Adverse Effects of Chronic Cardiac Denervation in Conscious Dogs with Myocardial Ischemia

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SUMMARY. The extent to which total chronic cardiac denervation protects the ischemic myocardium was investigated in conscious dogs. The major hemodynamic difference after coronary artery occlusion was that left ventricular end-diastolic pressure rose significantly more, \( P < 0.01 \), in the denervated group (12 ± 1.5 mm Hg) than in the normal group (4.4 ± 1.4 mm Hg). Blood flow (radioactive microspheres) in the ischemic endo- and epicardium fell to similar levels at 3–5 minutes after coronary occlusion, but was significantly less (\( P < 0.01 \)) in denervated dogs at 3 hours after occlusion in the endo- (0.05 ± 0.01) and epicardium (0.30 ± 0.02 ml/min per g), than in the endo- (0.13 ± 0.03) and epicardium (0.42 ± 0.05 ml/min per g) in the normal group. A subgroup of normal dogs was also studied, with left ventricular end-diastolic pressure increased by volume loading to levels similar to those observed in the denervated group after coronary occlusion; in these dogs, blood flow was similar to that in the other two groups 3–5 minutes after coronary artery occlusion, but, at 3 hours, was significantly more depressed (\( P < 0.01 \)) than that observed in normal dogs without volume loading in both endo- (0.03 ± 0.01) and epicardial (0.25 ± 0.03 ml/min per g) layers. Infarct size, as a fraction of the area at risk, was significantly greater (\( P < 0.05 \)) in the denervated group (60 ± 4.3%) and in the subgroup of normal dogs with elevated left ventricular end-diastolic pressure (73 ± 5.8%), compared with the normal group without volume loading (37 ± 8.1%). Thus, in conscious dogs, total chronic cardiac denervation exerts an adverse effect on infarct size which may be related to the sustained elevation in left ventricular end-diastolic pressure and consequent impairment of collateral perfusion. (Circ Res 57: 383–392, 1985)

TOTAL cardiac denervation is becoming an increasingly important clinical issue, since the heart in a patient with cardiac transplantation is denervated. Furthermore, since accelerated coronary atherosclerosis has been described in patients with cardiac transplantation (Alonso et al., 1977; Griepp et al., 1977; Lurie et al., 1981; Hess et al., 1983; Uys and Rose, 1984), it becomes more important to understand the consequences of myocardial ischemia in the denervated heart.

The extent to which cardiac nerves modify the response to myocardial ischemia remains controversial. Recent evidence indicates that sympathetically induced coronary constriction can compete with cardiac metabolic vasodilation in the presence of coronary artery stenosis, resulting in an acute imbalance between oxygen supply and demand (Buffington and Feigl, 1981; Heusch and Deussen, 1983). Others have suggested that cardiac sympathetic nerve stimulation following coronary artery occlusion can extend myocardial ischemic injury (Giudicelli et al., 1980). Furthermore, observations derived from anesthetized animal models with acute and chronic cardiac denervation suggest that this intervention exerts a protective influence on the acutely ischemic myocardium. Reductions of infarct size (Jones et al., 1978a, 1978b; Barber et al., 1982), and susceptibility to dysrhythmias (Ebert et al., 1968; Barber et al., 1980), improvement of collateral perfusion (Jones and Scheel, 1980; Scheel and Jones, 1983), and maintenance of epicardial ventricular function (Thomas et al., 1981) all have been reported after coronary artery occlusion in anesthetized animals with chronic cardiac denervation or ventricular sympathectomy, compared with anesthetized dogs with normal innervation.

Considering that anesthesia and recent surgery exert dramatic effects on the regulation of the cardiovascular system in general, and autonomic control in particular (Vatner and Braunwald, 1975; Manders and Vatner, 1976; Zimpfer et al., 1981, 1982), it becomes important to test the hypothesis that total cardiac denervation exerts a salutary effect on the ischemic myocardium in the conscious animal. Accordingly in this study, the specific goals were: (1) to examine in the conscious animal with chronic total cardiac denervation the extent to which myocardial necrosis develops following acute coronary artery occlusion and the extent to which collateral perfusion is distributed to the ischemic myocardium, (2) to characterize the hemodynamic responses to coronary artery occlusion following
total cardiac denervation, and (3) to compare these observations with data obtained from similar protocols in a group of normally innervated animals and in a subgroup of normally innervated animals with left ventricular (LV) end-diastolic pressure elevated by volume loading following coronary artery occlusion.

**Methods**

Mongrel dogs of either sex weighing between 22 and 32 kg were instrumented 2–3 weeks before experimentation. The dogs were anesthetized with sodium pentobarbital, 30 mg/kg, and underwent a left thoracotomy at the 4th intercostal space using sterile procedures. The pericardium was opened and Tygon catheters were implanted in the aorta and left atrium for pressure recordings with Statham (P23ID) pressure transducers. A solid-state miniature pressure gauge (Konigsberg Instruments) was implanted in the LV cavity through the apex. This transducer was used for measurements of LV systolic pressure and LV end-diastolic pressure, and to obtain the first derivative of LV pressure, LV dP/dt, using an operational amplifier connected as a differentiator, which has a frequency response of 700 Hz. We cross-calibrated the solid state pressure gauge against measurements of systolic aortic pressure and left atrial pressure, using the implanted catheters and Statham pressure transducers. A recording of LV pressure was obtained at high amplification for measurement of LV end-diastolic pressure. The left circumflex coronary artery was isolated 3–5 cm from its origin, and an ultrasonic Doppler blood flow transducer was implanted around the vessel to monitor coronary artery blood flow and to verify complete coronary artery occlusion. A snare occluder, made of polyethylene tubing and 2-0 silk, was placed distally to the flow probe to occlude the vessel completely.

