Cardiac function in hearts isolated from a rat model deficient in mast cells

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Kennedy, Richard H., Martin Hauer-Jensen, and Jacob Joseph. Cardiac function in hearts isolated from a rat model deficient in mast cells. Am J Physiol Heart Circ Physiol 288: H632–H637, 2005. First published September 23, 2004; doi:10.1152/ajpheart.00803.2004.—Several studies have examined the potential role of mast cells in the myocardial response to injury such as that caused by hypertension and ischemia-reperfusion. However, little is known about the influence of mast cells on normal myocardial structure and function. The present experiments examined cardiac function in Langendorff-perfused hearts isolated from 6- and 9-mo-old male mast cell-deficient (W/Wv) and mast cell-competent rats. A fluid-filled balloon catheter was used to measure left ventricular diastolic and systolic function at increasing preload volumes. At 6 mo of age, mast cell-deficient rats showed a slight cardiac hypertrophy (as monitored by heart weight and heart weight-to-body weight ratio) but no significant change in maximum observed systolic or diastolic function. In contrast, at 9 mo of age, the mast cell-deficient group showed no signs of hypertrophy but displayed a diastolic dysfunction characterized by decreased compliance without a significant decline in maximum observed basal –dP/dt max. There were no significant differences in maximum observed values for measures of systolic function (developed pressure and +dP/dt max). In summary, the results of this study in adult rats suggest that mast cells influence cardiac function in the absence of injury and that observed differences between mast cell-competent and -deficient animals vary with age. Thus it is important to consider these “physiological” actions and resulting changes in function when studying effects of insult in mast cell-deficient models.

MAST CELLS are part of the innate immune system that provides the first line of defense against tissue damage. In addition to their critical role in immunoglobulin E-dependent, histamine-mediated hypersensitivity, mast cells release and modulate cytokines, growth factors, chemokines, proteases, and other mediators, which in turn regulate a vast array of important biological processes. Many mast cell mediators (e.g., histamine, heparin, tryptase, chymase, TNF-α, and interleukin-4) are preformed and stored in large amounts in secretory granules, available for immediate release (21). Studies have demonstrated that mast cells reside in the myocardium even under physiological conditions, with their distribution along the coronary capillaries being more dense in the arteriolar section (30).

Several studies have examined the potential role of mast cells in the myocardial response to injury. However, in many cases, the results of these studies have been contradictory. For example, numerous investigators have monitored the contribution of mast cells to preconditioning as well as ischemic injury. Parikh and Singh (27, 28) suggested that ischemia- and nor-epinephrine-induced preconditioning are mediated in part by degranulation of resident mast cells in the rat heart, whereas Wang et al. (37) reported that mast cell degranulation is not involved in ischemic cardiac preconditioning in rabbits. Similarly, the role of mast cells in the acute tissue damage and cardiac dysfunction elicited by ischemia and ischemia-reperfusion (I/R) injury has not been resolved. Several reports suggest that mast cell stabilizers such as disodium cromoglycate and lodoxamide tromethamine diminish acute I/R damage in both rat and rabbit hearts (19, 27, 37) possibly via inhibition of TNF-α release (11), whereas other investigators found that antigen-evoked “mast cell depletion” or lodoxamide tromethamine has no effect on I/R or hypoxia/reoxygenation injury in the rat heart (8, 36). The possible role of mast cell-derived histamine in acute ischemia as well as ischemic preconditioning has been discussed; however, several investigators have concluded that mast cell-derived histamine is not involved (3, 22, 37).

In addition to their possible role in preconditioning and acute ischemic injury, mast cells have been proposed to play a role in postinfarction myocardial remodeling. Mast cell accumulation was observed in areas of collagen deposition in a canine model of I/R (9), in the subepicardium of infarcted and non-infarcted regions in rats (7), and in human hearts displaying ischemic as well as idiopathic cardiomyopathy (29). In contrast, it has been reported that mast cell accumulation is minimal in the infarcted mouse heart (5), although other investigators have indicated that mast cells are one source of the enhanced plaminogen activator inhibitor-1 (PAI-1) expression that occurs in the infarct border and fibrous regions in a mouse model (34). PAI-1 inactivates matrix metalloproteinases (MMPs) and may contribute to the increased fibrosis. Other investigators showed that mast cells can stimulate collagen expression by cardiac fibroblasts in vitro (4).

