Mast Cells in the Rat Heart During Normal Growth and in Cardiac Hypertrophy

Karel Rakusan, Kiriti Sarkar, Zdenek Turek, and Pierre Wicker

Mast cells in rat hearts were studied quantitatively during normal postnatal growth and in two types of cardiac hypertrophy. Normally, cardiac mast cell density in 11–12-day-old animals is very low, but increases markedly in the following 2–3 weeks to its highest values, with a subsequent decline toward adult values. At the peak of mast cell density, the percentage of mast cells in close proximity to capillaries is also highest. In adult animals, mast cell counts are significantly higher in the right ventricle than in the left. This relation is preserved even when the right ventricle is hypertrophic, as in rats born at simulated high altitude. Chronic hypertension and swimming have little effect on the mast cell density in rat hearts. Conspicuous changes in the mast cell density at the time of capillary proliferation seem to indicate a special role played by these cells in the formation of new vessels. (Circulation Research 1990;66:511–516)

It has been more than 100 years since a young medical student named Paul Ehrlich first described mast cells.1,2 Even now, however, their role in myocardial tissue is not clear. Judging by the experimental evidence from other tissues, they may play a role in the proliferation of new capillaries. For instance, tumor angiogenesis factor invokes a 40-fold increase in mast cell density of the choroidal membrane, which occurs within the 24 hours that precede the formation of new vessels, and suggests an intermediary role for mast cells in tumor angiogenesis.3

The possibility of a link between the mast cells and angiogenesis is also supported by our recent observation that the rapid postnatal formation of new capillaries in rat heart is hindered by repeated injections of protamine.4 The mechanism by which protamine inhibits angiogenesis is not clear. While we cannot exclude the direct effect of this compound on proliferation of capillary endothelial cells, a more probable explanation lies in its blockage of the effect of heparin released locally by mast cells. Mast cells are predominantly located in the tissues close to vascular structures. Many components contained in their granules (e.g., heparin, histamine, chymotrypsin, serotonin, platelet activating factor, and various chemotactic factors)1 can potentially contribute to the formation of new vessels.

An alternative role for cardiac mast cells has been proposed by Estensen,5 who suggested a possible cytotoxic function of mast cells in certain types of hypertrophy, leading to cardiac failure. Estensen, in his review article, also stressed the lack of reliable data on mast cell density in normal and hypertrophic hearts. This prompted us to examine the mast cell density in the rat heart and to look for correlations with other tissue parameters that could reveal functional dependence. This is the basis of the present study.

First, we studied cardiac mast cell density in conjunction with coronary capillary density during normal growth and development of the rat heart. The early postnatal period is characterized by a massive proliferation of new cardiac capillaries. If there is a link between the mast cells and angiogenesis, rapid capillary growth in this period should be accompanied by significant changes in the mast cell numbers. Subsequently, we examined the capillary–mast cell relation in two types of cardiac hypertrophy. In right ventricular hypertrophy, which occurs in rats exposed early in life to simulated high altitude, the growth of the muscle component is matched by a similar growth of the capillary bed. Therefore, cardiac samples originating from our recent studies on high altitude–adapted rats were reexamined and their mast cell densities recorded.6,7 The second type of cardiac hypertrophy studied was left ventricular hypertrophy.

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induced by pressure overload in older rats, which is characterized by decreased capillary supply. In this case, samples from our study of capillarization of the heart in rats with renal hypertension were used for quantitative analysis of the mast cells and for evaluation of the additional effect of exercise. Thus, we have studied mast cells in the rat heart during periods of rapid capillary formation during the early postnatal period, in the situation of concomitant growth of vascular and muscle components (as in high altitude--induced right ventricular hypertrophy), and finally, in the situation in which capillary growth did not match the muscle increase (as in left ventricular hypertrophy of renal hypertensive rats).