The instrumentation was implanted in 15 normal dogs and in eight dogs after cardiac denervation was completed. The technique utilized for intrapericardial cardiac denervation has been described in detail by Randall et al. (1980). The intrapericardial denervation technique consists of (1) section of the ventrolateral cardiac nerve, (2) adventitial stripping of the left superior pulmonary vein and of the right and common pulmonary artery, and (3) section of pericardial reflexions in the transverse sinus and around the superior vena cava, as well as ligation and section of the azygos vein. In addition to the intrapericardial denervation, the ansae subclaviae were cut bilaterally to ensure more complete sympathetic denervation. Completeness of cardiac denervation was confirmed at surgery by direct electrical stimulation of the left and right ansae subclaviae (10 Hz, 5 msec, 5–7 V) and the left and right thoracic vagi (20 Hz, 5 msec, 5–7 V). Absence of a change in heart rate and/or rhythm with electrical stimulation of these nerves at the time of surgery confirmed total denervation. Moreover, with the animals conscious, 2–3 weeks later, phenylephrine (10 μg/kg) raised mean arterial pressure by 27 ± 2 mm Hg and reduced heart rate by 33 ± 6 beats/min in the normal group, and increased mean arterial pressure by 56 ± 6 mm Hg while actually increasing heart rate by 9 ± 2 beats/min in the conscious dogs with total cardiac denervation. Nitroglycerin (15 μg/kg) reduced mean arterial pressure by 25 ± 2 mm Hg and increased heart rate by 71 ± 7 beats/min in normal dogs. In dogs with total cardiac denervation, nitroglycerin reduced mean arterial pressure by 34 ± 2 mm Hg, but did not change heart rate. In addition, at sacrifice, LV biopsies from the nonischemic zone were obtained for tissue catecholamine measurements using the radioenzymatic method of DaPrada and Zurcher (1976). Compared with the normal zone of innervated animals, tissue catecholamine levels were reduced by more than 98%, i.e., from 252 ± 38 pg/mg in normal dogs to 4.3 ± 0.9 pg/mg in dogs with total cardiac denervation.

Regional myocardial blood flow was measured by the radioactive microsphere technique, which has been utilized previously in our laboratory (Millard et al., 1977; Lavallee et al., 1983). Before left atrial injection, the 15-μm microspheres labeled with 95Nb, 85Sr, 111Ce, or 52Cr were suspended in a 0.01% Tween 80 solution (in 10% dextran) and were suspended sonically for 60 minutes. Immediately before use, microsphere suspensions were sonically dispersed for an additional 5 minutes and vortexed for 60 seconds. Before each injection of microspheres, a small quantity of Tween 80 solution was injected to test for potential adverse cardiovascular effects (Millard et al., 1977). One to 2 million microspheres in 1.0 ml of suspension medium were injected and flushed with 6 ml of saline over a 20-second period. Starting 30 seconds before injection, an arterial blood reference sample was withdrawn at a rate of 7.75 ml/min for a total of 150 seconds. In all, four regional blood flow determinations were made by this method. After the animals were killed, myocardial samples from the ischemic zone, i.e., within the area at risk, and the nonischemic contralateral normal zone, were obtained, divided in endocardial, mid-, and epicardial layers, weighed, and counted in a gamma counter (Searle Analytical) with appropriately selected energy windows. After correction of counts for background and cross-over, regional myocardial blood flow was obtained and expressed as ml/min per g of tissue. In addition, so as to eliminate variations in regional myocardial blood flow related to “microsphere loss” from the ischemic tissue generally observed after coronary occlusion, a correction factor was utilized (Reimer and Jennings, 1979; Jugdutt et al., 1979). Values for the ischemic tissue blood flow were multiplied by the ratio of blood flow in nonischemic myocardium to blood flow in the ischemic myocardium before coronary artery occlusion.

Three groups of dogs were studied: normal dogs, dogs with total cardiac denervation, and normal dogs with volume loading to elevate LV end-diastolic pressure to levels obtained in dogs with total cardiac denervation after coronary artery occlusion. In this latter group, 500 ml of blood were withdrawn 7–10 days before the experiment, and this blood was reinfused slowly during the experiment beginning at 30 minutes after coronary artery occlusion to elevate LV end-diastolic pressure to levels observed in dogs with cardiac denervation over the subsequent 24-hour period.

