Diminished Cardiac Hypertrophy and Muscle Performance in Older Compared With Younger Adult Rats With Chronic Atrioventricular Block

Gary D. Walford, Harold A. Spurgeon, and Edward G. Lakatta

The combined effect of advancing age and hemodynamic overload on cardiac muscle function has received little attention. In male, Sprague-Dawley rats, we studied the interaction of chronic atrioventricular heart block induced by transvenous electrocautery for 4–12 months (mean, 7 months) and age at study (12, 19 ± 0.7, and 24 ± 0.2 months) on cardiac hypertrophy and muscle function compared with age-matched, sham-operated controls. Hypertrophy was determined by the ratio of heart weight to tibia length. Muscle function was first determined from the mechanical variables of the isometric contraction of an excised, thin, left ventricular trabecular muscle bathed at 29°C under a variety of calcium concentrations and stimulation patterns. Then, in the same muscles after disruption of membranes with Triton X-100, the force-pCa curve of the myofibrils was obtained. No hypertrophy occurred with aging in the control group, but alteration in hypertrophy with age occurred in the block group such that the youngest animals with block had the most hypertrophy (170%) and the oldest animals with block the least hypertrophy (120%). The tension developed by cardiac muscle and the duration of the isometric contraction were not affected by age in the control group but were significantly affected by age in the block group. The young animals with block had a markedly prolonged contraction duration and almost twice the developed tension compared with the older animals with block or with controls. The age-related difference in muscle contraction duration in the block group was associated with, and may have only been secondary to, the age-related difference in the extent of cardiac hypertrophy. For developed tension, the age-related difference in the block group could not be explained by differences in the extent of cardiac hypertrophy. Rather, this difference was attributable to both an increased myofibrillar force-generating capacity in the young block and to an impairment in excitation-contraction coupling in the old block. The results show that during long-term block, age exerted not only a significant effect on the extent of cardiac hypertrophy but also an independent effect on the developed tension of cardiac muscle. (Circulation Research 1988;63:502–511)

Most of the data concerning contractile function in experimental, hypertrophied hearts is based on studies with short-term hypertrophy induced by a variety of methods. The effects of long-term hemodynamic overload in middle-aged or older animals is less clear because most studies have used relatively young animals or, when older animals were used, did not compare different age groups with the same duration of the hemodynamic load. There is good reason to believe that the adaptive process of hypertrophic growth may be modified by the age of the animal. Protein synthesis and protein synthetic reserves are altered with age in many organ systems, including the heart, and thus older hearts may be less able to produce or maintain the increased protein synthesis rates observed in younger hearts subjected to an increased workload. A decrease in myocardial transfer RNA and protein synthesis rates has been reported for aging rats under a prolonged, increased workload. In addition, cardiac protein degradation rates decrease with age, which leads to lower turnover rates of protein and yields different physiochemical properties of the protein itself, which in turn may affect cardiac function.
The extent of cardiac hypertrophy and changes in cardiac muscle function that result from cardiac work overload depend on the experimental stimulus used to cause the overload. A recent study of renovascular hypertension for 2½ months in rats of different ages showed that age affected the extent of hypertrophy, but that advancing age apparently did not affect biochemical, contractile, and electrical properties separate from effects that could be related to the differences in the extent of hypertrophy.10 Volume-overload hypertrophy by atrioventricular block or other methods is a stable model, extensively studied in young animals, that has resulted in cardiac cell growth in proportion to the increase in heart weight11 with maintenance of normal ventricular dimensions, that is, a symmetrically enlarged heart,11,12 contractile function per unit weight of cardiac muscle that in the absence of congestive heart failure was normal or increased10,13-16 and that was associated with normal energetics.17 There is presently no information on the effect of age in response to chronic volume-overload hypertrophy. To examine the effect of this type of chronic, hemodynamic overload in animals of different ages, we used closed-chest electrocautery to induce complete atrioventricular block for a mean duration of 7 months in male, Sprague-Dawley rats of 5, 12, and 16 months of age. Atrioventricular block by transvenous electrocautery was chosen as a model because 1) it is minimally invasive compared with extensive surgical techniques such as aorto-caval fistula; 2) it results in a volume-overload state that can be tolerated for long periods of time and thus could be present during a significant portion of an animal’s lifespan; and 3) it induces considerable cardiac hypertrophy that can be related to the amount that the heart rate is slowed.13,18,19 The purpose of our study was to examine the effect of cardiac work overload on both the extent of cardiac hypertrophy and on cardiac muscle function at different ages in the adult rat.

