Cardiac muscle mechanics in hyperosmotic solutions

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WILDENTHAL, KERN, C. LYNN SKELTON, AND HENRY NEAL COLEMAN III. Cardiac muscle mechanics in hyperosmotic solutions. Am. J. Physiol. 217(1): 302-306. 1969.—Alterations in cardiac muscle tension development have generally been found to be due to changes in contractile element (CE) shortening without changes in series elastic (SE) characteristics. The present study was designed to determine if alterations in tension development of cat papillary muscle bathed by hyperosmotic sucrose solutions were due to changes in the CE, the SE, or both. Isometric tension development was found to increase in solutions 100 mOsm/kg H2O above control and to decrease at 300 mOsm above control; changes were variable at 200 mOsm above control. The intrinsic velocity of CE shortening changed in a qualitatively similar manner at the three levels, but at the highest levels depression of shortening velocity tended to be more severe than was depression of tension development. Series elastic extensibility, as determined by an isotonic quick-release technique, decreased progressively as osmolalities increased; significant changes were observed at 200 and at 300 mOsm above control. The results demonstrate that hyperosmolality alters both CE and SE mechanical characteristics, with changes in tension development being influenced by both muscle components.

According to Hill's generally accepted model for active muscle (6-9), development of force or tension is the result of the interaction of two components: a contractile element (CE) capable of shortening and generating force and a passive series elastic element (SE). Theoretically, changes in the characteristics of tension development can be due to changes in CE shortening, SE extensibility, or both. Tension development is enhanced by increases in the rate and extent of CE shortening and by decreases in the extensibility of the SE. Conversely, tension development is depressed by decreases in CE shortening and by increases in SE extensibility.

A variety of interventions has been found to alter the mechanical state of cardiac muscle through changes in CE function, as analyzed by force-velocity relations (1, 19, 20, 22). Series elastic characteristics, on the other hand, appear usually to be unaffected by inotropic interventions (1, 17, 19, 20, 22). Recently, however, results of a study of the cardiac effects of acutely induced hyperosmolality in open-chest dogs (23) suggested the possibility that the alterations in tension development in mammalian ventricle known to occur under hyperosmotic conditions (12) might be influenced in part by a change in SE extensibility. Accordingly, the present investigation was undertaken to test such a possibility by analyzing the effects of varying degrees of hyperosmolality on the mechanical properties of isolated cat papillary muscle.

METHODS

Papillary muscles were excised from the right ventricles of 10 adult mongrel cats (1.5-2.5 kg) anesthetized with intraperitoneal sodium pentobarbital (25 mg/kg). Each muscle was rapidly suspended in a 20-ml Lucite bath containing Krebs-bicarbonate solution (Na+, 146; K+, 3.6; H2PO4−, 1.2; Mg++, 1.2; Cl−, 128; Ca++, 2.5; SO42−, 1.2; HCO3−, 25; and glucose, 5.6 mm; with a total osmolality of approximately 310 mOsm/kg H2O). The solution was continuously bubbled with 95% O2-5% CO2, giving a pH of 7.4. Temperature was maintained constant at 30°C. The base of the muscle was placed in a spring-loaded Lucite clip attached to a force transducer (Statham model G-1-3050) by a rigid stainless steel rod. The tendinous end of the muscle was tied by a short length of 0000 Ethicon surgical silk to a rigid wire connected to the tip of an isotonic lever system adapted for quick-release studies. Diagrams and complete descriptions of the isotonic lever and quick-release system have been published previously (13, 15). Muscle lengths varied from 3.7 to 8.5 mm (mean, 5.7) and cross-sectional area varied from .90 to 1.31 mm2 (mean, 1.08). The muscles were stimulated 12 times/min through platinum mass electrodes by an American Electronics Laboratory stimulator (model 104A) delivering 7- to 15-msec pulses at voltages 10-20% above threshold. Force and shortening of the muscles were recorded along with a stimulation artifact at 100 mm/sec paper speed on a Sanborn multichannel oscillographic recorder.

