Relation between local myocardial growth and blood flow during chronic ventricular pacing

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Abstract

Several studies have shown that, per unit mass, myocardial blood flow (MBF) and oxygen consumption are similar in hypertrophic and non-hypertrophic ventricles. This observation may be explained by the degree of myocardial growth matching the increase in oxygen demand. Such matching may, however, not be perfect at the local level, because substantial heterogeneity of MBF exists within the ventricular wall. We investigated to what extent local growth and MBF are matched after redistribution of workload within the left ventricular (LV) wall. Redistribution of workload was established by ventricular pacing at physiological heart rate, which induces asynchronous activation and contraction. Local wall mass (2D-echocardiography) and MBF (fluorescent microspheres) were determined in the canine LV wall before (t=0) and after 6 months of normal sinus rhythm (SHAM group, n=5) or 6 months of pacing at the LV free wall (PACE group, n=8). During acute pacing MBF (ml/min/g) increased with increasing distance to the pacing site. Local relative MBF (rMBF, local MBF normalized to mean MBF in the LV wall) varied from 0.8 adjacent to the pacing site to 1.2 in remote regions. After 6 months of pacing these regional differences had disappeared, probably due to changes in wall mass, which increased with increasing distance to the pacing site (by up to 39±13%). In SHAM animals rMBF at t=0 correlated well with rMBF 6 months later (r=0.71). In PACE animals, however, this correlation was poor (r=0.33), because rMBF increased in regions close to the pacing site with initial rMBF<1 and rMBF decreased in regions remote from the pacing site with initial rMBF>1. Conclusions: After redistribution of workload within the LV wall as induced by ventricular pacing, local load-regulated growth tends to equalize MBF distribution, but local adaptation of MBF also depends on initial MBF.

Keywords: Hypertrophy; Regional blood flow; Ventricular function

1. Introduction

Various studies have shown that MBF and oxygen consumption per unit mass are similar in hypertrophic and non-hypertrophic ventricles [1–6]. This suggests that at the level of the entire ventricle myocardial mass increased in proportion with metabolic demands. Mathematical simulations [7] and in vitro [8,9] and in vivo experiments [10,11] have shown that myocardial growth can be regulated locally by changes in local workload. For example, chronic ventricular pacing, an intervention known to induce asynchronous activation and contraction and pronounced redistribution of workload within the left ventricular (LV) wall [12–14], induces different degrees of hypertrophy and atrophy [11]. Acute ventricular pacing also redistributes MBF within the LV wall [12,13]. It is, however, not known...
whether during prolonged periods of pacing local growth restores the distribution of MBF towards the mean value of flow per gram of tissue.

That such matching may not be perfect, is indicated by the existence of substantial heterogeneity of MBF within the ventricular wall. In his recent review Deussen concluded that the MBF heterogeneity is largely independent of the layer within the LV wall, does not change with increasing workload and is related to aerobic metabolism [15]. Most of MBF heterogeneity is stable over 24 h [16], suggesting that some myocardial regions may have a systematically larger oxygen demand than others do.

The present study was designed to investigate (1) the temporal stability of the MBF distribution between large regions and at the local level over periods of months, (2) whether during chronic ventricular pacing local differences in hypertrophy tend to equalize local differences in MBF per unit mass and (3) whether adaptation in MBF is the same in all myocardial regions.

To answer these questions regional MBF measurements with fluorescent microspheres were performed at 6 months intervals in two groups of dogs: SHAM operated animals (SHAM group) and animals subjected to 6 months of ventricular pacing at intrinsic physiological heart rate (PACE group). Microspheres were injected during sinus rhythm and during ventricular pacing. Changes in local myocardial tissue mass, if any, were assessed by serial measurements of local LV wall mass with 2D-echocardiography.

2. Methods

Animal handling was performed according to the Dutch Law on Animal Experimentation (WOD) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol was approved by the Experimental Animal Committee of the Maastricht University. Experiments were performed on adult mongrel dogs, divided into two groups: dogs chronically paced from the LV free wall (PACE, n=8) and sham operated dogs (SHAM, n=5, Fig. 1).