Materials and Methods

Creation of Atrioventricular Block

Male, Sprague-Dawley, retired breeder rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) of 5, 12, and 16 months of age were anesthetized with sodium pentobarbital (60 mg/kg i.p.). When the procedure was prolonged, additional sedation was given by placing a cone that contained an ether-soaked gauze pad over the animal’s snout. Atrioventricular block was then produced by modifying a previously published method.20 The right jugular vein was exposed and was entered with a 4-in. long, 19-gauge stainless steel probe insulated with epoxy resin except at its distal tip, where it had been bent at a 45° angle. The proximal end was connected to a switch box, allowing the probe to be used as an intracavitary electrocardiogram displayed on an oscilloscope (model 5403, Tektronix, Beaverton, Oregon) or as an electrocautery device when connected to a diathermy unit (Bovie, Valleylab, Boulder, Colorado). The area of the atrioventricular node was located by its characteristic electrocardiographic signal. The probe was then removed if the animal was to be a control; otherwise, pressure was applied to cause mechanical block, and then a 1-second burst of electrical current was delivered to make the block permanent. The tip of the probe was withdrawn into the jugular vein, and if block persisted for 15 minutes, the probe was removed, the vein was ligated, the wound was closed, and the animal was returned to its cage. Animals were caged in pairs and allowed free access to water and standard rat chow (Ralston Purina, Richmond, Indiana). In the awake, restrained, and unanesthetized animal, heart rates were monitored by surface electrocardiogram at 24 hours, 72 hours, at 1 month after beginning the study, and also on the day before killing.

On the day of study, animals were examined for lethargy, edema, respiratory distress, and other signs of general or specific health problems and were killed by cervical dislocation. Body weight, wet and dry weights of the heart and of samples of liver and lung were determined. Cardiac size was evaluated by direct determination and by derived measurements that normalized heart weight to either body weight or tibial length.21

Studies of Intact Muscle

Immediately after death, the hearts were removed and placed in oxygenated Krebs-Ringer bicarbonate solution (bubbled with 95% O₂-5% CO₂, pH 7.4) containing 1.0 mM calcium concentration ([Ca²⁺]₀) and 4.2 mM [K⁺]. A left ventricular subendocardial muscle was dissected and mounted horizontally between two spring-loaded clamps in a 1.2-ml chamber perfused with Krebs solution at 29° C with a flow rate of 18 ml/min. One clamp was attached to a Statham UC-2 strain gauge (Gould, Cleveland, Ohio), which was coupled to a Gould Brush 480 recorder. Muscles were stimulated at 24 pulses/min by platinum electrodes with a Grass SD9 stimulator (Quincy, Massachusetts) at a pulse duration of 5 msec and a voltage 20% greater than that needed for maximum response. The muscles were allowed to equilibrate for 90 minutes in 2.5 mM [Ca²⁺], and then stretched to the peak of the developed force-length relation (L₀). In all muscles, after an additional equilibration of 60 minutes, resting force, developed twitch force, the maximum rate of rise of developed twitch force, time from stimulus artifact to peak force and time from peak force to half relaxation of force were recorded on line in digital form by an RDS-500 digital computer (Raytheon, Santa Ana, California) for eight consecutive beats and were averaged.

