Improved Distribution of Regional Oxygenation in Denervated Ischemic Dog Myocardium

Bat-Ami Acad, Joel Joselevitz-Goldman, Peter M. Scholz, and Harvey R. Weiss

The role of the adrenergic nervous system in the response to coronary artery occlusion has been examined using surgical and chemical denervation techniques. Experiments were conducted on four groups of dogs (n=18): 1) untreated controls; 2) intrapericardial denervation immediately prior to coronary ligation; 3) surgical denervation 2 weeks prior to the experiment; and 4) chemical sympathectomy 5 days prior to the experiment with 6-hydroxydopamine (50 mg/kg). Small artery and vein O2 saturations were obtained microspectrophotometrically and combined with radioactive microsphere blood flow determinations to calculate regional myocardial O2 consumption in open chest dogs. Denervation significantly reduced the preocclusion heart rate from 165 ± 16 beats/min in the control to 114 ± 13 in the chronic surgically denervated and to 137 ± 15 in the chemically sympathectomized groups. After 2 hours of occlusion, the O2 consumption and flow were similar in the nonischemic area except for lower values in the surgically denervated group. Total coronary blood flow and O2 consumption in the occluded regions were not significantly affected by chronic denervation. However, significant elimination of areas with low venous O2 saturation (less than 20%) were found in the ischemic myocardium of the chronically denervated groups as compared with the control or with the acutely denervated dogs. The mean venous O2 saturation was found to be significantly higher in all regions of these two groups as compared with the control. The O2 extraction was also lowered. Thus, chronic denervation reduced microregional heterogeneity of oxygenation in the ischemic myocardium. It is likely that the protective effect of denervation on the ischemic heart is related to the lowered levels of myocardial norepinephrine. (Circulation Research 1988;62:1041–1048)

There are experimental data to indicate that cardiac denervation may provide some protection to an ischemic myocardial region. Evidence indicates that cardiac denervation may reduce dysrhythmias following coronary artery occlusion and provide protection against the development of ischemia. Gregg et al1 have reported that O2 consumption of the chronically denervated heart is reduced compared with a nondenervated heart. Such a reduced O2 demand could be expected to help maintain the viability of the myocardium following occlusion of the coronary artery. Occlusion in a nondenervated heart caused contractile force in the muscle supplied by that artery to be reduced significantly more than in the denervated heart. It was also found that chronic sympathectomy led to a substantial reduction in coronary collateral resistance. Thus, compared with a control heart, the ischemic area perfusion in the chronically sympathectomized myocardium may be improved. Lavallee et al,7 however, reported an adverse effect on infarct size that may be related to elevation in left ventricular end-diastolic pressure and impairment of collateral perfusion. On the basis of these results, the present study was undertaken to examine more closely the effects of total cardiac denervation and chemical sympathectomy on myocardial O2 supply and O2 consumption balance in acute coronary occlusion.

Use of adrenergic blocking agents in therapy of the ischemic heart suggests that cardiac adrenergic nerves can play an important role in determining the viability of ischemic myocardium. β-Adrenoceptor blockade appears to reduce the size of a myocardial infarction in experimental animals. In man, the issue of whether β-adrenoceptor blockade can reduce the size of a myocardial infarction is more controversial. There is evidence that β-adrenoceptor blockade can reduce the heterogeneity of venous O2 saturation in normal and ischemic myocardium.11,12 It was found that the number of veins with low O2 saturation was reduced after β-adrenoceptor blockade. This could improve the distribution of available O2 supply to better meet O2 needs. Another purpose of the present study was to determine whether this change in venous O2 saturation heterogeneity also occurred after cardiac denervation.

It was previously reported by us that O2 extraction as well as coronary flow within the ischemic region was not uniform. The premise of the present study was that this heterogeneity might be related to the inhomogeneous distribution of norepinephrine or its release in the myocardial tissue. If norepinephrine levels were reduced then perhaps the distribution of O2 delivery and O2 needs could be improved in ischemia. Therefore, observations of O2 saturation in small myocardial arteries and veins were performed microspectrophotometrically. Local measurements of coronary blood flow with radioactive microspheres were also performed so that local O2 supply and O2 consumption...
could be determined. The degree of cardiac denervation was determined by high-performance liquid chromatography (HPLC) separation and electrochemical detection.

