Protective Effects of Long-term Bradycardial Pacing Against Catecholamine-Induced Myocardial Damage in Rabbit Hearts

Margaret D. Brown and Olga Hudlická

A high intravenous dose of norepinephrine (4 µg/kg/min for 60 minutes) to New Zealand Red rabbits produced patchy subendocardial damage (estimated stereologically in frozen sections) of about 5% of the heart volume 2 days after application. The damaged areas showed loss of staining for alkaline phosphatase, an enzyme present in normal capillary endothelium. Heart performance (cardiac output index, cardiac work [i.e., cardiac output x mean blood pressure], and dP/dt) were significantly lower than in control hearts. Capillary density distribution estimated in nondamaged areas of the left ventricular free wall was inhomogeneous favoring subepicardial regions, while homogeneous transmural distribution was found in control hearts. Bradycardial pacing (reduction of heart rate to 50% of normal) performed for 3—4 weeks prior to norepinephrine administration showed a protective effect against catecholamine damage manifested in a smaller extent of necrosis, in the maintenance of homogeneous transmural capillary distribution in nondamaged areas, and, most importantly, in the maintenance of normal cardiac pump performance at rest and during maximal work in response to acutely administered norepinephrine. (Circulation Research 1988;62:965–974)

Epidemiological studies have linked exercise with a low risk of ischemic heart disease. Experimentally, training has a beneficial effect on heart performance when the myocardium is subsequently exposed to ischemia and hypoxia. This effect may be exerted partly through improved vascularization since after coronary artery occlusion, more capillaries and smaller infarcts are found in trained than untrained rats and capillary-to-fiber ratio is increased, although not all exercise regimes result in increased capillary supply. Exercise is a complex intervention, and it is difficult to assess whether its beneficial effect on the improvement of heart performance is due solely to its effect on the heart or on the other cardiovascular parameters, such as total peripheral resistance, or even the hormonal changes involved in training. It results frequently in bradycardia, improved heart performance, and increased capillary density, explained by capillary proliferation. A significant increase in capillary density and improved maximal cardiac work were described in rabbit hearts paced to approximately 50% of normal heart rate. This procedure affects the heart directly. It, too, may offer protection to the myocardium at risk from a compromised vascular supply through its known effects on performance and capillary supply. Indeed, in hearts made hypertrophic by aortic valve lesion, subsequent bradycardial pacing for 4 weeks increased capillary density and improved cardiac performance.

The purpose of this study was to examine whether bradycardial pacing can protect against the impairment of myocardial structure and function that follows infusion of a high dose of catecholamine. The cardio-toxic effects of the catecholamines isoprenaline and norepinephrine have been extensively described in terms of the myocardial lesions produced, which appear to be similar despite the diverse hemodynamic effects of these two drugs. The underlying causes of catecholamine-induced necrosis are thought to include relative ischemia and a direct toxic effect. Trained rats have been shown to be more resistant than untrained animals to the cardiac damaging effects of isoprenaline. Since we found in preliminary experiments in rabbits that isoprenaline either did not produce damage or killed the animals, we used a modification of the protocol of Downing and Lee in which norepinephrine infusion resulted in reproducible quantifiable myocardial damage in the rabbit, together with impairment of function.

Materials and Methods

New Zealand Red rabbits of either sex, weighing between 2.0 and 3.6 kg, were used in four experimental groups: 1) control animals, 2) unpaced animals infused with norepinephrine (NE) 2 days prior to the final experiments, 3) bradycardially paced animals, and 4) bradycardially paced animals infused with NE as in Group 2.

Bradycardial Pacing Technique

The bradycardial pacing technique used in the present study has been described by Wright and Hudlická. Briefly, a portable stimulator delivered stimuli via a bipolar stainless steel electrode implanted in the right atrium at a frequency such that premature depolarization of the atrium was induced within the

From the Department of Physiology, University of Birmingham Medical School, Birmingham, England.
Supported by a grant from the British Heart Foundation.
Address for reprints: Dr. M.D. Brown, Department of Physiology, University of Birmingham Medical School, Birmingham, England B15 2TJ.
Received May 7, 1987; accepted December 11, 1987.
Hemodynamic Measurements

Acute experiments were performed either 2 days after NE infusion, 2 days after the cessation of pacing, or on unpaced control animals. Rabbits were anesthetized with sodium pentobarbital (35 mg/kg i.v.) supplemented as necessary through needle electrodes in- 
serted under the skin or from the implanted electrodes. During some infusions and under aseptic conditions, a cannula was placed in a superficial branch of the femoral artery of one leg with its tip positioned so as to record femoral artery blood pressure. The cannula was removed at the end of the infusion, the branch tied off, the wound closed, and the animal allowed to recover from anesthesia. Seven unpaced and six paced rabbits received NE infusions.

