Influence of Aging on Left Ventricular Hemodynamics and Stiffness in Beagles

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SUMMARY We studied the influence of aging on the contractile performance, stiffness, and contraction time of the canine left ventricle. Eight young (27 ± 2.5 months, mean ± SE) and seven old (128 ± 20.5 months) beagles were placed on complete cardiopulmonary bypass, the arterial pressure was adjusted to 80 mm Hg, and the heart contracted isovolumically at a paced rate of 120 beats/min. Diastolic pressure-volume curves were established for each unpaced left ventricle at the beginning of each experiment, and the volume at the knee of the curve was used during the subsequent data collection when the heart was paced. Stiffness was measured with a sinusoidal forcing function, which imposed a sinusoidal volume displacement of 1 ml at 20 Hz into a balloon placed in the left ventricle. In each ventricle, stiffness was related linearly to pressure during the cardiac cycle, and was greater for any given pressure in the older beagles. Contraction duration was prolonged in the older dogs. In an additional seven old beagles during right heart bypass, time and duration of contraction were longer than in seven young beagles. Aging of the beagle heart is associated with an increase in left ventricular systolic and diastolic stiffness and prolonged duration of contraction. Circ Res 44: 189-194, 1979

STUDIES of the dynamic stiffness of the canine left ventricle by a sinusoidal forcing function (Templeton et al., 1970) have shown the following: (1) There is a linear relationship between dynamic stiffness and pressure throughout an isovolumic contraction cycle. This relationship is not affected significantly by acute moderate changes in left ventricular volume or inotropic state (Templeton et al., 1972a). (2) Dynamic stiffness can be altered significantly by abnormal states of cardiac muscle such as ischemia, marked hyperosmolality, and hypothermia (Templeton and Nardizzi, 1974; Templeton et al., 1972b, 1974, 1975).

Aging of a variety of tissues has been characterized by an increase in stiffness. Connective tissue appears to exhibit increased stiffness with age (Vezar, 1969). This increase in stiffness is independent of the mass of connective tissue, and therefore appears to be at least in part a result of changes in the molecular structure and composition of connective tissue elements (Chavapil, 1966). Trabeculae carneae from the left ventricles of senescent rats demonstrate significantly greater resting tension than do trabeculae from sexually mature young adult rats (Weisfeldt et al., 1976). Because of the contradiction of these results by other investigations (Korecky et al., 1974; Kane et al., 1976) in which no age-related difference in stiffness was seen in anoxic arrested hearts, we planned the present studies to examine whether there is an age-related change in the dynamic stiffness of the intact beating left ventricle of the dog during rest or diastole. Since prior studies of dynamic stiffness (Templeton and Nardizzi, 1974; Templeton et al., 1974, 1975) show parallel changes in diastolic and systolic stiffness, the possibility of an age-associated increase in resting stiffness also raises the possibility of an age-associated increase in systolic dynamic stiffness. Such increases in systolic and diastolic dynamic stiffness would likely have significant consequences in terms of age-related changes in cardiac function, if, for instance, the increased stiffness represents a greater resistance to stretch as would be caused by increased connective tissue stiffness. Because trabeculae carneae from the aging rat heart have a prolonged duration of contraction (Lakatta et al., 1975a) and a decreased inotropic response to catecholamines (Lakatta et al., 1975b), these aspects of contractile performance also were studied in the left ventricles of old dogs.

Methods

Isovolumic Ventricular Preparation

The sinusoidal forcing technique has been used previously to measure ventricular dynamic stiffness. Descriptions of the apparatus and surgical procedure, an evaluation of the apparatus, and a theoretical analysis of the method have been published previously (Templeton and Nardizzi, 1974; Templeton et al., 1970, 1972a, 1972b). In brief, after sodium pentobarbital anesthesia (30 mg/kg, iv) and...
midline thoracotomy, eight young (27 ± 2.5 months, mean ± se) and seven old (128 ± 20.5 months) beagle dogs were placed on total cardiopulmonary bypass with retrograde perfusion of the aortic root. With the heart isolated from the rest of the circulation, complete heart block was produced by ligating the bundle of His, and the heart was subsequently paced with a stimulator at 120 beats/min through electrodes on the left ventricle. An isolumbar ventricle was prepared by occlusion of the atrial and aortic orifices with Teflon buttons and placement of a balloon within the ventricular cavity.

The balloon was attached to the end of a metal canula and inserted into the ventricular cavity through a stab incision in the apical dimple. After insertion, the volume of the balloon was adjusted so that the knee of the diastolic pressure-volume curve was reached; the knee of this curve is defined as the point of greatest inflection, and was easily identified on the experimental curves we obtained. Blood accumulating between the balloon and the ventricular endocardium was drained continuously through perforations in the mitral button and around the stab incision.