After control recordings of arterial pressure, LV systolic pressure, LV end-diastolic pressure, LV dP/dt, heart rate, and electrocardiogram (lead II), the first injection of microspheres was made. While continuously monitoring cardiovascular parameters, we tightened the snare occluder and periodically confirmed the absence of coronary blood flow by Doppler flow measurement over the following 48 hours. At 3–5 minutes after complete coronary artery occlusion, a second injection of microspheres was made, followed by additional injections of microspheres at 3 and 24 hours. Hemodynamics were monitored continuously for the first 6 hours, and at 24 and 48 hours after the
induction of ischemia. Dysrhythmias were treated with bolus injections of 2-3 mg/kg lidocaine for the first 150 minutes after coronary artery occlusion. Only one normal dog and one dog with total cardiac denervation showed any signs of discomfort during the procedure, and, accordingly, were immediately treated with morphine sulfate, 0.25 mg/kg, im. Serial measurements of serum creatine kinase (CK) were performed in normal dogs without volume loading and in dogs with cardiac denervation prior to coronary artery occlusion, and up to 36 hours after the induction of ischemia as previously described (Shell et al., 1971; Vatner et al., 1978). Samples were obtained hourly for the first 8 hours, then every 2 hours for the next 8 hours, every 4 hours for the next 8 hours, and, finally, every 6 hours for the next 12 hours. The samples were collected in tubes with ethylene glycol bis(β-aminoethoxy)ether)-N,N'-tetracetic acid and centrifuged. The plasma was decanted and frozen immediately at −70°C. The CK assay was performed using the method described by Rosalki (1967) and utilized previously in this laboratory (Vatner et al., 1978). CK values are expressed as international units per liter (IU/liter).

After recording hemodynamic data at 48 hours after coronary artery occlusion, we anesthetized the animals with sodium pentobarbital, and opened the chest through a median sternotomy. This procedure was carried out 24 hours after coronary artery occlusion in the four normal dogs in which LV end-diastolic pressure was elevated by volume loading. Biopsies were obtained for tissue catecholamine determination. The animals were killed with an overdose of barbiturates, and the heart was excised and placed on a perfusion apparatus. The method for dual perfusion employed in this study is similar to that previously employed by Lange et al. (1984). The ascending aorta was cannulated (distal to the sinus of Valsalva) and perfused retrogradely with warm (37°C) saline. The left circumflex coronary artery was cannulated at the site of occlusion with a short length of tubing and was also perfused with warm saline. The driving pressure for the perfusion apparatus was maintained via reservoir at 120-150 mm Hg and was the same for both cannulas. After perfusion for several seconds with warm saline, the perfusion media for the aorta was changed to include Evan’s blue dye (0.1% solution). Thus, the circumflex perfusion territory (anatomical area at risk) was perfused with saline, while the remainder of the heart was perfused with Evan’s blue solution. This simultaneous perfusion was maintained until the anterior free wall was stained a deep blue. After cardiac perfusion was complete, the heart was sectioned at the atrial-ventricular junction, the right ventricle was removed, and the left ventricle was divided into 6-7 rings, and individual ring weights were obtained. Photographs with calibration were made of both sides of individual rings for subsequent evaluation of perfusion bed size (anatomical area at risk) of the left circumflex coronary artery. After completion of this procedure, the rings were incubated in 1.0% triphenyl tetrazolium chloride (TTC) in phosphate buffer (pH 8.5) solution to reveal the normal tissue (stained in red) and the necrotic myocardium (TTC-negative) (Fishbein et al., 1981; Lavallee et al., 1984). Photographs were again prepared as described above. Slides (35 mm) were obtained and projected on a digitizing tablet at similar magnification for determination of area at risk and infarct surfaces. The tablet was calibrated with the tablet at similar magnification for determination of area at risk or infarcted. With those measurements and the individual weights of the myocardial rings, the following data were derived: area at risk as percent of LV weight, infarct weight as percent of LV weight, and infarct size as percent of area at risk. With the present techniques, our determination of area at risk for the left circumflex bed was similar to previously reported values (Reimer and Jennings, 1979; Lange et al., 1984). Nine normal animals, seven animals with total cardiac denervation, and four normal dogs with volume loading were analyzed for myocardial infarct size as percent of LV weight. However, one normal animal and one animal with total cardiac denervation could not be included in the analysis of infarct size as percent of area at risk, because more than 60 minutes elapsed between the time of death and dye perfusion.

Samples were obtained for regional myocardial blood flow determination from the area at risk, as indicated by the absence of Evans blue staining.

Values are reported as mean ± SEM throughout. Multiple comparisons to control within groups were assessed by analysis of variance and the Scheffé method. Analyses between groups were performed using Student’s t-test for group comparisons (Armitage, 1973). A probability level of 0.01 was chosen, according to the method of Bonferroni, to declare statistical significance between means, when multiple comparisons were made within or between groups (Miller, 1966).

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council [DHHS publication no. (NIH)78-23, revised 1978].

Results

Protocols were initiated in 15 normal dogs and eight dogs with total cardiac denervation. One animal in each group died of ventricular fibrillation less than 3 hours after coronary artery occlusion and could not be included in the final analysis. In addition, two normal animals without volume loading, one animal with total cardiac denervation, and one normal animal with volume loading died of ventricular fibrillation between 13 and 16 hours.