Materials and Methods

In this study, four groups of mongrel dogs weighing 12–18 kg were used: 1) control untreated dogs (n = 5); 2) acute surgically denervated dogs (n = 3) in which intrapericardial denervation of the heart was completed immediately prior to coronary occlusion; 3) chronic surgically denervated dogs (n = 5) in which the heart had been subjected to the same surgical procedure as that involved in acute denervation 2 weeks prior to the experiment; and 4) chemically denervated dogs (n = 5).

Surgical intrapericardial denervation was accomplished in the present study using a modified technique described initially by Randall et al.13 In the chronic, surgically denervated group, the procedure was performed under sterile conditions. A median sternotomy was used as the surgical approach. Both vagus nerves, the right and left stellate cervical ganglia, and ansa intrathoracic were isolated and marked with a silk tie for subsequent nerve stimulation. Skin ECG leads had been previously placed for recording of heart rate, and one femoral artery was cannulated percutaneously for direct arterial blood pressure recordings. A 5-msec square-wave stimulation pulse of 10–20 Hz and 5 V was applied for stimulation. Heart rate and systemic blood pressure responses were recorded during control nerve stimulations. After opening the pericardium in the midline and placing a silk tie on each atrial appendage for retraction, the ventrolateral nerve was divided. Dissection was continued around the entire circumference of the left superior pulmonary vein. The pericardial reflection was then divided on the front wall of the left atrium in the transverse sinus to the junction with the right atrium. The adventitia was removed circumferentially from the ascending aorta and the main pulmonary artery, including the fat pad with the dorsal nerves between the two. The dissection was carried beyond the pulmonary artery bifurcation onto both right and left pulmonary arteries. On the right side of the heart, the superior vena cava was freed of all adventitia and fatty tissue circumferentially from the pericardial reflection to the atrial-caval junction joining the previous dissection of the left atrium. The azygos vein was ligated and divided. The front surface of the right pulmonary artery was cleared of all connective and neural tissue. All surfaces of dissection were then painted with phenol solution. Both vagus nerves and stellate ganglia were electrically stimulated to verify the completeness of the surgical denervation procedure. The pericardium was loosely closed. The median sternotomy was repaired while evacuating the air with a temporary chest tube placed so it communicated with both pleural cavities. The endotracheal and chest tubes were removed when the dogs resumed spontaneous breathing. The animals received prophylactic antibiotic therapy perioperatively.

A chemical sympathectomy was performed by intravenous injection of 50 mg/kg of 6-hydroxydopamine, 5 days prior to the experiment.14 Reduction of some of the deleterious side effects of 6-hydroxydopamine administration was provided by previous oral administration of acepromazine maleate, 2 mg/kg. Since 6-hydroxydopamine produces a strong sympathomimetic effect, three fractional doses were administered over a period of 6 hours.

The denervated and the untreated (control) dogs were anesthetized by intravenous sodium pentobarbital (30 mg/kg), intubated and ventilated artificially to maintain end-tidal CO₂ constant. Polyethylene catheters were inserted into the femoral artery and vein for registration of blood pressure and for blood sampling. The chest was opened and a pericardial cradle was formed. The left anterior descending coronary (LAD) artery was isolated and a tie placed distal to its first main bifurcation. A catheter was inserted into the left atrium for radioactive microsphere injection and a large bore catheter was also inserted into the left ventricle for measurement of the left ventricular pressure and maximal positive derivative of pressure with respect to time (dP/dt). Blood samples taken from the femoral artery catheter were analyzed for pH, P O₂, and P CO₂ using a Radiometer BMS3 blood gas analyzer and also for hemoglobin concentration with a Fisher hemoglobinometer.

In all animals, control readings including heart rate, arterial and ventricular blood pressure, and blood gases were taken. Blood withdrawal for a microsphere reference sample from the femoral catheter was begun at a constant rate (7 ml/min). Thirty seconds after reference sampling was initiated, either 141 Ce- or 85Sr-labeled microspheres, 15 ± 3 μm in diameter, were injected into the left atrial catheter as a bolus and flushed with 3 ml of saline. The reference sampling continued for a total of 2 minutes. In all animals, the previously isolated LAD was then ligated. After 2 hours, hemodynamic parameters and blood gas determinations were repeated. In addition, regional blood flow measurements were obtained using alternatively labeled radioactive microspheres. The heart was excised below the atrioventricular ring and frozen in liquid nitrogen. The frozen hearts were stored in liquid nitrogen until analyzed. Multiple adjacent transmural samples of the ventricular free wall were cut from the center of the ischemic area and also from an area unaffected by occlusion of the LAD. The ischemic area was identified by the marked discoloration of the frozen tissue. Samples were prepared for microspectrophotometric determination of O₂ saturation and analysis of regional microsphere distribution and noradrenaline content analysis by HPLC technique. The sample used for microspectrophotometric analysis was taken from the center of a ring of samples used for the flow measurements.