To apply the sinusoidal forcing function to the left ventricle, a piston located at the external end of the stainless steel canula was used. Peak-to-peak displacement of the piston, and consequently of the ventricular cavity, was 1 ml, and the frequency of the volume change was held constant at 20 Hz. The system response to the externally applied forcing function was a sinusoidal component of left ventricular pressure, which was measured with a Konigsberg P22 pressure transducer. The dynamic properties of the balloon have been demonstrated previously not to influence the pressure measurements made in the left ventricle. Any friction between the balloon and the endocardium caused by the forcing function was assumed to be negligible.

To process the data, ventricular pressure and the sinusoidal displacement of the piston from 16 cardiac periods were recorded simultaneously on analog tape, and were averaged by computer to form two waveforms. The stimulus artifact was used to trigger the averaging procedure. To retain the sinusoidal information in the two waveforms, the forcing function was synchronized with the stimulus or trigger, i.e., the volume changes were made to occur at the same time during each cardiac cycle. Fourier analysis was used to separate the response to the sinusoidal forcing function from the ventricular pressure waveform. Once separated, this component of ventricular pressure is of varying amplitude. The peak amplitude of each pressure cycle (ΔP) was divided by the constant amplitude of the forcing function (ΔV) to yield an indicator of ventricular dynamic stiffness (ΔP/ΔV). Previous experience in our laboratory (Templeton and Nardizzi, 1974; Templeton et al., 1970, 1972a, 1972b, 1974, 1975) has shown that ventricular dynamic stiffness (ΔP/ΔV) is related linearly to ventricular pressure (P) by the equation, ΔP/ΔV = αP + β, where α and β are constants. The dimensional units for α and β are (ml)⁻¹ and (mm Hg/ml), respectively. In the present study, this linear relationship between dynamic stiffness and pressure occurred in both the young and the senescent groups of beagles during all conditions of the experimental protocol. The influence of aging and the interventions used in this study on ventricular stiffness were tested by determining the changes in the slope (α) and intercept (β) of this linear stiffness-tension relationship evoked by the different experimental conditions.

The experimental protocol for this study was initiated with a measurement of the resting pressure-volume relationship for the left ventricle. This relationship was obtained by infusing increments of fluid into the ventricle in a stepwise manner. Sufficient time was allowed between volume increments for stress relaxation to reach as minimal a value as could be obtained in a nonpaced ventricle. Upon completion of the measurement of the pressure-volume curve, the balloon was filled with that amount of fluid necessary to raise the ventricular volume to that value at the knee of the pressure-volume curve. Ventricular volume was maintained constant at this level for the rest of each experiment. Each beagle was paced at 120 beats/min, and the extracorporeal pump was set to perfuse the arterial system of the dog at a mean pressure of approximately 80 mm Hg. For each experimental condition, the diastolic pressure, maximal developed pressure, maximal LV dP/dt, contraction duration, and ventricular stiffness were measured. Contraction duration is the time from the rise of ventricular pressure from its diastolic level to its return to one-half of its maximal value. Studies of the young and old beagles were performed in a random order. After collection of control data, noradrenaline (Winthrop Labs) was infused at a dose level of 0.8 μg/kg per min. Measurements were made when steady state was reached.

The older dogs (Table 1) were retired breeders and were purchased from a breeding farm by the Gerontology Research Center, National Institute of Aging, Baltimore, Maryland. After their purchase, they were maintained in caged runs and fed ad libitum diets. Six of the eight young dogs were obtained from the same breeder. Two others were obtained from a second breeder.

Significance of change in the measured parameters was tested by Student's t-test for paired observations when each animal served as its own control, such as during norepinephrine infusion, or by Student's t-test for group comparisons when differences between the young and old groups were examined. Multivariate analysis was also used. Differences were considered significant when P < 0.05.

Right Heart Bypass Study

Seven young (16 ± 1.5 months) and seven old (119 ± 6 months) female beagles were anesthetized
Results

For the young and old groups, the mean values for the diastolic pressures and volumes obtained at the knee of the pressure-volume curves were 6 ± 0.9 (SEM) mm Hg and 14 ± 1.2 ml, respectively, for the young, and 8 ± 1.3 mm Hg and 15 ± 1.2 ml, respectively, for the old beagles. Neither of these differences was significant. Heart mass was 105.7 ± 3.96 (SEM) g and 89.8 ± 5.63 g for the young and old groups, respectively (P < 0.05), and the heart mass-body weight ratios were 10.6 ± 0.12 and 8.3 ± 0.72 g/kg, respectively (P < 0.01). This significantly greater heart mass in the young group raised the possibility that if the total heart stiffness were greater in the young group, the greater inertial component in the young animals could be partially responsible for this assumed age-related difference in stiffness. This result was not obtained; on the contrary, the older hearts were stiffer.

