SUMMARY We studied the effect of advanced age on the response to paired pacing and on the relationship between ouabain-induced inotropy and inhibition of (Na + K)-ATPase in hearts from young adult and senescent rats. In isometric trabeculae carneae, control values of developed tension (DT) and maximal rate of tension development (dT/dt) were not age-related. There was no age-related difference in the response to extrasystolic potentiation at prematurity intervals from 400 msec to the mechanical refractory period. The maximal response occurred at a prematurity interval of 200 msec and was above 200% of control. The inotropic response to ouabain occurred over a concentration range from 2 x 10^{-6} to 6 x 10^{-5} M. DT and dT/dt in muscles from the young adult group exhibited a greater response than those from the senescent group; e.g., at 6 x 10^{-5} M both parameters were approximately four times greater in the former group. There was no age-dependent difference in ouabain-induced enzyme inhibition, which occurred over the same concentration range as did ouabain-induced alterations in mechanical function. These data indicate that paired pacing, but not ouabain, results in similar increases in inotropy and therefore similar increases in myoplasmic calcium concentration in young adult and aged rat myocardium. The age-dependent difference in ouabain responsiveness appears to be related to an age-associated alteration in a step other than enzyme inhibition linking ouabain binding to increased myoplasmic calcium levels.

PREVIOUS WORK from this laboratory has demonstrated that cardiac muscle isolated from senescent as compared to that from adult rats exhibits a diminished inotropic response to catecholamines but a normal maximal inotropic response to an increased extracellular calcium concentration, [Ca^{2+}]_o (Lakatta et al., 1975). This finding suggests that the ability of the myofibrils to generate maximal force is not affected by age, but that sarcolemmal or intracellular structures and events that increase calcium delivery to the myofibrils in response to catecholamines may be altered with age. Paired pacing and ouabain are other inotropic interventions whose mechanisms of action are reported to be mediated by increasing the calcium concentration available to bind with troponin. Although considerable information is available concerning the effect of developmental changes on the volume of distribution of ouabain, and on the inotropic, electrophysiological, and biochemical responses to cardiac glycosides, (Langer et al., 1975; Akera et al., 1972; Inturrisi and Papaconstantinou, 1974; Rosen et al., 1975; Kelliher and Roberts, 1976; Berman et al., 1977; Glantz et al., 1976; Scott et al., 1971), the effect of advanced age on these responses has not been described. Adult rat myocardium generally has been reported to be insensitive to ouabain (Langer et al., 1975; Scott et al., 1971; Repke, 1963; Detweiler, 1967). However, preliminary experiments in our laboratory as well as in others have shown that under appropriate conditions a mechanical response to ouabain may occur (Masuoka and Saunders, 1950; Gerstenblith et al., 1975). There-
fore, ouabain-induced inotropy and inhibition of (Na + K)-ATPase and the relationship between these two effects were analyzed in young adult and senescent rat hearts to characterize further the response of the rat heart to ouabain and the effect of advanced age on that response.

Methods

Male, nonbreeder Wistar rats 6 and 24 months of age were supplied by the Gerontology Research Center Aging Colony. For the rat, 6 months of age is considered to be young adulthood and 24 months of age, when 50% mortality occurs, senescence (Schlettwein-Gsell, 1970). Considerable data concerning the absence of specific cardiovascular diseases, histological changes in the myocardium and in the vascular bed, and connective tissue content of rats from this colony have been summarized (Gerstenblith et al., 1976) elsewhere.

Mechanical Measurements

One muscle from a young adult and one from an aged heart were studied simultaneously. Hearts were removed from rats after a sharp blow was given to the head, and the hearts were immersed in iced, oxygenated, modified Krebs-Ringer bicarbonate solution (Umbreit, 1945). The heart then was slit and laid flat. A trabecular muscle from the posterior left ventricular wall was removed and suspended between two spring-loaded clamps inside a water-jacketed chamber. The volume of the chamber was 17 ml, and the perfusion rate was 13 ml/min with no recirculation. The muscles were equilibrated in the modified Krebs-Ringer bicarbonate solution, which contained calcium (1.0 mM), magnesium (0.6 mM), and glucose (16 mM).