For microspectrophotometric analysis,17 30-μm thick frozen sections were cut in a −25°C, N₂-flushed cold box. These sections were taken from the entire subepicardium or subendocardium after discarding the sections immediately adjacent to the epicardium or
endocardium. Each section was then transferred to a precooled slide, covered with degassed silicone oil, and rapidly transferred to the N2-flushed microspectrophotometer cold stage. Arteries and veins, 20–100 μm in diameter, were located in the regions of interest; absorbances at 560, 523, and 506 nm were obtained; and the O2 saturation of the blood contained within the vessel observed in transverse section was calculated. Between seven and 10 arteries and seven and 10 veins in the subepicardial and subendocardial regions of the occluded and the nonoccluded areas were examined microspectrophotometrically. The adjacent tissue samples were prepared for blood flow determination.

Tissue blood flow was determined as follows: the activity of the carbonized radioactively labeled microspheres 15 ± 3 μm in diameter was determined on a Hewlett-Packard Auto Gamma spectrometer. Appropriate corrections were made for activity overlap. Arterial blood samples obtained from the arterial reference sample and myocardial tissue were weighed and placed in tubes to be used in the spectrometer. Blood flows were calculated from the formula Fu = Fk x Cu/Ck, where Fu is the flow to any organ, Fk is the flow to the reference organ, Cu is the radioactivity in any organ, and Ck is the radioactivity in the reference organ. Blood flow was expressed in ml/min/100 g tissue.

Regional O2 extraction (ml O2/100 ml blood) was calculated in the control area as the local arteriovenous difference multiplied by the arterial hemoglobin concentration times the maximal O2 combining capacity of 1.36 ml O2/g hemoglobin. Oxygen extraction for the regions of interest was determined as the product of O2 extraction and regional blood flow.

An HPLC technique was used to electrochemically determine norepinephrine content in tissue. The heart tissue was prepared for HPLC and electrochemical analysis in four steps: 1) sonication and centrifugation of the tissue in 0.1N perchloric acid; 2) adjustment of the tissue homogenate to pH 8.6 and absorption of the catecholamines onto alumina; 3) washing of the alumina and filtering the supernatant; and 4) desorption of the catecholamines from the alumina into a minimal volume of acid. The acid solution was then directly injected into the HPLC column for quantitative readout. Concentration of norepinephrine was determined by measurement of the peak heights and comparing them to injected norepinephrine and dihydroxybenzylamine standard solutions.

A factorial analysis of variance was employed to determine whether differences existed between treatments, areas (occluded and nonoccluded), and regions (subepicardial and subendocardial) for arterial and venous O2 saturations, O2 extraction, blood flow, and O2 consumption after 2 hours of occlusion. Norepinephrine content was also compared. The statistical significance of differences was determined by Duncan's procedure. All values are expressed as mean ± SD. Differences in the frequency distribution of O2 saturations in vessels were determined using the χ2 test. A value of p < 0.05 was accepted as significant.

**Results**

The results obtained from hemodynamic recordings and blood gas analysis taken before and 2 hours following coronary artery ligation in all four groups of animals are presented in Table 1. No significant differences were observed in systolic or diastolic blood pressure among the groups before or following occlusion. Occlusion did not significantly affect blood pressure in any group. Preocclusion heart rate was significantly higher for the control group as compared with all the other groups. Postocclusion heart rate was still the highest for the control group, but significantly