Hemodynamics (Table 1)

Before coronary artery occlusion, only minor differences were noted between normal and cardiac-denervated groups. Heart rate tended to be lower in normal dogs (94 ± 3.9 beats/min), compared to dogs with total cardiac denervation (107 ± 4.2 beats/min), whereas LV systolic pressure and LV dP/dt tended to be less in the dogs with cardiac denervation. These differences did not reach statistical significance, using a probability level of 0.01. LV end-
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TABLE 1
Effects of Coronary Artery Occlusion (CAO) on Hemodynamics in Normal (N), Cardiac-Denervated (D), and Volume-Loaded (VL) Groups

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Pre-CAO baseline</th>
<th>Change with CAO at 3–5 min</th>
<th>3 hr</th>
<th>6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>N</td>
<td>94 ± 3.9</td>
<td>+36 ± 7.0*</td>
<td>+66 ± 7.0*</td>
<td>+36 ± 7.0*</td>
<td>+36 ± 7.0*</td>
<td>+36 ± 7.0*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>107 ± 4.2</td>
<td>+11 ± 2.8†</td>
<td>+11 ± 2.8†</td>
<td>+11 ± 2.8†</td>
<td>+11 ± 2.8†</td>
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<tr>
<td></td>
<td>VL</td>
<td>96 ± 2.7</td>
<td>+56 ± 9.1*</td>
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<td>+56 ± 9.1*</td>
<td>+56 ± 9.1*</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>N</td>
<td>108 ± 3.9</td>
<td>+8.9 ± 5.1</td>
<td>-9.6 ± 5.9</td>
<td>-9.6 ± 5.9</td>
<td>-9.6 ± 5.9</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>101 ± 4.5</td>
<td>-2.0 ± 3.9</td>
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<td>-10 ± 3.8</td>
<td>-10 ± 3.8</td>
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</tr>
<tr>
<td></td>
<td>VL</td>
<td>105 ± 2.1</td>
<td>+7.0 ± 5.4</td>
<td>+16 ± 4.5*</td>
<td>+16 ± 4.5*</td>
<td>+16 ± 4.5*</td>
<td>+16 ± 4.5*</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>N</td>
<td>134 ± 4.5</td>
<td>+3.9 ± 5.7</td>
<td>-16 ± 5.5*</td>
<td>-25 ± 6.1*</td>
<td>-25 ± 6.1*</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>119 ± 4.3</td>
<td>-6.6 ± 2.2*</td>
<td>-14 ± 4.2*</td>
<td>-22 ± 4.1*</td>
<td>-22 ± 4.1*</td>
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<tr>
<td></td>
<td>VL</td>
<td>123 ± 4.4</td>
<td>+5.7 ± 7.4</td>
<td>-5.0 ± 5.3</td>
<td>-25 ± 6.0*</td>
<td>-25 ± 6.0*</td>
<td>-25 ± 6.0*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>N</td>
<td>7.7 ± 0.5</td>
<td>+4.4 ± 1.4*</td>
<td>+2.8 ± 1.2</td>
<td>+1.8 ± 1.4</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>7.3 ± 1.2</td>
<td>+12 ± 1.5†</td>
<td>+7.9 ± 1.0†</td>
<td>+12 ± 2.5*</td>
<td>+12 ± 2.5*</td>
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<tr>
<td></td>
<td>VL</td>
<td>5.8 ± 1.1</td>
<td>+7.2 ± 2.5*</td>
<td>+6.5 ± 0.4*</td>
<td>+8.1 ± 0.9†</td>
<td>+8.1 ± 0.9†</td>
<td>+8.1 ± 0.9†</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg/sec)</td>
<td>N</td>
<td>3739 ± 232</td>
<td>+102 ± 221</td>
<td>-693 ± 269*</td>
<td>-950 ± 309*</td>
<td>-1441 ± 361*</td>
<td>-1327 ± 262*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3090 ± 153</td>
<td>-289 ± 129</td>
<td>-379 ± 65*</td>
<td>-782 ± 118*</td>
<td>-942 ± 111*</td>
<td>-941 ± 93*</td>
</tr>
<tr>
<td></td>
<td>VL</td>
<td>3555 ± 225</td>
<td>+287 ± 299</td>
<td>+7 ± 338</td>
<td>-543 ± 408</td>
<td>-1014 ± 502</td>
<td>-1014 ± 502</td>
</tr>
</tbody>
</table>

* Differences from baseline P < 0.01.
† Differences from N group P < 0.01.

Diastolic pressure and mean arterial pressure were similar in both groups. At 3–5 minutes after coronary occlusion, heart rate increased more (P < 0.01) in normal animals (+36 ± 7.0 beats/min) than in animals with total cardiac denervation (+11 ± 2.8 beats/min). However, between 3 and 48 hours after coronary artery occlusion, differences in heart rate were no longer statistically significant. Changes in mean arterial pressure, LV systolic pressure, and LV dP/dt were similar in normal and cardiac-denervated groups throughout the observation period. A major difference was noted for changes in LV end-diastolic pressure after coronary artery occlusion (Fig. 1). At 3–5 minutes after coronary occlusion, LV end-diastolic pressure increased by 4.4 ± 1.4 mm Hg in normal dogs and by 12 ± 1.5 mm Hg in the group with total cardiac denervation. This greater (P < 0.01) elevation in LV end-diastolic pressure for animals with total cardiac denervation was also observed at 1, 3, 6, and 48 hours after coronary artery occlusion.

