Beneficial Effect of Chronic Bradycardial Pacing on Capillary Growth and Heart Performance in Volume Overload Heart Hypertrophy

Andrew J.A. Wright, Olga Hudlicka, and Margaret D. Brown

We have previously reported that chronic bradycardial pacing increases both capillary density/mm² (CD) and maximal work output in normal rabbit hearts. This technique has now been applied to rabbits with volume-overload hypertrophy due to lesion of the aortic valve. Four groups of animals were studied: controls (C), paced (P), valve-lesioned (VL), and paced valve-lesioned (PVL). The aortic valve was lesioned 8 weeks before the acute experiments; pacing was started 4 weeks before the acute experiments, and thus, the PVL group had developed hypertrophy before pacing was started. The degree of hypertrophy was similar in VL hearts whether paced or not: heart wt/body wt ratio increased by 33.5±8.9% (mean±SEM) in VL and 25.2±8.2% in PVL versus control animals of similar body weight (p<0.001). The hearts of the PVL animals showed a higher CD (2,277±107) than VL hearts (1,383±43), CD in C hearts of similar weights being 1,595±103, and in P hearts 2,350±194. Thus, CD was lower by 14% in VL and higher by 43% in PVL than in C hearts. Valve-lesioning had a significant effect in reducing maximal cardiac minute work (p<0.001), whereas pacing significantly improved maximal cardiac minute work (p<0.001) to 2.467±0.206 J/gx10⁻⁴ in the P group versus 1.609±0.105 in the C group. In the valve-lesioned hearts, work levels were normal after pacing (1.613±0.152 J/gx10⁻⁴) compared with the lower maximal cardiac minute work in hypertrophy alone (1.102±0.162). Chronic bradycardial pacing, therefore, reversed what was a small deficit in capillary density to a substantial increase and significantly improved maximal cardiac minute work performance in hypertrophied hearts. (Circulation Research 1989;64:1205-1212)

Heart hypertrophy occurs either as a result of physiological stimuli such as postnatal growth¹² or exercise,³–⁵ stimuli on the borderline between the physiological and pathological such as exposure to high altitude,⁶–⁸ cold acclimatization,⁹ or hyperthyreosis,¹⁰,¹¹ or in pathological circumstances such as pressure or volume overload (for reviews, see Rakusan¹²,¹³). The adaptation of the vascular bed varies during the growth of myocytes, depending on the initial stimulus or age. Capillary supply was increased in young animals as a result of training,¹⁴–¹⁶ but in other experiments it did not change as a result of intensive training¹⁷ or was even decreased¹⁸ (for reviews, see Rakusan,¹³ Hudlicka and Tyler¹⁹). Hypertrophy elicited by exposure to cold was accompanied by a moderate growth of capillaries so that capillary density (CD, number of capillaries/mm²), capillary/fiber ratio, and the diffusion distances were maintained at a normal level.⁸ Increased capillary supply occurred in the right ventricle during exposure to high altitudes until hypertrophy was moderate. With further hypertrophy and imminent heart failure the intercapillary diffusion distances lengthened.⁹ A similar increase in diffusion distances occurs in cases of pathological heart hypertrophy where the growth of myocytes outstrips capillary growth and the final CD is decreased.²¹,²² There is some indication, however, that even though CD is lower, capillary length can increase, and thus, capillary surface area and volume per unit heart weight remain unchanged.²³,²⁴ An increase in diffusion distances indicates impaired supply of oxygen to hypertrophic myocytes. So far, attempts made to increase capillarization in various types of pathological heart hypertrophy have, in general, failed (for review, see...
Rakusan,13 Hudlicka and Tyler,19 although Crisman et al25 reported moderate capillary growth in hearts of young spontaneously hypertensive rats trained by running. We have shown that a chronic reduction in heart rate to about half by long-term atrial bradycardial pacing in rabbits increased CD by up to 70% compared with that found in control hearts of similar weights, without causing hypertrophy.26. The performance of the paced hearts in terms of maximal work output was also increased. The purpose of this study was to determine whether chronic bradycardial pacing can improve capillary supply and performance in the hearts of rabbits with volume-overload cardiac hypertrophy.