In a subset of experiments, changes in muscle length, stimulation frequency, or both stimulation frequency and [Ca²⁺]₀ were made. In these experiments, four separate muscle baths, each identical to that described above, were used and connected to a
common perfusate reservoir with a parallel arrangement of tubing. This allowed four separate muscles to be studied simultaneously. In these muscles, the twitch variables were measured in 2.5 mM [Ca\(^{2+}\)]\(_{0}\), at L\(_{\text{max}}\), 0.97 L\(_{\text{max}}\), 0.92 L\(_{\text{max}}\), and 0.87 L\(_{\text{max}}\); and at L\(_{\text{max}}\) ln (mM): 0.3, 0.5, 0.8, 1.0, 2.5, and 3.0 [Ca\(^{2+}\)]\(_{0}\) to determine a calcium dose-response curve. This was performed at both a stimulation rate of 24 pulses/min and also when stimulated once after a 2-minute pause in stimulation, that is, a rested-state contraction. The latter was performed because a single stimulus after a period without stimulation (2 minutes in our study) yields near-maximum developed tension in rat muscle. In an additional protocol in muscles at L\(_{\text{max}}\) bathed in 0.3 mM [Ca\(^{2+}\)]\(_{0}\) and stimulated at a rate of 24 pulses/min, a second electrical stimulus at a varying interval after the first (400, 300, 200, 170, 150, 120, 100, and 90 msec) was introduced continuously, and the parameters of the isometric contraction were determined for the first beat of the pair after developed twitch force had reached a steady level.

Studies in Chemically Skinned Muscle

After measuring the isometric twitch variables, stimulation was discontinued, and muscles in the subset described above were perfused for 30 minutes with the same buffer solution with 0 mM [Ca\(^{2+}\)]\(_{0}\), and 2 mM EGTA added. Muscle membranes were then disrupted by a 30-minute perfusion with Triton X-100 (1%) dissolved in a relaxing solution containing 100 mM KCl, 7 mM MgCl\(_2\), 5 mM ATP, 3 mM EGTA, 7.5 mM creatine phosphate, 0.05 mg/ml creatine phosphokinase, and 25 mM imidazole at pH 7.0. The criteria used to determine skimming have been previously reported by our laboratory and are similar to those published by others and include the demonstration of disrupted organelle membranes in the muscle core by electron microscopy. The muscle was subsequently washed with fresh relaxing solution without detergent, and then stepwise activation was produced by the addition of calcium to achieve a given pCa. After determination of the pCa that produced peak force, the perfusate was returned to the relaxing solution to ensure that force dropped to the original baseline. The Hill coefficient (N) and the pCa at half of maximal force (K\(_{50}\)) of the force-pCa relation in each preparation was determined by an iterative computer model as previously described.

After the above protocol, each muscle was removed from the bath and was blotted and weighed, and cross-sectional area was calculated assuming a cylindrical shape and density of 1.0 mg/mm\(^3\). Both the calcium-dependent force in the skinned preparation and the twitch-force measurements made before skinning in these muscles were normalized for cross-sectional area and thus expressed as tension, that is, resting tension, developed tension, and the maximum rate of rise of developed tension. The time course of the twitch was divided into two component intervals: 1) the time from the stimulus artifact to the peak of developed tension; and 2) the time from the peak of developed tension to the time the developed tension has relaxed to one half of this peak value, known as the one-half relaxation time. The overall index of the time course of the contraction was taken as the sum of time to peak developed tension and the time to one-half relaxation, and it was designated as the contraction duration.

Statistics

All results are expressed as mean ± SEM of muscles in the block or control group. Analysis of variance with a general factorial design (GLM, SAS, Cary, North Carolina) was used to determine the effect of block, age, or the interaction of block and age on the variables tested. Subsequent multiple t tests with Bonferroni’s correction were used to determine specific block or age effects. Linear regression analysis was used to determine relations between variables, for example, the extent of cardiac hypertrophy and developed tension or whether this relation differed among groups. A p value less than 0.05 was taken as the level of significance.

