Effect of Nifedipine on Coronary Capillary Geometry in Normotensive and Hypertensive Rats

Karel Rakusan, Nicholas Cicutti, Stanislav Kazda, Zdenek Turek

Abstract The aim of this study was to describe quantitatively changes in the coronary capillary network resulting from hypertrophy in spontaneously hypertensive rats (SHR) and a potential effect of long-term treatment of these animals with nifedipine. Age-matched male SHR and Wistar-Kyoto (WKY) rats were treated for 27 weeks. Four experimental groups were analyzed: (1) untreated SHR, (2) nifedipine-treated SHR, (3) untreated control WKY rats, and (4) nifedipine-treated WKY rats. Treatment significantly decreased systolic blood pressure in SHR, although normotensive pressures were not reached. SHR had significantly higher cardiac weight, which decreased in nifedipine-treated rats, but values remained above those in control animals. Morphometric evaluation revealed lower capillary density and larger capillary domain area in hearts from SHR, which were partially attenuated by treatment with nifedipine. Capillary domain area was also significantly larger at arteriolar portions compared with domains supplied at venular portions. Capillary segment length was consistently shorter on the venular than arteriolar portion of the capillary, whereas no differences were observed between hearts from WKY rats and SHR. Treatment with nifedipine resulted in a prolongation of segment length. Reconstruction of the three-dimensional capillary supply unit (capillary domain area times capillary segment length) revealed significant differences between the amount of tissue supplied by a capillary at its arteriolar portion than more distally, which was detectable in all experimental groups. In hypertrophic hearts from SHR this tissue volume is increased mainly because of longer intercapillary distances and larger domains, especially on arteriolar portions. The venular portions either support smaller growth of neighboring myocytes during hypertrophy or are subjected to additional branching. Treatment with nifedipine results in a moderate capillary growth along the entire pathway, as evidenced by smaller capillary domains, longer segment lengths, and unchanged proportion of proximal and distal capillaries in tissue cross sections. (Hypertension. 1994;24:205-211.)

Key Words • microcirculation • heart hypertrophy • calcium channel blockers • neovascularization • rats, inbred SHR

Cardiac hypertrophy induced by pressure overload in the adult organism is usually characterized by an increase in size of the individual myocytes and a decrease in capillary density.1,2 Recently, we observed that long-term administration of calcium channel antagonists attenuates the rarefaction of capillaries occurring in the hearts from spontaneously hypertensive rats (SHR). This was found in SHR treated with nifedipine3 and in SHR administered nisoldipine.4 Our results were subsequently confirmed for nifedipine by Mall and coworkers.5 These observations seem to indicate that additional capillary growth is possible even in adult mammalian hearts. This was not a unique observation. Several authors have already reported a similar reaction in adult hearts that resulted in partial or complete restoration of the capillary density in mammalian hearts.6-16 Nevertheless, the mechanism of this angiogenic response is still not fully elucidated. Moreover, the topographic features of this additional growth of capillary material within the terminal vascular bed have not been analyzed.

In the present study, we decided to reexamine our nifedipine investigations to learn more about the nature and localization of this additional capillary growth. We used recent morphometric methods based on dual staining of capillary walls that distinguished between the arteriolar and venular portions of capillaries by color. This approach allowed for a detailed analysis of the geometry of the capillary net in both longitudinal and cross sections of the heart.

Methods

Age-matched SHR and Wistar-Kyoto (WKY) rats were used in the study. Four groups of animals (n=8 for each group) were investigated: (1) untreated SHR, (2) nifedipine-treated SHR, (3) untreated control WKY rats, and (4) nifedipine-treated WKY rats. All animals were kept in Macrolon cages, fed commercial rat chow, and allowed to drink unlimited tap water. Nifedipine was mixed into the diet (0.3 g/kg) equivalent to a daily dose of 15 to 30 mg/kg body wt, depending on food consumption. Treatment started at the age of 24 weeks and lasted 27 weeks.