The solution in the reservoir and chamber was oxygenated with a 95% oxygen-5% carbon dioxide gas mixture. The muscles were excited via a Grass SD9 stimulator via platinum field electrodes at a rate of 24 beats/min at 29° C. The muscles were stretched to that length at which developed force was maximal (Lmax) and allowed to equilibrate for an additional 60 minutes. At that time the bathing solution was changed to one that was identical to the first except for [Ca2+]o, which was lowered to 0.25 mM, and the muscles then were allowed to equilibrate for an additional 30 minutes. At this [Ca2+]o, approximately 45% of maximal force development is present in the rat heart, whereas comparable force development is reached in dog and kitten hearts at a [Ca2+]o between 1 and 2 mM (Fig. 1). Under these conditions of lowered [Ca2+]o, rat cardiac muscle is an appropriate model in which to examine perturbations of excitation-contraction coupling, including changes in [Ca2+]o, paired pacing, and ouabain administration. The following baseline parameters were measured: resting force, developed force, maximal rate of force development, and contraction duration, taken as the time from the onset of active tension to the time required for tension to fall to 50% of its peak value. The muscles then underwent paired pacing at a rate of 24 pairs/min. The premature interval, that interval between the beats in a pair, ranged from 400 msec to the mechanical refractory period, the shortest interval at which the second stimulus of the pair elicited a mechanical response. In preliminary experiments, potentiation of the first to the fifth paired beat was recorded, and in all cases the fifth beat exhibited the greatest potentiation in both age groups. Therefore, a train of five paired pulses was delivered, and the degrees of potentiation of developed force and rate of force development were recorded for the fifth potentiated beat. A 2-minute period of regular stimulation sep-

![Figure 1](https://example.com/figure1.png)

**Figure 1** Normalized force production as a function of calcium concentration in the bathing fluid [Ca2+]o, in a typical isometric cardiac muscle from a rat (△), a kitten (●), and a dog (▲). The muscles were excited via field stimulation at a rate of 24 beats/min at 29°C. Resting muscle length was that at which isometric force development was maximal (Lmax).
arated each tested prematurity interval to allow performance to return to control. The initial prematurity interval was 400 msec, and it then was progressively decreased until the mechanical refractory period was reached.

Following recovery from paired pacing, ouabain was added to the bath in increasing concentrations. The ouabain response also was measured in additional muscles that had not been subjected to paired pacing. This was done to ensure that any age-related difference in the ouabain response was not secondary to the prior paced pacing. Ouabain solutions were prepared within 60 minutes of use and were infused continuously into the chamber to result in concentrations of from $2 \times 10^{-6}$ to $6 \times 10^{-4}$ M. The maximal isotropic effect of ouabain at each concentration occurred within 15 minutes, at which time parameters of contractile performance were measured and the next higher concentration introduced. At the end of the experiment the muscle was removed from the bath. The movable clamp was positioned so that it just touched the nonmovable clamp. The distance traversed was equal to the distance between the tips of the clamps, and that was taken to equal $L_{max}$. Cross-sectional area was determined with the assumption that the muscle had a cylindrical shape as previously described (Weisfeldt, 1971). Resting force, developed force, and $dF/dt$ were normalized for cross-sectional area and expressed as resting tension (RT), developed tension (DT), and the first derivative of tension with respect to time ($dT/dt$), respectively. Results are expressed as the mean ± standard error. Analysis of variance was used to determine whether significant age differences were present in the responses to paired pacing and ouabain administration (Snedecor, 1957). Student's $t$-test was also used when appropriate (Snedecor, 1957).

**Table 1**  

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>n</th>
<th>Cross-sectional area (mm$^2$)</th>
<th>Length (mm)</th>
<th>Resting tension (g/mm$^2$)</th>
<th>Developed tension (g/mm$^2$)</th>
<th>$dT/dt$ (g/mm$^2$ per sec)</th>
<th>Contraction duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>12</td>
<td>0.86 ± 0.08</td>
<td>5.98 ± 0.37</td>
<td>0.963 ± 0.12</td>
<td>0.79 ± 0.11</td>
<td>9.24 ± 1.37</td>
<td>199.5 ± 7.0*</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>0.85 ± 0.07</td>
<td>6.72 ± 0.67</td>
<td>1.143 ± 0.11</td>
<td>1.10 ± 0.15</td>
<td>12.86 ± 1.47</td>
<td>240.5 ± 12.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; $dT/dt$ = maximal rate of tension development.

$^*$ $P < 0.02$. 

Activity of (Na + K)-ATPase was measured by monitoring the oxidation of NADH in the presence of phospho-enolpyruvate, pyruvate kinase, and lactic acid dehydrogenase (Albers et al., 1968). The ATPase reaction was initiated by adding 10-20 μg of the enzyme to 1 ml of a reaction mixture containing 100 mm NaCl, 4 mM MgCl$_2$, 40 mM Tris-HCl (pH 7.5), 2.5 mM ATP, 0.35 mM phosphoenolpyruvate, 0.3 mM reduced β-nicotinamide adenine dinucleotide (NADH), and 1 μg each of lactic acid dehydrogenase and pyruvate kinase. The ouabain concentration was varied between 8 μM and 4 mM. All incubations were for 10 minutes at 25°C. The ouabain-sensitive activity, which averaged 4-6 μmol P/mg protein per hour, represented about 25% of the total ATPase activity.