Results

Hypertrophy Due to Atrioventricular Block

There were 228 attempts to create atrioventricular block, and there were 82 control procedures (Table 1). Operative mortality was higher in the block (18%) than in the control group (4%). One animal with block had a sustained tachyarrhythmia, two animals with block had uncontrolled hemorrhage, and two animals with block had perforated hearts. Otherwise, all deaths could be attributed to excessive anesthesia during block, which was required by a prolonged procedure and as evidenced by apnea, cyanosis, and adequate but gradually slowing heart rate and no evidence of perforation being subsequently found. After the operative procedure, but within 24 hours of the procedure, death occurred only in the block group. Although hemodynamic variables were not measured, these animals may not have tolerated the acute reduction in heart rate due to block. This early mortality was significantly greater in the 12-month block group than in either the 5-month or 16-month block groups. Once the perioperative period was over, subsequent mortality was greater in each block group compared with its age-matched control group, but no effect of age was found (Table 2).

Survivors of sustained block (n = 37) were compared with a random sampling of control animals (n = 35). Table 2 summarizes block duration, heart rate, body weight, tibial length, heart weight with its components of right ventricular weight and left ventricular weight (which includes the entire septum), and dry-to-wet ratios of pieces of tissue from the lung, heart, and liver for each block and control age group. Block decreased heart rate and
Table 1: Effect of Atrioventricular Block on Mortality

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at start</th>
<th>Procedures (n)</th>
<th>Operative mortality (%)</th>
<th>Successful block</th>
<th>Mortality in 24 hours (%)</th>
<th>Late mortality (%)</th>
<th>Late reversion</th>
<th>Available for study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>15</td>
<td>2 (13)</td>
<td>...</td>
<td>0 (0)</td>
<td>2 (15)</td>
<td>...</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>50</td>
<td>1 (2)</td>
<td>...</td>
<td>0 (0)</td>
<td>14 (29)</td>
<td>...</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>17</td>
<td>0 (0)</td>
<td>...</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>...</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>3</td>
<td>3 (4)</td>
<td>...</td>
<td>0 (0)</td>
<td>17 (18)</td>
<td>...</td>
<td>62</td>
</tr>
<tr>
<td>Block</td>
<td>5</td>
<td>28</td>
<td>1 (4)</td>
<td>24</td>
<td>3 (13)</td>
<td>3 (14)</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>125</td>
<td>31 (25)*</td>
<td>82</td>
<td>6 (7)</td>
<td>18 (23)</td>
<td>38</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>75</td>
<td>9 (12)*</td>
<td>65</td>
<td>5 (8)</td>
<td>20 (33)</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>41 (18)*</td>
<td>171</td>
<td>14 (8)</td>
<td>41 (26)</td>
<td>79</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

*Operative mortality was higher for the 12-month block group compared with either 5-month block group (*p = 0.01) or 16-month block group (p = 0.03) and for all animals with block compared with all controls (p<0.05). The 5-month block group and 16-month block group did not differ.

Table 3 summarizes the derived indexes of cardiac hypertrophy, which is heart and chamber weights normalized to body weight or tibial length. Tibial length was the same for block and control groups, whereas heart and chamber weights normalized to tibial length also show more hypertrophy for the younger than older block groups. In addition, regardless of the index used, right ventricular hypertrophy was greater than left ventricular hypertrophy. This has been observed previously and may be explained by the relatively greater wall stress imposed by the same volume load on the thinner-walled right ventricle compared with the thicker-walled left ventricle.

Isolated Cardiac Muscle Performance

Intact muscle. The results of the isometric contraction at the muscle length giving maximum developed tension, temperature of 29°C, stimulation rate of 24 pulses/min, and 2.5 mM [Ca2+]o for block and control animals at three ages are shown in Table 4. There is no effect of age within the control group. The control group showed a trend for prolonged contraction duration with age, but the magnitude of the increase was small and did not reach statistical significance as previously described for other strains.
This may be due to the fact that the Sprague-Dawley strain used in this study does not exhibit senescent effects until it is 2 years or older. 26 Block did not affect resting tension, but it did affect both developed tension and the maximum rate of rise of developed tension in an age-related manner. In the 12-month block group, developed tension and the maximum rate of rise of developed tension increased compared with the 19-month block, 24-month block, and control groups. Block prolonged the contraction duration and its components, the time to peak tension and one-half time to relaxation at all ages, and block exhibited an age-related effect. This effect was greater at 12 months than at 19 and 24 months.