2.1. Implantation procedure pace and sham groups

Anesthesia and preparation have been described in detail before [11]. In brief, under sterile conditions pacing leads were implanted in all dogs in the right atrial cavity (for sensing of sinus node activity) and at the epicardium of the LV free wall. Only in the PACE group a pacemaker was implanted and connected to the leads. In the SHAM group the acute effects of pacing were studied by temporarily connecting the leads to a pacemaker. LV cavity and ascending aortic pressure were measured with a dual tip micromanometer catheter and cardiac output was measured by thermodilution. Catheters for microsphere injection and reference sampling were introduced into the LV cavity and femoral artery, respectively [17]. Microsphere injections and hemodynamic measurements were performed, after closing the chest, under baseline conditions and 15 min after ventricular pacing was initiated (Fig. 1).

2.2. PACE and echo protocol

In the PACE group, ventricular pacing was started approximately 2 weeks after implantation. The heart was paced in the VDD mode (sensing of intrinsic rhythm by the atrial electrode and stimulation of the ventricular electrode with an A–V stimulation interval of 25 ms to ensure complete ventricular capture).

Two-dimensional echocardiographic short axis images of the left ventricle were made before (t=0) and 6 months after onset of pacing in the PACE group and at the same time points in the SHAM group (Fig. 1), as described before [11].

2.3. Terminal procedure

Six months after the implantation procedure microsphere injections and hemodynamic measurements were repeated while the chest was still closed (Fig. 1). After all measurements had been completed the heart was arrested in diastole by perfusion with ice-cold CdCl₂ (0.1 M). The heart was quickly removed and the left and right ventricles were separated and weighed. Subsequently, the LV wall was sectioned into five slices, the middle slice and the uppermost and lowermost slices were used for microsphere analysis, the other two slices were used for histological (see below) and biochemical analysis.

2.4. Capillary to fiber ratio

In transmural samples, taken from the LV free wall and
septum (close to and remote from the pacing site, respectively), myocyte thickness and collagen fraction were determined using AZAN and Sirius-Red staining, as described before [11]. Serial slices were embedded in paraffin and cardiomycocyte and capillary basement membranes were stained on 4 µm thick plastic sections by the Jones silver methamine method [18].

2.5. Analysis of echo images

In an off line analysis myocardial wall volume in 6–8 regions of the high papillary cross-section was estimated from the echo images [11]. Regional wall volume was defined as the product of the sector area and the mean LV radius, a measure of general dilatation [11].

2.6. Analysis of blood flow

The fluorescent microsphere method has been validated in short-term experiments [17] and proved to be superior to radioactive microspheres in chronic experiments [19]. Each injection contained 3·10^6 microspheres, labeled with blue, blue–green, yellow–green, orange, red or crimson fluorescent labels (15.5 µm±2%, Molecular Probes, Eugene, OR, USA). Reference blood samples were taken at a rate of 10.3 ml/min [17]. MBF (ml/min/g) was determined in high papillary, low papillary and apical slices. The two upper slices were divided into 6–8 sections, the apical slice into four sections. Each section was divided into epicardial and endocardial samples.

Microspheres were isolated from myocardial samples by tissue digestion and centrifugal sedimentation and fluorescence was determined by use of fluorimetry [17]. The tissue weights, as determined post mortem, were used for the flow measurements at t=6 months (W_v). Because of growth adaptation occurring over the 6 months period, local wall mass at t=0 (W_v,0) was calculated from the mass at t=6 months and the echocardiographically determined changes in local wall mass V_{t,0} as (see above) according to:

\[ W_{v,0} = W_{v,6} \cdot \left( V_{t,0} / V_{t,6} \right) \]  

The sites of tissue collection were matched with the echographic image using the papillary muscles and the pacing lead as landmarks. MBF at t=0 in the PACE group could only be calculated in the basal slice, because 2D-echo images were obtained only at this level. In the SHAM group MBF at t=0 was calculated assuming W_{v,0} = W_{v,6}, because changes in LV mass over the 6 months experimental period turned out to be <5%.