Hemodynamics before coronary artery occlusion and 3–5 minutes after the induction of ischemia were the same in the four normal dogs with volume loading and in the group of nine normal dogs with...
out volume loading. After volume loading was initiated at 30 minutes after coronary artery occlusion, measurements of heart rate, mean arterial pressure, LV systolic pressure, and LV dP/dt were not different from the group of normal dogs without volume loading. However, values of LV end-diastolic pressure were similar to those observed in dogs with total cardiac denervation and were significantly higher ($P < 0.01$) than in the group of normal animals without volume loading.

**Regional Myocardial Blood Flow**

**Nonischemic Zone**

Prior to coronary artery occlusion, regional myocardial blood flow levels in the nonischemic zone were similar in the three experimental groups. Endocardial blood flows averaged 0.98 ± 0.04, 1.00 ± 0.05, and 1.03 ± 0.06 ml/min per g in normal dogs, in dogs with total cardiac denervation, and in normal dogs with volume loading, respectively. Epicardial blood flows were 0.66 ± 0.04, 0.63 ± 0.04, and 0.76 ± 0.07 ml/min per g in normal dogs, in dogs with total cardiac denervation and in normal animals with volume loading, respectively. After coronary artery occlusion, myocardial blood flow to the nonischemic myocardium increased in all three groups. At 24 hours, regional myocardial blood flow in the nonischemic zone had returned to baseline in dogs with total cardiac denervation and normal dogs without volume loading, but remained significantly elevated ($P < 0.01$) in endocardial (1.54 ± 0.11) and epicardial (1.19 ± 0.11) layers of normal dogs with volume loading.

**Ischemic Zone (Fig. 2)**

Within the area at risk, as indicated by the absence of Evan’s blue staining, regional myocardial blood flow prior to coronary artery occlusion was identical to the values in the nonischemic myocardium, since a correction factor was employed to eliminate problems due to "microsphere loss."

At 3–5 minutes after coronary artery occlusion, regional endocardial blood flow fell to 0.05 ± 0.01, 0.03 ± 0.01, and 0.03 ± 0.01 ml/min per g, respectively, in normal dogs, in dogs with total cardiac denervation, and in normal dogs with volume loading. At 3 hours after coronary artery occlusion, regional endocardial blood flow rose ($P < 0.01$) in normal dogs to 0.13 ± 0.03 ml/min per g. In contrast, in dogs with total cardiac denervation, endocardial blood flow rose to 0.30 ± 0.02 ml/min per g. In normal dogs with volume loading, regional endocardial blood flow was significantly less ($P < 0.01$) than values observed in normal dogs without volume loading. In normal dogs with volume loading, regional endocardial blood flow remained at 0.03 ± 0.01 ml/min per g, which level is similar to that observed in dogs with total cardiac denervation, but significantly less ($P < 0.01$) than that observed in normal dogs without volume loading. At 24 hours after coronary artery occlusion, regional endocardial blood flows were slightly, but not significantly, higher in normal dogs (0.12 ± 0.02) than in dogs with total cardiac denervation (0.08 ± 0.01 ml/min per g). In contrast, endocardial blood flow was significantly less ($P < 0.01$) in normal dogs with volume loading (0.02 ± 0.01 ml/min per g).

In epicardial layers, regional blood flows at 3–5 minutes after coronary artery occlusion fell to 0.19 ± 0.02, 0.19 ± 0.01, and 0.17 ± 0.02 ml/min per g, respectively, in normal dogs, in dogs with total cardiac denervation, and in normal dogs with volume loading. At 3 hours after coronary artery occlusion, regional epicardial blood flow rose ($P < 0.01$) in normal dogs to 0.42 ± 0.05 ml/min per g. In contrast, epicardial blood flow at 3 hours after coronary artery occlusion was significantly less ($P < 0.01$) in dogs with total cardiac denervation (0.30 ± 0.02 ml/min per g). In normal dogs with volume loading, regional epicardial blood flow at 3 hours after CAO averaged 0.25 ± 0.03 ml/min per g, which was similar to that observed in dogs with

**FIGURE 2.** Mean ± SEM values for endocardial (solid lines) and epicardial (broken lines) blood flow levels are illustrated for normal dogs (circles), dogs with total cardiac denervation (triangles), and normal dogs with volume loading (squares) at 3–5 minutes, 3 hours, and 24 hours following coronary artery occlusion. At 3 hours after coronary artery occlusion, endocardial and epicardial blood flow levels were less ($P < 0.01$) in dogs with total cardiac denervation and in normal dogs with volume loading as compared to normal animals without volume loading.
total cardiac denervation, but significantly less ($P < 0.01$) than that observed in normal dogs without volume loading. At 24 hours after coronary artery occlusion, regional epicardial blood flows in normal dogs and in dogs with total cardiac denervation averaged $0.61 \pm 0.04$ and $0.67 \pm 0.03$ ml/min per g, respectively, and were similar to preocclusion baseline. In contrast, epicardial blood flow levels averaged $0.45 \pm 0.04$ ml/min per g in normal dogs with volume loading, which was significantly less ($P < 0.01$) than in dogs with total cardiac denervation or normal dogs without volume loading.

