Relaxation Properties of Mammalian Atrial Muscle

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SUMMARY The properties of relaxation, in particular the sensitivity of relaxation to load, were analyzed in isolated intact atrial muscle and in manually dissected, detergent-treated cellular preparations from cat, dog, and rat atria. Force and length traces under increasing afterloads and following load clamps were obtained using an electromagnetic lever-force transducer system for the intact muscles and a capacitance transducer system for the cellular preparations. In both types of preparations, the time course of relaxation was hardly affected by the load or by alterations in load (load clamps), unlike intact mammalian ventricular muscle. This load independence of relaxation, which was hardly influenced by variations of initial muscle length, resembled relaxation in intact frog ventricular muscle and in detergent-treated mammalian ventricular single cells. As relaxation of these ventricular preparations with poorly developed (frog) or absent (detergent-treated single cells) calcium-sequestering systems was shown to be governed by the dissipation of activation, these results suggest a similar control mechanism for relaxation in mammalian atrial muscle. Furthermore, load independence of relaxation of mammalian atrial muscle in late diastole may promote optimal filling of the ventricle. Circ Res 48: 352-356, 1981

ALTHOUGH the contractile properties of the contraction phase of mammalian atrial muscle have been well studied and compared with the contractile performance of ventricular muscle (Fabiato and Fabiato, 1972; Tarr et al., 1979; Urthaler et al., 1975), its properties during the relaxation phase are less well understood. Because of the unquestionable contribution of atrial contraction to the pump function of the ventricle, and because of the significant differences between the contractile properties of atrial and ventricular muscles (Blinks and Koch-Weser, 1963; Koch-Weser and Blinks, 1963), we have analyzed, in the present study, the mechanical behavior of mammalian atrial muscle during relaxation and in particular, its sensitivity to load or load alterations and its possible role in the functioning of the heart. Two kinds of preparation were used: (1) intact atrial muscle strips from cat and dog and (2) single atrial cells of rat, obtained by manual dissection after treatment with detergent to destroy the membranous systems.

Methods

Atrial muscle strips were dissected from cat (n = 9) and dog (n = 2). The basic characteristics of these preparations are summarized in Table 1.

Long thin strips of longitudinally oriented bundles of muscle fibers were cut from the free left atrial wall. The muscle strips were mounted vertically, the lower end being held by a force transducer (compliance, 0.3 μm/mN; resonant frequency in aqueous solution, 250 Hz) and the upper end being tied (7.0 braided thread, Deknatel, Surgical Tevdak, Code 103-T) to an electromagnetic lever system (compliance 0.2 μm/mN, equivalent moving mass 155 mg, step response 3 msec). The current through the coil of the electromagnet determined the load on the muscle. It was controlled by a current source, calibrated for step changes of 0.98 mN and 9.8 mN and could be switched from one level to another by means of two reference voltage sources. A detailed description of force transducer and electromagnetic lever system and their response characteristics to abrupt load alterations has been published previously (Brutsaert and Claes, 1974; Claes and Brutsaert et al., 1971). Abrupt (<5-msec) changes in load during muscle shortening are imposed by switching from one level of the current source to another at a predetermined time after the initial stimulus by a delayed signal from the stimulator. The abrupt imposition during shortening of a total load which is different from that previously applied is termed a load clamp, which is to be distinguished from a quick release, where the force applied to the isometrically contracting muscle is reduced abruptly so that the muscle then shortens at the new load. The physiological and methodological aspects of the "load clamp" technique have also been reported (Brutsaert et al., 1971; Brutsaert and Claes, 1974).

For each contraction, length and force were recorded simultaneously and displayed as functions of time on a Storage Display Unit (Tektronix 611).
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Table 1

<table>
<thead>
<tr>
<th>lmax</th>
<th>Mean cross-sectional area (mm²)</th>
<th>Resting force (R) (mN/mm²)</th>
<th>Total force of isometric twitch (T) (mN/mm²)</th>
<th>R/T</th>
<th>Maximal shortening (%)</th>
<th>Time to peak tension (msec)</th>
<th>Time to half isometric relaxation (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.7 ± 0.5</td>
<td>0.91 ± 0.08</td>
<td>14</td>
<td>34</td>
<td>0.42 ± 0.04</td>
<td>9</td>
<td>121</td>
<td>212</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE; n = 9.

and photographed with a Hard Copy Unit (Tektronix 4601).