Measurements of systolic blood pressure were done using the tail-cuff method in conscious animals on a weekly basis in all groups. Afterwards, the animals were weighed and killed, and their hearts were removed. The ventricles were isolated from the atria, weighed, and transversely sliced. The middle portions between apex and base were frozen in liquid nitrogen and used for quantitative morphology.
Morphometric Studies

The staining protocol has been described elsewhere. Briefly, tissue sections (16 μm) were incubated in a solution sensitive to dipeptidyl peptidase IV, which stained the venular portions of capillaries red. Next, the sections were transferred to a solution sensitive to alkaline phosphatase, which stained the arteriolar portion of capillaries blue.

Tissue cross sections of the midmyocardium were prepared by dissecting a block of tissue parallel to the principal axis of the heart. Longitudinal portions of the capillary net were used for measuring segment length, defined as a distance between two consecutive branching points. Capillary segments were measured with a Bioquant Image Analyzer (RM Biometrics, Inc). From each section, 150 capillary segments were chosen at random. Recently, we compared the results of this short-cut method with those obtained from complete reconstructions of capillary sets. Both methods yield similar results.

The intercapillary distance between parallel capillary segments was also recorded in places where three or more parallel segments were available. The segment lengths were distinguished according to their staining: blue (arteriolar), red (venular), and, occasionally, mixed. Similarly, the intercapillary distances were divided into three groups: distances between arteriolar, venular, or mixed segments.

Quantitative data obtained from both longitudinal and cross sections enabled us to calculate the capillary supply unit. We proposed this concept recently as the smallest tissue supply volume that can be modeled in three dimensions. It is defined as the product of the average capillary domain area and average capillary segment length. The use of this double-staining method helped us to distinguish between the capillary supply units derived from the arterial and venous capillary portions.

Statistical Analysis

All results are presented as mean±SEM. Basic data, summarized in Table 1, were evaluated with one-way ANOVA and subsequent Bonferroni post hoc tests. The remaining data, which display a log-normal distribution, were first log-transformed. The standard deviation of this log-normal distribution, SD log, was used as an index of the heterogeneity of capillary spacing. The higher the index, the more variable is the capillary spacing and vice versa. The heterogeneity of capillary spacing directly influences the local tissue PO2; this effect is independent of the average intercapillary distance, another important oxygen determinant.

Longitudinal parameters were recorded using a short-cut method that we proposed recently. Their collection may be described as follows: Longitudinal tissue sections of the midmyocardial wall were prepared by cutting sections of tissue perpendicular to the principal axis of the heart. Longitudinal quantitation of the capillary net was used for measuring segment length, defined as a distance between two consecutive branching points. Capillary segments were measured with a Bioquant Image Analyzer (RM Biometrics, Inc). From each section, 150 capillary segments were chosen at random. Recently, we compared the results of this short-cut method with those obtained from complete reconstructions of capillary sets. Both methods yield similar results.

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All results are presented as mean±SEM. Basic data, summarized in Table 1, were evaluated with one-way ANOVA and subsequent Bonferroni post hoc tests. The remaining data, which display a log-normal distribution, were first log-transformed, and then a nested three- or four-way ANOVA design was used with Bonferroni post hoc tests, as well as least-squares pairwise contrasts when applicable. Calculations were performed with SYSTAT software.

TABLE 1. Basic Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY Controls</th>
<th>Nifedipine-Treated WKY Rats</th>
<th>SHR Controls</th>
<th>Nifedipine-Treated SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>403±8</td>
<td>388±4</td>
<td>389±9</td>
<td>392±7</td>
</tr>
<tr>
<td>Total heart mass, mg</td>
<td>997±22</td>
<td>1005±30</td>
<td>1251±18*</td>
<td>1173±50*</td>
</tr>
<tr>
<td>Left ventricle + septum, mg</td>
<td>829±18</td>
<td>830±23</td>
<td>1090±15*</td>
<td>996±52*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>145±3</td>
<td>137±3</td>
<td>237±3*</td>
<td>197±3*</td>
</tr>
</tbody>
</table>

WKY indicates Wistar-Kyoto; SHR, spontaneously hypertensive rats. Values are mean±SEM, eight rats in each group.