Materials and Methods

Protocol

Developmental studies were carried out on four age groups of male Sprague-Dawley rats: 11–12, 21–25, and 30–34-day-old and 9-month-old rats. Histological techniques have been previously described. Briefly, the rats were anesthetized with sodium pentobarbital and then their hearts were stopped in diastole, perfused in situ with heparinized saline, and fixed with 1.5% glutaraldehyde buffered to pH 7.4 with 1.5% phosphate buffer. After fixation and dehydration in alcohol, the samples were embedded in historesin. Capillaries were evaluated from 1-μm-thick sections stained by Avallone’s modification of Jones’ silver methenamine method for staining basement membranes. Mast cells were counted in 3-μm-thick sections stained by modified Nach’s azure eosin method. Density of coronary capillaries was measured in tissue cross-sections from midmyocardium and endomyocardium. In each region, five or six photomicrographs were taken, each photomicrograph representing an original area of 28,500 μm². The rule of Gundersen to correct for edge effect (“forbidden line” rule) was applied.

Mast cells were counted in 3-μm-thick sections of the left ventricle and the interventricular septum stained specifically for these cells. Depending on the size of the heart, four to eight cross-sections were evaluated per heart (150–400 mm²). First, the area of the cross-section was determined with an image analyzer, and then the number of mast cells was counted and the cell density per square millimeter was calculated. Thus, for the sake of convenience, the mast cell density was evaluated in all regions across the ventricular wall, whereas capillary density was measured in only the midmyocardium and endomyocardium. Epicardium, however, comprises only 10–15% of the whole cross-section, and its capillary density is usually the same as in midmyocardium. In addition, we also recorded the position of each mast cell with respect to the nearest vascular wall; cells within a radius of approximately 12–15 μm from the wall were considered to be “close” to the vessels. The occurrence of mast cell clusters was also recorded. A cluster was defined as a group of three or more mast cells in close proximity to one another.

Similar morphometric studies were applied to histological specimens of hearts originating from our previous studies dealing with left and right ventricular hypertrophy.

Left ventricular hypertrophy samples were obtained from renal hypertensive rats. Female Sprague-Dawley rats were made hypertensive by the two-kidney, one-clip Goldblatt procedure, while control animals were sham operated. One week later, half of the animals were subjected to a moderate swimming exercise and the other half remained sedentary. Thus, four experimental groups, each comprising 14 rats, were formed: control animals that were exercised or kept sedentary and the corresponding renal hypertensive animals either exercised or sedentary. The exercise consisted of two phases: an acclimation period of 4 weeks, followed by a stable training period of 5–6 weeks during which the rats swam 2 hours a day, 5 days a week. For details of experimental designs and methods, see our previous publications.

Right ventricular hypertrophy samples originated from our study on high-altitude animals. Male rats of the Wistar strain were used to study the effect of simulated high altitude on myocardial capillarization. Rats were born and raised for two generations at a simulated altitude of 3,500 m. These rats had similar ventilatory responses to hypoxia, changes in blood hematocrit, right ventricular hypertrophy, and heart capillarization as newcomers of the same strain acclimatized to the same altitude. Our sample consisted of 14 rats divided equally into groups of animals born and raised at sea level or at simulated high altitude for two generations. At the age of 3 months, the rats were anesthetized, and their hearts were perfused and evaluated as described above. For more details, see our previous studies.

Statistical Analysis

The average sampling error for capillary and mast cell counting was kept at less than 5% by the rather conservative approach for sampling error estimates described by Loud and Anversa. This is well below the biological variability, usually assumed to be around 10%. Statistical evaluation was performed using analysis of variance (ANOVA) with subsequent Bonferroni test, where applicable. Two levels of statistical significance, p < 0.05 and p < 0.01, were considered.

Results

Results are summarized in Tables 1–3 and in Figure 1.

Normal Development

Data on normal development may be found in Table 1. Mast cell density was very low in hearts from the youngest animals (11–12 days) but increased markedly in the following 2–3 weeks to the highest values in 1-month-old rats, with a subsequent decline
toward adult values. (The overall effect of age as well as differences among individual age groups was significant at \( p<0.01 \).) At the peak of mast cell density (1-month-old animals), the percentage of mast cells "close" to the capillaries is also the highest \( (p<0.01) \). Postnatal development of both of these parameters is displayed in Figure 1. Due to distinctive changes in the capillary density during the same postnatal period, the youngest hearts were characterized by a very large number of capillary profiles in a cross-section compared with the number of mast cells. This ratio decreased precipitously during the subsequent 3 weeks to the lowest values in animals 30–34 days of age, followed by a modest increase in the capillary/mast cell ratio in hearts from adult rats. Occasionally, we observed clusters of mast cells. These "nests" of mast cells were usually located in the proximity of larger vessels. They were found in all hearts from 21-day-old animals and in approximately 50% of the hearts from the remaining age groups.