Each study was performed at a preload of 0.50 g (0.38-0.55 g/mm2). Although, with shortening, the load initially supported by the parallel elastic element of the muscle contributes to the force across the SE, such a contribution is minimal at the light preload used (5, 15, 21), and no correction for the effect of the parallel elastic element was included in the calculations. No significant changes in resting length were observed at the constant preload during the course of the experiments. The use of a model with a negligible mechanical contribution from the parallel elastic element allowed consideration of the muscle as a simple two-component system (CE and SE), and obviated the need to choose between the various possible locations of the parallel
TABLE 1. Effect of hyperosmolality on isometric force ($P_o$) and intrinsic velocity of shortening ($V_{max}$)

<table>
<thead>
<tr>
<th></th>
<th>$P_o$ (g/mm²)</th>
<th>$V_{max}$, muscle length/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control (5 muscles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$+100$ mOsm/kg H₂O</td>
<td>2.9± .39</td>
<td>0.95±.093</td>
</tr>
<tr>
<td>Difference</td>
<td>+0.7±.30</td>
<td>+0.14±.048</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B. Control (7 muscles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$+200$ mOsm/kg H₂O</td>
<td>3.1±.31</td>
<td>0.93±.068</td>
</tr>
<tr>
<td>Difference</td>
<td>+0.2±.29</td>
<td>-0.02±.055</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C. Control (6 muscles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$+300$ mOsm/kg H₂O</td>
<td>2.8±.25</td>
<td>0.94±.062</td>
</tr>
<tr>
<td>Difference</td>
<td>-0.5±.19</td>
<td>-0.35±.078</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

A $\times 10^{-6}$

The equipment was determined as previously described (15), and used as a correction factor for the SE extension curves.

The osmolality of the solution did not influence the equipment extensibility.

Statistical analysis of the data was performed using the Student $t$ test for paired observations. Results are expressed as the mean ± 1 SEM.

RESULTS

Effect of hyperosmolality on force-velocity relations. Abruptly changing the bathing solution from the control solution to any of the three hyperosmotic solutions caused an initial augmentation of muscle shortening and force development in all instances. After 2–5 min this enhancement of the contractile state usually ceased, and muscle function either stabilized at the new level or began declining. In all three hyperosmotic solutions satisfactory stabilization of mechanical function occurred within 30 min. The final functional state varied with the degree of hyperosmolality. Effects on isometric tension ($P_o$) and on intrinsic velocity of shortening ($V_{max}$), as determined by extrapolation of the force-velocity curve to zero load, are summarized in Table 1 for each of the three levels.

An increase of 100 mOsm above control shifted the force-velocity curve upward and to the right in all cases, and resulted in moderate increases in $V_{max}$ and $P_o$.

Hyperosmolality of 200 mOsm above control caused variable changes in the force-velocity relation. In three muscles $V_{max}$ and $P_o$ decreased; in two, they increased; and in one, there was no significant change in either.

The muscles, contracting isotonically at 12/min, were allowed to stabilize in the control medium for 1 hr, after which force-velocity relations were determined (1, 19, 20). Series elastic characteristics were then analyzed by determination of SE extension (stress-strain) curves from isotonic quick-release experiments, as described by Wilkie (24). The procedures utilized were those of Parmley and Sonnenblick (15). The stimulated muscle was initially prevented from shortening by an air jet which stabilized the isotonic lever against the preload stop. After 350–425 msec (a period which coincided with the attainment of at least 90% of peak isometric tension) a solenoid attached to a second stimulator suddenly diverted the air jet from the lever, which was then free to move. Rapid shortening of the muscle and a simultaneous fall in tension were recorded (15), and these changes were assumed to represent the effects of shortening of the SE component (1, 3, 11, 13, 24). A series of quick releases were recorded, with afterload varying from zero to peak isometric force, and multiple trials were observed at each condition. The SE extension curve was determined from the relation of afterload to the amount of quick-release shortening.

After initial control measurements were completed, the medium was changed to one of three hyperosmotic solutions: 1) the control Krebs-bicarbonate solution + 100 mOsm/kg H₂O of sucrose, 2) control solution + 200 mOsm sucrose, or 3) control solution + 300 mOsm sucrose. Following stabilization of the muscle in the new medium for 45 min, force-velocity and SE extension curves were redetermined in an identical manner. The control medium was then re-established and measurements were repeated after another 45-min equilibration period. In most muscles the procedure was repeated using one or both of the other hyperosmotic solutions. Of the 10 muscles, 5 were tested at 100 mOsm above control, 7 at 200 mOsm, and 6 at 300 mOsm. Control measurements were always made before and after each intervention. The results were not affected by previous interventions or by the order in which the solutions were tested. At the conclusion of the experiments, extensibility of the equipment was determined as previously described (15), and used as a correction factor for the SE extension curves.

Fig. 1. Quick-release SE extension curves (uncorrected for equipment compliance) in one muscle. Determinations were performed in following order: control (O), $+200$ mOsm/Kg H₂O ( ), control (△), $+300$ mOsm (▲), control (□). Equipment compliance curve is shown by dotted line.
seventh muscle $V_{\text{max}}$ was unchanged, whereas $P_0$ increased by 25%. The average changes in $V_{\text{max}}$ and $P_0$ were not significant.