2.7. Data analysis

MBF heterogeneity within the LV wall (%) was defined as the standard deviation (S.D.) of MBF values times 100, divided by the mean value of all measured MBF values. To facilitate comparison between experiments regional MBF at a particular site was expressed as relative MBF (rMBF), defined as local MBF (ml/min/g) relative to the mean MBF value observed in all samples in the same heart and under the same conditions.

Regional differences in MBF and growth were evaluated in three ways. Differences in MBF between regions close to and remote from the pacing site were analyzed using the LV free wall/septum blood flow ratio. The pattern of distribution of regional growth and MBF, as induced by ventricular pacing, was evaluated by plotting rMBF and % growth as a function of the distance of each sample to the pacing site.

Finally, long-term changes in rMBF in individual regions were evaluated by plotting rMBF at t=6 months as a function of rMBF at t=0.

2.8. Statistical analysis

Differences within the groups were evaluated for statistical significance with one-way analysis of variance (ANOVA), followed by appropriate post-hoc testing, differences between the groups with the Mann–Whitney U-test. The level of significance was set at p<0.05. Data are presented as mean values with S.D.s.

3. Results

3.1. Hemodynamics

Acute (15 min) ventricular pacing significantly reduced stroke volume (10%), dPiv/dt_{max} (8%) and dPiv/dt_{min} (15%) and significantly increased heart rate (5%), but did not change end-diastolic LV pressure, systolic LV pressure and cardiac index significantly as compared to sinus rhythm. Similar, but opposite changes were observed when pacing was stopped after 6 months. In the PACE group no significant changes in hemodynamics were observed between 15 min and 6 months of pacing, except for a 12% higher heart rate and a lower end-diastolic LV pressure (10±3 vs. 5±4 mmHg, respectively).

3.2. Structural changes

The dogs in the PACE group showed significant global LV hypertrophy. The LV/body weight (LV/BW) ratio was on the average about 25% higher than in the SHAM group (Table 1). LV cavity and wall volume did not change significantly in the SHAM group, but increased by 27±16 and 16±17%, respectively, after 6 months in the PACE group. In this group septal wall sector volume increased by 39±13% but LV free wall (FW) sector volume did not change significantly as compared with t=0 (Table 1). The ratio of LV FW and septum sector volume decreased
significantly by 36±14% as compared to baseline. Intermediate degrees of growth were observed for regions in between the mid-FW and mid-septum (Fig. 2). Histological analysis showed that in the PACE group myocyte diameter was 18±7% larger in the septum than in the LV FW (close to pacing site). Despite the thicker myocytes in the septum of the PACE group, the number of capillaries per fiber in the septum was not different from that in the LV FW or in the septum of the SHAM group. Collagen fraction was not significantly different between and within groups (Table 1).

### 3.3. Blood flow in LV free wall and septum

During initial sinus rhythm at $t=0$ MBF was not significantly different between the LV free wall and septum, in both the SHAM and the PACE group (Table 2). In both groups acute LV FW pacing reduced MBF in the early-activated LV free wall as compared with sinus rhythm and as compared with the septum (Table 2). As a consequence, the LV free wall to septum (FW/S) MBF ratio was close to one during initial sinus rhythm and decreased by approximately 30% to values significantly lower than unity during acute pacing (Table 2).

At $t=6$ months absolute MBF values in the LV free wall and septum of both groups were not significantly different from those at $t=0$. However, in the PACE group the MBF distribution between septum and free wall at $t=6$ was significantly different from that at $t=0$. This difference is illustrated by the higher FW/S blood flow ratios at $t=6$ months than at $t=0$, so that during pacing at $t=6$ this ratio was not significantly different from unity anymore, whereas it was significantly higher than unity during sinus rhythm (Table 2).