Infusion of Norepinephrine

Animals were anesthetized with halothane. Norepinephrine bitartrate (Levophed) in sterile saline solution was infused for 60 minutes at a dose of 4 μg/kg/min via a cannula attached to a 21-gauge needle inserted into the marginal ear vein. The total volume of infusate was 12 ml containing, for a 3.0-kg rabbit, 720 μg NE. This volume represents under 6% of circulating blood volume administered over 1 hour. ECG recordings were made periodically either from needle electrodes inserted under the skin or from the implanted electrodes. During some infusions and under aseptic conditions, a cannula was placed in a superficial branch of the femoral artery of one leg with its tip positioned so as to record femoral artery blood pressure. The cannula was removed at the end of the infusion, the branch tied off, the wound closed, and the animal allowed to recover from anesthetic. Seven unpaced and six paced rabbits received NE infusions.

Histochemistry

Blocks were cut with a razor blade through the thickness of the left ventricular free wall from midway between the base and apex, oriented on cork pieces, and frozen in isopentane cooled in liquid nitrogen in such a way that sections could be obtained showing subepicardial and subendocardial layers in cross-section (Figure 1). Twelve-micron transmural sections were taken at 240-μm depths through each block, providing 4–6 sections/block. At each depth, one section was stained with hematoxylin and eosin to identify myo-

FIGURE 1. Diagram of anterior view of heart showing sites for sampling from the left ventricular wall for histology. The surface parallel to the vertical heart axis of the block from site A provides sections with subendocardial myocytes in good cross-section, while the similar surface of the block from site B provides sections with subepicardial myocytes in good cross-section.
cardiac damage. Two serial sections were stained, one for alkaline phosphatase (ALP) by an indoxyl tetrazolium method to visualize capillaries and one for succinic dehydrogenase (SDH) to identify any areas of infarction. The indoxyl tetrazolium method stains all capillaries along their entire length (as visualized in longitudinal sections in skeletal or cardiac muscle). The staining was quite homogeneous in normal and paced hearts. However, in paced and unpaced hearts damaged by NE, there were scattered areas of myocardial tissue that lacked any staining for ALP, and hence, capillaries could not be visualized in these areas. Therefore, numerical counts of capillary density were made only in those regions of myocardium that did stain.

The number of capillaries in both subepicardial and subendocardial layers was counted at a magnification of $\times 400$ using an eyepiece with a grid in seven square fields of 0.143 mm$^2$ each. These fields were randomly selected from at least four different sections to give myocytes sectioned in good cross-section, and covered a total observed area of 1 mm$^2$. Capillary density/mm$^2$ was calculated from the mean of the seven individual capillary counts and thus was derived from direct observation of between 2,000 and 4,000 capillaries. The distribution of capillaries between subepicardial and subendocardial layers was shown by the ratio of subendocardial/subepicardial capillary density. A ratio of 1 indicated that capillaries were distributed homogeneously across the left ventricular wall.

In five unpaced and five paced hearts treated with NE, 150 myocytes (30 in each of five fields sampled at random) in undamaged myocardium were outlined in cross-section at a magnification of $\times 400$ using a microscope with a drawing arm in both subepicardial and subendocardial regions. The outlines were then digitized using a Summagraphics pad (Reflex Ltd., Reading, United Kingdom), and fiber cross-sectional areas were calculated using a program devised for a PDP11 computer.