At 300 mOsm above control $V_{\text{max}}$ fell significantly in all cases. $P_0$ fell less markedly, in general, and it actually rose slightly (8%) in one of six muscles.

Effect of hyperosmolarity on series elastic extensibility. The stiffness of the series elastic component tended to be increased by hyperosmotic solutions, and was greatest at the highest osmolality. Typical effects of hyperosmolarity on SE extensibility, as determined from isotonic quick releases, are shown for one muscle in Fig. 1. Shifts of the load-extension (stress-strain) curve downward signify decreases in extensibility. It is apparent that alterations in extensibility occurred at both 200 and 300 mOsm above control. It should also be noted that return to control osmolality was accompanied by a return to the original SE characteristics.

In five muscles in which hyperosmolality of 100 mOsm above control was induced, three showed no change in SE extensibility and two showed a slight decrease. At a load of 2 g/mm², quick release resulted in shortening of the muscle by an average of 3.14% of the initial muscle length under control conditions and by 2.98% with hyperosmolality. The difference (0.16 ± 0.12%) was not significant ($P > 0.10$).

At 200 mOsm above control, definite stiffening of the SE was observed in six of seven muscles. Quick-release shortening at 2 g/mm² averaged 3.10% during the control condition and 2.37% during hyperosmolarity. The difference was 0.73 ± 0.24% (approximately a 25% decrease) and was statistically significant ($P < 0.05$).

Series elastic extensibility was markedly reduced in six muscles tested at 300 mOsm above control. Each curve may be described by the exponential equation $P = C(e^{KL} - 1)$, where $P = \text{load}$ and $L = \text{SE extension}$. Such an equation also defines a linear relation between load and the rate of change of load with respect to extension ($dP/dL$, or the modulus of elasticity), so that $dP/dL = KP + C$. Values for the $K$ and $C$ constants for control and hyperosmotic conditions, as determined by least squares analysis, were:

$$K(\text{control}) = 1.0 \pm 0.16/\% \text{ muscle length};$$
$$K(+300 \text{ mOsm}) = 1.9 \pm 0.25/\% \text{ muscle length};$$
$$C(\text{control}) = 0.2 \pm 0.06 \text{ g}/\% \text{ muscle length};$$
$$C(+300 \text{ mOsm}) = 0.2 \pm 0.05 \text{ g}/\% \text{ muscle length}.$$  

The $C$ constants are similar in each case ($P > 0.10$), whereas the $K$ constants differ significantly ($P < 0.01$).

**DISCUSSION**

The influence of hyperosmolality on cardiac muscle has received surprisingly little attention. Koch-Weser (12) has provided most of the available information on changes in mechanical characteristics of mammalian ventricle in hyperosmotic solutions. His work established that increases in osmolality up to twice normal are accompanied by the following: 1) no significant change in the resting length-tension relation at light preloads; 2) no change in time-to-peak tension until the total osmolality is greater than 500 mOsm/kg H₂O, after which a slight delay in the time-to-peak tension occurs; 3) a progressive decline in the rate of relaxation at high osmolalities; and 4) an increase in tension development at lower degrees of hyperosmolality (up to 100 mOsm total) and a progressive decline thereafter, with developed tension falling below normal at osmolalities above 500 mOsm total. It remained unclear, however, whether the observed alterations in tension development resulted from changes in contractile element shortening, in series elastic element extensibility, or in both.

The experiments performed in the present study yielded results in agreement with the findings of Koch-Weser. In addition, it has been demonstrated that $V_{\text{max}}$ increases at low levels of hyperosmolality (100 mOsm above control), returns toward or past original values at 200 mOsm above control, and is markedly depressed at 300 mOsm above control. Simultaneously, the extensibility of the SE decreases progressively as osmolality increases. Small decreases in SE extensibility are apparent in some muscles at 100 mOsm/kg H₂O above control under the in vitro conditions studied. Significant decreases in SE extensibility are present at 200 and 300 mOsm above control. Thus, changes in tension development of cardiac muscle in hyperosmotic media are influenced by alterations in both the CE and the SE. The change in SE stiffness would, in effect, result in an internal shift along the CE force-velocity curve, causing the CE to utilize more of the available energy in exerting tension, rather than in shortening internally and stretching a more...
compliant SE. The external mechanical manifestations of this are a tendency for the muscle to maintain its capacity to exert a relatively large isometric tension, while velocity of shortening is more severely limited.

Most interventions that influence myocardial function have been shown to affect the CE without altering the SE. Previous studies have demonstrated that norepinephrine (19, 20), digitalis (22), calcium (19), hyperthyroidism (17), paired electrical pacing (1), changes in frequency of stimulation (1, 19), pulmonary artery constriction induced heart failure and hypertrophy (17), and variations in temperature (1) do not change SE extensibility. Only following actual death or traumatic injury of cells, as in postinfarction fibrosis (4) or segmental contracture induced by physical compression (16), has SE extensibility been demonstrated unequivocally to change with alterations in the contractile state.