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**Table 1. Effect of Denervation and Occlusion on Hemodynamic and Blood Gas Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Untreated control</th>
<th>Acute surgically denervated</th>
<th>Chronic surgically denervated</th>
<th>Chemically sympathectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>145 ± 34</td>
<td>136 ± 2</td>
<td>145 ± 18</td>
<td>130 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>114 ± 28</td>
<td>118 ± 29</td>
<td>112 ± 12</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>165 ± 16</td>
<td>156 ± 11</td>
<td>143 ± 15†</td>
<td>148 ± 3</td>
</tr>
<tr>
<td>Left ventricular end-diastolic blood pressure (mm Hg)</td>
<td>7 ± 5</td>
<td>11 ± 4</td>
<td>5 ± 3</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec)</td>
<td>2,452 ± 326</td>
<td>2,371 ± 249</td>
<td>1,877 ± 454</td>
<td>1,652 ± 541</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>69 ± 4</td>
<td>72 ± 2</td>
<td>69 ± 5</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>PCO2 (mm Hg)</td>
<td>35 ± 5</td>
<td>37 ± 5</td>
<td>36 ± 6</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.06</td>
<td>7.42 ± 0.02</td>
<td>7.43</td>
<td>7.37</td>
</tr>
</tbody>
</table>

*Significantly different from the preocclusion value.
†Significantly different from the untreated control value.
The effect of denervation and coronary occlusion upon the mean O₂ saturation of hemoglobin in small vessels of various myocardial regions is given in Table 3. Arterial O₂ saturations were not significantly different between treatments in the nonoccluded regions. Occlusion lowered arterial O₂ saturations in all groups. There were no significant subepicardial versus subendocardial differences in arterial O₂ saturations in any treatment or region. In the occluded region, many small arteries had reduced O₂ saturations.

Venous O₂ saturations were lowered in both the subepicardium and subendocardium following occlusion in all groups (Table 3). In comparison with the control values, significantly higher values of venous O₂ saturations were recorded for both of the chronically denervated groups in the occluded and in the nonoccluded regions. Subepicardial venous O₂ saturations were higher than subendocardial values in both occluded and nonoccluded areas in all treatment groups. The frequency of individual readings of venous O₂ saturations in the control group and in the denervated dogs are presented in Figure 2. A greater number of high O₂ saturation vessels were seen in the subepicar-

**TABLE 2. Effect of Denervation on Regional Myocardial Blood Flow (ml/min/100 g) in Subepicardium and Subendocardium of Nonoccluded and Occluded Regions**

<table>
<thead>
<tr>
<th>Region</th>
<th>Untreated control</th>
<th>Acute surgically denervated</th>
<th>Chronic surgically denervated</th>
<th>Chemically sympathectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Nonoccluded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>74.7±11.2</td>
<td>109.8±41.8</td>
<td>97.2±20.0</td>
<td>124.7±36.0</td>
</tr>
<tr>
<td>Endo</td>
<td>90.1±20.0</td>
<td>115.9±43.3</td>
<td>102.6±21.1</td>
<td>117.8±15.5</td>
</tr>
<tr>
<td>Occluded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>61.0±18.1</td>
<td>19.1±11.1*</td>
<td>77.9±18.2</td>
<td>10.4±7.3*</td>
</tr>
<tr>
<td>Endo</td>
<td>73.0±16.6</td>
<td>10.9±6.1*</td>
<td>80.5±8.8</td>
<td>1.1±0.5*†</td>
</tr>
</tbody>
</table>

*Significantly different from the preocclusion value.
†Significantly different from the untreated control value.
Epi, epicardium; endo, endocardium.

different only from the chronically denervated groups. Maximum positive dP/dt values did not significantly change as a result of the occlusion, nor were there significant differences between the groups. However, left ventricular end-diastolic pressure increased significantly following occlusion in the acute surgically and chronically denervated dogs. Arterial blood gas values were in the normal range in all four groups under all experimental conditions.

According to the norepinephrine content analysis, myocardial norepinephrine tissue concentration was reduced in the chronic surgical denervation group from 679±283 to 292±196 ng/g wet wt (Figure 1). Chemical sympathectomy by 6-hydroxydopamine caused almost complete depletion of the endogenous myocardial norepinephrine to 2.5% of the control group value. However, the catecholamine content in most of the myocardial regions of the acute surgically denervated group was not significantly different from the control group. The subendocardial norepinephrine content of the nonoccluded region tended to be higher than the subepicardial in the control and the acutely denervated areas. In the acutely denervated group, occlusion significantly reduced the level of the endogenous catecholamines in the subendocardium. No significant differences in norepinephrine content between the examined layers were found in the control or chronically denervated groups.