Quantitative Estimation of Myocardial Damage

Sections stained for ALP were used to estimate the extent of damage caused by NE in both paced and unpaced hearts (see "Results" for reasons). For each heart, five subepicardial and five subendocardial fields, 2.60 mm$^2$, were randomly selected from at least four sections from different blocks. A total area of myocardium of 67.6 mm$^2$ was thus sampled for each region. Within each field, areas of myocardium that lacked ALP stain were outlined at low magnification ($\times 40$) using an Olympus microscope with a drawing arm. Drawings were then covered with a grid, and points overlying unstained myocardial tissue were counted relative to total myocardial tissue counts, excluding extracellular space, large vessels, and non-myocardial tissue.

Volume fraction (Vv) of unstained tissue was given by $Vv = Pu/Pt$, where $Pu$ is the number of points counted over unstained tissue and $Pt$ is total number of tissue points counted. Replicate counts were made of each field to yield $Pt > 400$ so that minimal resolution, defined as $(1/Pt \times 100)$, was $< 0.25\%$.

Statistics

Statistical comparisons were made by Student’s $t$ test (paired or unpaired as appropriate), and comparisons of treatment effects were made by analysis of variance according to Sokal and Rohlf. Values quoted in "Results" are mean ± SEM unless otherwise stated.

Results

Acute Effects of 60-Minute Norepinephrine Infusion

Mean arterial blood pressure rose approximately 50% within 5 minutes and declined gradually during the course of the infusion (Figure 2A). All animals exhibited cardiac arrhythmias shown by ECG recordings during the infusion. Disturbances of rhythm included periods of tachycardia lasting several minutes interspersed with bradycardia with or without elevation of the ST segment (Figure 2B). Immediately after the infusion ended, cardiac arrhythmias ceased and blood pressure dropped 10–15% below resting levels, returning to normal after 15–20 minutes.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** a) Record of arterial blood pressure recorded during intravenous infusion under halothane anesthesia of norepinephrine (4 µg/kg/min) in a 3.0 kg rabbit. b) Examples of typical changes in ECG observed at intervals during norepinephrine infusion as above.
TABLE 1. Body Weights, Heart Weights, and Heart-to-Body Weight Ratios for Control, Paced, Unpaced Norepinephrine-Treated and Paced Norepinephrine-Treated Animals at Final Experiment (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Body weight</th>
<th>Heart weight</th>
<th>Heart weight (g X 100)/body weight (g)</th>
<th>Heart weight to body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>2.58 ± 0.08</td>
<td>6.22 ± 0.25</td>
<td>0.241 ± 0.004</td>
<td>0.241 ± 0.004</td>
</tr>
<tr>
<td>Paced (n = 6)</td>
<td>2.86 ± 0.12</td>
<td>6.81 ± 0.36</td>
<td>0.239 ± 0.010</td>
<td>0.239 ± 0.010</td>
</tr>
<tr>
<td>NE-treated, unpaced (n = 7)</td>
<td>2.96 ± 0.13</td>
<td>6.57 ± 0.23</td>
<td>0.213 ± 0.007*</td>
<td>0.213 ± 0.007*</td>
</tr>
<tr>
<td>NE-treated, paced (n = 6)</td>
<td>2.77 ± 0.06</td>
<td>6.32 ± 0.16</td>
<td>0.228 ± 0.004*</td>
<td>0.228 ± 0.004*</td>
</tr>
</tbody>
</table>

NE, norepinephrine.
*p<0.05 vs. control.

Heart and Body Weights

At the final experiments, mean heart weights and body weights did not differ among the four groups (Table 1), but heart-to-body weight ratios for both NE-treated groups were significantly lower than those of controls. Also, the significant correlation between heart and body weight in control and paced animals (r = 0.94 and r = 0.89, respectively, p < 0.01 for both in agreement with the findings of Wright and Hudlicka) was absent in both NE-treated groups (unpaced r = 0.306, paced r = 0.323, NS), indicating a disturbance in the normal heart-to-body weight relation.

Cardiac Performance

Resting cardiac pump function was impaired in both paced and unpaced hearts by NE treatment (Table 2). Mean arterial blood pressures were similar for the four groups, but NE treatment significantly lowered the resting COI, minute work per gram of heart weight, and heart rates (p < 0.05 by analysis of variance for treatment effect). Resting dP/dt max was significantly lower in unpaced hearts after NE treatment while paced hearts had dP/dt max values similar to those in control hearts.