**Infarct Size vs. Area at Risk (Fig. 3)**

Similar LV weights averaging $171 \pm 10.6$, $164 \pm 8.5$, and $161 \pm 11.8$ g and body weights averaging $25.2 \pm 2.8$, $26.2 \pm 0.7$, and $23.2 \pm 1.3$ kg were obtained in normal dogs, in dogs with total cardiac denervation, and in normal dogs with volume loading, respectively. The amount of tissue at risk as determined by postmortem dye perfusion was also similar for normal dogs ($59.5 \pm 5.9$ g), for dogs with total cardiac denervation ($65.1 \pm 6.1$ g), and for normal dogs with volume loading ($61.5 \pm 0.7$ g). Expressed as percent of the area at risk, the group with total cardiac denervation showed significantly more ($P < 0.05$) necrotic tissue ($59.9 \pm 4.3$%) than the normal group ($37.4 \pm 8.1$%). Similarly, the percentage of left ventricle that became necrotic was significantly greater ($P < 0.05$) in dogs with total cardiac denervation ($22.4 \pm 1.7$%) than in normal dogs ($14.0 \pm 2.6$%). Thus, despite similar amounts of tissue at risk in both groups, the ultimate extent of necrosis induced by coronary artery occlusion was greater in dogs with total cardiac denervation than in the normal group.

In normal dogs with volume loading, infarct size expressed as percent of the area at risk averaged $72.9 \pm 5.8$% and $28.2 \pm 2.8$% as percent of the left ventricle. These values were significantly greater ($P < 0.05$) than those observed for normal dogs without volume loading, but were not significantly greater than infarcts in dogs with total cardiac denervation.

**Plasma Creatine Kinase Measurements**

Before coronary artery occlusion, plasma CK levels were similar for normal dogs ($36.8 \pm 3.50$ IU/liter) and dogs with total cardiac denervation ($36.9 \pm 4.38$ IU/liter). Peak CK levels occurred $12.2 \pm 0.8$ hours after coronary artery occlusion in normal dogs, and at $11.4 \pm 0.6$ hours in dogs with total cardiac denervation. However, peak values were greater ($P < 0.05$) in the group with total cardiac denervation ($1658 \pm 153$ IU/liter), compared with the normal group ($1008 \pm 183$ IU/liter). Integrated areas of CK curves vs. time in the group with cardiac denervation yielded values $87\%$ greater than those in the normal group.

**Discussion**

The technique utilized in the present study to perform total cardiac denervation has been previously demonstrated to be effective in abolishing cardiac responses to vagal and stellate nerve electrical stimulation (Randall et al., 1980). In addition to this approach, total cardiac denervation was confirmed in the present study, by the absence of reflex cardiac responses to pressor agents in conscious animals and by reductions of tissue catecholamine content by more than $98\%$ in denervated as compared to normal animals. The mean LV tissue noradrenaline levels for the nonischemic zone ($252 \pm 38$ pg/mg) of innervated dogs is somewhat lower than values recently reported by Pierpont et al. (1984) for normal dogs. However, reductions of
myocardial norepinephrine levels of 33–39% at 2 days after coronary artery occlusion have been reported for the nonischemic myocardium of dogs (Mathes et al., 1971). Furthermore, other LV tissue norepinephrine determinations from our laboratory from innervated animals with chronic instrumentation, but not subjected to coronary artery occlusion, yielded values of 575 ± 87 pg/mg of tissue (Vatner et al., 1985).

The present investigation revealed several important features concerning the influence of cardiac nerves on the development of myocardial ischemia and consequent infarction in conscious animals. First, in this model, total chronic cardiac denervation does not exert a protective effect on the ischemic myocardium and does not prevent the development of myocardial injury and necrosis following acute coronary artery occlusion. In fact, observations made in the present study indicate that the ultimate amount of myocardium that becomes necrotic following coronary artery occlusion is greater in totally denervated hearts than in normal hearts. Both in terms of absolute mass of ventricular myocardium irreversibly injured, and in terms of percent of the area at risk that became necrotic following coronary artery occlusion, the denervated heart demonstrated a greater vulnerability to ischemia and myocardial necrosis. This was substantiated with measurements of infarct size using the TTC method and was further documented by greater peak levels and total amounts of CK released into the blood following coronary artery occlusion in the group of dogs with cardiac denervation. Moreover, the similarity in time-to-peak CK levels suggests that the infarct process evolved at similar rates in normal and totally denervated hearts undergoing myocardial ischemia.

Second, a positive effect of cardiac denervation on collateral perfusion following coronary artery occlusion was not demonstrated in this model. The totally denervated heart showed a pattern similar to the innervated myocardium, in terms of reduction of regional perfusion at 3–5 minutes after coronary artery occlusion, but showed less recovery of regional blood flow at 3 hours after occlusion. This suggests that functionally, preformed collateral vessels of the totally denervated heart are less effective following coronary artery occlusion than in normally innervated animals.