Table 2 presents the regional blood flow both preocclusion and postocclusion in subepicardial and subendocardial areas of both occluded and nonoccluded regions. No significant differences in the initial flows were found between the examined groups. A drop in flow as a consequence of ligation of the LAD was recorded in all of the occluded areas regardless of treatment. The flow decrements tended to be greater in the occluded subendocardium than in the subepicardium. Furthermore, the myocardial blood flow values of the occluded areas were not statistically different between groups, except for the acute denervated subendocardial region, which was found to be lower than the control. The nonoccluded regions of both of the chronically denervated (chemical and surgical) groups had lower blood flow than the control nonoccluded areas after the LAD region had been occluded for 2 hours.

The effect of denervation and coronary occlusion upon the mean O₂ saturation of hemoglobin in small vessels of various myocardial regions is given in Table 3. Arterial O₂ saturations were not significantly different between treatments in the nonoccluded regions. Occlusion lowered arterial O₂ saturations in all groups. There were no significant subepicardial versus subendocardial differences in arterial O₂ saturations in any treatment or region. In the occluded region, many small arteries had reduced O₂ saturations.

Venous O₂ saturations were lowered in both the subepicardium and subendocardium following occlusion in all groups (Table 3). In comparison with the control values, significantly higher values of venous O₂ saturations were recorded for both of the chronically denervated groups in the occluded and in the nonoccluded regions. Subepicardial venous O₂ saturations were higher than subendocardial values in both occluded and nonoccluded areas in all treatment groups. The frequency of individual readings of venous O₂ saturations in the control group and in the denervated dogs are presented in Figure 2. A greater number of high O₂ saturation vessels were seen in the subepicar
### Table 3. Effect of Denervation on Regional O$_2$ Extraction and O$_2$ Consumption in Subepicardial and Subendocardial Layers of Nonoccluded and Occluded Regions

<table>
<thead>
<tr>
<th></th>
<th>Untreated control</th>
<th>Acute surgically denervated</th>
<th>Chronic surgically denervated</th>
<th>Chemically sympathectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi Endo</td>
<td>Epi Endo</td>
<td>Epi Endo</td>
<td>Epi Endo</td>
</tr>
<tr>
<td><strong>Nonoccluded region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SaO$_2$%</td>
<td>86.5±6.8</td>
<td>85.6±5.3</td>
<td>88.1±0.4</td>
<td>82.6±3.6</td>
</tr>
<tr>
<td>SvO$_2$%</td>
<td>40.4±2.3</td>
<td>37.0±5.2</td>
<td>37.9±7.2</td>
<td>33.5±9.8</td>
</tr>
<tr>
<td>O$_2$ extraction (ml O$_2$/100 ml)</td>
<td>7.8±0.8</td>
<td>8.6±0.9</td>
<td>7.7±0.8</td>
<td>8.0±1.8</td>
</tr>
<tr>
<td>O$_2$ consumption (ml O$_2$/min/100 g)</td>
<td>8.34±2.80</td>
<td>10.22±4.87</td>
<td>9.37±1.83</td>
<td>9.50±2.79</td>
</tr>
<tr>
<td><strong>Occluded region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SaO$_2$%</td>
<td>72.7±15.0</td>
<td>76.4±12.9*</td>
<td>77.3±4.5*</td>
<td>70.7±6.5*</td>
</tr>
<tr>
<td>SvO$_2$%</td>
<td>24.7±8.1*</td>
<td>22.5±8.6*</td>
<td>24.6±6.6</td>
<td>21.0±9.2</td>
</tr>
<tr>
<td>O$_2$ extraction (ml O$_2$/100 ml)</td>
<td>10.0±1.9</td>
<td>11.1±0.8*</td>
<td>9.7±0.9</td>
<td>10.0±2.6</td>
</tr>
<tr>
<td>O$_2$ consumption (ml O$_2$/min/100 g)</td>
<td>2.04±1.58*</td>
<td>1.20±0.62*</td>
<td>1.05±0.77*</td>
<td>0.11±0.06*†</td>
</tr>
</tbody>
</table>

*Significantly different from the nonoccluded region value.
†Significantly different from the untreated control value.

Epi, epicardium; Endo, endocardium; SaO$_2$, arterial O$_2$ saturation; SvO$_2$, venous O$_2$ saturation.

dium compared with the subendocardium as determined by $\chi^2$ test. It can be seen that in the untreated dogs and in the acute surgical denervation group, almost half of the vessels observed in the occluded region had a venous O$_2$ saturation in the range of 0–20%, which is significantly different from the distribution of the nonoccluded area. In both of the chronic denervated groups, there was a significant reduction in the number of vessels with low saturation. Acute denervation had no effect on the distribution of venous O$_2$ saturation following occlusion.