During acute norepinephrine infusion used as a stimulus to increase cardiac work, the mean arterial pressure increase was similar in all groups (p < 0.001 vs. resting) over the range of doses used (Figure 3), indicating that peripheral responses to the drug had not been affected either by pacing or by the initial NE treatment. By contrast, NE treatment resulted in the depression of maximal cardiac output compared with untreated groups; however, maximal increases in COI were significantly greater in paced than in unpaced NE-treated hearts (Table 2).

As blood pressure increased during acute NE administration, heart rates fell significantly in all groups (p < 0.05 vs. resting) through baroceptor activation (the animals were vagally intact) and stroke volume increased in all groups. The decrease in heart rate was similar in all four groups but the absolute levels were

TABLE 2. Resting and maximum hemodynamic parameters in four experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Paced (n = 6)</th>
<th>NE-treated (n = 7)</th>
<th>Paced NE-treated (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output index (ml/min/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>192.9 ± 18.7</td>
<td>204.0 ± 17.3</td>
<td>152.9 ± 15.2*</td>
<td>152.0 ± 9.8*</td>
</tr>
<tr>
<td>Maximum</td>
<td>244.7 ± 10.5</td>
<td>296.8 ± 17.5</td>
<td>169.5 ± 20.9*</td>
<td>220.2 ± 16.1*</td>
</tr>
<tr>
<td>+52.0 ± 10.5</td>
<td>+92.1 ± 16.1</td>
<td>+21.8 ± 16.3†</td>
<td>+67.1 ± 16.3</td>
<td></td>
</tr>
<tr>
<td>Cardiac minute work index (/g heart wt X 10^-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>0.970 ± 0.085</td>
<td>0.979 ± 0.045</td>
<td>0.851 ± 0.111*</td>
<td>0.849 ± 0.101*</td>
</tr>
<tr>
<td>Maximum</td>
<td>1.854 ± 0.154</td>
<td>2.464 ± 0.206</td>
<td>1.308 ± 0.232</td>
<td>1.865 ± 0.286</td>
</tr>
<tr>
<td>+0.884 ± 0.119</td>
<td>+1.473 ± 0.249</td>
<td>+0.447 ± 0.116†</td>
<td>+1.005 ± 0.280</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>91.3 ± 2.9</td>
<td>85.9 ± 8.3</td>
<td>97.9 ± 7.2</td>
<td>93.9 ± 5.6</td>
</tr>
<tr>
<td>At maximum COI</td>
<td>136.5 ± 5.1</td>
<td>134.5 ± 4.5</td>
<td>129.9 ± 13.4</td>
<td>134.7 ± 8.7</td>
</tr>
<tr>
<td>At maximum MWI</td>
<td>138.1 ± 5.4</td>
<td>139.9 ± 4.9</td>
<td>159.1 ± 12.6</td>
<td>142.0 ± 9.6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>316.5 ± 12.1</td>
<td>318.6 ± 7.2</td>
<td>303.6 ± 8.3*</td>
<td>286.0 ± 9.8*</td>
</tr>
<tr>
<td>At maximum COI</td>
<td>273.0 ± 6.9</td>
<td>295.0 ± 9.2</td>
<td>266.0 ± 9.4*</td>
<td>254.0 ± 20.7*</td>
</tr>
<tr>
<td>At maximum MWI</td>
<td>290.0 ± 15.3</td>
<td>290.5 ± 6.8</td>
<td>257.0 ± 9.5*</td>
<td>263.0 ± 14.7*</td>
</tr>
<tr>
<td>dP/dt max (mm Hg/sec X 10^-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>4.06 ± 0.43</td>
<td>3.60 ± 0.74</td>
<td>2.80 ± 0.40†</td>
<td>4.68 ± 0.62</td>
</tr>
<tr>
<td>Maximum</td>
<td>7.43 ± 1.16</td>
<td>7.43 ± 1.06</td>
<td>5.70 ± 0.48</td>
<td>7.41 ± 0.77</td>
</tr>
</tbody>
</table>

Cardiac minute work index (MWI) is calculated from cardiac output and mean blood pressure but related to heart weight. Maximum minute work index did not always correspond with maximal cardiac output index (COI) due to different increases in blood pressure. NE, norepinephrine. Values are mean ± SEM.