Mast cells have also been implicated in the cardiac remodeling and dysfunction elicited by other types of insult. Stewart et al. (33) presented data suggesting that increases in mast cell density and mast cell chymase activity play a role in the MMP activation, extracellular matrix degradation, and adverse remodeling caused by mitral regurgitation, a model of volume overload, in dogs. This was supported by Chancey et al. (2), who demonstrated that even the relatively low density of mast cells in normal myocardium is capable of mediating ventricular dilatation and a significant decrease in collagen volume fraction in the isolated rat heart. Mast cells may also play a role in hypertensive heart disease. Although changes in mast cell number were not observed in hearts from a Goldblatt model of

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hypertension in rats (30), studies in spontaneously hypertensive rats led investigators to suggest that mast cells play a role in the cardiac fibrosis and hypertrophy that occur in hypertension, possibly due to the secretion of cytokines and growth factors (26, 32). Experiments also showed that the progression from compensated hypertrophy to decompensated failure in a pressure overload model is largely prevented in mast cell-deficient mice or in wild-type mice treated with tranilast, a mast cell stabilizer (15). In vitro studies demonstrated that mast cell granules and chymase promote cardiomyocyte apoptosis and the proliferation of noncardiomyocyte intramyocardial cells, both of which could contribute to the progression of heart failure (14). Similarly, SUNC8257, a chymase inhibitor, decreased the angiotensin II levels, transforming growth factor-β expression, left ventricular (LV) end-diastolic pressure, collagen expression, and fibrosis elicited by chronic tachycardia in dogs (23). A study by Briest et al. (1) suggests that mast cell degranulation is not involved in the hypertrophy elicited by chronic treatment with norepinephrine.

In contrast to literature addressing the possible role of mast cells in myocardial injury, little is known about the physiological importance of mast cells in the uninjured heart. It has been reported that cardiac mast cell density increases during the first month of life in rats and then declines to a lower adult level (30); however, the role of mast cells in normal cardiac development and function is unknown. The present experiments using a mast cell-deficient rat model were designed to determine whether mast cells affect cardiac function in adults in the absence of insult.

MATERIALS AND METHODS

Animal model. All procedures in this study were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Male 3-mo-old mast cell-deficient (Ws/Ws) and mast cell-competent control (+/+ ) rats were obtained from Japan SLC (Hamamatsu, Japan) and maintained in our Division of Laboratory Medicine on a 12:12-h light-dark cycle with free access to food and water until they reached 6 or 9 mo of age.

Mast cells arise from CD34 + hematopoietic precursors that acquire the tyrosine kinase transmembrane receptor for stem cell factor, c-kit. A mutant strain of rats having a 12-bp deletion in the tyrosine kinase domain of the c-kit gene was identified by Tsujimura et al. (35). The mutant allele was designated Ws (white spotting), and homozygous (Ws/Ws) rats were found to be deficient in mast cells as well as melanocytes and interstitial cells of Cajal (17). In addition, these Ws/Ws rats exhibit a hypoplastic anemia at birth that, in contrast to c-kit mutant mice, resolves somewhat by 10 wk of age (24). Ws/Ws rats have <1% of normal mast cell numbers in the skin and are, for all practical purposes, completely devoid of both mucosal and connective tissue mast cells in internal organs (25). In contrast to mast cell-deficient mice, Ws/Ws rats have normal numbers of germ cells and are fertile. The mast cell-competent control animals (+/+ ) were of the Donryu strain (an inbred strain developed in Japan). Langendorff-perfused hearts. Hearts were isolated from rats in each of the four groups (mast cell-competent and -deficient rats at 6 and 9 mo of age) and perfused via the aorta with an oxygenated Krebs-Henseleit solution (37°C) of the following composition (in mM): 118.0 NaCl, 27.1 NaHCO3, 3.7 KCl, 1.8 CaCl2, 1.2 MgCl2, 1.0 KH2PO4, and 11.1 glucose. As reported previously (18), the flow rate was set at 8.0 ml g heart-1 min-1, a value similar to that observed when flow is examined at a constant pressure of 70 mmHg; coronary pressure was monitored continuously by a Statham pressure transducer. Both atria were removed, and the ventricles were paced electrically at 250 beats/min by platinum contact electrodes positioned on the interventricular septum. Preliminary studies showed that the measured coronary pressure was not affected significantly by removal of the atria (data not shown). A fluid-filled balloon catheter (connected to a pressure transducer) was placed in the LV to measure intraventricular pressure, and the heart was enclosed in a humidified, temperature-controlled chamber. Cardiac function was monitored by measuring diastolic pressure, peak pressure, +dP/dt max, and −dP/dt max at various preload balloon volumes (20–300 μl, a range that elicited maximum contractility in all preparations). In addition to a polygraph recording, all data were digitized and analyzed with the use of acquisition and analysis software (CODAS, DataQ Instruments; Akron, OH).

