeks after latex injection showing homogeneous uptake (Panel C). Angular circumferential profiles of myocardial activity obtained preoperatively (red), 7 weeks postoperatively (yellow), and 14 weeks postoperatively (blue) (Panel D).
trast, the yellow curve is derived from Figure 5B and shows a decrease in myocardial activity beginning at 180° corresponding to the anteropapical defect seen on the analogue image. Finally, the blue curve, derived from Figure 5C, shows activity that parallels the red curve (preoperative study) and no longer shows the anteropapical defect, supporting the impression of reinnervation based on image analyses alone.

Figure 6 demonstrates similar findings for the latex infarction model. Figure 6A shows a preoperative left lateral MIBG image, whereas an anteropapical defect is evident in Figure 6B (arrows) 7 weeks after infarction. Figure 6C shows the corresponding left lateral image obtained 14 weeks after infarction showing essentially complete resolution of the defect, consistent with reinnervation. Circumferential myocardial activity curves are shown in Figure 6D. The red (preoperative) and blue (reinnervation) curves are parallel, whereas the yellow curve derived from Figure 6B shows decreased activity beginning at 180°, corresponding to anteropapical defect. These findings support the image interpretation of denervation at 7 weeks after infarction followed by reinnervation at 14 weeks after infarction.

Electrophysiological Data

Eight dogs with phenol application and 10 dogs receiving latex injection underwent electrophysiological studies after an MIBG scan showing evidence of anteropapical denervation (Figure 7).

After transection of the ansae subclaviae, effective refractory period increased equally at both apical and basal sites (9 ± 7 vs. 12 ± 8 msec, respectively, p = NS). During ansae subclaviae stimulation, the effective refractory period at the apex shortened less than at the base (−13 ± 11 vs. −23 ± 11 msec, respectively, p < 0.001). During both norepinephrine infusions, effective refractory period shortened more at the apex than at the base (0.5 µg/kg/min: −30 ± 10 vs. −19 ± 10 msec, respectively, p < 0.001; 1.0 µg/kg/min: −37 ± 7 vs. −29 ± 7 msec, respectively, p < 0.001). A plot of the dose-response curve (Figure 8) reveals a leftward shift, which is consistent with a hypersensitive response for the apical sites. These findings are consistent with apical sympathetic denervation and supersensitivity.

Nineteen dogs underwent electrophysiological studies after a normal MIBG scan. This group included the eight dogs treated with phenol and six dogs given latex injections with apparent reinnervation, one dog injected with latex without a transmural myocardial infarction, and the four dogs undergoing sham operation. The electrophysiological...
cal data from the four dogs undergoing sham operation (Figure 9) showed that changes in effective refractory period at the apex and base were similar in response to ansae subclaviae transection (15±10 vs. 13±5 msec, respectively, p=NS), ansae subclaviae stimulation (−26±9 vs. −26±8 msec, respectively, p=NS), and norepinephrine infusions (0.5 μg/kg/min: −20±5 vs. −19±5 msec, respectively, p=NS; 1.0 μg/kg/min: −32±8 vs. −32±4 msec, respectively, p=NS).

Finally, electrophysiological data from the dogs with apparent reinnervation were analyzed as a subgroup. The two phenol dogs in whom it was unclear when reinnervation may have taken place were omitted from this analysis. Also, data from one dog with latex injection were omitted from this analysis because the norepinephrine data were incomplete. The results for 11 dogs are illustrated in Figure 10. After transection of the ansae subclaviae, the effective refractory period increased equally at the apex and base (15±9 vs. 15±12 msec, respectively, p=NS). During ansae subclaviae stimulation, effective refractory period shortened equally at the apex and base (−26±8 vs. −27±8 msec, respectively, p=NS). However, both norepinephrine infusions shortened the effective refractory period more at the apex than at the base (0.5 μg/kg/min: −30±11 vs. −22±8 msec, respectively, p=0.004; 1.0 μg/kg/min: −38±12 vs. −32±9 msec, respectively, p=0.02). These data for norepinephrine infusion are also shown in a dose-response curve demonstrating a leftward shift of the curve for the apical sites (Figure 11). These results are consistent with apical sympathetic reinnervation, judged by the results of response to ansae subclaviae stimulation, but with a persistent supersensitive response to infused norepinephrine.