*Both NE-treated groups significant vs. both untreated groups, p < 0.05 by ANOVA.
†NE-treated p < 0.05 vs. paced NE-treated.
‡Paced p < 0.05 vs. control.
§Paced p < 0.05 vs. control.
lower in both NE-treated groups. Stroke volume could therefore have been maintained in spite of significantly lower cardiac output. This, however, was not the case. At maximal cardiac output, the stroke volume index was $0.90 \pm 0.08$ ml/min/kg in control hearts, $1.06 \pm 0.11$ in paced hearts, and $0.63 \pm 0.07$ ($p < 0.05$ vs. control) and $0.90 \pm 0.11$ in un paced and paced hearts after NE treatment, respectively. Thus, pacing had prevented the significant decrease in stroke volume index after NE damage. Moreover, the stroke volume was significantly higher in paced than un paced hearts for the same decrease in heart rate (Figure 4). This suggests that contractility in paced hearts may have been depressed less by NE treatment than in un paced hearts. Un paced NE-treated hearts did tend to have slightly lower maximum dP/dt values during work (Table 2), and generally required larger doses of NE to elicit maximal dP/dt ($7.2 \pm 4.5$ µg/kg/min) compared with $5.6 \pm 0.4$ µg/kg/min for paced NE-treated, and $3.3 \pm 0.7$ and $5.3 \pm 0.9$ µg/kg/min for control and paced hearts, respectively.

Cardiac minute work per gram of heart weight increased during NE infusion due to a combination of raised peripheral resistance and increased cardiac output. The respective proportions of each component are shown in Table 2. While the control and both paced groups showed similar values for blood pressure during maximal cardiac output and cardiac work, it is obvious that the NE-treated un paced group increased cardiac work because of a greater increase in blood pressure. Minute work increases in paced NE-treated hearts were significantly greater than those of un paced NE-treated hearts, exceeded those achieved by control hearts, and were not significantly different from those of paced untreated hearts (Table 2).

The improved performance of paced hearts even after NE damage is summarized in Figure 5, in which proportional changes in cardiac output index and minute work are shown.

Capillary Density in the Left Ventricular Free Wall

Figure 6A shows the capillary density (CD) increase in both subepicardial and subendocardial regions of paced hearts, with a mean transmural CD of $2,455 \pm 548$ mm$^2$ (mean ± SD) as compared with $1,963 \pm 504$ mm$^2$ in control hearts. Distribution of capillaries across the left ventricular wall was homogeneous in both groups as shown by the subendocardial/subepicardial CD ratios close to one (Figure 6B).

Capillary density in NE-treated hearts was evaluated only in the undamaged parts of the myocardium where all capillaries could be visualized. Mean CDs (± SD) in paced and un paced NE-treated hearts were not different from each other ($2,229 \pm 305$ and $2,227 \pm 285$ mm$^2$, respectively) nor from control values. However, in un paced NE-treated hearts, the ratio of
FIGURE 6. a) Capillary density in undamaged regions of control, paced, unpaced NE-treated, and paced NE-treated hearts in subepi- and subendocardial layers. b) Subendocardial/subepicardial ratio of capillary density in the same groups. Values are mean ± SEM.

subendocardial/subepicardial CD was reduced significantly from 1 to 0.863 ± 0.020 (Figure 6B) since subepicardial CD was significantly higher than that in controls (2,388 ± 127 compared with 1,954 ± 176/ mm²), whereas subendocardial CDs were similar (2,066 ± 94 and 1,979 ± 168, respectively). In paced NE-treated hearts, both subepicardial and subendocardial CDs (2,300 ± 153 and 2,158 ± 111/mm²) were slightly but not significantly higher than those of control hearts, and subendocardial/subepicardial CD ratio was 0.982 ± 0.056, which is close to 1.