Statistical analysis. Data were evaluated by ANOVA with a Student-Newman-Keuls post hoc test using SigmaStat (SPSS; Chicago, IL). The criterion for significance was P < 0.05. Data are reported as means ± SE.

RESULTS

Animal model. As shown in Table 1, body weight did not differ among the four groups. Heart weight and heart weight-to-body weight ratio did not differ when mast cell-competent and -deficient rats were compared at 9 mo of age; however, both of these parameters were increased in mast cell-deficient compared with mast cell-competent rats at the younger age.

LV diastolic function. As shown in Fig. 1, the diastolic pressure-volume relationship was not affected by mast cell deficiency at the younger age; however, the curve was shifted leftward/upward in mast cell-deficient compared with mast cell-competent hearts from the older age group. For example, diastolic pressures at a balloon volume of 300 μl were 33.0 ± 5.4, 33.4 ± 6.7, 17.2 ± 3.0, and 42.4 ± 5.2 mmHg in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively (n = 4–8 rats/group).

The decreased compliance observed in 9-mo-old mast cell-deficient hearts was not paralleled by a decrease in −dP/dt max. In fact, as indicated in Fig. 2, at lower balloon volumes (20–160 μl) −dP/dt max was greater in hearts isolated from 9-mo-old mast cell-deficient compared with 9-mo-old mast cell-competent hearts (e.g., at 140 μl, values for −dP/dt max were 1,244 ± 135 and 1,608 ± 50 mmHg/s in 9-mo-old mast cell-competent and 9-mo-old mast cell-deficient hearts, respectively; n = 4 rats/group). However, maximum observed values for −dP/dt max were not significantly different when compared between the two old groups (1,540 ± 113 and 1,723 ± 70 mmHg/s in 9-mo-old mast cell-competent and 9-mo-old mast cell-deficient hearts, respectively). In contrast to the older

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6-Mo-Old Rats</th>
<th>9-Mo-Old Rats</th>
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<tbody>
<tr>
<td></td>
<td>Competent</td>
<td>Deficient</td>
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<tr>
<td>Body weight, g</td>
<td>411 ± 9</td>
<td>427 ± 19</td>
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<tr>
<td>Heart weight, mg</td>
<td>1,272 ± 34</td>
<td>1,450 ± 54</td>
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<tr>
<td>Heart weight-to-body</td>
<td>3.09 ± 0.06</td>
<td>3.41 ± 0.11</td>
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<td>weight ratio, mg/g</td>
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<tr>
<td>Coronary pressure, mmHg</td>
<td>55.1 ± 4.2</td>
<td>60.5 ± 6.3</td>
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Values are means ± SE; n = 4–8 rats/group. *Significantly different from corresponding competent group.
Similar to 9-mo-old mast cell-deficient hearts, respectively; 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively; n = 4–8 rats/group).

Coronary pressure. As shown in Table 1, coronary perfusion pressure did not differ when the four groups were compared.

DISCUSSION

The results of this study suggest that mast cells influence cardiac function in the absence of injury in adult rats and that observed differences between mast-cell-competent and -deficient animals vary with age. The two ages included in the study (6 and 9 mo) were selected as mature adult models that do not express aging-associated changes. At 6 mo of age, mast-cell-deficient rats showed slightly but significantly greater heart weights and heart weight-to-body weight ratios than the mast-cell-competent group. These parameters, which are suggestive of hypertrophy, did not differ when the mast-cell-competent and -deficient rats were compared at 9 mo of age. In contrast, systolic and diastolic function did not differ when the two groups at 6 mo of age were compared, whereas the 9-mo-old mast cell-deficient group displayed a decreased compliance without a significant decline in maximum observed −dP/dt_max.

In fact, at some submaximal preload volumes, values for −dP/dt_max as well as developed pressure and +dP/dt_max were greater in 9-mo-old mast cell-deficient hearts compared with the other groups. As with −dP/dt_max, maximum observed values for measures of systolic function (developed pressure and +dP/dt_max) did not differ among the four groups. As discussed in a review by Zile and Brutsaert (38), diastolic function is monitored by the onset, rate, and extent of relaxation as well as by the stress-strain relationship during diastole. Thus these data suggest that 6-mo-old mast cell-deficient animals express a slight cardiac hypertrophy without diastolic dysfunction, whereas at 9 mo of age they exhibit a rather pronounced diastolic dysfunction marked by decreased compliance without significant changes in heart weight-to-body

groups, −dP/dt_max did not differ between the two younger groups at any balloon volume, and values for the younger hearts were not significantly different from those in the 9-mo-old mast cell-competent group. At a balloon volume of 140 μl, values for −dP/dt_max were 1,219 ± 119 and 1,339 ± 58 mmHg/s in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 6-mo-old mast cell-deficient hearts, respectively (n = 7–8 rats/group; the corresponding values for maximum observed −dP/dt_max were 1,539 ± 79 and 1,544 ± 61 mmHg/s).