Discussion

New Observations

The major observation from this study is the demonstration that regional sympathetic denervation in the canine heart can be established noninvasively with a scintigraphic technique. The electrophysiological data provide proof of the regional denervation shown by the MIBG images. Thallium images exclude abnormalities in myocardial blood flow as a cause of
the defect in the MIBG image. Also noted for the first time in this model is that sympathetic reinnervation after phenol- and infarction-induced denervation occurs at 8–17 weeks. The final new observation is that denervation supersensitivity is present even though reinnervation has occurred.

Previous work from our laboratory has established the interplay between various experimental models of myocardial injury and disruption of autonomic neural innervation.6–10 It appears that this disruption may be arrhythmogenic.11 Whether such nonhomogeneous sympathetic innervation occurs in humans and plays a role in the genesis of ventricular arrhythmias is hypothetical and potentially important. The first step to prove this hypothesis would be to establish reliably the presence of regional sympathetic denervation noninvasively. Therefore, we sought to validate the MIBG technique by correlating it with the known electrophysiological characteristics of denervated myocardium.

From the scintigraphic, electrophysiological, and morphological findings presented in this study, it appears that 123I-labeled MIBG can reliably demonstrate areas of sympathetic denervation produced by epicardial phenol application and experimental transmural myocardial infarction resulting from coronary latex injection. The correlation between the scintigraphic and electrophysiological findings was excellent.

Five dogs with unexpectedly normal MIBG scintigraphy postoperatively helped verify the technique. Two of these dogs received coronary latex injections but, unusually, did not develop transmural myocardial infarction. Nontransmural myocardial infarction would not be expected to produce sympathetic denervation. The other three dogs that were expected to develop apical sympathetic denervation from epicardial phenol application (two dogs) or transmural myocardial infarction (one dog) demonstrated electrophysiological findings during ansae subclaviae stimulation indicative of apical innervation. Thus, the scintigraphic results correctly predicted normal sympathetic innervation as verified by the electrophysiological findings. Normal innervation was either due to reinnervation or to a failure to achieve denervation initially. We found no dog with a normal MIBG scan that showed electrophysiological evidence of denervation or any dog that had a defect on MIBG scan and had electrophysiological evidence of innervation.

Reinnervation

Peiss and colleagues27 reported that sympathetic reinnervation occurs in autotransplanted canine heart as early as 74 days after denervation.19 Those findings on sympathetic reinnervation after postganglionic interruption are consistent with our scintigraphic and electrophysiological evidence of reinnervation after both the phenol and latex interventions that caused postganglionic sympathetic disruption. Several observations regarding the time course and scintigraphic findings of sympathetic reinnervation can be made. It became apparent that scintigraphic proof of early myocardial sympathetic reinnervation was not always clear cut. The scans obtained soon after surgery had, for the most part, easily detected defects representing denervation. However, with serial imaging near the time of reinnervation, the defect occasionally was equivocal and was sometimes detected only by tomographic analysis. When this occurred, the dog had follow-up MIBG imaging within a week. Each time, the follow-up scan was interpreted as normal and thus consistent with reinnervation. Because it is not likely that sympathetic reinnervation was absent one week and present the next, the equivocal scans probably were due to a gradual regrowth of the postganglionic sympathetic fibers that became more obvious in a scan taken 1 week later.

Although the mean time to achieve reinnervation shown scintigraphically was similar for the epicardial phenol and latex injection models (14.5 and 13.0 weeks, respectively), there was much greater variability in the time course of reinnervation in the phenol model. For the phenol-treated dogs, reinnervation occurred from perhaps as early as 8 weeks to as late as 19 weeks postoperatively. For the dogs with latex-induced myocardial infarction, reinnervation was always demonstrated between 12 and 14 weeks postoperatively. The reason for the variability
in the phenol model is not clear but may represent variable degrees of neuronal disruption due to slight differences in the amount and width of the phenol stripe applied to the epicardium. The fact that the time course of reinnervation found in this study correlates fairly well with the time course noted by Peiss and colleagues suggests that the rate of regrowth and reinnervation by the postganglionic sympathetic neurons is similar regardless of what method is used for neuronal disruption.

