

Prolonged Contraction Duration in Aged Myocardium

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ABSTRACT Isometric performance at 29°C was measured in left ventricular trabeculae carneae from young adult (6-mo) and aged (25-mo) rats ($n = 18$ in each group). Active tension and maximal rate of tension development did not differ with age, but contraction duration was 255 ± 6 ms in the young adult and 283 ± 6 ms in the aged group ($P < 0.001$). Although catecholamine content per gram heart weight was less in the aged myocardium, additional experiments showed that neither 1×10^{-6} M propranolol nor pretreatment with 6-hydroxydopamine eliminated the age difference in contraction duration. To determine if this age difference resulted from a prolonged active state, electromechanical dissociation and the overshoot of contraction duration during recovery from hypoxia were measured. During paired stimulation greater mechanical refractoriness was found in aged muscles ($P < 0.01$), but intracellular action potential recordings showed no age difference in the electrical refractory period. On recovery from hypoxia, contraction duration overshoot was $117 \pm 4\%$ of control in the young and $138 \pm 4\%$ of control in the aged muscles ($P < 0.01$). The greater electromechanical dissociation and greater overshoot in contraction duration following hypoxia in aged myocardium suggests that prolonged contraction duration in aged myocardium results from a prolonged active state rather than changes in passive properties or myocardial catecholamine content.

INTRODUCTION

While the ability of isolated mammalian myocardium to develop force under isometric conditions appears to be unaltered with age (1-3), prolonged contraction duration

(CD)¹ in aged myocardium has been demonstrated by some investigators (1, 2). This prolongation is reflected as either prolonged time to peak tension (1, 2), prolonged relaxation time (1), or both (1). Prolonged CD could result from changes in myocardial passive properties, active properties, or both. Studies using indirect polygraphic techniques have found that isovolumic contraction and relaxation are prolonged in aged man and it has been postulated that this is due to age differences in passive visco-elastic properties (4). Alternatively, age differences in active properties, possibly resulting from decreased catecholamine levels in aged myocardium (5, 6), may also account for prolongation of CD. This mechanism is suggested by the facts that catecholamines shorten the duration of active state (7) and that catecholamine depletion with reserpine prolongs CD (8).

The present study confirmed that under isometric conditions CD is prolonged in isolated aged rat myocardium. Additional experiments were then undertaken to investigate whether this age-related change results from a prolongation of active state and whether this change is mediated by myocardial catecholamine content. The time-course of active state has been characterized by measurements of electromechanical dissociation during paired stimulation (9) and of CD overshoot above baseline during recovery from hypoxia (10-12). Therefore, electromechanical dissociation, defined as mechanical refractoriness in the absence of electrical refractoriness (9), and the overshoot in CD during recovery from hypoxia were

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¹Abbreviations used in this paper: AT, active isometric tension; CD, contraction duration; CSA, cross-sectional area; dF/dt , the first derivative of force with respect to time; dT/dt , maximal rate of tension development; L_{max} , length at which active tension is maximal; RT, resting tension; $RT_{\frac{1}{2}}$ (half-relaxation time), time for tension to fall to 50% of its peak value; TCA, trichloroacetic acid; TPT, time to peak tension.

measured in young adult and aged myocardium. To determine if the age-associated prolongation of CD results from a catecholamine-mediated or from a more intrinsic change in active state, additional experiments were performed in which CD was measured in the presence of propranolol and after tissue catecholamine depletion by pretreatment with 6-hydroxydopamine.

METHODS

Male, nonbreeder, Wistar rats, aged 6-, 12-, and 25-mo, were selected from the Gerontology Research Center aging colony. 6-mo of age, as body weight begins to plateau, is considered young adulthood, 12-mo of age is considered middle age, and 25-mo of age, when approximately 50% colony mortality occurs, is considered senescence (13). Previous studies of animals from this colony have failed to detect evidence of specific cardiovascular disease or hypertension, in either the middle-aged or the aged rat (14). The aged heart weighs more, the cavity is larger, and estimated wall thickness is the same in the middle-aged and aged heart (15, 16). The connective tissue constitutes 3.5% more of the endocardial tissue in the aged than in the middle-aged animals (1). Studies of cardiac performance revealed that there is no significant age change in the ability to extract oxygen under normal and hypoxic conditions (17), or in the ability to respond to an acute volume load (16). However the response to an acute pressure load was less in the aged rat (16).