LV systolic function. Maximum observed systolic function was not significantly different among the four experimental groups. Although somewhat elevated in the 9-mo-old mast cell-deficient hearts, maximum observed values for developed pressure (peak systolic − diastolic) were not significantly different when the four groups were compared by ANOVA (103.1 ± 5.2, 107.2 ± 3.4, 103.7 ± 4.5, and 117.2 ± 4.1 mmHg in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively; n = 4–8 rats/group; Fig. 3). Similar to −dP/dt_max, developed pressure at intermediate balloon volumes (80–160 μl) was greater in the 9-mo-old mast cell-deficient hearts compared with the other groups (e.g., at 140 μl, developed pressures were 80.6 ± 6.7, 87.9 ± 4.2, 79.0 ± 7.3, and 103.8 ± 6.6 mmHg in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively; n = 4–8 rats/group).

Values for +dP/dt_max (Fig. 4) showed similar differences in terms of systolic function. There were no significant differences in the maximum observed values for +dP/dt_max (3,133 ± 154, 3,070 ± 184, 3,009 ± 166, and 3,477 ± 210 mmHg/s in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively; n = 4–8 rats/group). However, values observed at intermediate balloon volumes (40–140 μl) were greater in the 9-mo-old mast cell-deficient hearts compared with the other groups (e.g., at 120 μl, +dP/dt_max was 2,119 ± 222, 2,404 ± 191, 2,090 ± 201, and 2,904 ± 109 mmHg/s in 6-mo-old mast cell-competent, 6-mo-old mast cell-deficient, 9-mo-old mast cell-competent, and 9-mo-old mast cell-deficient hearts, respectively; n = 4–8 rats/group).

Fig. 1. Effects of balloon volume on left ventricular (LV) diastolic pressure in Langendorff-perfused hearts isolated from 9-mo-old mast cell-competent (●, n = 4), 6-mo-old mast cell-competent (○, n = 8), 9-mo-old mast cell-deficient (■, n = 4), and 6-mo-old mast cell-deficient (□, n = 7) male rats. Hearts were perfused with oxygenated Krebs-Henseleit buffer (37°C) at a flow rate of 8.0 ml·g tissue − 1·min − 1 and paced at 250 beats/min. The balloon volume was increased in 20-μl increments, with values being recorded after steady state was achieved. Values for diastolic pressure were not detectable at balloon volumes <120 μl. Values are means ± SE.

Fig. 2. Effects of balloon volume on LV −dP/dt_max in Langendorff-perfused hearts isolated from 9-mo-old mast cell-competent (●, n = 4), 6-mo-old mast cell-competent (○, n = 8), 9-mo-old mast cell-deficient (■, n = 4), and 6-mo-old mast cell-deficient (□, n = 7) male rats. Hearts were perfused with oxygenated Krebs-Henseleit buffer (37°C) at a flow rate of 8.0 ml·g tissue − 1·min − 1 and paced at 250 beats/min. The balloon volume was increased in 20-μl increments, with values being recorded after steady state was achieved. Values are means ± SE.
weight ratio, maximum basal $-dP/dt_{\text{max}}$, or systolic function. Further studies are required to elucidate the cause of the diastolic dysfunction at 9 mo of age and to explain the absence of decreased compliance in the slightly hypertrophied 6-mo-old heart. Nonetheless, these differences in function between mast cell-deficient and mast cell-competent animals, which were observed under near-physiological conditions and in the absence of inotropic stimulation, need to be considered when using mast cell-deficient models to study effects of cardiac injury.