To further examine the age-related differences in the effect of block on developed tension and on the maximum rate of rise of developed tension, the results of the 12-month block group across a range of conditions that alter excitation-contraction coupling and the extent of myofilament activation were compared with those of a subset of the 19-month block group that was equivalent for mortality and duration of block. The effect of muscle length on resting, developed, and maximum rate of rise of developed tension for each age group of block is shown in Figure 1. Resting tension was similar between groups at all lengths (Panel A). Developed tension and the maximum rate of rise of developed tension were not only greater at the peak of the length-tension curve in the younger block than in the older block groups, but remained greater at shorter muscle lengths; that is, the relative decrease in both developed tension and the maximum rate of rise of developed tension brought about by decreasing muscle length was equivalent for both block groups (Panel B).

The response to changes in calcium concentration at a stimulation rate of 24 pulses/min or in the rested-state contraction (see page 10 for definition) for the same two block groups is presented in Figure 2. The absolute developed tension and maximum rate of rise of developed tension of the 12-month block group were greater than those of the 19-month block group across the entire range of calcium concentrations during both stimulation rates (Panel A). As is typical in isolated rat muscles, the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Block</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at finish (months)</td>
<td>12±0.0</td>
<td>19±0.7</td>
</tr>
<tr>
<td>Muscles (n)</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>0.50±0.06</td>
<td>0.39±0.10</td>
</tr>
<tr>
<td>RT (g/mm²)</td>
<td>1.09±0.16</td>
<td>0.77±0.07</td>
</tr>
<tr>
<td>tDT (g/mm²)</td>
<td>4.82±0.72</td>
<td>1.94±0.20</td>
</tr>
<tr>
<td>tDT/dt (g/mm²/sec)</td>
<td>72±7.30</td>
<td>29±4.20</td>
</tr>
<tr>
<td>tCD (msec)</td>
<td>263±12</td>
<td>224±6</td>
</tr>
<tr>
<td>*tTPT (msec)</td>
<td>142±6</td>
<td>123±5</td>
</tr>
<tr>
<td>*tRTH (msec)</td>
<td>120±8</td>
<td>102±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
CSA, cross-sectional area; RT, resting tension; DT, developed tension; DT/dt, maximum rate of rise of tension; CD, contraction duration; TPT, time to peak of developed tension; RTH, one-half of the time to relaxation.
Significant effect of *atrioventricular block and †age within atriopulmonary block.

This may be due to the fact that the Sprague-Dawley strain used in this study does not exhibit senescent effects until it is 2 years or older. Block did not affect resting tension, but it did affect both developed tension and the maximum rate of rise of developed tension in an age-related manner. In the 12-month block group, developed tension and the maximum rate of rise of developed tension increased compared with the 19-month block, 24-month block, and control groups. Block prolonged the contraction duration and its components, the time to peak tension and one-half time to relaxation at all ages, and block exhibited an age-related effect. This effect was greater at 12 months than at 19 and 24 months.

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The response to changes in calcium concentration at a stimulation rate of 24 pulses/min or in the rested-state contraction (see page 10 for definition) for the same two block groups is presented in Figure 2. The absolute developed tension and maximum rate of rise of developed tension of the 12-month block group were greater than those of the 19-month block group across the entire range of calcium concentrations during both stimulation rates (Panel A). As is typical in isolated rat muscles, the
FIGURE 1. Effect of age on the response of developed tension (DT), maximum rate of rise of developed tension (DT\(\text{Idt}\)), and resting tension (RT) changes in muscle length in isolated muscles from hearts of animals subject to block. Length is the muscle length giving maximum DT. Absolute values (A) and relative values (B) as percentage of maximum value for each muscle. For 12-month block (O—O, n=5; muscle length at \(L_{\text{max}}\), 8.6±0.6 mm; cross-sectional area, 0.50±0.06 mm\(^2\)); and 19 month block: (●—●), n=5, muscle length at \(L_{\text{max}}\), 6.5±1.1 mm; cross-sectional area, 0.59±0.10). RT is NS for both absolute and relative values; DT and DT\(\text{Idt}\) p<0.05 by multiple t test for absolute values (A) but NS for relative values (B).