Third, differences were noted in overall ventricular function and hemodynamics between normal and cardiac-denervated groups. After coronary artery occlusion, the totally denervated heart differed in terms of responses of heart rate and of LV end-diastolic pressure. The initial tachycardia observed in normal dogs after coronary artery occlusion was blunted in cardiac-denervated dogs. However, the inability to demonstrate prolonged differences in responses of heart rate in the two groups at 3, 6, 24, and 48 hours after coronary artery occlusion may have been due to the presence of denervation supersensitivity to catecholamines in the dogs with chronic cardiac denervation (Trendelenberg, 1966; Dempsey and Cooper, 1968; Vatner et al., 1985), which could have been responsible for the late increase in heart rate and appearance of arrhythmias. It is unlikely that supersensitivity to circulating catecholamines is responsible for the larger infarcts observed in conscious dogs with total cardiac denervation, since, in prior studies in anesthetized animals, this mechanism of denervation supersensitivity was also present, but infarcts were smaller in the anesthetized dogs with cardiac denervation (Jones et al., 1978a, 1978b; Barber et al., 1982).

There was a more striking and sustained difference in measurements of LV end-diastolic pressure. Compared to the normal group, the animals with total cardiac denervation responded to coronary artery occlusion with significantly greater increases in LV end-diastolic pressure at 3–5 minutes and at 1, 3, 6, and 48 hours after coronary artery occlusion. It is possible that either the sustained elevation of LV end-diastolic pressure reflected the more extensive area of necrosis observed in the dogs with total cardiac denervation, or was in fact, responsible for the augmented infarct size. The experiments in the normal animals with volume loading following coronary artery occlusion support the latter possibility. Since infarct size was augmented in both the cardiac-denervated dogs and the normal dogs with elevated LV end-diastolic pressure, it is more likely that the increased LV end-diastolic pressure was responsible for, rather than a consequence of, the increased amount of necrosis. In further support of this concept is the fact that areas at risk were similar, yet, even at 3–5 minutes after coronary artery occlusion, LV end-diastolic pressure was significantly higher in dogs with total cardiac denervation. This increase could have been facilitated initially by the smaller increases in heart rate at 3–5 minutes after coronary artery occlusion in dogs with total cardiac denervation, compared to normal dogs, since increases in heart rate reduce diastolic filling time. However, it cannot be excluded that the more intense ischemia observed 3 hours after coronary artery occlusion and the greater infarct size observed in cardiac-denervated animals could have been responsible for maintaining elevated LV end-diastolic pressure at a later time, when statistically significant differences in heart rate were no longer observed. In this connection, it is important to realize that the elevated LV end-diastolic pressure preceded the initiation of necrosis, and preceded any differences in intensity of ischemia.

Prior studies by Dunn and Griggs (1983) and by Kjekshus (1973) suggested that increases in left ventricular preload exert a deleterious effect on coronary blood flow during ischemia. To test further the hypothesis that the increased LV end-diastolic pressure is an important mechanism for the larger infarcts in dogs with total cardiac denervation, we also
studied a subgroup of four normal dogs with LV end-diastolic pressure raised to levels similar to those attained in dogs with cardiac denervation. In those animals, infarct sizes were similar to those of dogs with total cardiac denervation and significantly greater than in normal dogs without volume loading, supporting the view that an increase in preload in dogs with total cardiac denervation is a potential important determinant of infarct size.

The conclusions of the present study indicating that total cardiac denervation has deleterious effects on the response to acute coronary artery occlusion are in sharp contrast with most previous reports which suggest that cardiac denervation protects against the ischemic insult (Jones et al., 1978a, 1978b; DuPont et al., 1979; Thomas et al., 1981; Barber et al., 1982). Despite the fact that the surgical technique for total cardiac denervation utilized in the present study is similar to that used in some of the prior studies (Jones et al., 1978b; Thomas et al., 1981), an important feature of the present study is that myocardial ischemia was induced in the conscious animal model where the complicating influences of anesthesia and recent surgery were excluded. It is important to note that most of the studies reporting beneficial effects of chronic cardiac denervation following coronary artery occlusion were conducted in anesthetized animals. When conscious, instrumented animals were used in a previous study by Randall et al. (1981), the protective effects of denervation became less apparent, as reflected by similar reductions in global ventricular function following transient coronary artery occlusion in normal animals and animals with cardiac denervation. However, Randall et al. (1981) studied 5-minute coronary artery occlusions in conscious dogs and, accordingly, could not address the major findings of the current investigation. Changes in regional myocardial blood flow and myocardial infarct size have not been reported previously for experiments in conscious animals with total cardiac denervation.

