Diminished Inotropic Response of Aged Myocardium to Catecholamines

By Edward G. Lakatta, Gary Gerstenblith, Charles S. Angell, Nathan W. Shock, and Myron L. Weisfeldt

ABSTRACT

The effect of advanced age on the response of active tension, maximal rate of tension development (dT/dt), and contraction duration to catecholamines and to calcium was evaluated in isometric trabeculae carneae from young adult (6-month-old), middle-aged (12-month-old), and aged (25-month-old) rats. Control values were not age dependent except for that for contraction duration which was prolonged in the aged group. At a norepinephrine concentration of 8 x 10^{-5}M, dT/dt increased to 163.8 ± 5.3% of control in the young adult group and to 125.9 ± 6.3% of control in the aged group (P < 0.001). Active tension increased to 121.3 ± 4.0% of control in the young adult muscles but did not increase in the aged muscles (P < 0.01). Contraction duration shortened proportionately in both age groups. Similar results were obtained with isoproterenol. In contrast to the response to catecholamines, there was no age difference in the response of active tension and dT/dt to increasing concentrations of calcium. It is concluded that the intrinsic inotropic response to catecholamines is diminished in the aged myocardium. This finding does not appear to result from differences in tachyphylaxis, tissue uptake of catecholamines, or the ability of the contractile proteins to respond to increasing concentrations of calcium but instead may result from a decreased ability of catecholamines to increase the intracellular calcium available for contraction.

KEY WORDS rat myocardium isometric performance active state norepinephrine isoproterenol calcium

The cardiovascular performance of animals (1, 2) and man (3, 4) during stress is diminished with advancing age. Augmentation of myocardial contractile ability during stress appears to be importantly related to increased sympathetic activity (5). Since certain age differences in the cardiovascular response to stress are obliterated by propranolol (3), it appears that there are important age changes in either the elaboration or the effectiveness of the sympathetic response. This study utilized an isolated cardiac muscle preparation to examine whether the age changes in response to stress result from a decreased intrinsic response of the aged myocardium to catecholamines. The inotropic response of isometric left ventricular trabeculae carneae from young adult and aged rats was examined during exposure to catecholamines. Since it has been postulated that the inotropic response to catecholamines is mediated by an increase in the amount of calcium available for contraction (6-11), the inotropic response to calcium was also measured in an effort to further characterize the mechanism for the age-associated changes.

Methods

Male, nonbreeder, Wistar rats 6, 12, and 25 months of age were selected from the Gerontology Research Center aging colony. The rat at 6 months of age, as body weight begins to plateau, is considered to be a young adult, that at 12 months of age is considered middle-aged, and that at 25 months of age, when approximately 50% colony mortality occurs, is considered aged (12).

Previous studies have revealed some specific age-associated changes in the cardiovascular system of rats from this colony. Hearts from aged rats weigh more and have a larger left ventricular cavity but the same estimated wall thickness as middle-aged hearts (1, 2). The histological appearance of the coronary bed as judged by light microscopy does not distinguish aged hearts from middle-aged hearts (13). Hydroxyproline content indicates that connective tissue constitutes 3.5% more of the total subendocardial tissue in the aged heart than it does in the middle-aged heart (14). Functionally, there is no age difference in the ability to extract oxygen even under extremely hypoxic conditions (13). The blood pressure of unanesthetized rats is not changed with age (15). Studies of cardiac performance have revealed no age difference in the response to...
volume loads, but they have shown a decrease in the response of the aged heart to pressure loads (1).