slower stimulation rate of the rested-state contraction, which is equivalent to one pulse every 2 minutes, shifts the relation of developed tension and calcium concentration to the left compared with that found at the faster steady-state stimulation rate of 24 pulses/min. Thus, the maximum value was reached at a lower calcium concentration in the rested-state contraction.\(^{22}\) This leftward shift was observed in both groups but was more pronounced in the 19-month group. This is best understood when the absolute developed tension or maximum rate of rise of developed tension at each calcium concentration is expressed as a percentage of the maximum absolute developed tension or maximum rate of rise of developed tension, respectively (Figure 2, Panels B and C). Note that as the calcium concentration is lowered, the curves for both stimulation rates reach lower percentages in the old animals with block than in young animals with block. These differences in the calcium dependence of developed tension and maximum rate of rise of developed tension are greater at 24 pulses/min (Panel C) than in the rested-state contraction (Panel B). Thus, muscle from the 19-month block group was less able than muscle from the 12-month block group to maintain its developed tension or maximum rate of rise of developed tension at lower calcium concentrations, especially at the faster stimulation rate, indicating an age-related effect on excitation-contraction coupling within the block group.

Excitation-contraction coupling was further examined by evaluating the effect of paired-stimulation on the developed tension in a reduced calcium concentration. The absolute values of developed tension are shown in the upper panel, and the relative increases over the single stimulus control value in each muscle are shown in the lower panel of Figure 3. The relative increase was greater for the 19-month block group than for the 12-month block group. However, this greater relative increase for the 19-month block group was not sufficient to increase the absolute value of developed tension to that achieved by the 12-month block group (upper panel). Because the pairing interval at which maximum potentiation occurred varied slightly, we also examined the maximum absolute developed tension regardless of the pairing interval. For the 12-month block group, developed tension was 2.91±0.4 g/mm\(^2\), and maximum rate of rise of developed ten-

FIGURE 2. A: Effect of bathing calcium concentration ([Ca\(^{2+}\)\(_\text{o}\)]\(_{0}\)) on developed tension (DT) and maximum rate of rise of developed tension (DT\(\text{Idt}\)) in 12-month block muscles stimulated at either 24 beats/min (Ο—Ο) and for rested state contraction (Ω—Ω) and in 19-month block muscles for 24 beats/min (●—●) and for rested state contraction (●—●). B: Effect of [Ca\(^{2+}\)]\(_{0}\) on DT and DT\(\text{Idt}\) expressed as a percent of the maximum absolute DT or DT\(\text{Idt}\) obtained using stimulation by a rested state contraction for 12-month block (Ω—Ω) and 19-month block (●—●). C: Effect of [Ca\(^{2+}\)]\(_{0}\) on DT and DT\(\text{Idt}\) expressed as a percent of the maximum absolute DT or DT\(\text{Idt}\) obtained using stimulation at 24 beats/min for 12-month block (Ο—Ο) and 19-month block (●—●).
Effect of paired-stimulation on absolute developed tension (DT) (Panel A) and relative DT (Panel B) in muscles bathed in calcium of 0.3 mM for 12 month block, (O—O), and 19 month block (■■■). Relative DT is determined after dividing the absolute DT obtained at each paired-stimulation interval by the DT obtained without paired-stimulation and then multiplying by 100 to yield a percentage value. N=4; in each p<0.05 for all intervals in Panel A and for 200-msec and 170-msec intervals in Panel B.