The present evidence that hyperosmotic sucrose solutions can alter SE extensibility of papillary muscle and that return to the control medium is accompanied by a return to the original SE characteristics constitutes, to our knowledge, the first direct demonstration of a reversible intervention which increases the stiffness of the SE of cardiac muscle. Changes in initial muscle length, although not basically altering the SE, will affect the quick-release response of cardiac muscle tested with the technique used in the present study, altering the $C$ constant in the equations $P = C(e^{kz} - 1)$ or $dP/dz = KP + C$, but not the $K$ constant (15, 17). That the $K$ constant changes significantly with hyperosmolality indicates that the alteration in the SE is different from that occurring with changes in initial length.

The observed changes in CE and SE properties of cardiac muscle in hypertonic sucrose solutions are similar in many regards to those of skeletal muscle in hypertonic solutions. Reductions in SE extensibility occur in frog sartorius exposed to hypertonic media (11), and progressive decreases in the intrinsic velocity of CE shortening are also seen (10). A significant difference between cardiac and skeletal muscle exists, however, in that the initial increase in $V_{max}$ seen at low levels of hyperosmolality in cardiac muscle is not seen in skeletal muscle. Rather, $V_{max}$ of skeletal muscle is maximal in physiologically isotonic solutions and declines even at minimal degrees of hyperosmolality (10).

The mechanisms responsible for contractile changes in muscle bathed in hypertonic solutions have not been adequately defined. Sucrose diffuses easily through muscle but does not freely enter cells, resulting in an osmotic loss of intracellular water (2, 3, 14). Both the cellular dehydration itself and the resultant relative increase in electrolytes may play a major role in the mechanical changes observed. Koch-Weser (12) has suggested that the increase in contractile force observed at lower degrees of hyperosmolality may be the result of an increase in calcium ion concentration near the muscle membrane, a condition which is well known to increase $V_{max}$ in cardiac muscle (19). Reduction of the velocity of shortening (and of relaxation) at still higher osmolalities may be the result of increased viscosity and the development of friction between closely packed contractile filaments of the dehydrated fibers (10). The work of Podolsky and Sugi (18) has demonstrated that decreases in velocity of shortening in hypertonic solutions are effected through the contractile apparatus itself, independent of effects on excitation-contraction coupling.

It seems likely that the apparent decrease in SE extensibility, as analyzed in quick-release experiments, might result at least in part from an increase in the viscosity of the muscle. Just as a reduction in shortening velocity could occur simply as a consequence of close packing of the contractile filaments (10), so too an increase in viscosity might tend to damp the SE and decrease its apparent extensibility indirectly, simply as a result of passive interference with the motion of whatever intracellular components constitute the SE. Such an effect would be especially noticeable in quick-release conditions, since the influence of changes in internal damping would be augmented at high velocities. The results of the present study do not allow differentiation of the observed effects of hyperosmolality on the SE stress-strain curve into changes mediated through alterations in viscosity and changes caused by other, nonviscous factors. As emphasized by Hill (7) and Jewell and Wilkie (11), because of the problem of internal viscosity and damping it is impossible to make quantitative extrapolations about SE extensibility from quick-released muscle to the normally contracting muscle. It is even conceivable theoretically that an effect noticeable during quick releases would be negligible or absent when contractions were more physiological. It seems extremely unlikely that the effects of hyperosmolality on SE extensibility are present solely during quick releases, however, in view of the results obtained during isotonic, afterloaded, and isometric contractions. Rather, as discussed earlier, the noticeably disproportionate fall in velocity of shortening, relative to peak isometric tension, suggests that a reduction in SE extensibility during severe hyperosmolality is mechanically important under conditions of normal contraction.

Whatever may be the complete explanation of the effects of hyperosmolality, the results of the present study emphasize the need for a detailed description of the mechanical properties of myocardium if the effects of agents which change contractile function are to be understood fully. Although the SE usually may be assumed to remain unchanged with inotropic interventions, the present study establishes that it does not always do so. Such a finding has more than theoretical pertinence when one considers that in the intact experimental animal exposed to serum osmolalities up to 450 mOsm/kg H2O, left ventricular tension development may be enhanced at a time when the rate of ejection is not (23), an occurrence which suggests that diastolic extensibility may decrease in the intact heart at levels of hyperosmolality which occur clinically in man.

The authors wish to express their appreciation to Dr. Eugene Braunwald for his advice and encouragement during the study.


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Received for publication 6 September 1968.
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