**Results**

The baseline performance of trabeculae at a calcium concentration of 0.25 mM is presented in Table 1. The length, cross-sectional area, RT, DT, and $dT/dt$ did not differ with age. Contraction duration, however, was 20.6% greater in the aged than in the young adult muscles ($P < 0.02$).

The effect of age on the response of DT and $dT/dt$ to paired pacing is illustrated in panels A and B, respectively, of Figure 2. The interval tested ranged from 400 msec to that at which a significant number of muscles in both age groups failed to exhibit a mechanical response to the extrasystolic stimulus. The shortest interval was 180 msec. Over the entire range of prematurity intervals there was no age-dependent difference in the response of both DT and $dT/dt$. Therefore, at a calcium concentration of 0.25 mM there is no age-dependent difference in the isotropic response to extrasystolic potentiation.

The effect of age on the isotropic response to ouabain is presented in Figures 3-5. The response to ouabain occurs over a concentration range from $2 \times 10^{-6}$ M, at which a 20% increase in DT occurred in the muscles from the young adult rats, to $6 \times 10^{-4}$ M, at which all muscles exhibited contracture. There was no difference between muscles that were exposed to ouabain after paired pacing and those that did not undergo paired pacing, and the combined results are presented.

Figure 3 depicts the response of RT in muscles of both age groups to the entire range of ouabain concentrations employed. No increase in RT occurred at ouabain concentrations at which the isotropic effect was observed. At $2 \times 10^{-4}$ M, the mean increase in RT in the muscles from the young adult group was significantly greater than control ($P <$...
The effect of advanced age on the response of DT (A) and dT/dt (B) to extrasystolic potentiation at prematurity intervals from 400 msec to the mechanical refractory period. Analysis of variance disclosed no age effect. For this and the following figures, each point represents the mean ± SEM for the number of rats given in parentheses next to that point.

Whereas that in the muscles from the senescent group was not. All muscles in both groups exhibited contracture at a ouabain concentration of $6 \times 10^{-5}$ M. Analysis of variance revealed no significant age-related difference in the response of RT over the entire range of ouabain concentrations.

When contracture occurred in a given muscle, a decline rather than an increase in DT and dT/dt occurred. This resulted in a mixed population of responses at the two highest concentrations of ouabain. DT and dT/dt therefore were analyzed only over the range of concentrations at which contracture did not occur, i.e., from $2 \times 10^{-6}$ to $6 \times 10^{-5}$ M (Fig. 4). A significantly greater inotropic effect occurred in the young adult muscles over this entire range of concentrations. At $6 \times 10^{-5}$ M, the increase in DT and dT/dt was approximately four times greater in the young adult muscles than in the senescent muscles.

When performance is measured as total tension, the sum of DT and RT, similar age-related differences are noted (Fig. 5). The peak response in both age groups occurred at $2 \times 10^{-4}$ M and is significantly greater in the young adult group. At $2 \times 10^{-4}$ and $6 \times 10^{-5}$ M, the response in both age groups is distributed over a wider range than at lower concentrations, reflecting the fact that 50% of the muscles in both groups are in contracture.

The ouabain-sensitive ATPase, expressed as a percent of the total ATPase activity isolated from young adult and aged rats, was not significantly different, averaging $27.1 \pm 5.42\%$ (n = 5 pools) in the young adult group and $24.6 \pm 5.6\%$ (n = 4 pools) in the aged group. The concentration dependence of the ouabain-induced inhibition of (Na + K)ATPase activity is shown in Table 2. In both age groups, ouabain inhibition begins to be observed at $8 \times 10^{-6}$ M and approaches maximal levels at $10^{-3}$ M. Analysis of variance disclosed that there is no significant effect of age on ouabain-induced inhibition of (Na + K)-ATPase. A review of the data presented in Figures 3-5 with that presented in Table 2 shows that inhibition of (Na + K)-ATPase by guest on June 26, 2017 http://circres.ahajournals.org/ Downloaded from
and increased inotropy occur over the same range of ouabain concentrations. This relationship is illustrated in Figure 6 in which dT/dt and inhibition of (Na + K)-ATPase in the young adult group are plotted as percent of maximal response vs. ouabain concentration. The ouabain concentrations at which the biochemical and mechanical effects occur are similar.

Discussion

This study demonstrates that ouabain produces an inotropic effect in rat myocardium that occurs over the same concentration range as that at which (Na + K)-ATPase inhibition occurs. The results also show that, although there is no age-associated difference in response to paired pacing, aged rat myocardium exhibits a diminished response to ouabain as compared to young adult myocardium. Ouabain-induced inhibition of (Na + K)-ATPase is not age-dependent.