In the present study, hearts were removed under light ether anesthesia and immediately immersed in iced oxygenated modified Krebs-Ringer’s solution. The heart was then slit and laid flat: a trabecular muscle from the posterior ventricular wall was removed. At Lmax, the length at which active tension is maximal, trabeculae averaged 5.5 mm in length and less than 1.0 mm² in cross-sectional area (Table 1). The muscle was suspended between two spring-loaded Lucite clamps, which had been roughened to avoid slippage, inside a water-jacketed chamber at 29°C. The chamber was bathed with a Krebs-Ringer’sbicarbonate solution (16) modified by decreasing CaCl₂ to 1.0 mM and MgSO₄ to 0.6 mM and adding glucose to a concentration of 16 mM. The solution was oxygenated both in the reservoir and directly in the muscle chamber with 95% O₂-5% CO₂ gas mixture; the resulting oxygen tension (P₀₂) was approximately 680 mm Hg and the resulting pH was 7.45. The volume of the muscle chamber was 17 ml, and the chamber perfusion rate was 13 ml/min with no recirculation. The muscles were stimulated at a rate of 24 beats/min unless otherwise stated with a Grass SD9 stimulator via platinum field electrodes. Voltage was 20% above threshold, and the duration of the stimulus was 5 msec. Under these conditions, the muscles exhibited stable performance for at least 4-5 hours, as has been previously demonstrated (17). Muscle length was varied by raising or lowering a lever to which the bottom muscle clamp was firmly attached by a thin rod. The gear mechanism was calibrated to change length in 0.01-mm increments. The upper clamp was fixed to a Hewlett-Packard PTA 100 force transducer that was linear over the range of 0.1 to 10 g of force. The compliance of the entire apparatus was 0.007 mm/g force between 0 and 10 g of force. The first derivative of force (dF/dt) was obtained with a Hewlett-Packard 8805B preamplifier. Force, dF/dt, and stimulus artifact were recorded on a Hewlett-Packard 7888A ink-writing polygraph at a speed of 100 mm/sec. Force and dF/dt were normalized for cross-sectional area and expressed as tension and the first derivative of tension (dF/dt), respectively. Cross-sectional area was determined as previously described (14).

Except as indicated, results are expressed as means ± SE and compared using Student’s t-test for nonpaired values (18).

**EXPERIMENTAL PROCEDURE**

Two muscles of different age were studied simultaneously on a given day. During an initial 90-minute equilibration period, resting force was maintained at approximately 1 g. The muscle was then stretched to Lmax and allowed to equilibrate for an additional 60 minutes. The following base-line parameters were then measured: resting tension, peak active isometric tension, maximal rate of tension development (dF/dt), time to peak tension measured as the time from the onset of active tension to the peak of active tension, and the half-relaxation time measured as the time required for tension to fall to 50% of the peak value. The sum of the time to peak tension and the half-relaxation time was defined as contraction duration.

After these base-line measurements, increasing concentrations of l-norepinephrine were added to the bath. Norepinephrine solutions were prepared within 60 minutes prior to use and were continuously infused into the chamber so as to establish bath concentrations ranging from 1 x 10⁻⁸M to 8 x 10⁻⁵M. Each concentration was maintained for 8 minutes. The concentration was then increased to the next level. Concentrations greater than 8 x 10⁻⁵M were not employed because of the frequent occurrence of spontaneous tachyarrhythmias; any muscle that exhibited such arrhythmias was discarded. The response to 1 x 10⁻⁷M isoproterenol was also determined in other muscles under identical conditions.

The contractile response to calcium was measured in additional muscles from young adult and aged rats. Preliminary results indicated that maximal inotropy occurred at a calcium concentration of 2.6 mM and varied directly with heart rate. These findings are consistent with reports that rat myocardium is saturated with calcium at concentrations normally used in isolated muscle preparations (19) and that calcium significantly augments contractility at higher frequencies of contraction and lower initial external calcium concentrations (20). Therefore, the response to calcium was examined at a base-line calcium concentration of 0.5 mM and a heart rate of 48 beats/min. In additional muscles, the response to 1 x 10⁻⁷M norepinephrine was measured at a heart rate of 48 beats/min and a calcium concentration of 0.5 mM.

**Results**

The composite base-line data for muscles subjected to increasing concentrations of norepinephrine or to a single concentration of isoproterenol are presented in Table 1. The base-line data for the muscles in each of these subgroups did not differ

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**TABLE 1**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Cross-sectional area (mm²)</th>
<th>Length (mm)</th>
<th>Resting tension (g/mm²)</th>
<th>Active tension (g/mm²)</th>
<th>dT/dt (g/mm² sec⁻¹)</th>
<th>Contraction duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>19</td>
<td>0.86 ± 0.20</td>
<td>5.50 ± 0.38</td>
<td>1.33 ± 0.15</td>
<td>2.92 ± 0.25</td>
<td>33.7 ± 3.06</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>0.82 ± 0.10</td>
<td>5.79 ± 0.71</td>
<td>1.30 ± 0.50</td>
<td>2.77 ± 0.55</td>
<td>35.2 ± 1.82</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>0.96 ± 0.07</td>
<td>5.76 ± 0.42</td>
<td>1.12 ± 0.20</td>
<td>3.31 ± 0.31</td>
<td>34.5 ± 3.88</td>
</tr>
</tbody>
</table>

Values are means ± SE. dT/dt = maximal rate of tension development.