In general, higher O$_2$ extraction was recorded for the subendocardial than for the subepicardial layers in both the occluded and nonoccluded regions (Table 3). The occluded region had a higher O$_2$ extraction than the nonoccluded area in all groups. The O$_2$ extraction was significantly lower in the nonoccluded regions of the chronically denervated groups as compared with the corresponding regions in the control group. In the occluded regions, only the O$_2$ extraction of the subendocardial layer of both chronically denervated groups was significantly lower than the control group occluded subendocardium.

Occlusion significantly decreased the O$_2$ consumption in both the subepicardium and subendocardium compared with the corresponding nonoccluded region.

![Figure 2. Venous O$_2$ saturation histograms for the occluded and nonoccluded areas of dogs in which the LAD was occluded for 2 hours. The groups are control, acute surgical denervation, chronic surgical denervation, and chemical sympathectomy. Note the reduced number of veins with O$_2$ saturations below 20% in the occluded region of the chronic surgical and chemical sympathectomy groups compared with control.](http://access.ahajournals.org/)}
in all experimental groups (Table 3). There were no significant differences in regional O₂ consumption in the occluded area between the experimental groups except that O₂ consumption in the occluded subendocardium of the acutely denervated group was lower than control. Myocardial O₂ consumption in the chronically denervated animals was lower in the nonoccluded regions compared with the corresponding regions in the control group.

Discussion

Ligation of a coronary artery causes a decrease in both local O₂ supply and local O₂ consumption. This can lead to cell death and necrosis. There is evidence that the ischemic area is not homogeneous. Since the capillary density in various regions is not homogeneous and the contraction at different sites of the myocardium is not uniform, occlusion of an artery could cause microregional differences in tissue distress. We found that the frequency of veins with low O₂ saturation was greater in the occluded region of the control group as a consequence of coronary ligation compared with the nonoccluded area, but we still found veins in the ischemic area with high O₂ saturations, indicating a heterogeneous distribution of vessel O₂ saturations. Similar venous O₂ saturation values have been found in the acutely denervated dogs and in other reports from our laboratory. Heterogeneous O₂ delivery to the cellular cytochrome chain has been found by Chance et al and by Steenbergen et al in the hypoxic heart. Since β-adrenoceptor blockade has been shown to both improve the relation between O₂ supply and O₂ consumption as well as reduce the number of veins found with low O₂ saturations in the ischemic area, we wished to test whether reduction in myocardial tissue norepinephrine levels would have a similar effect.

Catecholamines increase heart rate and contractile tension. When coronary blood supply is reduced, the ischemic heart becomes unable to support this increased demand. Since norepinephrine is released from the myocardial tissue following coronary artery occlusion, the severity of coronary occlusion could depend on the level of the adrenergic supply in specific regions of the myocardium and on the adrenergic response of each area. Moreover, it has been accepted recently that the distribution of the adrenergic function in various regions of the myocardium, and particularly within the left ventricle, is heterogeneous. Regional variations in norepinephrine concentration were found in the left ventricle. The distribution of β-adrenoceptors in the myocardium is also heterogeneous.

In the current study, the norepinephrine level of the myocardium was reduced in both of the chronic denervated groups. Similar effects of chronic denervation on norepinephrine content have been reported previously. The lack of effect of acute denervation on the catecholamine tissue level reported here is also in accordance with another report. Destruction of noradrenergic nerve terminals with 6-hydroxydopamine or in chronic cardiac denervated animals can lead to an increase in the density of β-adrenergic receptors. Thus, the denervated animals are more sensitive to sympathetic stimuli. There is also evidence that the adrenal medulla increases its catecholamine secretion after 6-hydroxydopamine treatment. However, circulating levels of catecholamines return to control levels within a few days of chemical sympathectomy. Despite potential adrenoceptor up-regulation, an increase was observed in the present study in ischemic region oxygenation in the denervated groups.