*P<.01 vs WKY groups.

†P<.01, effect of treatment.
TABLE 2. Capillary Density

<table>
<thead>
<tr>
<th></th>
<th>WKY Controls</th>
<th>Nifedipine-Treated WKY Rats</th>
<th>SHR Controls</th>
<th>Nifedipine-Treated SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium, n/mm²</td>
<td>2436±39</td>
<td>2593±108</td>
<td>2247±16</td>
<td>2277±46*</td>
</tr>
<tr>
<td>Midmyocardium, n/mm²</td>
<td>2510±24</td>
<td>2716±70†</td>
<td>2359±52</td>
<td>2488±53†</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. Values are mean±SEM, eight hearts per group. Overall results in the effect of strain significant at P<.0001, treatment at P=.01, and region at P=.01.

*P<.01, effect of strain in the same region and with the same treatment.
†P<.05, effect of nifedipine in the same strain.
‡P<.05, effect of region in the same experimental group.

**Results**

Table 1 summarizes basic results. Total body mass was similar in all four experimental groups. SHR were characterized by higher systolic blood pressure than WKY rats (P<.01), the difference being significantly smaller in nifedipine-treated animals (P<.01). SHR also had significantly higher total heart mass and left ventricular mass compared with WKY controls. The increase was higher in untreated animals (25% for the total heart and 31% for the left ventricle plus septum) than in nifedipine-treated SHR (17% and 20%, respectively).

Table 2 contains capillary density values in all four experimental groups arranged according to cardiac regions (subendomyocardium versus midmyocardium). Three-way ANOVA revealed a highly significant overall effect of strain (SHR having lower capillary densities), treatment (nifedipine increasing the capillary density), and region (higher values in midmyocardium than in subendomyocardium).

Differential staining enabled us to distinguish between the proximal portions of capillaries located close to the arterioles, which stained blue, and distal portions on the venular side, which stained red. More capillaries stained blue than red (55% to 61% of the total number), with no significant differences in this proportion among the four experimental groups. Our capillary domain data were evaluated by a four-way nested ANOVA according to strain, treatment, region, and color of capillary supplying a particular domain. Hearts were regarded as a nested factor within (strain, treatment).

The differences were statistically highly significant for the effect of strain (P<.0001) and were more moderate (P<.03) for the remaining comparisons. These results are summarized in Table 3 and Fig 2. They reveal a larger area supplied by an individual capillary on a cross section (domain) (1) in SHR than in WKY rats, (2) in untreated animals than in nifedipine-treated animals, (3) in the subendocardial region than in the midmyocardium, and (4) on the arteriolar portion of the capillary than on the venular side.

The distribution of domain areas and of various subsequent measurements was log-normal, as demonstrated in Fig 1. From these domain areas, an equivalent radius of the Krogh cylinder with the same area was calculated. Since these radii also followed a log-normal distribution, the standard deviation of their log distributions provided an index of the heterogeneity of capillary spacing. Values ranged from 0.058 to 0.070, with higher values in subendomyocardium than in midmyocardium (P<.01) and higher values in WKY rats than in SHR (P<.05).

Table 4 contains measurements obtained from longitudinal sections, ie, capillary segment lengths and intercapillary distances. The differences among segment lengths of various colors were highly significant (P<.001). Blue-colored arteriolar segments were always significantly longer than red, venular segments. Occasionally, we noticed segments containing both or transitional colors, designated as mixed segments. These mixed segments were the longest; their number, however, was relatively small. Nifedipine treatment also resulted in longer segments (P<.05), whereas no significant differences were found when SHR were compared with WKY rats.