**Effect of High Altitude**

Measurements on left and right ventricles from high-altitude rats and their sea-level controls revealed the following results (Table 2). Mast cell density was significantly higher in the right ventricle compared with the left ventricle of the same animal (two-way ANOVA, \( p<0.01 \)), despite the sizeable degree of right ventricular hypertrophy (+108%) and moderate increase (22%) of left ventricular weight in high-altitude rats. No significant overall effect of high altitude was detected in the mast cell density by two-way ANOVA. The left ventricles from high altitude–adapted rats seemed to have higher values (significant Bonferroni's test, \( p<0.05 \)), but a small decline was noted in the right ventricle. Thus, only significant interaction between the effects of region and treatment was obtained. The number of capillaries per square millimeter was approximately the same in both ventricles of rats belonging to the high altitude–adapted and control groups. Consequently, the capillary/mast cell ratio was significantly higher in the left ventricle than in the right ventricle. It was also higher in the left ventricles of control rats than in the left ventricles of high altitude–adapted rats \( (p<0.05) \). The overall effect of altitude exposure did not reach significant levels when ANOVA was used because there were no changes in the right ventricle. However, significant interaction between the effects of region and treatment was detected \( (p<0.05) \).

**Effect of Renal Hypertension and Swimming**

Finally, the mast cell density was not influenced by chronic hypertension or swimming (Table 3). Hypertrophic hearts (+44–49%) from rats with chronic hypertension were, however, characterized by signif-
Table 2. Heart Development in Sea Level- and High Altitude-adapted Rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>C-LV</th>
<th>C-RV</th>
<th>HA-LV</th>
<th>HA-RV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>439±12</td>
<td>439±12</td>
<td>398±15</td>
<td>390±20</td>
<td>NS</td>
</tr>
<tr>
<td>Heart wt (mg)</td>
<td>1,440±46</td>
<td>259±13</td>
<td>1,757±84</td>
<td>540±46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mast cells/mm²</td>
<td>1.04±0.08</td>
<td>2.46±0.24</td>
<td>1.61±0.17</td>
<td>2.14±0.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% Mast cells close to capillaries</td>
<td>75.6±2.2</td>
<td>78.4±2.4</td>
<td>76.4±2.3</td>
<td>65.8±3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Mast cells close to capillaries/mm²</td>
<td>0.76±0.07</td>
<td>1.98±0.20</td>
<td>1.24±0.15</td>
<td>1.39±0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Capillaries/mm²</td>
<td>3,280±115</td>
<td>3,391±88</td>
<td>3,449±175</td>
<td>3,018±272</td>
<td>NS</td>
</tr>
<tr>
<td>Capillaries/mast cells</td>
<td>3,338±311</td>
<td>1,425±135</td>
<td>2,446±211</td>
<td>1,507±279</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Seven rats per group. C, control rats; HA, high altitude-adapted rats; LV, left ventricle + septum; RV, right ventricle.
Significance refers to the difference between LV and RV; no significant effect of HA was detected, although the interaction between region and treatment was significant at p<0.05 in the case of mast cells/mm² and percentage of mast cells close to capillaries and at p<0.01 for mast cells close to capillaries/mm² and capillaries/mast cells. NS, not significant.

Data on body weight, heart weight, and capillary density are subsets of results reported in Reference 6.

...icantly decreased capillary density (p<0.01) and a significantly lower percentage of mast cells located in the proximity of terminal vessels (p<0.01). No significant effect of chronic swimming was detected.

The number of mast cell clusters was also recorded in both types of cardiac hypertrophy. The incidence and numbers were only slightly (nonsignificantly) higher than in normal hearts. Thus, a possible contribution of the mast cells present in focci of myocardial damage was negligible.