*P < 0.001 vs. 25 months.
from the data given in Table 1. The cross-sectional area and the length were not different among age groups. Resting tension, active tension, and dT/dt were also not age related. However, the contraction duration of the aged group was 17% and 19% greater than that of the young adult and the middle-aged group, respectively (P < 0.001). The effect of age on the response of dT/dt expressed as percent of control to increasing concentrations of norepinephrine is illustrated in Figure 1. At norepinephrine concentrations greater than $10^{-5}$ M, the response of dT/dt in the aged group was significantly less than that in the two younger groups. At a norepinephrine concentration of $8 \times 10^{-7}$ M, the response of dT/dt in the young adult muscles was more than twice that in the aged muscles (P < 0.001). Contraction duration (Fig. 2) shortened at norepinephrine concentrations greater than $10^{-7}$ M. In contrast, the extent of shortening was similar in the young adult and aged groups; however, comparing the young adult muscles with the middle-aged muscles, the difference in the extent of shortening of the contraction duration was significant at the two highest concentrations of norepinephrine. Active tension (Fig. 3) was augmented in the young adult and middle-aged groups, but in the aged group active tension was not significantly changed from the base-line value at any norepinephrine concentration employed. Although dT/dt increased in all muscles, active tension fell below base-line levels in four of the eight muscles in the aged group. The response of one of these muscles is presented in Figure 4.

Table 2 presents the base-line data for the muscles subjected to increasing concentrations of calcium (six in each age group) or to $1 \times 10^{-5}$ M norepinephrine (nine young adult and eight aged rats) at a base-line calcium concentration of 0.5 mM and a heart rate of 48 beats/min. The base-line data for the muscles in each of the subgroups did not differ from the data given in Table 2. There was no age difference in any parameter listed in Table 2. Contraction duration tended to be longer in the muscles from the aged rats than it was in the muscles from the young adult rats, but the difference was not statistically significant. The effect of age on the response of dT/dt to increasing concentrations of calcium is shown in Figure 6;
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Effect of age on the response of active tension to increasing concentrations of norepinephrine. Means ± SE are shown. n = number of rats tested.

there was no age difference at any concentration. Active tension, like dT/dt, was augmented similarly in both age groups at all calcium concentrations (Fig. 7). Increasing the calcium concentration also caused a small but similar increase in the contraction duration in both age groups (Fig. 8).

The response to a single concentration of norepinephrine administered under identical conditions at a calcium concentration of 0.5 mm and a heart rate of 48 beats/min (Fig. 9) demonstrated age differences similar to those previously noted in the norepinephrine dose-response curves (Figs. 1–3).

Discussion

The data on base-line performance indicated that active tension and dT/dt were not changed with age. Contraction duration was prolonged in the aged myocardium except at a calcium concentration of 0.5 mm; at this concentration, only a trend toward prolongation was observed. Other investigators have previously found that active tension (14, 21, 22) and dT/dt (22) do not vary with age and that contraction duration taken as the sum of time to peak tension and relaxation time (14) is prolonged in aged myocardium.

This study demonstrated that the inotropic response to both single and cumulative doses of norepinephrine was decreased in aged myocardium. That the decreased responsiveness was specific for aged myocardium was emphasized by the similar greater response in the young adult and middle-aged groups compared with the response in the aged group.

Endogenous catecholamine content is lower in aged myocardium (23, 24). It might be postulated that the deficient store of catecholamines in the aged muscles causes greater uptake of norepinephrine by storage sites with the result that less of the administered norepinephrine is available at the receptor site to produce an inotropic effect (25, 26). However, the age changes in performance persist after exposure to isoproterenol, an agent which has a high receptor affinity and which is negligibly taken up by storage sites (27). Therefore, it is unlikely that the age difference in the response to norepinephrine can be attributed to an age difference in storage site uptake. Greater tachyphylaxis

Figure 3

Effect of age on active tension (A.T.), maximal rate of force development (dT/dt) and contraction duration (C.D.) after exposure to 1 × 10^-8 M isoproterenol. Means ± SE are shown. n = number of rats tested.

Figure 4

Effect of norepinephrine (NE) (1 × 10^-6 M) infused for 8 minutes on the isometric twitch of an aged trabecula carnea. The rate of force development (dF/dt) was increased slightly, contraction duration was shortened, and the resulting force development was decreased to less than control. Paper speed for the rapid twitch recording is 100 mm/sec.