As a potential mechanism for the protective effect of denervation observed in anesthetized animals, reduction of collateral resistances (Jones and Scheel, 1980; Scheel and Jones, 1983) and cardiac oxygen requirements have been postulated (Gregg et al., 1972, Jones et al., 1982). In the prior studies of Jones and Scheel, assessment of collateral perfusion in the chronically denervated myocardium was performed using a perfusion technique in the isolated heart preparation. These studies revealed substantial reductions of collateral resistances in the chronically sympathectomized heart. Supportive data were obtained from the same group of investigators in anesthetized open-chest animals with chronic ventricular sympathectomy, where regional blood flow, measured with microspheres, was greater in ischemic regions after coronary artery occlusion (Jones et al., 1978a; DuPont et al., 1979). This was not observed in the conscious animals with total cardiac denervation in the present investigation. In fact, at 3 hours after coronary artery occlusion, regional blood flow was significantly lower in endo- and epicardial layers in the dogs with total cardiac denervation, compared with normal dogs. Thus, whereas prior studies have demonstrated that the chronically sympathectomized heart has more collateral reserve (Jones and Scheel, 1980; Scheel and Jones, 1983), these potential salutary mechanisms are masked during the ischemic process in the intact, conscious animal. In support of this concept, as pointed out by Kjekshus (1973), Hoffman (1978), Griggs (1981), and Dunn and Griggs (1983), LV end-diastolic pressure is an important determinant of coronary driving pressure and collateral blood flow. The sustained rise in LV end-diastolic pressure after coronary artery occlusion in conscious animals with total cardiac denervation most likely reduced the pressure gradient for collateral perfusion, inhibited full opening of collateral channels, and resulted in more extensive myocardial necrosis. In further support of this concept are the data on regional myocardial blood flow for the group of normal dogs with volume loading. In those animals, regional endocardial and epicardial blood flows were significantly depressed at 3 and 24 hours after coronary artery occlusion, compared to the normal group of animals without volume loading. Thus, the deleterious effects of increased LV end-diastolic pressure on collateral blood flow and infarct size in the dogs with total cardiac denervation probably represent the mechanisms responsible for the differences from the control group.

There are several differences between the present investigation and prior reports concerning the protective effects of cardiac denervation on myocardial ischemia. As noted above, with the exception of the study by Randall et al. (1981), which did not find a protective effect of cardiac denervation and was conducted in conscious animals, the remaining studies demonstrating protective effects were conducted in anesthetized animals or in isolated hearts (Ebert et al., 1968; Jones et al., 1978a, 1978b; Barber et al., 1980; Jones and Scheel, 1980; Thomas et al., 1981; Barber et al., 1982; Scheel and Jones, 1983). Another difference between the current data and some of the prior studies showing beneficial effects of denervation on collateral perfusion is that, in some of those prior experiments, the vagi remained intact (Jones et al. 1978a; DuPont et al., 1979). It is conceivable that the vagus exerts a beneficial influence during the development of myocardial ischemia, and that vagal denervation would tend to blunt the reflex heart rate response to coronary artery occlusion, which might be important in preventing the deleterious effects of abnormally elevated LV end-diastolic pressure. Thus, chronic sympathectomy in the absence of parasympathectomy might be beneficial for the response to ischemia, even in the conscious animal. There are two other minor points of difference between the present and prior investigations which
also should be mentioned. First, in those studies demonstrating the favorable effects of cardiac denervation on responses of ventricular function and necrosis following coronary artery occlusion (Jones et al., 1978a; 1978b; Thomas et al., 1981; Barber et al., 1982), the left anterior descending, rather than the left circumflex coronary artery, was occluded. Second, the denervation employed in the current investigation differed slightly from that described by Randall et al. (1980), in that we also transected the ansae subclaviae.

Previous estimations of metabolic requirements of the chronically denervated myocardium indicated that lower myocardial perfusion and reduced oxygen consumption (Gregg et al., 1972; Jones et al., 1982) can explain the resistance of the denervated heart to ischemia. In fact, Elson et al. (1981) noted that cardiac denervation exerts a transient protective effect against myocardial necrosis by increasing the time required for necrosis to develop after brief (40-minute) coronary occlusion, but not when coronary occlusion was maintained for 80 minutes. In the present model, since coronary artery occlusion was maintained throughout the study, a potential transient benefit of total cardiac denervation was excluded. In fact, the severity of ischemia observed in dogs with total cardiac denervation at 3–5 minutes after coronary artery occlusion and the limited recovery of myocardial blood flow noted at 3 hours suggest that even a reduction in cardiac metabolic demand would not be sufficient to prevent the development of necrosis observed in the denervated hearts. Moreover, based upon the measurements of plasma CK following coronary artery occlusion, the similarity in time required to achieve maximal plasma CK levels in normal and cardiac-denervated groups suggests that total cardiac denervation does not substantially delay myocardial injury.

In conclusion, total chronic cardiac denervation does not exert a protective influence on the ischemic myocardium in the conscious dog model. In fact, this procedure resulted in a significant increase of myocardial necrosis following coronary artery occlusion associated with greater elevation in LV end-diastolic pressure and reduction in collateral blood flow after coronary artery occlusion. Thus, neither the initial severity of ischemia nor the subsequent opening of collateral channels was influenced favorably by total cardiac denervation in this model. The adverse effects observed in dogs with total cardiac denervation may have been due to the elevated LV end-diastolic pressure, since the group of normal dogs in which LV end-diastolic pressure was elevated by volume loading following coronary artery occlusion behaved similarly in terms of reduced collateral blood flow and increased infarct size.

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References


Jones CE, Beck LY, DuPont E, Barnes GE (1978a) Effects of...
Manders WT, Valter SF (1976) Effects of sodium pentobarbital anestheia on left ventricular function and distribution of cardiac output in dogs, with particular reference to the mechanism for tachycardia. Circ Res 39: 512–517
Scheel KW, Jones CE (1983) Reduced resistances of septal artery collateral channels after cardiac sympathectomy. Basic Res Cardiol 78: 373–383

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