Table 4 also summarizes measurements of intercapillary distances, which were longer between capillaries from proximal (arteriolar) portions of capillaries than

**Table 3. Area of Capillary Domain**

<table>
<thead>
<tr>
<th></th>
<th>WKY Controls</th>
<th>Nifedipine-Treated WKY Rats</th>
<th>SHR Controls</th>
<th>Nifedipine-Treated SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium, µm²</td>
<td>400±3</td>
<td>377±2*</td>
<td>440±3</td>
<td>438±3</td>
</tr>
<tr>
<td>Midmyocardium, µm²</td>
<td>384±3</td>
<td>358±3*</td>
<td>424±4</td>
<td>407±3†</td>
</tr>
<tr>
<td>Artiolar</td>
<td>390±2</td>
<td>361±2*</td>
<td>434±3</td>
<td>410±3*</td>
</tr>
<tr>
<td>Venular</td>
<td>366±3</td>
<td>341±3*</td>
<td>399±3</td>
<td>374±3*</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. Values are mean±SEM, eight hearts per group. Statistics calculated after logarithmic transformation of the data. Overall results: Significant effect of strain (P<.0001), treatment, region, and capillary portion (all P<.03).

*P<.01, †P<.05, significant effect of nifedipine in the same strain, zone (region), and on the same capillary portion.
on the distal, venular side ($P<.05$). They were also longer in the hearts from hypertensive rats ($P<.02$), and there was a significant interaction of these factors ($P<.003$). This significant interaction term (color x strain x treatment) indicated that the potential effect of any one of the above factors differed depending on the value or level of the other factor or factors in the interaction.

Finally, Fig 3 depicts the estimates of the capillary supply units. Capillary supply units were significantly larger on the arteriolar than the venular side of coronary capillaries ($P<.0001$), and they were significantly larger in SHR than in WKY rats ($P<.0001$). On the other hand, the differences caused by nifedipine treatment did not reach statistical significance.

Discussion

The morphometric techniques used in the present investigation have served as discriminative indicators of the geometric conditions for oxygen supply in the left ventricular myocardium of both WKY rats and SHR. Furthermore, the differential staining of capillary walls that differentiate between proximal and distal capillary regions by color allowed for the precise localization of capillary growth processes occurring as a result of nifedipine administration.

Basic data summarized in Table 1 are consistent with results from our previous studies of the effect of nifedipine showing that nifedipine-treated SHR had significantly lower systolic blood pressure compared with untreated SHR, thus confirming the efficacy of calcium channel antagonists as antihypertensive drugs. The decrease in blood pressure was accompanied by a moderate decrease in cardiac mass. Nifedipine treatment of WKY control rats did not influence these parameters.

Capillarization of the hearts was evaluated using several morphometric methods. Our initial approach, yielding general information, was based on estimation of the overall capillary density. In this case, significant effects of strain, cardiac region, and treatment were found (Table 2). A more discriminating analysis was provided by our method of capillary domains. Measurements of the capillary domains (see Table 3 and Fig 2), representing the tissue areas supplied by each individual capillary, were subdivided according to the position of each capillary along the capillary pathway into arteriolar (proximal) and venular (distal) regions.

<table>
<thead>
<tr>
<th>TABLE 4. Capillary Segment Length and Intercapillary Distance</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Segment length, µm</strong></td>
</tr>
<tr>
<td>Arteriolar</td>
</tr>
<tr>
<td>Venular</td>
</tr>
<tr>
<td>Mixed</td>
</tr>
<tr>
<td><strong>Intercapillary distance, µm</strong></td>
</tr>
<tr>
<td>Arteriolar</td>
</tr>
<tr>
<td>Venular</td>
</tr>
<tr>
<td>Mixed</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. Values are mean±SEM, eight hearts per group, statistics calculated after logarithmic transformation of the data. Differences among segment lengths from different capillary portions (arteriolar, venular, and mixed) were highly significant in all experimental groups ($P<.001$). Similarly, differences between arteriolar and venular intercapillary distances were highly significant in all experimental groups ($P<.001$).