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TABLE 2

Base-line Data Obtained at a Heart Rate of 48 beats/min and a Calcium Concentration of 0.5 mM

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Cross-sectional area (mm²)</th>
<th>Length (mm)</th>
<th>Resting tension (g/mm²)</th>
<th>Active tension (g/mm²)</th>
<th>dT/dt (g/mm² sec⁻¹)</th>
<th>Contraction duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>15</td>
<td>0.80 ± 0.06</td>
<td>5.89 ± 0.34</td>
<td>1.67 ± 0.14</td>
<td>1.61 ± 0.19</td>
<td>21.7 ± 2.7</td>
<td>226.7 ± 5.0</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>0.73 ± 0.05</td>
<td>5.79 ± 0.31</td>
<td>1.53 ± 0.12</td>
<td>1.84 ± 0.26</td>
<td>22.6 ± 3.3</td>
<td>235.6 ± 6.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. dT/dt = maximal rate of tension development.

to cumulative doses also cannot explain the decreased responsiveness to norepinephrine in the aged myocardium, because the age difference is seen with a single high concentration of norepinephrine or isoproterenol.

Some insight into the mechanism responsible for the age difference can be obtained by examining the way in which catecholamines are thought to modify the characteristics of an isometric contraction. The two major actions of catecholamines on the isometric twitch are to shorten the duration of and to increase the intensity of the active state (28). These actions are not necessarily interdependent and need not occur through a common mechanism (29, 30). The duration of the active state is believed to reflect the length of time the contractile proteins are activated by calcium (7). It has been postulated that catecholamines shorten the duration of the active state via a cascade of events initiated at the beta receptor, mediated by cyclic 3', 5'-adenosine monophosphate (31) and protein kinase (32), and resulting in an increased rate of accumulation of calcium by the sarcoplasmic reticulum. Since catecholamines shorten the duration of the active state equally in the young adult and aged groups, as evidenced by equal shortening of the contraction duration, it would appear that this entire cascade from beta receptor to sarcoplasmic reticulum is as responsive to catecholamines in the aged myocardium as it is in the young adult myocardium.

It has been postulated that catecholamines increase the intensity of the active state by increasing the amount and the rate of arrival of calcium at the contractile site (6-11). One of the mechanisms by which beta-adrenergic agonists accom
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Effect of age on the response of contraction duration to increasing concentrations of calcium (Ca++) Means ± SE are shown. n = number of rats tested.

Under identical conditions, the response of dT/dt in the young adult muscles was greater after exposure to $1 \times 10^{-5} \text{M}$ norepinephrine (Fig. 9) than it was after exposure to 2.6 mM calcium, that concentration which elicits a maximal response (Fig. 6). This finding is consistent with previous observations that the maximal catecholamine effect on dT/dt is greater than the maximal effect induced by increasing the external calcium concentration (36). It has been hypothesized that this greater effect is mediated by a catecholamine-induced increase in the proportion of the total calcium which dissociates to the myofilaments to influence contraction (11). Under identical conditions, the response of dT/dt in the aged muscles after exposure to norepinephrine (Fig. 9) was not different from the maximal response after exposure to calcium (Fig. 6). Therefore, the diminished response to catecholamines in aged myocardium

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exposure to $1 \times 10^{-5} \text{M}$ norepinephrine.
probably results from an intrinsic alteration in the aged myocardium whereby the proportion of total calcium which dissociates to the myofilaments cannot be increased by catecholamines.

Active tension is directly related to the intensity and the duration of the active state (28). Catecholamines shortened the duration of the active state, reflected as contraction duration, equally in both aged and young adult muscles. However, the increase in the intensity of the active state, reflected as dT/dt, after catecholamine administration was greater in the young adult group than it was in the aged group. Therefore, active tension increased more in the young adult group than it did in the aged group; in fact, in several experiments, it fell below the base-line level in the aged muscles.

In summary, we have demonstrated a diminished intrinsic responsiveness to catecholamines in the aged myocardium. The decreased cardiovascular responsiveness of the intact organism to stress (1-4) is due at least in part to a decreased intrinsic inotropic responsiveness to catecholamines in the aged myocardium itself. This phenomenon can be explained by an age difference in the mechanism whereby catecholamines increase the amount of calcium available to the myofilaments. Since augmented tension development is the usual expected result of catecholamine administration, it is important to note that in the aged myocardium the combined effects of catecholamines on contraction duration and dT/dt in some circumstances do not result in significant augmentation of tension development.

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