**Materials and Methods**

Experiments were performed on 39 New Zealand Red rabbits of 2.1-4.3 kg body weight, of either sex, with a similar proportion of male and female rabbits in each group. All animals had rabbit pellets and water available ad libitum. Four groups of animals were compared: controls (C), paced (P), valve-lesioned (VL), and paced valve-lesioned (PVL).

**Aortic Valve Lesioning and Pacing Procedures**

Volume-overload hypertrophy was successfully induced in 19 rabbits by lesion of the aortic valve via a carotid artery, as described by Fizelova and Fizel27 under halothane anesthesia. This was accomplished using a length of stainless steel needle (1.1 mm diameter) with a blunt, slightly curved tip to facilitate movement around curves and into the cusps of the valve. This metal tubing was connected to a pressure transducer using nylon cannula; pressure at the tip was measured throughout the procedure, showing when the tip was pressed against an obstruction and when it was in the left ventricle. The ventricle was initially entered gently to confirm the tip location; then, the tubing was withdrawn slightly into the aorta and reintroduced forcibly. This usually damaged the valve, causing pulse pressure to approximately double. If this was not the case, the valve penetration was repeated. Post-mortem examination normally showed that one cusp was severely damaged or sometimes completely absent.

Half of the rabbits lesioned in this way were kept for 8 weeks in the animal house. The others were kept for 4 weeks and then bradycardially paced for 4 weeks. We have previously described the basic method26 by which heart rate was kept at about 55% of the normal rate for 24 hr/day using transvenous right atrial pacing. The method was slightly modified in that lightweight portable pacemakers were used,28 carried by the animals on a harness, rather than a larger device with leads, as previously employed. Bipolar pacing electrodes were used, rather than a unipolar electrode and an indifferent electrode, since this simplified the surgery. These, as well as ECG recording electrodes, were implanted under halothane anesthesia.

Nine rabbits, not valve-lesioned, were paced for 4 weeks, and 11 rabbits were used as controls without any intervention.

**Measurement of Heart Performance**

The rabbits were anesthetised with sodium pentobarbital (Sagatal, May & Baker Ltd, Dagenham, England) 35 mg/kg i.v. supplemented as necessary. Cannulae were inserted into a femoral vein for administration of drugs and into a brachial artery for measurement of blood pressure. Heart rate was determined from the blood pressure record. The animals breathed spontaneously through a tracheal cannula. Rectal temperature was maintained in the range 37.5–38.5° C.

The thermodilution method was used to measure cardiac output to allow repeated measurements to be made within a fairly short time. Bolus 0.7 ml injections of room temperature (20° C) saline were made into the left ventricular cavity through a cannula introduced via the left carotid artery. A purpose-built probe containing an STC U23US thermistor (Electronic Services, Harlow, England) was introduced into the descending aorta via the right femoral artery to measure blood temperature. The off-balance voltage of the Wheatstone bridge circuit in which the thermistor was included was amplified by a Devices (Welwyn, Garden City, England) sub IC preamplifier for display on a Devices M8 pen recorder, together with the integral of the temperature signal. The temperature signal was set to zero, and the integrator was reset immediately before each injection. The integrated area reached by the time the temperature had returned to a constant value (slightly lower than before) was used in the calculation of cardiac output as described by Beillin and Bhattacharya.29 The results obtained by this method were indistinguishable from those obtained by extrapolation of initial slope of the decaying thermodilution curve.

As an index of heart performance, maximal cardiac minute work values were obtained from each animal during increasing rates of norepinephrine infusion in the range of 2.5-125 μg/min/kg. Norepinephrine was used for its positive inotropic effect as well as its effect on the peripheral resistance of increasing afterload. The dose was increased until minute work began to decline, and the response to the previous dose was then taken to be maximal. There was a great variability in the dose of norepinephrine necessary to elicit the maximal response in individual hearts; but the average maximal dose of 35 μg/kg/min was similar in all groups. Minute work was calculated as cardiac output times mean blood pressure and was standardized per gram of heart weight and expressed as Joules per gram.