**Denervation Supersensitivity**

Denervation supersensitivity was first described by Cannon in 1939. Kammerling and others demonstrated sympathetic denervation supersensitivity in apical segments of canine hearts subjected to latex-induced transmural myocardial infarction. Dogs with scintigraphic and electrophysiological evidence of sympathetic denervation in the present study also showed greater shortening of effective refractory period at apical than at basal sites in response to norepinephrine infusion, which is consistent with supersensitivity. Further, this supersensitive response was also noted in dogs with scintigraphic reinnervation and electrophysiological evidence of sympathetic innervation. It is possible that partial denervation existed in these dogs, which we have suggested may still produce supersensitivity. Whether this persistence of supersensitivity is temporary and associated with incomplete reinnervation or whether it is a permanent characteristic of reinnervation cannot be determined from the present study. Kaye and colleagues noted that catecholamine content of the denervated heart failed to reach normal values by 2 years after functional reinnervation, which may account for the persistence of the supersensitivity. The norepinephrine storage mechanism lags behind the specific membrane mechanism for uptake in the reinnervated heart after surgical denervation.

**Consideration of the Model**

Although latex induction of transmural myocardial infarction is obviously nonphysiological, we have verified repeatedly the use of this technique. Also, in these studies, we have shown that a careful dissection of the coronary artery adventitia does not interrupt sympathetic fibers. Finally, we have shown that electrophysiological data similar to those achieved with latex injection can be obtained if multiple coronary arteries are occluded to produce a transmural myocardial infarction.

**Clinical Applications**

Preliminary studies with MIBG scintigraphy in humans have shown abnormalities in MIBG uptake that may have been caused by either disruption or alterations in myocardial sympathetic innervation after myocardial infarction and in association with dilated cardiomyopathies. It is important to caution that such MIBG defects may have also arisen from some physiological change in the uptake pathway for MIBG such as might occur if there were competition for the uptake mechanism from a sympathetic agent such as norepinephrine. Nevertheless, it appears that a similar interplay between myocardial injury and autonomic nervous system innervation may exist in humans. Whether myocardial reinnervation occurs in these conditions is not known, although it does not appear to occur after human cardiac transplantation. Based on data presented in this study, it should be possible to use MIBG to demonstrate noninvasively the disruption of myocardial sympathetic innervation in humans after a variety of disease states, such as transmural myocardial infarction, and cardiac surgery including coronary artery bypass grafting, left ventricular aneurysmectomy, and cardiac transplantation. We have found no cardiac sympathetic neural uptake shortly after cardiac transplantation (unpublished results from our laboratory). The MIBG imaging technique could also be used to detect reinnervation if it occurs. Should such an interplay exist between myocardial damage and the autonomic nervous system, it may in part contribute to arrhythmias and sudden cardiac death associated with these pathophysiological conditions. MBG scans in patients with arrhythmias directly attributable to sympathetic abnormalities such as the idiopathic long QT syndrome or dysautonomias might shed further light on the pathogenesis of arrhythmias in these disease states.

**Acknowledgments**

We acknowledge the expert technical assistance of Monica Beal, Paul Kreitl, and John Krepskaw for the scintigraphic imaging; and Naomi S. Fineberg, PhD, for statistical analysis. Hiroshi Inoue, MD, for help with some of the experiments; and Doctor D.M. Wieland for supply of the nonlabeled MIBG.

**References**

8. Barber MJ, Mueller TM, Davies BG, Zipes DP: Phenol topically applied to canine left ventricular epicardium inter-
11. Inoue H, Zipes DP: Results of sympathetic denervation in the canine heart: Supersensitivity that may be arrhythmogenic. Circulation 1987;75:877
33. Stanton MS, Tuli M, Heger JJ, Miles WM, Mock BH, Wellman HN, Zipes DP: Comparative Spect 1-123 metaiodobenzylguanidine (MIBG) and thallium 201 cardiac imaging following myocardial infarction in patients (abstract). J Am Coll Cardiol 1988;11:81A

KEY WORDS • sympathetic denervation • sympathetic scintigraphy • sympathetic reinnervation • myocardial infarction • phenol
Scintigraphic and electrophysiological evidence of canine myocardial sympathetic
denervation and reinnervation produced by myocardial infarction or phenol application.
J D Minardo, M M Tuli, B H Mock, R E Weiner, H P Pride, H N Wellman and D P Zipes

Circulation. 1988;78:1008-1019
doi: 10.1161/01.CIR.78.4.1008

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 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/78/4/1008

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/