Discussion

Normal Development

The results of mast cell density found in the hearts from rats during normal cardiac growth and in two types of cardiac hypertrophy are comparable with data found in the literature. For instance, Keller and coworkers recently reported measurements in two hearts from adult rats; they found the average number of mast cells identified in 5-μm-thick sections to be 2.09 and 1.25/mm², respectively. The average value recounted from the data of Estensen on mast cell numbers in adult human hearts is 1.3/mm². Higher, but still in a similar range, are the older data of Fernex on human hearts and of Constantinides and Rutherdale on rat myocardium. The difference is probably explainable by their use of formalin fixation and paraffin embedding, which leads to a certain degree of shrinkage.

We found only a few mast cells in the hearts from the youngest rats. The mast cell density, however, was rapidly increased and reached the highest values in 1-month-old animals. This is in agreement with the observation of Hellström and Holmgren, who reported exceptionally low values of mast cell density in human hearts from children under 2 years of age. According to these authors, mast cell counts rise sharply in older children and then drop continually with increasing age. Similarly, low values in newborn hearts are mentioned as an unpublished observation in the article of Constantinides and Rutherdale. These authors reported 5–6 mast cells/mm² in the

Table 3. Heart Development in Renal Hypertensive and/or Exercised Rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Sham-Sed</th>
<th>Sham-Swim</th>
<th>RHR-Sed</th>
<th>RHR-Swim</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>249±6</td>
<td>245±6</td>
<td>236±5</td>
<td>224±9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left ventricular wt (mg)</td>
<td>539±16</td>
<td>599±24</td>
<td>802±37</td>
<td>776±30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mast cells/mm²</td>
<td>1.15±0.11</td>
<td>1.23±0.12</td>
<td>1.21±0.09</td>
<td>0.93±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>% Mast cells close to capillaries</td>
<td>75.8±1.6</td>
<td>76.5±1.7</td>
<td>74.0±1.3</td>
<td>69.0±1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mast cells close to capillaries/mm²</td>
<td>0.88±0.35</td>
<td>0.94±0.10</td>
<td>0.90±0.07</td>
<td>0.65±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Capillaries/mm²</td>
<td>4,652±159</td>
<td>4,682±130</td>
<td>3,968±137</td>
<td>3,993±118</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Capillaries/mast cells</td>
<td>4,679±570</td>
<td>4,357±466</td>
<td>3,551±291</td>
<td>4,872±548</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Fourteen rats per group. Sham-operated and renal hypertensive rats (RHR) were either sedentary (Sed) or swimming (Swim).
Significance refers to the effect of chronic hypertension; no significant effect of swimming was detected (two-way ANOVA). NS, not significant.

Data on body weight, left ventricular weight, and capillary density were reported previously in reference 8.
hearts of 1-month-old rats, which decreased to 1.7/mm² in the oldest age group.

Very low values in the youngest hearts of our morphometric study probably cannot be entirely explained by smaller mast cell size, which in turn would decrease the chances of finding the mast cell in the cross-section. While there is a moderate increase in the volume of the mast cell with age, the increase is on a much smaller scale than are numerical changes. We cannot, however, exclude the possibility of missing in our counts any mast cells that may have been completely degranulated.

It is probably not coincidental that a rapid increase in mast cell density takes place in the early postnatal period, which is also characterized by a substantial proliferation of new capillaries. According to our estimates, close to one half of all the existing capillaries in the adult rat heart are formed during the first 3–4 postnatal weeks. After this period, proliferation of new capillaries slows down remarkably, and both capillary and mast cell densities decrease as a result of an increasing size in cardiac myocytes. This may be illustrated by the following simple comparison: from day 11 to day 30, left ventricular mass increased 2.7 times. At the same time, the estimated total number of mast cells (assuming no changes in cell size) increased 29 times, and the estimated total capillary length (assuming no changes in capillary orientation) increased 19 times. In contrast, from day 30 to day 270, further increase in left ventricular mass amounted to an additional 217% compared with only 18% for mast cells and 59% for capillary length.

During the early postnatal period, characterized by a rapid growth of capillaries, not only do mast cell numbers increase but their distribution changes as well, with a significantly higher proportion of mast cells being found close to terminal vessels. A potential relation between mast cells and capillary growth has already been suggested in the introduction. Recently, Norrby and coworkers reported on mast cell–mediated angiogenesis, using rat mesentery as the experimental model. After activation of mast cells by repeated intraperitoneal injections of the mast cell–secretagogue compound 48/80, they observed that the number of blood vessels in the mesentery increased six- to sevenfold.