### 3.4. Distribution of myocardial blood flow within the LV wall

The distribution of MBF in the LV wall was depicted by plotting relative MBF (rMBF) as a function of the distance to the site of pacing (Fig. 3). During initial sinus rhythm ($t=0$, open triangles in Fig. 3A and B) rMBF was independent of the distance to the pacing site. During acute pacing (open circles in Fig. 3A and B) rMBF increased with increasing distance from the pacing site ($p<0.05$ by ANOVA). In regions adjacent to the pacing site rMBF was significantly lower than one (mean MBF in the entire LV wall) and significantly lower than in the same region during initial sinus rhythm. Remote from the pacing site rMBF was significantly higher than one and significantly higher than during sinus rhythm. In the SHAM group
values of rMBF were comparable at \( t = 0 \) and \( t = 6 \), during both sinus rhythm and pacing. In the PACE group the distributions of rMBF during sinus rhythm and acute pacing at \( t = 0 \) were similar to the corresponding distributions in the SHAM group (compare Fig. 3A and B). After chronic pacing (\( t = 6 \)), however, rMBF distribution was significantly different (Fig. 3C, closed circles), because rMBF did not depend on the distance to the pacing site anymore. However, 15 min after restoring sinus rhythm following chronic pacing rMBF distribution was almost a mirror image of that during acute pacing, with values significantly decreasing with increasing distance from the pacing site (closed triangles).

At \( t = 6 \) months endo/epicardial blood flow ratios in the various regions in both groups, ranged from 1.09±0.20 to 1.4±0.56 and were not significantly different between the sites. Moreover, endo/epicardial blood flow ratios were not significantly different between sinus rhythm and pacing in any of the regions.

### 3.5. Blood flow heterogeneity

MBF heterogeneity at \( t = 6 \) months was calculated as S.D./mean of MBF values. In the SHAM group acute ventricular pacing increased MBF heterogeneity in 5/5 experiments from 21.2±9.2% during sinus rhythm to 26.5±6.2% during acute ventricular pacing (Fig. 4). In the PACE group MBF heterogeneity was 29.5±12.4% after chronic pacing and 25.0±5.1% 15 min after restoration of sinus rhythm. These values were not significantly different from each other or from those in the SHAM group (Fig. 4).

### 3.6. Long-term rMBF changes in individual regions

In order to investigate the local changes in rMBF over time in individual regions rMBF at \( t = 6 \) months was plotted as a function of rMBF at \( t = 0 \) during sinus rhythm and during pacing. In a representative SHAM experiment rMBF values, obtained in endocardial and epicardial samples, were highly correlated during both sinus rhythm and ventricular pacing (Fig. 5A). Pooling all SHAM experiments also showed significant correlations between rMBF at \( t = 0 \) and \( t = 6 \) months (Fig. 5B). In the PACE group comparison of rMBF values between \( t = 0 \) and \( t = 6 \) months was only possible in transmural samples, because of the correction for local wall mass changes during the experiment (see Methods). In the SHAM group pooling endocardial and epicardial MBF to transmural MBF caused a slight decrease in the correlations between rMBF at \( t = 0 \) and \( t = 6 \) months (from \( r = 0.54 \) to \( r = 0.34 \) during ventricular pacing). In the PACE group, however, correlations between rMBF at \( t = 0 \) and \( t = 6 \) months were considerably poorer (\( r = 0.15 \) during sinus rhythm and \( r = 0.34 \) during ventricular pacing, Fig. 5C).

The cause of these poor correlations was investigated in more detail by dividing the regions adjacent to and remote from the pacing site into regions where MBF values during initial sinus rhythm were <1 and >1 (low and high flow regions, respectively. During ventricular pacing rMBF at \( t = 6 \) was 133±25% of rMBF at \( t = 0 \) (\( p < 0.05 \)) in low flow adjacent regions, but 101±13% (N.S.) in high flow adjacent regions. Six months of pacing decreased rMBF in remote regions; this decrease reached the level of significance in the high flow (84±17% of \( t = 0 \)) but not in the low flow remote regions (87±18%, Fig. 6). The differences in chronic rMBF changes between low and high blood flow regions were not caused by differences in regional growth. As compared to \( t = 0 \) local wall mass increased by −5.4±10.5 and 9.6±10.3% in low- and high-flow adjacent regions, respectively, and by 34.7±12.8 and 42.1±15.6% in low and high flow remote regions, respectively.
Fig. 4. MBF heterogeneity in the SHAM (open symbols) and PACE group (closed symbols). Data are derived from all epicardial and endocardial samples for the microspheres injections at \( t = 6 \). Note that in the SHAM group the animals were in sinus rhythm continuously, followed by 15 min of ventricular pacing (VP, indicated by the hatched bar), whereas in the PACE group the animals had been paced for 6 months, followed by 15 min of sinus rhythm.