The decreased compliance of the 9-mo-old mast cell-deficient group compared with the 9-mo-old mast cell-competent group is demonstrated by the greater slope of the diastolic pressure-volume relationship shown in Fig. 1. However, Fig. 1 also shows that the slopes of the curves for the two 6-mo-old groups, which do not differ, are greater than those in the 9-mo-old mast cell-competent hearts. This is explained, at least in part, by the difference in LV chamber diameter. Preliminary echocardiographic analysis in three to four rats of each group (data not shown) indicate that midwall LV diastolic diameters at 6 and 9 mo of age are ~0.78 and 0.88 cm, respectively, with no marked differences between mast cell-competent and -deficient animals. This suggests that the LV volume at an end-diastolic pressure of 0 mmHg is at least 30% less in the slightly hypertrophied 6-mo-old heart that resolves somewhat by 10 wk of age (24). In addition, mast cell-deficient mice have been reported to display both hypertriglyceridemia and hypercholesterolemia, which may be due in part to a deficiency in heparin (13). The lipid profile has not been examined in the mast cell-deficient rat model. Thus it is possible that mast cell deficiency acts indirectly as well as directly on the myocardium to elicit observed changes in cardiac structure and function. In addition to the more well-understood cardiac effects of hyperlipoproteinemia and ane-

In conclusion, the decreased compliance in hearts isolated from 9-mo-old mast cell-deficient rats is a mutant strain having a 12-bp deletion in the tyrosine kinase domain of the c-kit gene (35). In addition to being essentially devoid of mast cells (25), these rats are deficient in melanocytes and interstitial cells of Cajal (17), and they exhibit a hypoplastic anemia at birth that resolves somewhat by 10 wk of age (24). In addition, mast cell-deficient mice have been reported to display both hypertriglyceridemia and hypercholesterolemia, which may be due in part to a deficiency in heparin (13). The lipid profile has not been examined in the mast cell-deficient rat model. Thus it is possible that mast cell deficiency acts indirectly as well as directly on the myocardium to elicit observed changes in cardiac structure and function. In addition to the more well-understood cardiac effects of hyperlipoproteinemia and ane-

Further studies are required to determine the changes underly-

ing the diminished compliance in hearts isolated from 9-mo-old mast cell-deficient rats. In many disease states, a decrease in compliance is mediated by changes in the content or physical properties of collagen; however, other factors may contribute such as amyloidosis, cellular disarray, coronary vascular engorgement, myocardial ischemia, and the cardiomyocytes (10, 16). In addition, it is unclear why the 9-mo-old mast cell-deficient hearts displayed somewhat enhanced slopes when the effects of balloon volume on developed pressure, $+dP/dt_{\text{max}}$, and $-dP/dt_{\text{max}}$ were examined. It would appear that elements controlling the length-active tension relationship, such as the myofilaments, are acted upon at lower balloon volumes in these hearts.

The mechanisms by which mast cells affect cardiac structure and function are largely unknown, even during disease states such as hypertension and myocardial infarction. However, mast cells release a variety of mediators, such as histamine, proteases, cytokines, proteoglycans, and arachidonic acid metabolites (21), that may influence the heart during injury, repair, or even normal physiological states. Among other actions, these agents may be either pro- or antifibrogenic. For example, chymase may elicit antifibrinogenic actions by activating pro-MMP-1 (31), and tryptase can activate pro-MMP-3, which initiates activation of multiple MMPs (12). In contrast, other mast cell-derived mediators such as cytokines promote fibroblast proliferation and collagen production (15, 26).

Further work is required to verify that the changes in cardiac function observed in this study are mediated entirely by the absence of mast cells in the myocardium. As discussed previously, the Ws/Ws mast cell-deficient rat is a mutant strain having a 12-bp deletion in the tyrosine kinase domain of the c-kit gene (35).

Fig. 3. Effects of balloon volume on LV developed pressure (peak systolic – diastolic) in Langendorff-perfused hearts isolated from 9-mo-old mast cell-competent (●, $n = 4$), 6-mo-old mast cell-competent (○, $n = 8$), 9-mo-old mast cell-deficient (■, $n = 4$), and 6-mo-old mast cell-deficient (□, $n = 7$) male rats. Hearts were perfused with oxygenated Krebs-Henseleit buffer (37°C) at a flow rate of 8.0 ml/g tissue$^{-1}$·min$^{-1}$ and paced at 250 beats/min. The balloon volume was increased in 20-μl increments, with values being recorded after steady state was achieved. Values are means ± SE.
nia, it was recently suggested that interstitial cells of Cajal serve as stretch receptors for the vagal afferent pathway that can modulate myocardial function (20). Additional work is obviously required to address these possibilities, especially in available mouse models where the hyperlipoproteinemia is documented and the hypoplastic anemia does not resolve by 10 wk of age (24). Nonetheless, it is important that observed changes in cardiac function be considered when utilizing these animal models to study myocardial response to insult.

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