Table 1 summarizes control data from both the young and old groups of dogs. The specific ventricular parameters, end-diastolic pressure, peak systolic pressure, contraction duration, maximal rate of pressure change with time (dP/dt), and the slope (a) and intercept (b) of the linear stiffness-tension relationship are presented and were measured from the waveforms containing the sinusoidal component induced by the forcing function. Comparison of the two groups shows that only end-diastolic pressure and the slope of the stiffness-pressure relationship were not significantly different. Significantly lower ventricular pressures, maximal LV dP/dt, and longer contraction durations were found in the hearts from the old beagles. A change in the intercept of the stiffness-pressure relationship indicates that the older beagle hearts were stiffer for any given pressure during the entire contraction cycle.

To determine whether there was a difference between the groups in their ability to respond to catecholamines, norepinephrine was infused into the blood reservoir in the extracorporeal circuit. The directional changes of ventricular pressure, contraction time, and maximal LV dP/dt during the norepinephrine infusion were the same for the young and the old beagles, as shown in Table 2. A significant difference between the two groups in their percent response to norepinephrine was not detected by multivariate analysis. The ventricular stiffness parameters shown in Table 2 were unaffected by norepinephrine in either the young or the old group of beagles.

In the second portion of our study, on seven young and seven old beagles, right heart bypass was
used to determine whether there were age-related differences in the hemodynamics of ejecting ventricles. Table 3 shows left ventricular end-diastolic pressure, left ventricular developed pressure, mean arterial pressure, contraction time, and contraction duration for heart rates of 180, 200, 240, and 280 beats/min. Using multivariate analysis of variance to compare differences in the control data of the two groups and the differences in their response to changes in heart rate, we found significant differences (P < 0.02) between the two groups in contraction time and duration at 180 beats/min (Table 3). Increases in heart rate from 180 to 240 and from 180 to 280 beats/min evoked greater declines in the contraction times and contraction durations of the old group as compared to the young group (P < 0.02), but the magnitudes of both parameters at both 240 and 280 beats/min were still greater for the old group than were the comparable values for the young group.

### Discussion

Conclusions drawn from previous investigations (Weisfeldt et al., 1976; Korecky et al., 1974; Kane et al., 1976) do not agree on whether or not senescence is characterized by a change in myocardial stiffness. Weisfeldt and collaborators (1976) showed an age-associated increase in resting tension in left ventricular trabeculae carnea from 12- and 24- to 27-month-old rats. The 24- to 27-month-old rats were senescent, since in this colony 50% mortality occurred at approximately 25 months. Resting tension was higher at Lmax in muscles from the older group and was also significantly higher at comparable points on the ascending portion of the length-tension curve. Korecky and associates (1974) have studied pressure-volume relationships of excised rat left ventricles during anoxic arrest. The ages of the rats studied were 1, 7, and 17 months. The 7-month-old rat is quite comparable to the 12-month-old rat used in the previous study (Weisfeldt et al., 1976), since at 7 months the rat is sexually mature, and cardiac muscles from 6- and 12-month-old rats show similar functional characteristics (Lakatta et al., 1975). Rats of 17 months, though, may well not be comparable to senescent rats of 24-27 months.

The data of Kane and coworkers (1976) were interpreted as showing no age-associated change in stiffness in the anoxic-arrested hamster ventricle. Two strains of hamster were used in this study, one between 1 and 11 months of age, and the second from 17 to 24 months old. The hamster from the first strain showed widely varying body and heart weights, with relatively constant heart weight-body weight ratios, suggesting widely varying conditions for growth and development. This makes the interpretation of data with regard to age changes per se difficult. However, if one compares the 24-month-old age group with other groups, there are apparent age changes. For example, left ventricular elastic modulus at 3.5 mm Hg of left ventricular pressure for the 24-month-old group is significantly different.
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from that of the 8-month-old group ($P < 0.005$) and the 11-month-old group. ($P < 0.01$). Of course, care should be taken in comparing moduli with stiffness values, since the former is devoid of geometrical considerations whereas the latter is not.

Limitations of the previous studies may explain the lack of consistency of their conclusions. Two of the investigations used rats, which with their relatively short life may have differences from those species with a longer life-span. With the recent evidence that, in isolated muscle preparations, there are inactive or less active sarcomeres at the clamped ends (Pollack and Huntsman, 1974), the possibility is raised that senescent muscle could be more traumatized in such a way as to influence overall stiffness measurements. Further, trabecular muscle is subject to the maximal amount of fibrosis with aging, and its stiffness may respond differently to aging than the entire ventricle (Weisfeldt et al., 1976). Likewise, anoxic arrest may differentially modify left ventricular stiffness in old vs. young hearts. Further, experiments in one study (Korecky et al., 1974) attempted to subtract the viscus contribution to total stiffness, and thereby may have limited the apparent age change, since age changes in viscosity may contribute substantially to the total stiffness.