4. Discussion

The findings in the present study show that in the normal heart in sinus rhythm MBF heterogeneity in the LV wall is highly stable over 6 months. Acute pacing at the LV free wall redistributes MBF from that region towards the septum and increases MBF heterogeneity. After chronic ventricular pacing regional differences in myocardial growth tend to return regional flows toward the mean MBF for the whole ventricle. The restoration of MBF uniformity is associated with variable changes in relative MBF (rMBF, local MBF relative to mean LV flow) in individual regions, the response to early- or late-activation being dependent on the initial rMBF value. Therefore, regional growth may serve to maintain uniformity of MBF between large parts of the LV wall, but locally the adaptation of MBF is also dependent on the level of initial MBF.

4.1. Changes in MBF distribution between adjacent and remote regions

The redistribution of MBF during acute ventricular pacing, demonstrated in the present study in transmural samples, is similar to that in subepicardial samples in previous studies [12,13]. Long-term continuation of ventricular pacing grossly returns the MBF distribution to normal again. The change in the free wall/septum blood
Fig. 6. Relative myocardial blood flow (rMBF) during initial sinus rhythm (SR) and acute and chronic ventricular pacing in the PACE group. Presented are mean values and S.D. in adjacent (distance to pacing site <1/8 of the LV circumference, open symbols), and remote regions (distance to pacing site >5/8 of LV circumference, closed symbols). Within adjacent and remote regions distinction was made between low and high flow regions [rMBF during initial <1 (circles) and >1 (squares), respectively]. Data are derived from the transmural samples in the basal slice of the LV. * = p<0.05 compared with SR, # = p<0.05 compared with acute pacing.

Fig. 5. Relative MBF (rMBF) values at the end of the experiment (t=6) as a function of the values at the beginning of the experiment (t=0). Data from all endocardial and epicardial samples of the LV wall from one animal (panel A) and all animals (panel B) of the SHAM group and data from all transmural samples in the PACE group (panel C). Presented are the data obtained during sinus rhythm (dots) and acute ventricular pacing (open circles). In panel A regression equations are \( y = 0.21 + 0.80x \) \( (r = 0.90) \) for sinus rhythm and \( y = 0.13 + 0.87x \) \( (r = 0.85) \) for ventricular pacing.

The flow ratio during acute ventricular pacing (approximately 30%, Table 2) is similar to the difference in myocardial growth between free wall and septum during chronic pacing (Table 1 and 2). Local myocyte growth can be expected to decrease workload per unit volume, whereas local myocyte atrophy will increase workload per unit volume. Therefore, the local growth response to altered local workload may be regarded as the mechanism by which the uniformity of MBF distribution is restored during chronic ventricular pacing. Such a mechanism may also explain the observation that MBF and oxygen consumption per unit mass in globally hypertrophic ventricles are similar to those in non-hypertrophic hearts [1-6].

While after 6 months of ventricular pacing global MBF distribution during pacing was uniform, the return to sinus rhythm caused a non-uniform blood flow distribution with a free wall/septum blood flow ratio above unity. This observation may be explained by the fact that during the 6 months of pacing the local growth regulation has created a
new steady state in MBF distribution with a free wall/septum blood flow ratio of one. Compared to LV free wall pacing, during sinus rhythm the workload becomes lower in the septum and higher in the LV free wall, leading to corresponding changes in MBF, thus causing a free wall/septum blood flow ratio above one.

In contrast to the present experimental study, studies in chronically paced patients showed low MBF adjacent to the pacing site [20,21]. These locally depressed MBF values were not due to the abnormal contraction patterns or coronary obstructions, because MBF increased to the same extent in all myocardial regions when the ventricular pacing rate was increased [20]. The absence of the uniform MBF distribution in chronically paced patients could be explained by the incapability of the myocardium to grow in proportion to the local differences in work. This could be related to pre-existing hypertrophy in the patients, due to sinus node disease. In a recent study in canine hearts we recently showed that the local differences in growth were smaller in hypertrophic than in non-hypertrophic paced ventricles [22]. However, other conditions, like differences in pacing site and use of anesthesia in the present study might also play a role.