There were no significant differences between mean fiber cross-sectional areas in undamaged regions of unpaced and paced hearts after NE treatment, either in subepicardial (373 ± 13 vs. 415 ± 15 µm²) or subendocardial layers (406 ± 19 vs. 429 ± 18 µm²). However, these values are considerably lower than had been expected from previous measurements of fiber diameters from electron micrographs of control and paced hearts.38 Despite high intra- and inter-animal variation, cross-sectional areas of subepicardial fibers were smaller than subendocardial in all five unpaced NE-treated hearts by around 8 ± 3%. In paced NE-treated hearts, subepicardial fibers were slightly smaller in three hearts, but in two hearts the reverse was the case so that, overall, subepicardial fibers were only 2 ± 7% smaller than subendocardial fibers.

Quantification of Myocardial Damage in NE-Treated Hearts

Both paced and unpaced hearts showed structural damage after NE treatment, evident as irregular patchy disintegration of myocytes and cellular infiltration in sections stained with haematoxylin and eosin (Figure 7A). Associated with this focal type of damage was patchy loss of staining reaction for ALP, an enzyme normally demonstrable histochemically in the endothelium of all capillaries present using our method of staining (Figure 7B). SDH staining in serial sections was no different in damaged regions, except in a few cases of more severe damage where cellular disintegration had progressed further and staining was fainter. However, SDH was not a good indicator of damage. Since delineation of damaged areas lacking ALP stain was clearer than in haematoxylin and eosin sections, quantification of the extent of damage as Vv of unstained tissue was made from ALP-stained sections.

Within individual fields, Vv% of unstained tissue corresponding to damage varied considerably, from 0% to a maximum of 32%. In subepicardial regions, there was no difference between paced or unpaced hearts in the very small extent of damage after NE treatment, on average less than 2% (Figure 7A). In subendocardial regions, the proportion of fields without damage was higher in all six paced hearts (26.7%) than in seven unpaced hearts (11%) (Figure 8B). There was thus a shift to the left in the frequency distribution of Vv% damage in paced hearts, indicating a trend towards smaller areas of damage.

Discussion

Treatment of rabbits with high doses of NE 2 days prior to experiments resulted in patchy subendocardial necrosis, lower heart-to-body weight ratio, impaired cardiac performance, and a disturbance of the homogeneous transmural capillary distribution with a lower capillary density in undamaged subendocardial than subepicardial regions. Most of these changes were diminished or prevented by previous bradycardial pacing for 3-4 weeks. The extent of damage was smaller, cardiac pump function was preserved at a level comparable with that of control animals, and the transmural capillary density was homogeneous. The only parameter not affected by previous pacing was the heart-to-body weight ratio.

The main difference between paced and unpaced NE-treated hearts was in cardiac pump performance — in the ability of the former to sustain increases in cardiac output and perform greater work against similar pressor effects of acute administration of NE. This could be explained by 1) smaller changes in heart rate in paced than unpaced animals, 2) different increases in peripheral resistance and, consequently, different afterload, and 3) better myocardial contractility.

NE treatment resulted in a depression of heart rate. Absolute heart rate was lower in both paced and unpaced animals, and this was more obvious during the
blood pressure increase induced by acute administration of NE. Thus, the contribution of heart rate to cardiac output was not affected by previous pacing.

The increase in blood pressure and consequently the extent of afterload at maximum cardiac output was not affected by pacing.

Despite similarly lower heart rates, paced NE-treated hearts were able to sustain cardiac output through greater increases of stroke volume.

Pacing also helped to maintain dP/dtmax at normal levels and counteracted the impaired contractility produced by NE treatment, shown in the present experiments as well as in those reported by Lee and Downing. The impaired contractility of the unpaced NE-treated group was also obvious from the fact that the dose of NE necessary to produce maximal cardiac work was higher than in any other group and that the maximal cardiac work in this group was achieved to a greater extent by increased afterload rather than increased cardiac output.

It is not quite clear what factors could explain the beneficial effect of bradycardial pacing on cardiac performance in NE-treated hearts. The mechanism of catecholamine-induced cardiac damage is not fully understood. Possible contributory factors include impairment of microcirculation, hypoxia, exhaustion of energy supply, altered myocyte membrane permeability leading to electrolyte disturbances (including increased intracellular acidity and cytosolic Ca2+ concentration), deposition of Ca2+ in mitochondria, and consequently inefficient oxygen utilization and perhaps formation of free radicals (see Rona20). The increased cytosolic concentration of Ca2+ would be responsible for hypercontraction of myofibrils in the affected cells, which occur next to areas with completely normal ultrastructure in rat hearts damaged by NE. Similar heterogeneous damage was seen in the present experiments at the light microscopic level and was somewhat different from that reported by Lee and Downing, who found a very uniform cellular disruption in the hearts.