In the present study, hearts were removed under light ether anesthesia and immediately immersed in iced oxygenated modified Krebs-Ringer solution. The heart was then slit, laid flat, and a trabecular muscle from the posterior ventricular wall was removed. At L_{max} , the length at which active tension is maximal, the trabecular cross-sectional area (CSA) averaged less than 0.9 mm² and the length averaged 5.5 mm (Table I). Cross-sectional area was determined as previously described (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 bicarbonate solution (18) modified by decreasing the CaCl₂ to 1.0 mM, the MgSO₄ to 0.6 mM, and by adding glucose to a final concentration of 16 mM. The solution was oxygenated both in the reservoir and directly in the muscle chamber with a mixture of 95% oxygen-5% carbon dioxide resulting in a P_{O₂} of approximately 680 mm Hg and a pH of 7.45. The volume of the muscle chamber was 17 cm³ and the chamber perfusion rate was 13 cm³/min with no recirculation. The muscles were stimulated at a rate of 24 beats per min with a Grass stimulator (model SD9, Grass Instrument Co., Quincy, Mass.) via platinum field electrodes. Voltage was 20% above threshold and duration of stimulus was 5 ms. Under these conditions, the muscles exhibited stable performance for at least 4-5 h as has been demonstrated previously (19). 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 thin rod which was attached to a force transducer (Hewlett-Packard Co., Waltham, Mass.), which gave a linear response over the range of 0.1-10 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 by using a Hewlett-Packard preamplifier model 8805B.

Force, dF/dt , and stimulus artifact were recorded on a Hewlett Packard ink writing polygraph, model 7888A, at a speed of 100 mm/s.

Experimental procedure. Two muscles, each of different age, were studied simultaneously on a given day. During an initial 90-min equilibration period, resting force was maintained at approximately 1 g. The muscle was then stretched to L_{max} and allowed to equilibrate for an additional 60 min. The following base-line parameters were then measured: resting force, peak active isometric force, and maximal rate of force development. Force measurements were normalized to tension by dividing by the cross-sectional area and the results expressed as resting tension (RT), peak active isometric tension (AT), and maximal rate of tension development (dT/dt). The time to peak tension (TPT) was measured as the time from onset of active tension to peak active tension and the half-relaxation time (RT_{1/2}) was measured as the time for tension to fall to 50% of its peak value. The sum of TPT and RT_{1/2} was used as contraction duration (CD). After base-line stabilization, one group of these muscle underwent paired stimulation to determine mechanical refractoriness and subsequent hypoxia and reoxygenation to determine CD overshoot during recovery from hypoxia. A second group of muscles was exposed to propranolol and CD was remeasured.

Mechanical refractoriness. Paired stimulation was performed on trabeculae which were paced at the rate of 24 beats/min. A second stimulus was introduced at decreasing intervals after the initial stimulus. The interstimulus interval was shortened in a stepwise fashion from 400 to 80 ms. A second mechanical event was defined as either a discrete second twitch, which if present was easily discernable from the dF/dt tracing, or as an increase in RT_{1/2} following the second stimulus (20).

Hypoxia and reoxygenation. Hypoxia was induced by abruptly changing to a bathing fluid equilibrated with 95% N₂-5% CO₂. The chamber was also bubbled with 95% N₂-5% CO₂ and the entire transition was accomplished in approximately 5 s. The P_{O₂} fell progressively and after 8 min was approximately 20 mm Hg until the termination of the 20-min hypoxic period. Reoxygenation was accomplished by reversing the above sequence and after 1 min of reoxygenation P_{O₂} had increased to the base-line value of 680 mm Hg.

Propranolol. In preliminary experiments, it was determined that 1×10^{-6} M *dl*-propranolol was able to reduce the response to exogenous 1×10^{-6} M norepinephrine by 50%. In the second group of muscles, *dl*-propranolol, was continuously added to the bathing fluid to result in a concentration of 1×10^{-6} M. After 20 min of exposure, the parameters of the isometric twitch were remeasured.