The major finding of the present investigation is that there is an age-associated increase in the dynamic stiffness of the left ventricle of the beagle. The unique aspects of the present study are: (1) an intact beating preparation was used in demonstrating an age-associated increase in dynamic diastolic stiffness of the left ventricle. (2) Dynamic stiffness was measured during contraction, and an age-associated increase in systolic stiffness was found as well.

The protocol for our study required filling each ventricle to the volume at the knee of the diastolic pressure-volume relationship. This particular volume was chosen for two reasons. The first was that the point was reproducible and well-defined for our experimental setup. The second was based on a previous study in our laboratory with the same setup, which showed that, within the same heart, the slope ($\alpha$) and intercept ($\beta$) of the linear stiffness-pressure relationship were independent of changes in ventricular volume around this point on the diastolic pressure-volume curve (Templeton et al., 1972a). Based on this information, we assumed for the present study that operating at the knee of the curve would permit a meaningful comparison of stiffness and functional data from the two groups.

In a review by Sonnenblick and Skelton (1974), the sources of resting tension were cited to be the cardiac sarcolemma, collagenous connective tissue, cross-bridges during rest, and S-filaments (linkages between actin filaments across the H zone). Of these, only collagen has been shown to become stiffer with age (Verzar, 1969). This may involve not only the interstitial connective tissue, which at least in the rat shows some increase in quantity with age (Weisfeldt et al., 1976), but also the connective tissue structures of the fibrous skeleton and valves of the heart. Recent studies by Spurgeon and associates (1977) have examined the dynamic stiffness of trabecular collagen from the left ventricles of 8- and 28-month-old female rats. These studies demonstrate an age-associated increase in dynamic stiffness, and suggest therefore that a contribution to the overall dynamic stiffness changes with age is made by elements within the left ventricular myocardium. The increase in dynamic stiffness during contraction implies that there is a change in the stiffness properties of the active contractile elements. This increase in stiffness during contraction appears proportional to the overall change in dynamic stiffness of the resting heart. The age-related increase of stiffness in collagen could explain the increased stiffness seen by us in the older hearts, and also could explain the diminished contractile function, if the assumption is made that the stiffer connective tissue in the older beagles altered the diastolic pressure-volume relationship such that shorter sarcomere lengths prevailed throughout the hearts. The shorter sarcomeres would result in diminished contractile performance and our observations of altered contraction duration and developed force.

In addition to the difference in connective tissue stiffness, another reason for supposing that sarcomere lengths in the young and old groups may have been different is recent evidence of inhomogeneity in sarcomere length and performance between different parts of individual fibers, between different fibers, and between different parts of the heart (Pollack and Huntsman, 1974; Nassar et al., 1974; Winegrad, 1974; Krueger and Pollack, 1975). In view of these data, it may be impossible to obtain populations of sarcomeres at the same length in two groups of animals.

The observed age-related decline in developed pressure and maximal rate of pressure development is consistent with the observations in the rat of an age-associated decrease in $V_{\text{max}}$ and myosin ATPase activity (Alpert et al., 1967). Unlike the rat data, which indicate an age-related decline in inotropic contractile function, this conclusion cannot be drawn from the beagle data, because of the possible difference in sarcomere length between the senescent and young groups.

Prolonged contraction duration appears to be a characteristic of the myocardium of the aged dog as well as of the aged rat and perhaps of man (Harrison et al., 1964). Although catecholamine stores have been shown in other species to be decreased with age (Lakatta et al., 1975), the prolonged contraction duration in the old beagles does not appear to be entirely accounted for on the basis of decreased norepinephrine stores. At an infusion rate of norepinephrine of 0.8 $\mu g$/kg per min, the hearts of the old beagles were at levels of peak
isovolumic left ventricular pressure and maximum left ventricular dP/dt similar to those of the young beagles under control conditions. Despite the similarity of the levels of dP/dt and peak pressure, contraction duration remained significantly prolonged in the hearts from the older age group (257 ± 3.9 msec vs. 294 ± 6.1 msec, P < 0.001).

Although the absolute magnitude of the increase in left ventricular dP/dt and peak isovolumic left ventricular pressure is substantially less in the hearts from older dogs, there is no significant age-associated decrease in inotropic response when the data are presented as percents of the control values. This contrasts with the clear age-associated decrease in the inotropic response to catecholamines in rat trabeculae carneae (Lakatta et al., 1975). In the rat, baseline values were not significantly different.

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References
Sonnenblick EH, Skelton CL: Reconsideration of the ultrastructural basis of cardiac length-tension relations. Circ Res 35: 517-526, 1974
Winegrad S: Resting sarcomere length-tension relation in living frog heart. J Gen Physiol 64: 343-356, 1974
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