At the end of the experiment, animals were killed by an overdose of pentobarbital. The hearts were removed, rinsed in saline, and firmly blotted. The vessels were trimmed flush, and any large deposits of fat were removed before weighing to the nearest
0.05 g. They were then immersed in cold saline to achieve a similar state of contraction in all groups.

Estimation of Capillary Density

Capillary density was estimated using a tetrazolium indoxyl method for alkaline phosphatase, which stains capillary endothelium specifically and thus reveals all the capillaries in the tissue. Apart from the use of a more rapid stain, the preparation of the tissue and the evaluation of the capillary density was as previously described. Samples were taken from subendocardial and subepicardial regions close to the base of the heart in positions that yielded good cross sections.

Statistical Evaluation

The analysis was done using program 7d from the BMDP statistical package 1988 version (BMDP Statistical Software Ltd, Cork, Ireland). For each set of results, the data were analyzed by performing a two-way analysis of variance with factors paced versus nonpaced and valve-lesioned versus non-valve-lesioned. In no case was interaction between the factors significant.

Bonferroni significances were obtained for comparisons of pairs of data.

Results

Degree of Hypertrophy

Cardiac hypertrophy is usually evaluated on the basis of the heart wt/body wt ratio. However, this value also changes with body weight. We, therefore, first compared the heart wt/body wt ratio in 93 control rabbits of different body weight (Figure 1); as a rule, heavier rabbits had a lower ratio, probably because they had a greater proportion of fat than light rabbits. Although there was no significant difference in the means of body weights among the four experimental groups (Table 1), the individual differences might have affected the heart wt/body wt ratio. Therefore, the data are presented both as

<table>
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<th>Parameters</th>
<th>Comparison groups</th>
<th>Probability values</th>
</tr>
</thead>
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<tr>
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<td>Heart wt/body wt ratio</td>
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<td>P/PVL&gt;C/VL</td>
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<tr>
<td>CD total</td>
<td>C/P&gt;VL/PVL</td>
<td>&lt;0.05</td>
</tr>
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</table>

C, control; P, paced; VL, valve-lesioned; PVL, valve-lesioned and paced; CD, capillary density. Numbers of animals in each group are in parenthesis. Values are mean±SEM for C, P, VL, and PVL.
Percentage deviation of the expected heart weight from controls of the same body weight range in the experimental groups. \( *p<0.001 \) vs. control hearts.

Means of absolute values for heart weight and heart wt/body wt ratio (Table 1) and as a deviation from the expected value using the appropriate value for a control animal of similar body weight as a baseline (Figure 2). Both hypertrophied groups had a similar increase in the heart wt/body wt ratio of 33.5±8.9% in VL group and 25.2±8.2% in PVL group.

Capillary Density

Previously, we have published data showing an inverse relation between heart weight and capillary density in normal rabbits for hearts weighing less than about 8 g, with a relatively constant CD in heavier hearts.\(^2^6\) This data was used to enable comparison of the observed CD in experimental animals with the expected CD for a control heart of similar weight. Figure 3 shows CD in hearts ranging in weight from 7.5 to 10 g (the heaviest heart observed in any normal animal), from a group of very large control animals. The CDs for the VL hypertrophied hearts were similar to those of the heavy control hearts, whereas the paced hypertrophied hearts showed a considerably greater capillary density than expected.

Comparison of capillary density in subendocardial and subepicardial regions showed a homogeneous transmural distribution in all groups (Table 1). CD in paced hearts was increased by about 25% in both regions compared with control hearts. Hypertrophied VL hearts had lower CDs (\( p<0.05 \)) in both regions than control hearts used in the present study whose weights were less than 7 g and hence had higher CDs according to the inverse relation described above. Pacing resulted in a highly significant increase in CD in both regions of these hypertrophied hearts (Figure 4).

Heart Performance

Data in Table 2 show that there were no significant differences among the four groups in heart rate and mean blood pressure either at rest or during

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**Figure 2.** Percentage deviation of the expected heart weight from controls of the same body weight range in the experimental groups. \( *p<0.001 \) vs. control hearts.