Electrical refractory period. In a third group of muscles the action potential of a ventricular muscle cell was recorded by an impaled microelectrode while the muscle fiber was stimulated by extracellular electrodes at a rate of 24 beats/min under conditions identical to those described above. The duration of the action potential at 80% repolarization was measured. The electrical effective refractory period was then measured by delivering a second stimulus at progressively shorter intervals until a conducted action potential was no longer recorded (21).

6-Hydroxydopamine. A fourth group of rats was pretreated with 6-hydroxydopamine, 20 mg/kg, administered subcutaneously 24-48 h before the experiment, in the manner described by Roberts (22). This dose effected an average catecholamine depletion of 95% of control in the young adult animals and 97% of control in the aged animals.

TABLE I
Base-line Performance

Age	No.	CSA	Length	RT	AT	dT/dt	TPT	RT _‡	CD
mo		mm ²	mm	g/mm ²	g/mm ²	g/mm ² /s	ms	ms	ms
6	18	0.90±0.06	5.22±0.28	1.36±0.15	2.92±0.22	34.8±2.8	134±2.2*	121±4.2*	255± 6.1‡
12	8	0.81±0.11	5.25±0.61	1.36±0.21	2.85±0.22	36.1±2.2	134±4.5	118±6.9§	252±10.7§
25	18	0.84±0.07	5.71±0.36	1.45±0.18	3.40±0.26	38.8±3.6	142±2.0	141±5.0	283± 6.2

* $P < 0.01$ vs. 25 mo.
‡ $P < 0.001$ vs. 25 mo.
§ $P < 0.05$ vs. 25 mo.

Control values for myocardial catecholamines in each age group are presented below. The same base-line parameters of isometric performance were measured in this group and these muscles subsequently underwent hypoxia and reoxygenation.

Catecholamine assay. A fifth group of rats were decapitated and the hearts were quickly removed, rinsed in iced saline, blotted, weighed, and then rapidly frozen in liquid nitrogen. The heart was then pulverized, blended with 5% trichloroacetic acid (TCA), centrifuged, and filtered. An aliquot of the TCA filtrate, or of standard catecholamine solution, was combined with disodium EDTA and the pH was adjusted to 6.5. Catecholamines were isolated on pre-filled Bio-Rad ion exchange columns (Bio-Rad Laboratories, Richmond, Calif.), eluted with sodium hydroxide, and analyzed by a fluorometric procedure using a modified trihydroxyindole method (23). Recovery of standard solution of catecholamine added to the column was 97±1% and recovery of catecholamines added to tissue homogenates in the range of 0.5–1.01 µg/heart was 91±4%.

Except as indicated results are expressed as mean ± standard error and compared by using Student's *t* test for nonpaired values (24).

RESULTS

Base-line mechanical performance. The base-line mechanical data from all trabeculae except those from hearts which had been depleted of catecholamine by 6-hydroxydopamine are presented in Table I. There was no significant age difference in mean cross-sectional area or muscle length at L_{max} . There was also no significant age difference in RT, AT, or dT/dt. However TPT, RT_‡, and CD were significantly prolonged in the 25-mo age group. The CD in the aged group was 11% longer than in the 6-mo group and 12% longer than in the 12-mo group.

Mechanical refractoriness. Fig. 1 depicts the percent of muscles in each age group exhibiting a second mechanical event at each coupling interval. At long coupling intervals (400–300 ms) all muscles responded. As the coupling interval decreased, significantly fewer aged than young adult muscles exhibited a second mechanical response ($P < 0.01$ [X^2]). Fig. 2 shows the typical responses of an aged and young adult muscle at coupling intervals from 400 to 120 ms.

Hypoxia and reoxygenation. The effect of hypoxia and reoxygenation was examined in muscles from 6-, 12-,

and 25-mo-old rats. AT, dT/dt, and CD expressed as percent of control shortened proportionately in all three age groups after 20 min of hypoxia (Table II). 2 min after reoxygenation, the overshoot above baseline in CD was significantly greater in the old muscles. Fig. 3 illustrates the change in CD expressed as percent control during the entire hypoxia and reoxygenation sequence. The 12-mo group was not different from the 6-mo group and has been omitted from the chart for clarity. It can be seen that CD shortens during hypoxia and overshoots during early reoxygenation before returning towards control values at 15 min. The age differences in overshoot prolongation of CD is maximal during early reoxygenation and diminishes with time.