* $P<.01$, † $P<.05$, significant effect of nifedipine in the same strain and on the same capillary portion.
† † $P<.05$, † † † $P<.01$, significant effect of strain with the same treatment and on the same capillary portion.
In the subendomyocardium, the increase in domain area in hearts from SHR was approximately the same at both arteriolar and venular portions of capillaries (approximately 10%). On the other hand, in the midmyocardium, the increase in domain area was larger at arteriolar than venular portions. A similar preferential increase on the arteriolar portion of the capillary was found previously in cardiac hypertrophy due to pressure overload induced by experimental aortic constriction. Arteriolar capillary portions containing blood with high oxygen tension can probably sustain oxygen delivery and nutrient support to a larger cardiac myocyte volume than venular capillary portions. The discrepancy is thus reflected by a larger degree of myocyte hypertrophy in the vicinity of arteriolar capillaries. On the other hand, and possibly in defense of tissue oxygenation, venular capillary domains do not appear to increase in hypertrophy. This may be because of either a lack of myocyte growth in their vicinity or an increased venular capillary proliferation. Thus, the supply region of these distal capillary portions is commensurate with their lower Po2. On the other hand, the effect of nifedipine treatment seemed to be equally distributed between the two capillary portions and two cardiac regions examined.

Theoretically, the size of the capillary domain area on the tissue cross section should be reflected in measurements of intercapillary distances obtained from longitudinal sections. Indeed, results from the intercapillary distance measurements (Table 4) were comparable to findings for domain measurements (Table 2), i.e., significantly larger distances between capillaries on the proximal (arteriolar) side and larger distances between capillaries in hearts from SHR.

The segment length was always shorter on the venular side of the capillary. We found no significant differences between hearts from WKY rats and SHR (Table 4). This is in agreement with our previous studies on cardiac hypertrophy induced by pressure and volume overload, in which the segment length was also found unchanged. In contrast, nifedipine treatment resulted in a small elongation of the segment lengths, especially in WKY rats.

Finally, we reconstructed the three-dimensional capillary supply units as a product of capillary domain areas and capillary segment lengths. If one envisages the capillary network as an infinite mesh in which the segment length is the only clearly defined unit, the capillary supply unit may be considered as the smallest tissue supply volume that can be modeled in three dimensions with some degree of accuracy. It is of interest to note that the average values of the capillary supply units (varying among the experimental groups between 27 200 and 48 100 μm^3) were very similar to the values of the cardiac myocyte cell volumes, as measured previously in isolated myocytes from WKY rats and nifedipine-treated SHR. Campbell and coworkers reported the average myocyte volumes in this experimental situation to vary between 39.8 and 47.7×10^3 μm^3. The similarity of these basic units of muscle and of the capillary bed is demonstrated in Fig 3.

Our measurements of the tissue capillary supply units are summarized in Fig 3. We found highly significant differences (P<.001) between proximal and distal portions of capillaries in all experimental groups. This was due to a larger capillary domain area and longer segment length on the proximal (arteriolar) portion of the capillaries. Similarly, capillary supply units were significantly larger in the hearts from SHR, mainly because of increases in the size of capillary domains. On the other hand, a decrease in the domain area in nifedipine-treated rats was accompanied by an increase in capillary segment length. Thus, the resulting capillary supply unit remained more or less unchanged. Nevertheless, smaller domain areas and longer segment lengths in nifedipine-treated rats with presumably greater blood flow and increased red blood cell velocity might be more favorable for oxygen supply than capillary supply units of the same volume composed of larger domains and shorter segments.