Before the actual experiments, the strips were allowed to equilibrate for at least 2 hours. Equilibration and experiments were performed in a bathing solution, containing (mm) NaCl, 118; KCl, 4.7; MgSO₄·7H₂O, 1.2; KH₂PO₄, 1.1; NaHCO₃, 24; CaCl₂·6H₂O, 2.5; and glucose, 4.5. The solution was gassed with a mixture of 95% O₂-5% CO₂ and the bath temperature was maintained at 29°C. Homogenous electrical stimulation (12 beats/min) was obtained by rectangular pulses of 5 msec duration through two platinum electrodes arranged longitudinally along the entire strip.

Single atrial cells from rat (n = 30) were isolated by manual dissection using a method similar to the one previously described for ventricular muscle (De Clerck et al., 1977). The characteristics of the cellular atrial preparations are summarized in Table 2.

These cells were incubated for about 40 minutes in the following solution at room temperature (mM): NaCl, 132; MgCl₂·6H₂O, 4; Na₂ATP, 5; imidazol, 18; glucose, 7; EGTA (ethyleneglycol-bis(beta-amino-ethyl-ether)N,N'-tetraacetic acid) 0.5; and 0.5% Brij-58(polyethylene-20-cetyl-ether), a detergent which has been shown to eliminate the sarcoplasmic reticulum and the sarcolemma (Orlentlicher et al., 1974); pMgATP averaged 2.43 ± 0.00; pMg, 3.59 ± 0.00; and pCa, 9.05 ± 0.09. ATP, EGTA, and Brij-58 were obtained from the Sigma Chemical Company, imidazol from Aldrich-Europe, and the other reagents (all of analytical grade) from Merck. After perfusion with the Brij-containing solution, the actual experiments were performed in a solution containing all the ingredients as listed above, but with only 125 × 10⁻³ mM EGTA and no Brij-58 and with an average pMgATP of 2.43 ± 0.00, pMg of 3.58 ± 0.00, and pCa of 8.39 ± 0.13. The isolated cells were activated directly by calcium ions released iontophoretically from micropipettes filled with calcium chloride (0.1 M) (De Clerck et al., 1977).

One end of each cell was fixed by a glass microtool, while the other freely movable end was attached to a miniature transducer. A detailed description of this capacitance transducer has been published previously (Brutsaert et al., 1978a). It allows simultaneous measurement of (1) shortening (a resolution smaller than 0.2 μm) and velocity of shortening and (2) force development (a resolution of 1 μg). It is also possible to change the load (load clamp) abruptly in the course of a contraction. (De Clerck et al., 1977).

For both kinds of preparations, the results were the same in all experiments studied and are illustrated by representative examples.

Results

Mammalian Atrial Strips

Afterloaded Contractions

In mammalian ventricular muscle, comparative analysis of the time course of a series of afterloaded isotonic contractions against various loads up to full isometric twitch, proved that early deactivation occurred when the muscle was allowed to shorten against a load, resulting in a load-induced premature isometric relaxation (Brutsaert et al., 1978b). Figure 1 shows the length and force traces of an atrial strip that went through a similar course of

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Length (l/lmax) and force (f) traces of a series of afterloaded contractions against various loads, i.e., from an isotonic twitch at preload only, then with different afterloads up to a full isometric twitch, in a cat atrial muscle strip at lmax (muscle characteristics at lmax: length, 7.5 mm; cross-sectional area, 0.8 mm²; and resting force at lmax, 9.8 mN/mm²).
increasingly afterloaded contractions. In contrast to ventricular muscle, atrial muscle does not reveal load dependence: the isometric phase of relaxation of the isotonic afterloaded contractions almost fully coincides with the isometric twitch; however, a negligible degree of early deactivation cannot be denied. Atrial muscle thus proved to be relatively load independent. The same experiment was repeated at different initial muscle lengths (Fig. 2, upper traces) both shorter and longer than $l_{\text{max}}$, i.e., the initial muscle length at which maximal isometric force is developed. At the longer initial muscle length, load dependence was always complete.