Electrical refractory period. The action potential duration at 80% repolarization was 73.8±6.0 ms in the 6-mo, $n = 10$, and 76.0±5.8 ms in the 25-mo age group, $n = 10$ (NS).

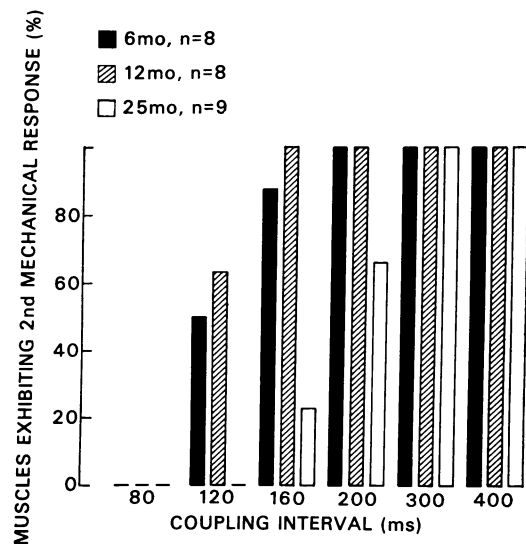


FIGURE 1 Effect of age on the ability of muscles to respond to a second stimulus during paired pacing at varied coupling intervals. As the coupling interval shortens fewer muscles in the aged group exhibit a second mechanical response, ($P < 0.01$ [X^2]).