**Figure 3.** Capillary density/mm\(^2\) (CD) (combined subendocardial and subepicardial values) against heart weight in 17 heavy hearts (7.5–10.5 g) from large control animals. Mean CD is shown by the middle line and the hatched areas represent ±1 SD. \( \Delta \), individual values for valve-lesioned animals; \( \bullet \), individual values for paced valve-lesioned animals.

**Figure 4.** Capillary density/mm\(^2\) in valve-lesioned and paced valve-lesioned hearts. Values are mean±SEM; \( *p<0.01 \) vs. valve lesioned.
Table 2. Heart Rate, Mean Blood Pressure, Cardiac Output Index, and Cardiac Minute Work in Four Groups of Rabbits During the Final Experiment (Under Sodium Pentobarbital Anesthesia)

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Cardiac output index (ml/min·kg⁻¹)</th>
<th>Cardiac minute work (J/g·10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest  Max</td>
<td>Rest Max</td>
<td>Rest Max</td>
<td>Rest Max</td>
</tr>
<tr>
<td>C (n=11)</td>
<td>315±11 313±11</td>
<td>95±5 134±4</td>
<td>127±13 191±15</td>
<td>0.822±0.043 1.609±0.105</td>
</tr>
<tr>
<td>P (n=9)</td>
<td>290±9 318±10</td>
<td>105±5 132±6</td>
<td>146±10 292±29</td>
<td>0.979±0.045 2.467±0.206</td>
</tr>
<tr>
<td>VL (n=10)</td>
<td>315±7 324±9</td>
<td>101±6 128±5</td>
<td>151±18 186±23</td>
<td>0.699±0.077 1.102±0.162</td>
</tr>
<tr>
<td>PVL (n=6)</td>
<td>309±8 322±8</td>
<td>108±5 127±10</td>
<td>215±8 275±6</td>
<td>1.022±0.108 1.613±0.152</td>
</tr>
</tbody>
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Analysis of variance

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<th>Parameter</th>
<th>Comparison groups</th>
<th>Probability values</th>
</tr>
</thead>
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<td>No significant differences</td>
<td></td>
</tr>
<tr>
<td>Blood pressure rest and max</td>
<td>No significant differences</td>
<td></td>
</tr>
<tr>
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<td>&lt;0.01</td>
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<tr>
<td></td>
<td>VL/PVL&gt;C/P</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac minute work rest</td>
<td>P/PVL&gt;CVL</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>VL/PVL&gt;C/P</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac minute work max</td>
<td>P/PVL&gt;CVL</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>C/P&gt;VL/PVL</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values at rest and during maximal cardiac minute work (Max) (during norepinephrine infusion) are given as mean±SEM. C, control; P, paced; VL, valve-lesioned; PVL, valve-lesioned and paced. Numbers of animals in each group are shown in parentheses.

Discussion

Measurement of capillary density is dependent on the reliability of the method and is, of course, related to fiber size—a heart with smaller fibers having higher capillary density and vice versa. The method used in this study—demonstration of capillary endothelium on the basis of the reaction for alkaline phosphatase (ALP)—could be criticized since it was shown recently that the venular ends of capillaries lack this reaction. However, our method is based on a different substrate and different staining reaction. All capillaries were counted under norepinephrine infusion. Their relative increases in blood pressure during norepinephrine infusion were also similar. Any changes in cardiac minute work were therefore due to differences in cardiac output.

Resting cardiac minute work was higher in both paced (P and PVL) than unpaced groups (C and VL), and this was supported by higher cardiac output indexes (Table 2). However, analysis of variance also showed that valve lesioning had resulted in a significant increase in resting cardiac output index in VL and PVL. This was not associated with greater resting minute work as this parameter was calculated per unit heart weight rather than body weight. Thus, despite cardiac hypertrophy, resting work output/g was not changed by valve lesioning.

Maximal cardiac minute work was also significantly improved in the paced (P and PVL) versus nonpaced (C and VL) groups, and again, this was due to much greater cardiac output indexes in the paced groups (Table 2). In addition, valve lesioning led to a significant (p<0.001) impairment of maximal minute work because in both the VL and PVL groups, cardiac output index increased by only 22% during norepinephrine infusion, compared with a 50% increase in the C group and a doubling in the P group. However, the beneficial effect of pacing on the performance of hypertrophied hearts was shown by the fact that the lower maximal work levels found after valve lesioning were restored to those comparable with control hearts (Figure 5).