Our results clearly indicate that hearts from SHR have all the expected morphological features of pressure-overload hypertrophy: decreased capillary density, resulting in an increased capillary domain area, which is more pronounced on the arteriolar portion of the capillaries. At the same time, the segment length more or less remained the same. The difference between pressure-overload cardiac hypertrophy and control hearts becomes even more pronounced if one compares the size of the capillary supply unit conceived as a product of capillary domain area and segment length. These data are presented here for the first time (see Fig 3). Most previous studies, typically using the simple index of capillary density, reported decreased capillarization of the hearts from SHR. In contrast, Tomanek and coworkers found these differences to disappear in the hearts of older SHR, and Anversa and coworkers found no differences in the early postnatal period.

Our detailed analysis of capillary net geometry enabled us to speculate about the topology of myocyte and capillary growth. Capillary supply units were reconstructed from measurements in the midmyocardium. In cardiac hypertrophy from SHR, the increase in tissue volume supplied by a capillary segment was more pronounced on the proximal portion of the capillaries. As the segment length was unchanged, the increase was due to an increased intercapillary distance and thus larger capillary domain area. Changes on the distal portion were less pronounced, which may be due to the fact that the distal segments, containing less oxygenated blood, support smaller growth of neighboring myocytes, or alternatively, it may be explained by an additional branching in the distal regions of capillaries. In contrast, nifedipine treatment results in a moderate capillary growth along the entire pathway, as supported by the finding of smaller capillary domains, longer segment lengths, and an unchanged proportion of proximal and distal capillaries in tissue cross sections. Values of the tissue volume supplied by a capillary unit are very close to the values of the myocyte volume, and both parameters are changing in various situations in parallel, as
demonstrated in Fig 3. The reason for this intriguing resemblance is not immediately clear.

Most investigators accept the fact that capillary growth capacity decreases with age.1,3,12 Thus, pathological growth of the heart occurring in the adult organism would be characterized by a decreased capillarization. Often, there are certain indicators of additional moderate capillary growth, albeit at a much lower rate than the growth rate of the total heart and its myocytes. This is in contrast to cardiac hypertrophy occurring early in life, which is characterized by an unchanged capillarization.13,14,32 Reports about possible angiogenesis after specific interventions in the adult organism are becoming more common. For instance, Hudlicka4 and Wright and Hudlicka3 documented capillary growth response in rabbit hearts with bradycardial pacing. Several authors have reported a similar response in hearts exposed to chronically increased coronary blood flow caused by various interventions: long-term administration of dipyridamole,10 ethanol,11 thyroxine,12 propranolol,13 and adenosine14 and in dogs with experimental emphysema.15

Treatment with a calcium channel blocker such as nifedipine is apparently an additional angiogenic stimulus. Our detailed analysis seems to demonstrate that the effect is not limited to any particular portion of the capillary net. Data obtained from tissue cross sections would indicate creation of new channels either by capillary branching or by increased length density caused by a more obtuse angle of branching. Alternatively, the effect may be due to an increased tortuosity of existing channels. Data obtained from longitudinal sections would also indicate an increase in the length of existing segments.

Our finding of a more pronounced angiogenic response in WKY rats seems to suggest that the major effect of nifedipine is due to mechanical stimuli resulting from chronically increased coronary blood flow. This decreases the probability of the second possible stimulus, tissue hypoxia, which would be more pronounced in hypertrophic hearts characterized by impaired conditions for oxygen supply. In addition, tissue hypoxia would have its main effect on venular portions of capillaries where the capillary blood PO2 would be lower. Nevertheless, the hypoxia effect is a plausible explanation in a variety of experimental situations, and although we feel that the magnitude of this influence in our model would be minimal, it should not be completely discounted.

In conclusion, the present study confirmed previously described decreased capillarization of hearts from SHR and its improvement by long-term treatment with calcium channel blockers. Using discriminating morphometric methods we were able to provide a more detailed picture of this phenomenon in tissue cross sections and longitudinal sections. In both cases, we were able to distinguish between the effect occurring at the proximal and distal portions of capillaries.

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