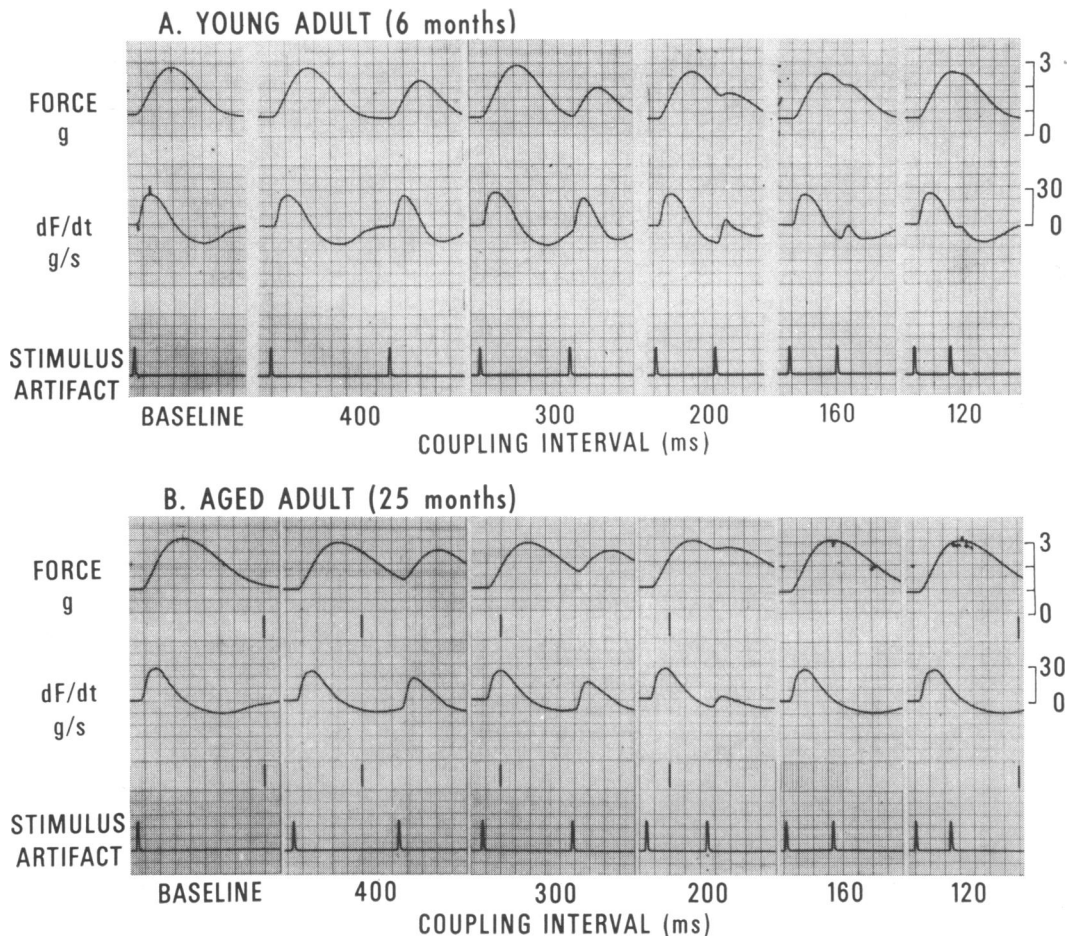


FIGURE 2 Comparison of the response of a typical young adult (panel A) and aged (panel B) muscle to paired pacing at varied coupling intervals. The muscle from the young adult rat exhibited a second mechanical response evident in both the force and dF/dt tracings at coupling intervals from 400 to 120 ms inclusive. The muscle from the aged rat did not generate a second mechanical response at coupling intervals of 160 ms or less.

The electrical effective refractory period was 83.3 ± 5.6 ms in the 6-mo-old group, $n = 9$, and 86.5 ± 5.4 ms in the 25-mo-old group, $n = 10$. Failure of all muscles to generate a second mechanical response at the 80-ms coupling interval, as seen in Fig. 1, probably represents electrical refractoriness.

Relation of catecholamines to CD with age. The catecholamine content per gram wet heart weight was significantly less in the 25-mo group than in the 6- or 12-mo groups (Table III). This decrease in content per gram heart weight is in part attributable to a greater heart weight in the old rat.

CD and other parameters of isometric contraction were examined before and after exposure to propranolol in muscles from nine 6- and nine 25-mo-old rats (Table IV). Although propranolol tended to decrease CD in

each age group, the prolongation of CD persisted in the aged group.

Nine muscles from 6-mo and nine muscles from 25-mo-old rats were studied after depletion of tissue catecholamines with 6-hydroxydopamine (Table V). Although CD tended to increase in both age groups (NS), the age-associated prolongation of CD was not obliterated. After 6-hydroxydopamine pretreatment in the aged muscles, dT/dt was less and TPT was greater when compared to the aged muscles in the control group in Table I ($P < 0.05$). Similar differences did not occur in the young muscles after 6-hydroxydopamine.

After 20 min of hypoxia dT/dt fell to a similar extent in both groups; AT fell less in the aged than in the young group and was accompanied by less shortening of CD (Table VI). After 2 min of reoxygenation the

overshoot in CD above baseline was, as in the non-catecholamine-depleted muscles, significantly greater in the old group and again represented significantly greater overshoot in both TPT and RT₁.

DISCUSSION

This study demonstrates that CD is prolonged in aged myocardium and confirms previous observations (1, 2). Since CD did not vary between 6 and 12 mo, prolongation of CD represents a phenomenon of late adulthood. Since appreciable variation in CD occurs within age groups, a substantial number of muscles must be studied to demonstrate a significant age difference. The failure of some investigators (3) to find this difference may be explained by a small sample size. Our findings that RT, AT, and dT/dt do not change with age are consistent with the results of previous studies (1-3).

Prolonged CD could result from age changes in the active state, visco-elasticity, or both. The measurements of mechanical and electrical refractoriness and those of CD overshoot during reoxygenation suggest that prolonged CD in the aged myocardium is due to an age-associated prolongation of active state. During paired stimulation experiments with short coupling intervals fewer old than young muscles were able to respond with a second mechanical event. Since the electrical effective refractory period is unchanged with age, the greater mechanical refractoriness indicates greater electromechanical dissociation in the old muscles (9) and therefore a change in active properties of aged myocardium. The overshoot in CD during recovery from hypoxia has been ascribed to a prolongation of active state (10-12) and the greater overshoot in old myo-