Figure 5. Minute work per gram of heart weight in the four groups of animals. The open columns show resting values, and the hatched columns show the maximum value obtained during an increasing infusion of norepinephrine (paced vs. control p<0.01, valve-lesioned vs. control p<0.05, paced valve-lesioned vs. valve-lesioned p<0.05).
Considered to be angiogenic by Folkman et al., there is considerable evidence that the angiogenic stimulus arises from mechanical factors connected with increased blood flow.

Capillary proliferation was shown in normal hearts of animals treated with dipyridamole, adenosine, or xanthine derivative, all of which increase coronary blood flow. Long-term treatment with nifedipine, a potent coronary dilator, slowed the decrease of capillary supply in hearts of SHR. Changes in the pattern of blood flow could thus represent a stimulus for capillary growth.

In bradycardially paced hearts, flow/beat was found to be significantly higher in P than C hearts. It has been suggested that changes in the vessel wall geometry and consequently wall tension might induce growth of new vessels. Tillmans et al. demonstrated a greater diameter of arterioles and capillaries in dog and turtle hearts in diastole than systole. As diastole is prolonged with bradycardial pacing, it is possible that higher vessel wall stress, connected with increased blood flow during this period, might produce slight damage to the capillary endothelium, which would lead to the release of proteases, degradation of the basement membrane, and subsequent endothelial migration and mitosis. Capillary wall tension could also make the flattened endothelial cells more susceptible to any growth factors possibly present in the heart as suggested by Folkman and Greenspan for endothelial cells in tissue culture.

In rabbit hearts with a relatively low percentage of perfused capillaries, pacing might improve perfusion before it resulted in capillary proliferation, and this improved perfusion could represent an angiogenic stimulus. Studies on capillary permeability times surface area showed a more homogeneous extraction of Cr^51-EDTA in paced hearts and an improvement in oxygen extraction. This indicates a more homogeneous perfusion of a larger capillary bed in paced hearts.

While better perfusion may promote capillary growth, it may also contribute to the greater work output of paced hearts. In hypertrophic hearts, a decrease in capillary density and consequent lengthening of diffusion distances are usually considered the cause of impaired cardiac performance. In the present study, hypertrophied hearts had significantly lower maximal cardiac minute work than control hearts, but this deterioration was completely arrested by bradycardial pacing. Thus, pacing is capable of improving performance not only in normal but also in hypertrophied hearts.

Although the effects of pacing on performance may be attributed to the increased capillary supply and better oxygen delivery, it was found that cardiac work increased before CD. The possibility of some other effects of pacing to explain improved performance should therefore be considered. In pathological hypertrophy, there was a shift in the pattern of myosin isozymes from high to low Ca^2+ ATPase activity forms which conserve energy by having lower oxygen consumption. It seems...
unlikely, however, that pacing affects performance by altering isozymes as these were no different in P than unpaced control hearts (Hudlicka and Zak, unpublished observations). The inotropic state (dP/dtMax) was also not changed by bradycardial pacing.33 Another possibility is that paced hearts might use metabolic substrates more efficiently; adaptations in oxidative capacity were found in paced hearts.33 Whatever the mechanisms involved, the increase in CD achieved by bradycardial pacing in hearts where hypertrophy had already developed is much greater than changes (if any) induced by training. Thus, bradycardial pacing offers the possibility of improving capillary supply and cardiac performance not only in normal but also in pathologically hypertrophied hearts.

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**KEY WORDS** • capillary density • left ventricular hypertrophy • cardiac minute work • heart rate • rabbit
Beneficial effect of chronic bradycardial pacing on capillary growth and heart performance in volume overload heart hypertrophy.
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_Circ Res._ 1989;64:1205-1212
doi: 10.1161/01.RES.64.6.1205

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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http://circres.ahajournals.org/content/64/6/1205