Ouabain and paired pacing act in cardiac muscle by enhancing the loading and/or the release of calcium from the superficial storage site so as to result in a higher calcium concentration surrounding the myofilaments (Nayler, 1973; Koch-Weser and Blinks, 1963). There is only a minimal response to positive inotropic interventions in the rat or in other species under conditions at which this storage site is saturated (Allen et al., 1976; Forester and Mainwood, 1974; Speirs, 1959). As is evident on examination of Figure 1, rat myocardium behaves as if the capacity of the storage site were saturated at a [Ca²⁺]o of 2.5 mM. Inotropic responses do occur when the storage site is unloaded (Masuoka and Saunders, 1950; Forester and Mainwood, 1974; Meijler et al., 1962), and this was accomplished in the present study by lowering the [Ca²⁺]o to 0.25 mM. Under these conditions, baseline DT and dT/dt are not age-related.

Previous studies have reported that there is no age-dependent difference in these parameters as the calcium concentration is increased up to 2.5 mM, indicating that the releasable pool can be equally and maximally loaded by an increase in [Ca²⁺]o. The lack of an age-related difference in response to paired pacing suggests that this intervention also results in a similar increase in myoplasmic calcium in both young adult and senescent rat muscle.

Aged myocardium exhibits a diminished inotropic response to ouabain. This suggests that oua-
bain does not enhance loading of the releasable calcium pool in senescent muscle as effectively as it does in young adult cardiac muscle. The response to ouabain is paralleled by binding of ouabain to the (Na + K)-ATPase receptor and by a sequence of intermediate steps that link binding to the enhanced loading of this calcium pool (Akera, 1977). Previous investigations have reported that the dose-response curve of ouabain-induced (Na + K)-ATPase inhibition in the rat is shifted to the right by two to three log units as compared with other species (Akera et al., 1969). The results presented indicate for the first time that in rat cardiac muscle the inotropic effect occurs over the same concentration range as that at which enzyme inhibition occurs (Fig. 6). Thus both the inotropic response and the ATPase inhibition caused by ouabain in rat myocardium are shifted to the right relative to other species. The relationship between the biochemical and inotropic responses is similar to that observed in the dog (Besch et al., 1970; Akera et al., 1970); inotropy closely follows inhibition in the early and middle concentration ranges, with maximal inotropy occurring at about 90% of maximal inhibition. Further inhibition is associated with the toxic contractile effect of ouabain. Ouabain-induced inhibition of (Na + K)-ATPase isolated from young adult and aged rat hearts does not differ and is similar to that reported in young adult rats by other investigators (Akera, et al., 1972; Inturrisi and Papaconstantinou, 1974). Therefore, the age-dependent difference in inotropic response to ouabain is not due to differences in (Na + K)-ATPase inhibition. The age-associated changes responsible for the diminished response of the aged myocardium to ouabain therefore must occur in other stages of ouabain-induced inotropy, possibly the sodium-calcium exchange and/or conformation changes in the enzyme protein (Langer, 1972; Schwartz, 1976).

In summary, the data presented indicate that rat myocardium exhibits a significant inotropic response to paired pacing and to ouabain at an extracellular calcium concentration of 0.25 mM. Aged myocardium exhibits an identical response to paired pacing but a diminished response to ouabain when compared with young adult myocardium. This age-dependent difference is not due to a change in ouabain-induced inhibition of the Na + K dependent ATPase. These findings provide further definition of the relationship between the inotropic and biochemical responses to ouabain in the rat heart and the effect of advanced age on that response.

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SOMETIMES excess fluid accumulates in the lung primarily because of changes in the lung circulation (Brigham et al., 1974). When humans develop pulmonary edema while lung vascular pressures are low, it is inferred that lung exchanging vessels are leaking too much fluid and protein (increased permeability; see Robin et al., 1968, 1973). Diagnosing increased permeability in this way poses two problems. First, the diagnosis cannot be made until enough lung fluid has accumulated to make pulmonary edema obvious on chest x-ray or physical examination. Second, the diagnosis does not involve a measurement of lung vascular permeability per se, because there has been no way to make that measurement.

In animals, we found a multiple-indicator method useful for measuring lung water and vascular permeability (Harris et al., 1976; Brigham et al., 1977; Harris et al., 1978; McKeen et al., 1978b). We have now used the method in humans with normal and increased pulmonary vascular pressures due to stable heart failure. We found that the lung permeability-surface area product (PS) for ¹⁴C-urea correlated well with alveolar volume (Vₐ) not correlated with Pmv (r = 0.51, P = 0.02) and even better with Pmv - plasma oncotic pressure (r = 0.63, P = 0.007). We therefore conclude that ¹⁴C-urea PS is a measure of lung vascular permeability in humans, and that, as in animals, permeability is unaffected by Pmv. EVLW may be a more useful measure of lung water in humans than previously thought, when interpreted in light of the measurable forces affecting fluid exchange. Circ Res 44: 523-530, 1979
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