Chemically Skinned Muscle

To determine whether part of the difference in developed tension between the 12-month block and 19-month block groups (Figures 1–3) could have been due to differences in the myofilament response to calcium, the same muscles had their membranes disrupted with Triton X-100 so that the myofibrils could be directly subjected to a graduated, controlled calcium-induced activation. Control muscles of the two ages were also studied in this protocol. The calcium-force dose-response (force-pCa curve) described by 1) the peak force and the calcium concentration at which it occurred, 2) the calcium concentration at which half of the peak force occurred ($K_m$, used as a measure of the sensitivity of the myofibrils to Ca$^{2+}$), and 3) the slope of the calcium concentration-force curve as described by its Hill coefficient ($N$, used as a measure of the cooperative interaction of the myofibrils during exposure to different calcium concentrations) were measured, and the results are shown in Table 5 for all groups. To simplify this presentation, the curves for only the two block groups are shown in Figure 4. Similar to the results for absolute developed tension in the intact muscle, 1) there was no effect of age on the absolute calcium-dependent force in chemically skinned muscles in the controls; 2) there was a significant effect of age on absolute calcium-dependent force in the animals with block, with the younger animals with block having more force than the older animals with block; and 3) the younger animals with block had significantly more absolute calcium-dependent force than that of the control group (Figure 4, Panel B; Table 5). This indicates that at least part of the increased developed tension by the intact muscle of the 12-month block group may be due to increased force-generating capacity by the myofibrils. The Hill coefficients and pCa at half-maximum force were not altered by age, block, or age within block (Figure 4, Panel C; Table 5). Thus, although peak force was increased in the young block, all other aspects of the chemically skinned preparation’s force-pCa curves were similar among the groups.

Relation of Hypertrophy to Isolated Muscle Contraction

Because hypertrophy was greater in the young animals with block, the effect of age on developed tension and the maximum rate of rise of developed tension in muscle with block may in fact have been secondary to an age-related difference in the extent of hypertrophy. However, neither developed tension nor the maximum rate of rise of developed tension was significantly correlated with the extent of hypertrophy (p>0.5 for linear regression correlation coefficients). In addition, a 19-month block subgroup (n=5) selected for the same hypertrophy as that of the 12-month block group (heart weight: tibial length = $10.6 \pm 0.6$ g/cm x $10^{-2}$) still had developed tension ($2.16 \pm 0.36$ g/mm$^2$) and maximum rate of rise of developed tension ($32 \pm 7.1$ g/mm$^2$/sec)

<table>
<thead>
<tr>
<th>TABLE 5. Results of Chemically Skinned Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Age at finish (months)</td>
</tr>
<tr>
<td>Muscles (n)</td>
</tr>
<tr>
<td>Hill coefficient ($N$)</td>
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<tr>
<td>$K_m$ (pCa)</td>
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<td>pCa at peak force</td>
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<tr>
<td>Peak force (g/mm$^2$)</td>
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Values are mean±SEM.
*Significant effect of age within block.
Figure 4. Effect of pCa on force in Triton-treated muscle showing a representative tracing for a single muscle (A) and the results for the relative force (B) and absolute force (C) of the 12-month block (O-O) and the 19-month block (•-•) groups. Relative force is determined by designating the maximum absolute value as 100% and then expressing the lower absolute values in the terms of a percentage of this maximum (n=5). Same muscles as Figures 1-3. p<0.05 for absolute peak values.

Discussion

The results show that chronic atrioventricular block caused 1) cardiac hypertrophy that was greater in the younger than the older rats; 2) prolonged contraction duration that was correlated with the extent of hypertrophy regardless of age; and 3) age-related alterations in contractile function of isolated intact and chemically skinned muscle that could be attributed to differences between younger and older block groups in both excitation-contraction coupling and myofilament force production.

These results cannot be explained by obvious problems with the experimental model such as gross differences in survival, which was uniformly lower for all block compared with control groups, or by different durations of block. Age-related differences in the extent of cardiac hypertrophy may be explained by other aspects of the experimental model. These include age-related differences in afterload based on age-related differences in vascular impedence; the mechanism of adaptation, acute or chronic, to a hemodynamic load due to differences in myosin isoenzymes and the ability to maintain a response to a chronic hemodynamic load due to age-related differences in protein turnover rates.

This age-related difference in the extent of cardiac hypertrophy may explain the age-related difference in contraction duration of the isometric twitch. Prolonged contraction duration is a nearly uniform finding in animal models of hypertrophy induced by hemodynamic overload. If there is an effect of age on contraction duration, it may be indirectly through those age-related factors that modify the extent of cardiac hypertrophy.