**Effect of High Altitude**

In that part of our study dealing with the effect of high altitude on myocardial capillarization and mast cells, the most striking finding was a significant difference in the mast cell density between right and left ventricles, with the right ventricle having a much higher value than the left (p < 0.01). This is evident in both sea level– and high altitude–adapted rats, despite a much higher right ventricular mass in the latter. Thus, normal levels of both capillary and mast cell densities are maintained in animals born at high altitude, despite considerable right ventricular hypertrophy. The reason for this reaction is probably that the stimulus for the accelerated growth of the right ventricle occurred during the early stages of development when the plasticity of myocardial tissue was still present. The left ventricular mass increased only slightly in high altitude–adapted rats and was accompanied by a moderate increase in the mast cell density and a decrease in the number of capillary profiles per mast cell in a cross-section.

Using the same approach as in the case of normal development, we can compare the data from high altitude–adapted with those from sea level–adapted animals as follows: the increase in the right ventricular mass was 108% in high-altitude animals, accompanied by an 86% increase in estimated capillary length and an 81% increase in estimated number of mast cells. The increase in left ventricular mass was 72%, with a 28% increase in capillary length and an 89% increase in the number of mast cells. We are not aware of any similar study on the effect of high altitude or right ventricular hypertrophy on cardiac mast cells. Regional differences, namely, higher mast cell density in the right than in the left ventricle, have also been observed by Hellström and Holmgren in normal human hearts, indicating that our rat model is apparently applicable to the human situation.

**Effect of Hypertension and Exercise**

Finally, the effects of renal hypertension and/or swimming on cardiac mast cells and coronary capillaries were determined in the last part of our study. Left ventricular hypertrophy due to chronic pressure overload was characterized by a lower capillary density, but the mast cell density was not affected. The only difference observed was a significantly lower percentage of mast cells in close proximity to the capillaries, which probably reflects hypertrophy of the individual myocytes. No significant effect of swimming was detected in either sham-operated or renal hypertensive rats, so exercise does not seem to be a determining factor in capillary or mast cell density. The average increase in left ventricular mass of renal hypertensive rats (sedentary and swimming groups combined) was 47%, which was not fully compensated for by an increase in total capillary length (29%) and an increase in total number of mast cells (36%).

**General Discussion**

It is rather difficult to compare directly the quantitative data obtained in the three projects reported here, as they originate from animals of different sex and various strains. Nevertheless, most of the results seem to indicate the same geometric relation between mast cells and capillaries, with implications for capillary proliferation. Direct comparison of the total capillary length and the mast cell counts would probably be an oversimplification for several reasons: 1) the timing is not clear as to when the new capillary material and the mast cells were formed with respect to one another; 2) reliable estimates of the total number of mast cells would also require measurement of cell size; and 3) determining the number of...
mast cells does not give any indication of the number and composition of the granules, which may contain angiogenic substances.

In spite of the reservations mentioned above, it is interesting that an early postnatal increase in cardiac mass is proportionately much smaller than an increase in total capillary length and number of mast cells, whereas the opposite is true in later stages of postnatal development. In high altitude–adapted animals the right ventricular hypertrophy is almost matched by the increase in capillary length and mast cell number, whereas in the left ventricular hypertrophy of renal hypertensive rats the increase in cardiac mass significantly exceeds the increases in capillary length and mast cell count.

In summary, our results reinforce indirectly the role of mast cells in angiogenesis generally, and in the heart muscle specifically. Substantial formation of new capillaries during the early postnatal period is accompanied by profound changes in the mast cell density and spatial distribution. Similarly, changes during hypertrophic cardiac growth in rats born at simulated high altitude are accompanied by formation of additional capillaries and mast cells. On the other hand, no major changes in mast cell parameters are found in left ventricular hypertrophy in adult hypertensive rats that were either sedentary or swimming. Therefore, we suggest that these results taken together indicate a role by mast cells in angiogenesis but that more studies are necessary to pinpoint the precise nature of the causative mechanism(s).

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