**Figure 7.** Hematoxylin and eosin stained section (a) of left ventricular myocardium from rabbit heart infused 2 days previously with NE (4 μg/kg/min × 60 minutes) and serial section stained for alkaline phosphatase (b). Scale bars represent 100 μm for both. Capillaries are visible as black dots except where damage is evident. Comparison at higher magnification of control heart (c) and damaged region of a NE-treated heart (d) both stained for alkaline phosphatase. Scale bars represent 10 μm for both. Reaction product is no longer visible in this region of the NE-treated myocardium.
The obvious disturbance in heart-to-body weight relation would affect measurements of absolute capillary density. In maximally contracted control and paced hearts, there was an inverse relation between capillary density and heart weight. Therefore of a similar size must be satisfied before comparisons can be made. Although our histological procedure was intended to ensure this, the values obtained for fiber cross-sectional areas in undamaged regions of paced and unpaced NE-treated hearts showed large variation both within and between animals, corresponding to minimum and maximum fiber diameters of 17.0–28.5 μm with a mean of around 22 μm. These are smaller values than those found in four control and six paced hearts (19.7–32.5 μm, mean 28.5 μm) measured from electron micrographs of resin-embedded left ventricular wall myocardium. These smaller fibers could be a feature of the histopathological changes that occur after NE treatment and that are related to the loss of enzymes, or alterations in the Ca²⁺ fluxes could mean that postmortem contraction did not occur in NE-treated hearts the same way as in control hearts. Alternatively, Vetterlein and Schmidt found smaller fiber cross-sectional areas in both subepicardial and subendocardial regions of rat hearts after epinephrine treatment and related it to an increased ventricular dilatation that stretched the fibers, making them thinner and causing an apparent increase in capillary density in these hearts. Our study does not allow us to differentiate between these possibilities.

Whatever the cause of the smaller fiber diameters after NE treatment, they represent a decrease in fiber cross-sectional area of around 40%, which would be expected to result in a concomitant increase in capillary density. The observed increase of only 15–20% over values in control hearts of similar weight implies that capillary density has not increased proportionately to the decrease in fiber area. This could result if alterations in extracellular matrix prevented the smaller fibers from becoming closer together. This may be the case because the decrease in fiber cross-sectional area is disproportionately greater than would be expected from the loss of heart weight (around 5–10% compared with control hearts in animals of similar body weight). The fact that subepicardial fibers had smaller cross-sectional areas than subendocardial fibers in all unpaced hearts corresponds with the apparently significant increase in subepicardial capillary density and the reduced subendocardial/subepicardial capillary density ratio. In paced hearts, fiber areas were still smaller after NE treatment but differences between subepicardial and subendocardial fiber cross-sectional areas were less and transmural capillary density distribution was more homogeneous. Similar effects were observed in rabbit hearts after chronic propranolol treatment by Tasgal and Vaughan-Williams. They reported that heart-to-body weight correlation was lost, significant transmural differences between the volume fraction of myocytes were evened out, the fractional volume of extracellular matrix was significantly increased, and capillary volume density distribution was more homogeneous.

Homogeneous distribution was also found in the total number of capillaries visualized by staining for ALP in both control and bradycardially paced hearts but not in hearts made hypertrophic because of volume

![Figure 8](http://circres.ahajournals.org/)
overload where the relative decrease in capillary density was more obvious in subendocardial regions. Long-term administration of vasodilating drugs also resulted in inhomogeneous transmural distribution with adenosine producing a greater increase in capillary density in subepicardial regions and the xanthine derivative HWA 285 a greater increase in subendocardial regions. Whatever the reasons for this inhomogeneity—and it might be related to relative perfusion resulting from different changes in blood pressure—hearts with lower CD in the subendocardium showed worse performance than hearts with uniform transmural CD or higher CD in the subendocardium. This consideration can, of course, only be applied in regions where all capillaries are visualized, and great care was taken in the present experiments to count capillaries solely in sections with no loss of staining. Although we cannot say to what extent these capillaries are functional, the importance of a homogeneous transmural distribution of “perfused” capillaries for heart performance can be inferred from studies where the use of fluorescent-labeled plasma proteins shows no difference between capillary perfusion of the subepicardium and subendocardium at rest in rabbit hearts and at rest and under conditions of increased or decreased heart rate and blood pressure in the rat heart.