Regardless of how age may have further influenced the cardiac workload due to block or the hypertrophic response to it, and thus affected the contraction duration, the lesser extent of cardiac hypertrophy in the 19-month block group compared with the 12-month block group produced a functionally superior form of cardiac muscle compared with the older animals with block. The finding of increased cardiac muscle function is not unique to our study and has been found for exercise-trained animals compared with sedentary animals at any given age and for rats with spontaneous hypertension. This increased function could have resulted from increased myofilament density or from altered characteristics of the myofilaments at the same density. Our results favor the former interpretation because the Triton-treated
muscle had increased tension per cross-sectional area in the young animals with block compared with the older animals with block; that is, there were a greater number of force generating sites per unit of muscle, but the pCa values were similar at half-maximum force, and the Hill coefficients were similar, indicating these unaltered characteristics of the myofibrils regarding calcium affinity and cooperativity among myofilament calcium binding sites. In another model of hypertrophy, the spontaneously hypertensive rat, a similar increase in force production by isolated muscle has been attributed to just such an increase in myofibrillar density.

However, not all of the effects of age noted on the intact muscle contraction of the animals with block can be explained by the presence of an increased myofibrillar density in the young animals with block; rather, some of the effects indicate that abnormalities in excitation-contraction coupling have occurred in the older animals with block. The first evidence for this is in the calcium dose-response curve of the intact muscle (Figure 2). As the calcium concentration is reduced, developed force is reduced to a greater extent in the old animals with block than in the young animals with block in either the rested-state contraction (Figure 2B) or at 24 pulses/min (Figure 2C). This relative insensitivity to the low calcium concentration of the bathing solution in the older animals with block compared with the younger animals with block was not noted in the myofibrillar preparation, indicating that this effect of age on the muscle of blocked animals would more likely have been the result of differences in subcellular components other than the myofibrils involved in excitation-contraction coupling. It appears that in the intact muscle of the older animals with block these other subcellular components may have delivered less calcium to the myofibrils. The second evidence for an abnormality in excitation-contraction coupling can be related to the first and is found in the results of paired stimulation (Figure 3). This intervention increases internal calcium delivery to and activation of the myofibrils. If routine, regular contraction at 24 pulses/min does not deliver enough calcium to result in full activation of the myofibrils, then paired stimulation will boost the developed tension in proportion to the additional myofibrillar activation that was obtained. This also means that a muscle with less myofibrillar activation during a routine, regular contraction will show a greater relative increase in developed force during paired stimulation. This was the case with the older animals with block. At a low bathing calcium concentration, the relative developed tension was less for the older than the younger animals with block (Figure 2C and the paragraph above). Paired stimulation overcame this difference because there was a greater relative increase in developed tension in the older compared with the younger animals with block (see Figure 3). This indicates that the older animals with block had relatively less myofibrillar activation than the younger animals with block during routine regular stimulation. Still, the absolute developed tension during paired stimulation remained greater in the younger than in the older animals with block. Thus, although an age-related difference in the excitation-contraction coupling process was present in the block group and explains some of the age-related difference in absolute developed tension, a significant portion remains attributable to the differences in calcium-dependent myofibrillar force generation as shown in the chemically skinned preparation.

In conclusion, the younger animals with block had more cardiac hypertrophy than the older animals with block. An age-related difference in the prolongation of the contraction duration of the twitch was also seen in the younger compared with the older animals with block, but it was associated with and was probably secondary to the age-related difference in the extent of cardiac hypertrophy. More significantly, the younger animals with block also had increased developed tension and rate of rise of developed tension in isolated cardiac muscle. This was independent of the extent of cardiac hypertrophy and attributable to a combination of age-related differences in both myofibrillar force generating capacity and excitation-contraction coupling. Ultimately, the explanation for these findings will require a better understanding of how the myocardium "restructures" itself under the influence of hemodynamic stress and how aging affects this process.

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