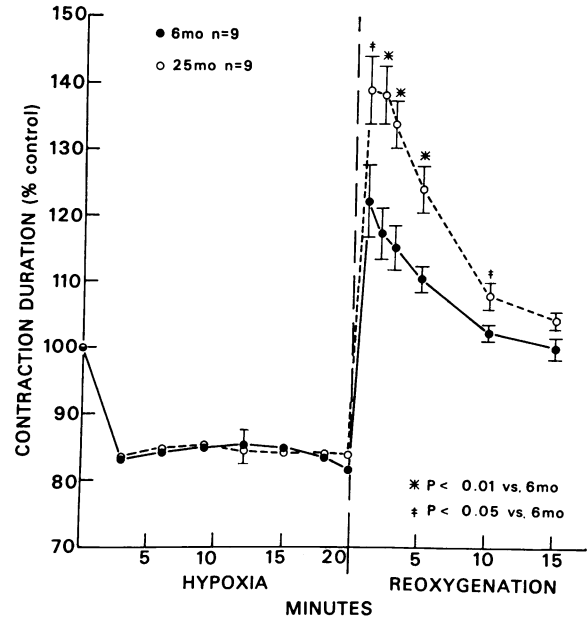


FIGURE 3 Effect of age on CD expressed as percent control during hypoxia and reoxygenation. During reoxygenation, the CD overshoot above baseline is greater in the aged myocardium. Base-line data is given in Table II.

cardium would therefore appear to result from a greater prolongation of active state.

Alternatively, if prolonged CD in the aged myocardium is explained solely on the basis of a change in visco-elasticity, stiffness would have to be decreased. But such a muscle would generate less active tension and active tension does not decrease with age. In addition, it has been demonstrated that although stiffness increases during hypoxia it decreases towards baseline without an overshoot during reoxygenation (25, 26). Therefore, the overshoot prolongation of CD during reoxygenation cannot be explained by a change in stiffness properties. Furthermore, any age difference in recovery rate of stiffness would necessitate an age difference in recovery rate of tension development and there was none. Therefore, prolonged CD in aged myocardium must involve

TABLE II
Hypoxia and Reoxygenation

Age	No.	AT	dT/dt	CD
mo		g/mm ²	g/mm ² /s	ms
Control				
6	9	2.78±0.38	34.4±4.3	254.2±10.39
12	8	2.85±0.22	36.1±2.2	251.6±10.67
25	9	3.54±0.41	42.7±5.0	282.0± 9.99
% Control hypoxia (20 min)				
6	9	35.9±1.70	48.1±1.98	81.3±2.66
12	8	33.2±2.83	43.2±3.09	79.48±1.49
25	9	35.5±2.54	40.9±3.32	83.50±1.81
% Control reoxygenation (2 min)				
6	9	78.3±6.71	69.4±4.75	117.6±3.70*
12	8	73.0±7.16	63.5±5.84	122.1±3.67‡
25	9	84.6±5.63	69.3±4.91	138.1±4.47

* $P < 0.01$ vs. 25 mo.

‡ $P < 0.05$ vs. 25 mo.

TABLE III
Myocardial Catecholamine Content

Age	No.	Total catecholamine	Heart wt	Catecholamine
mo		μg/heart	g	μg/g heart wt
6	10	0.693±0.057	1.27±0.022*	0.569±0.063*
12	11	0.687±0.040	1.50±0.087	0.474±0.040‡
25	10	0.555±0.061	1.73±0.097	0.325±0.037

* $P < 0.01$ vs. 25 mo.

‡ $P < 0.05$ vs. 25 mo.

TABLE IV
Effect of Propranolol on Isometric Contraction

Age	No.	AT	dT/dt	TPT	RT _‡	CD
<i>mo</i>		<i>g/mm²</i>	<i>g/mm²/s</i>	<i>ms</i>	<i>ms</i>	<i>ms</i>
Prepropranolol						
6	9	3.06±0.24	35.4±4.0	133±3.5*	123±3.9*	256±6.9*
25	9	3.26±0.34	34.9±4.9	142±1.9	141±7.1	284±7.7
Postpropranolol						
6	9	2.89±0.24	34.7±3.8	129±2.6‡	118±4.8*	248±5.4‡
25	9	3.12±0.34	33.7±4.8	140±2.1	137±7.0	277±7.6

* $P < 0.05$ vs. 25 mo.

‡ $P < 0.01$ vs. 25 mo.

prolonged active state and not just a change in visco-elasticity. It is possible that changes in visco-elasticity occur concurrently with this change in active state.