**Load Clamps**

Load dependence at the end of the contraction was tested further by imposing load clamps (Fig. 2, middle and lower traces). Both loading and unloading steps of the same magnitude were imposed during an afterloaded twitch contraction, and small additional loads were imposed during a preloaded

**Figure 2**  Length ($l/l_{\text{max}}$) and force ($f$) traces of a cat atrial muscle strip performing at an initial muscle length below (panel A), at (panel B) and above (panel C) $l_{\text{max}}$. Length and force scales shown for the upper traces are the same for the other traces of the panel. The upper two sets of traces of each panel show a series of afterloaded contractions against various loads. The middle two sets are traces of a loading (c) and an unloading (b) step of the same magnitude and imposed at the same time during afterloaded isotonic contractions. Both contractions were clamped to the same afterload. The control afterloaded contraction (a) to which the muscle was clamped, the control preloaded isotonic, and the control isometric contraction are shown. The lower two sets of length and force traces represent load-clamped contractions with clamp steps of various magnitudes imposed near peak shortening of a preloaded isotonic contraction. The control preloaded isotonic and the control isometric contraction are included (same muscle as in Fig. 1).

**Figure 3**  Force ($f$) and length ($l$) traces of a series of afterloaded contractions in a single Brij-pretreated rat atrial cell. The activating calcium pulses ($Ca^{2+}$) are also recorded ($65 \times 16 \mu m$).
twitch contraction. Again the time course of relaxation in mammalian atrial muscle showed a relative load independence, which was most pronounced at the longer initial muscle lengths.

Single Mammalian Atrial Cells

Afterloaded Contractions

Figure 3 shows a series of increasingly afterloaded contractions up to full isometric twitch from an isolated Brij-pretreated atrial cell of the rat. Each contraction was activated similarly by a pulse of calcium ions of the same size. The time course of relaxation of this preparation is completely load independent: all isometric relaxation curves coincide with the isometric twitch.

Load Clamps

Again, abrupt alterations in load, both loading (Fig. 4) and unloading, were imposed during an isotonic afterloaded contraction. These load clamps did not reveal any influence of the instantaneous or preceding load on the time course of relaxation. Accordingly, relaxation of isolated atrial cells, pretreated with Brij-58 seems to be determined solely by the decay of activation, as was also shown in pretreated mammalian ventricular cells.

Discussion

In the intact heart, the atrium functions not only as a passive conduit and reservoir, but also as an active pump, thus adding to the filling of the ventricle, particularly in pathological conditions. To optimize this booster pump function, the atrium displays a few specific features. First, it does not develop as much force as the ventricle (Urthaler et al., 1978) since the load to be carried is much less. Instead, the atrial contraction has to proceed at a rate fast enough, not only to cause the atrioventricular blood flow to be over before the valves close, but also to give this blood flow a final acceleration. This induces a Venturi effect on the valve leaflets and sucks them closely together. This enhanced velocity of shortening of the atrial muscle as compared to the ventricular muscle (Urthaler et al., 1975) has been correlated with a difference in ATP-ase activity of atrial vs. ventricular myosin (Long et al., 1977; Yazaki et al., 1979). On the other hand, the isotonic phase of the contraction and thus the shortening phase, albeit faster, should be maintained as long as possible to allow the atrium to squeeze its content into the ventricle at end diastole, thus causing the ventricular muscle to be additionally lengthened, and thus put at a higher level of function. The load-independent relaxation of intact atrial preparations demonstrated here meets the need of the atrium to sustain isotonic contraction as long as possible, without unduly prolonging the overall contraction duration.

Relaxation of cardiac muscle previously has been shown to be governed by the interplay of the decay of activation and the load, the relative contribution of which differs among various species (Brutsaert et al., 1978; Chuck et al., in press; Lecarpentier et al., 1979). In ventricular muscle, load dependence of relaxation became manifest only in the presence of a well-functioning calcium-sequestering system (Lecarpentier et al., 1979) and explains the explosive character of the early diastolic filling phase (Brutsaert et al., 1980). The variety of preparations studied demonstrated that the preponderance of load dependence or of activation dependence is not determined by the architectural nature of the preparation. Intact atrial preparations were now shown to be largely load independent; relaxation of the atrium thus seems to be governed mainly by the underlying inactivation mechanisms. After destroying the membranous systems with detergent (Or-entlicher et al., 1974) in single atrial cells, no influence of load on relaxation could be demonstrated. Yet, some early load-induced deactivation cannot be denied in the intact atrial strip at shorter initial muscle lengths, suggesting a weakly functioning sarcoplasmic reticulum. Indeed, histological studies of atrial muscle show less numerous tubules and a less-developed sarcoplasmic reticulum, with a smaller calcium content (McNutt and Fawcett, 1969). Thus, load independence of relaxation of the intact mammalian atrial strip could result from a sarcoplasmic reticulum which is functioning at too low a level to make relaxation dependent on the loading conditions and which causes the decaying calcium concentration to be the main factor governing relaxation.

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