Significantly reduced numbers of veins with low O₂ saturation were found in both of the chronically denervated groups. Thus, the number of low O₂ saturation microregions following occlusion was reduced in these groups as compared with the ischemic area of the untreated dogs. It should be noted that the variance of venous O₂ saturation did not significantly change in the acutely denervated group in comparison with the control group, indicating that the beneficial effect of the chronic groups is related to the lowered endogenous catecholamine content. This result is similar to that found by Conway and Weiss, who examined the effect of propranolol in myocardial ischemia. They found a reduced number of veins with low O₂ saturations in the ischemic area after propranolol treatment. This indicates that norepinephrine and β-adrenoceptors play an important role in the control of venous O₂ saturation heterogeneity.

Myocardial performance in cardiac denervated animals has been shown to be similar to that of nondenervated animals. Gregg et al, however, have reported that O₂ consumption of the chronically denervated heart is reduced compared with a non-denervated heart. In our study after chronic surgical or chemical denervation, the heart rate under control conditions and following occlusion was significantly lower than in the control group. Similar behavior of the heart rate during denervation has already been described. We also found significantly reduced O₂ consumption in the nonischemic regions of the chronically denervated hearts compared with the same regions in the nondenervated group. Thus, the nonoccluded regions of the denervated heart tend to function at a lowered metabolic level. Regional O₂ consumption is reduced in ischemia. This effect on myocardial O₂ consumption is not altered by chronic denervation, although with acute denervation the ischemic region subendocardial O₂ consumption was below the control group value. Thus, reductions in tissue norepinephrine levels do not significantly alter total ischemic regional O₂ consumption, although they do redistribute myocardial oxygenation better, for example, reduced number of veins with low O₂ saturation.

In contrast to the intrapericardial denervation that causes the destruction of cardiac adrenergic and cholinergic nerves, chemical sympathectomy using 6-hydroxydopamine has been shown to have a destructive effect only on adrenergic nerve endings. Chemical sympathectomy resulted in a depletion of the
myocardial norepinephrine to 2.5% of control group values. A similar decrease in norepinephrine content following 6-hydroxydopamine injection has been reported. The decrease in catecholamine levels in the chronic surgically denervated dogs was less marked although others have reported lower levels of norepinephrine. Both denervation procedures seemed to cause similar hemodynamic changes and almost equal effects on the ischemic myocardium. Although there was a beneficial effect on local oxygenation in the chronically denervated groups, left ventricular end-diastolic pressure rose significantly following occlusion in the acute and the chemically denervated groups.

Coronary artery ligation was accompanied by a significant decrease in blood flow to the region of the left ventricle supplied by the LAD, regardless of treatment. No significant changes in the myocardial postocclusion perfusion among the groups were recorded, indicating the absence of any significant contribution of mean flow to the beneficial effect of the chronic denervation on the ischemic myocardium. However, it should be pointed out that the proportion of coronary blood flow going toward the nonoccluded area in the control and in the acute surgical denervation group was greater than that found in the chronically denervated groups. Within the ischemic region of the chronic denervation groups, there appeared to be some microregional rearrangements of $O_2$ supply and consumption as can be noted from the reduced number of veins with low $O_2$ saturations.

Several studies have demonstrated enhanced tolerance to myocardial ischemia associated with denervation. A decreased ventricular fibrillation threshold following coronary occlusion during stellate ablation has been reported by Kliks et al. Chronic denervation was found to improve perfusion to the ischemic myocardium. Occlusion of the coronary artery caused a smaller decline in contractile force in the denervated dogs than in the untreated dogs. Previous treatment with reserpin improved the hemodynamic course of recovery from infarction. The results of Jones et al also demonstrate that the protective effect of cardiac denervation is enhanced with time. Their suggestion of collateral proliferation is not borne out by our results. Thus, the infarct produced by coronary occlusion in the chronically denervated heart is usually smaller, although not in all reports, than in control hearts. In the present study, we report a decrease in the number of veins with low $O_2$ saturation in the ischemic myocardium of denervated dog. We have no evidence whether this could reduce the size of the developing infarction.

Results of the present study help to explain why $\beta$-adrenoceptor blockers may be useful in myocardial ischemia. Reduction in infarct size with denervation was found to be similar to that observed following $\beta$-adrenergic blockade alone. There are, however, some reports that denervation and $\beta$-adrenergic blockade may be ineffective in reducing infarct size. Denervation in the present study and propranolol in our other report reduced microregional differences in ischemic region oxygenation. Thus, the number of veins with low $O_2$ saturations in the ischemic area is reduced and this may reduce the number of microregions that are ischemic after chronic adrenergic denervation.

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Improved distribution of regional oxygenation in denervated ischemic dog myocardium.
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