Depletion of endogenous catecholamines increases CD without affecting the length-tension curve (12). It is also known that catecholamines are liberated from heart muscle during hypoxia (27) and reoxygenation (28) and therefore may modify recovery from hypoxia (29, 30). Since myocardial catecholamine content per gram heart weight in our study as well as others (5, 6) decreased significantly over the same age range in which CD increased, an attractive hypothesis might be that diminished catecholamine levels in aged myocardium causes the prolonged active state. Although catecholamine depletion with 6-hydroxydopamine tended to prolong CD, the age difference persisted and therefore cannot be explained by changes in tissue catecholamine content. Furthermore, the age difference in overshoot of CD during recovery from hypoxia persisted after catecholamine depletion with 6-hydroxydopamine and hence cannot be explained by a catecholamine mechanism. Propranolol likewise did not obliterate the age change in CD. Therefore it appears that increased duration of active state in aged myocardium results not from a catecholamine-mediated change in active state but from a more intrinsic alteration. However, unlike 6-hydroxydopamine, propranolol tended to decrease CD in both groups as well as to decrease AT and dT/dt. Although

propranolol was employed because of its beta blocking properties, it is apparent that its direct negative inotropic effect (31, 32) predominated.

The duration of active state is thought to be determined by the length of time calcium is present on the contractile proteins and reflects a balance between the time-course of calcium delivery onto and the time-course of calcium removal from the contractile proteins (33). Prolonged duration of active state in old myocardium can theoretically result from prolonged calcium delivery to or slower calcium removal from the contractile proteins or both. In other mammalian species it has been postulated that changes in the transmembrane delivery of electrogenic calcium are influenced by the duration of the phase two plateau of the action potential (34). There is no distinct plateau in rat myocardial action potential but insofar as action potential duration may reflect this movement it appears that there is no age difference. Release from intracellular sites may play a more important role in the delivery of calcium to the contractile proteins (35). However, electromechanical dissociation at short coupling intervals would not result from prolonged delivery of calcium from intracellular sites to the contractile proteins but results rather from inadequate time to recharge the releasing site with calcium (9). Inadequate time to recharge these sites could be due to delayed calcium removal from the contractile protein or to delayed translocation of calcium from

TABLE V
Base-line Performance after 6-Hydroxydopamine Pretreatment

Age	No.	CSA	Length	RT	AT	dT/dt	TPT	RT _‡	CD
<i>mo</i>		<i>mm²</i>	<i>mm</i>	<i>g/mm²</i>	<i>g/mm²</i>	<i>g/mm²/s</i>	<i>ms</i>	<i>ms</i>	<i>ms</i>
6	9	0.85±0.06	6.33±0.41	1.44±0.19	3.15±0.35	34.7±3.3	140±2.5*	133±6.4	270± 8.4‡
25	9	0.75±0.06	6.85±0.57	1.41±0.13	2.66±0.29	27.8±3.0	156±5.0	143±6.5	299±10.9

* $P < 0.01$ vs. 25 mo.

‡ $P < 0.05$ vs. 25 mo.

TABLE VI
Hypoxia and Reoxygenation after 6-Hydroxydopamine Pretreatment

Age	No.	AT	dT/dt	CD
mo		g/mm ²	g/mm ² /s	ms
% Control hypoxia (20 min)				
6	9	31.96±1.47*	43.98±1.88	78.38±1.41‡
25	9	37.10±1.91	45.40±1.34	84.20±1.31
% Control reoxygenation (2 min)				
6	9	76.32±5.15	70.02±4.55	113.0±1.97*
25	9	81.79±3.29	74.23±2.10	121.4±2.93

* $P < 0.05$ vs. 25 mo.

‡ $P < 0.01$ vs. 25 mo.

storage to releasing sites. The fact that CD is prolonged in aged myocardium indicates that the delay occurs in the removal of calcium from the contractile proteins themselves. This sequestration of calcium from the contractile proteins is thought to be mainly a function of the sarcoplasmic reticulum, and delayed sequestration in the aged myocardium, therefore, could result from either tighter binding of calcium to the contractile proteins or to a slower calcium sequestering mechanism.

In summary, we have demonstrated that CD is prolonged in aged myocardium and that this prolongation cannot be explained solely by age changes in visco-elastic properties but must reflect a prolonged active state. Although catecholamines are thought to enhance calcium sequestration (36) and although myocardial tissue catecholamine levels are decreased with age, it appears that slow removal of calcium from the contractile proteins, independent of myocardial catecholamine content, is at least in part responsible for the increased duration of active state in aged myocardium.

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