In the hypertrophied region (remote from the pacing site) of the chronically hypertrophied hearts the capillary/myofiber ratio was unchanged whereas myofiber diameter was increased. This indicates that during hypertrophy, induced by locally increased workload, capillary density decreases, like in ventricles hypertrophied due to pressure overload [23,24] and in surviving myocardium of infarcted ventricles [25]. This inability to grow capillaries in proportion to myocardial tissue does, most likely, not affect baseline MBF values, because these values are well within the autoregulatory range. However, the hypertrophied regions of paced hearts may be more susceptible to ischemia under more compromising circumstances, as has been for globally hypertrophied ventricles [26].

4.2. Heterogeneity of myocardial blood flow

The heterogeneity of MBF in the normal heart is well recognized [15,27–29]. The present study considerably extends the information on the stability of MBF heterogeneity in time. King et al. [16] compared microsphere deposition distributions over a period of a day; we extended this period to 6 months. During this long time interval the correlation between blood flow at \( t=0 \) and \( t=6 \) months is significant and shows considerable stability of MBF heterogeneity over time. Because a recent publication showed a good correlation between MBF and oxygen uptake levels in the normal ventricular myocardium [30] our data support the idea that some regions within the heart have a continuously higher aerobic metabolism than others do, without evidence for ischemia [15,31]. Until now, it is not known whether the heterogeneity in oxygen uptake is caused by local differences in mechanical work or local differences in efficiency of conversion of metabolic to mechanical energy.

4.3. Changes in blood flow in individual regions

The present study shows that the pattern of MBF heterogeneity does change during the process of ventricular remodeling caused by ventricular pacing. During this process on average differences in rMBF between adjacent and remote regions become smaller, but in individual regions the changes appear to depend on the level of rMBF during initial sinus rhythm. Consistent long-term changes in rMBF are only observed in regions where pacing changes rMBF to values away from the mean flow (rMBF = 1): in low flow adjacent regions (where acute pacing further decreases rMBF) and in high flow remote regions (where acute pacing further increases rMBF). These data suggest that within the myocardium a certain degree of MBF heterogeneity is tolerated, but that a compensatory response is evoked when local MBF deviates from the mean MBF by more than a certain threshold value.

The nature of this response is not clear. Several studies have shown that low and high flow regions are equally susceptible to ischemia [32,33]. Therefore, it seems unlikely that a change in workload evokes different metabolic stimuli in low and high flow regions, which could lead to different MBF changes on the long term. A possible explanation for our observations could be that the remodeling process in the LV wall (the locally different degrees in hypertrophy and the ventricular dilatation) causes local differences in stress and/or strain, which on their turn, changes the distribution of energy demands within the myocardial wall. Further investigations are required to support this theory.

4.4. Comments on the experimental set-up

Ventricular pacing at physiological heart rate caused significant, although moderate, hemodynamic effects, as indicated by the small increase in heart rate and small decreases in indices for contractility, relaxation and preload.

The endo/epicardial blood flow ratios above 1.0 and the absence of changes in endo/epicardial blood flow ratio indicate that no transmural MBF redistribution occurs and suggests absence of subendocardial ischemia. This is in keeping with previous findings that acute ventricular pacing at physiological heart rate does not cause lactate production and does not change myocardial oxygen extraction [13]. These data also indicate that the redistribution of MBF during acute ventricular pacing is induced by local demands rather than that it is imposed by the abnormal contractions.
5. Conclusions

The heterogeneous distribution of MBF is highly stable over a period of months. After redistribution of workload within the LV wall, induced by ventricular pacing at physiological heart rate, regional differences in myocardial growth tend to equalize MBF distribution within the LV wall. However, the local adaptations of MBF depend on the change in local rMBF induced by pacing and on the initial rMBF value. These data suggest that a certain degree of MBF heterogeneity is tolerated, but that a too large deviation from the mean MBF leads to a compensatory response.

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References