The indoxyl tetrazolium method for ALP seems to stain the entire length of capillaries (seen in preliminary experiments in longitudinal sections in skeletal and cardiac muscle), unlike the method used by Lojda in a number of tissues including rat heart and by Mrazková et al., who used a different substrate and found no staining in the venular parts of skeletal muscle capillaries. Our method gives maximal results in heart after 10–15 minutes of incubation and is not dependent on longer incubation times for demonstration of greater reactivity, as is the case with the method employed by both Lojda and Mrazková et al. Moreover, values for capillary density in normal and paced hearts estimated from sections stained for alkaline phosphatase were in good agreement with values estimated from electron micrographs.

The patchy loss of ALP staining of the capillary endothelium in areas of NE-treated hearts where myocardial damage was demonstrated by hematoxylin and eosin staining was unexpected. It might have been the long-term result of disturbed microcirculation. A similar loss of ALP stain was reported for the cardiomyopathic Syrian hamster in skeletal muscle by Jasmin and Bajusz and in hearts, at a time when myocyte damage was not yet evident, by Factor et al. The latter group demonstrated spasm of small arteriolar vessels in these hearts by silicone rubber casts and proposed that transient obstruction of small vessels and their capillaries, sufficient to cause ischemia, could lead to myopathic necrosis upon reperfusion. Although the microvascular spasms and necrosis could be prevented by use of the calcium channel blocker verapamil, loss of ALP stain was not prevented and would therefore seem to indicate a specific failure within the endothelium. Figulla et al. subsequently demonstrated unperfused areas preceding the occurrence of necrosis in these hearts, and Vetterlein and Schmidt found a similar patchy perfusion with a lower overall perfusion in subendocardium than in subepicardium in rat hearts during infusion of isoproterenol. It is not known whether capillaries lacking ALP in NE-treated hearts are perfused and functional and whether loss of ALP is consequent upon prior ischemia or is representative of some alteration in capillaries likely to initiate myopathic necrosis. In a study on papillary muscles from hearts damaged by NE, a number of capillaries showed swollen endothelium, and it is possible that the loss of stain is in these capillaries, which would obviously be poorly perfused. Therefore there appears to be some microcirculatory defect after NE treatment, evidenced by loss of alkaline phosphatase staining in the capillary endothelium.

Animal models of myocardial damage due to catecholamines are quite important since morphological changes are similar to those found in cases of sudden cardiac death that were ascribed to an increased endogenous release of catecholamines. Consequently, any procedure that reduces the extent of damage and improves the cardiac pump performance is of great importance. We showed that prior bradycardial pacing confers upon the rabbit heart a measure of resistance to the damaging effects of NE, and cardiac performance is better maintained under these conditions, especially during work. This protective effect may be related to the enhancement of myocardial capillary supply and its diffusion capacity and improved oxidative capacity, which are brought about by such pacing. However, the complete mechanism by which bradycardial pacing protects the heart remains obscure. Stangeland et al. have also identified heart rate as the dominant predictor of infarct size in cats treated with alinidine, which reduces heart rate by a nonreceptor mechanism.

Acknowledgments
Margaret Glover provided excellent technical assistance, and we are grateful to Hamish Ross, who devised the PDP11 computer program for calculation of fiber areas.

References
7. Rakušan K: Microcirculation in the stressed heart, in Legato MJ Brown and Hudlická Chronic Bradycardial Pacing Protects the Heart 973


Key Words • noradrenaline • cardiac output • cardiac work • heart rate • capillary density
Protective effects of long-term bradycardial pacing against catecholamine-induced myocardial damage in rabbit hearts.

M D Brown and O Hudlická

doi: 10.1161/01.RES.62.5.965

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/62/5/965