ABSTRACT

The influence of osmolality of the external medium on the calcium (Ca) dependency of contractility of isolated electrically excited cat papillary muscle was examined. Maximum unloaded velocity of shortening was directly measured by load clamping the muscle from the preload (at the length, L_m;iv, at which maximum active tension was developed) to zero load (zero load clamp). Peak velocity of shortening at the L_m;iv preload, peak total force, peak rate of force development, time to peak force, and time to half relaxation were also recorded. The performance-Ca response curves (Ca concentration between 1.25 nM and 10 nM) for maximum unloaded velocity of shortening, peak shortening velocity at L_m;iv preload, total force, and peak rate of force development were shifted to the left when osmolality was increased (from 290 mosmoles to 410 mosmoles) with sucrose, and to the right when osmolality was increased with NaCl. The sensitivity for Ca, as determined from the slopes of these response curves, appeared essentially unaltered by either sucrose or NaCl, except for the high Ca concentrations (above 5 mM) at the higher osmolalities (above 370 mosmoles) especially with sucrose.

KEY WORDS force-velocity-length relation cat papillary muscle maximum unloaded velocity of shortening sucrose NaCl zero load clamp

In a previous study on electrically stimulated cat papillary muscles, myocardial contractility, determined from the time-independent portion of the force-velocity-length relation (1), was markedly influenced by alterations in the external calcium (Ca) concentration in the bathing solution (2). Similarly, recent studies on isolated glycerinated skeletal muscle fibers (3, 4) have indicated that both velocity and tension are Ca dependent. However, other investigations on skinned skeletal muscle fibers have shown that only tension is Ca dependent (5). This apparent discrepancy between studies of skeletal muscle fibers has been resolved by the finding (6) that Ca sensitivity is greatest at lower ionic strengths and disappears at higher ionic strengths. Accordingly, the effect of Ca on intact heart muscle should be considered in relation to the intracellular ionic strength and, hence, in relation to the osmolality of the external medium. In intact skeletal muscle, an increase in the osmolality of the external medium depresses mechanical performance (7, 8); however, in heart muscle, contractility is augmented at a moderately elevated osmolality and depressed at higher levels (9). In the present study, the Ca dependence of the contractility of cat papillary muscles on the osmolality of the external medium was investigated.

Methods

Thirteen papillary muscles were removed from the right ventricle of cats anesthetized with sodium pentobarbital (40 mg/kg, ip). The muscles were suspended vertically in a bath containing modified Krebs-Ringer's solution (mM): NaCl 118, KCl 4.7, MgSO_4 · 7H_2O 1.2, KH_2PO_4 1.1, NaHCO_3 24, CaCl_2 · 6H_2O 2.5, and glucose 4.5. The solution was bubbled with 95% O_2:5% CO_2. The temperature was maintained at 29°C and the pH at 7.4. The muscles were stimulated at 12/min with 5-msec rectangular pulses about 10% above threshold. The stimulus was provided through two platinum electrodes arranged longitudinally one on each side of the muscle. The lower nontendinous end of the muscle was held by a light phosphor bronze...
clip soldered to the middle of the spring of the force transducer (2). The tendinous end of the muscle was tied with a short thread (7.0 braided, noncapillary, moisture- and serum-resistant, surgical thread) extending upward to the electromagnetic lever system that was mounted on a Palmer stand immediately above the bath. The electromagnetic lever system and the control units were the same as those previously described (2). Zero load clamps with the required optimal damping to obtain maximum unloaded velocity of shortening in the length range around 0.9 $L_{\text{max}}$ have also been extensively described previously (10).

In all experiments, the preload on the muscle was adjusted so that the initial muscle length corresponded to $L_{\text{max}}$. Table 1 summarizes the mean basic characteristics of all muscles at $L_{\text{max}}$. Under these conditions, perfect stabilization was obtained after 2–3 hours. The mean ratio of resting tension to total tension at $L_{\text{max}}$ was then 10.80 ± 0.73% (SE) in 2.5 mM Ca.

The effects of various external Ca concentrations (1.25, 2.5, 5.0, 7.5, and 10.0 mM) were studied in normal modified Krebs-Ringer's solution. Ca was augmented by adding 0.5, 1, 1, and 1 ml sequentially of a concentrated Ca solution containing 125 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to the bath (content 50 ml). The experiment was started with a solution containing 1.25 mM Ca; a lower Ca concentration was used so that the stability of contractility of the muscle could be maintained. Concentrations higher than 10.0 mM were not tested because of precipitation in the bathing solution. At any Ca concentration, stabilization of the muscle was obtained within 10–15 minutes. In the same group of muscles, this dose-response curve for Ca was repeated at various osmolalities (290 [Krebs-Ringer's solution], 330, 370, 410 mosmoles/kg) by adding either sucrose or NaCl to the solution. At any osmolality, the bath was washed three times with the new solution which contained a Ca concentration of 1.25 mM and had been equilibrated for temperature and pH. The osmolality was directly verified by cryoscopy (Osmette A, Precision Systems). The sequence of the addition of solutions of different osmolalities was randomized. To verify stability of the muscle after each experiment at a different osmolality, a dose-response curve for Ca was obtained in control Krebs-Ringer's solution.

For any given condition in each group of muscles, a mean value ± SE was calculated. For clarity, the SE was not presented in the figures, except in Figure 1; the SE is given in Results when necessary. The curves in Figures 2 and 4 illustrate the results obtained in the control solution and represent the means of all control curves obtained in all experiments. The values obtained at any higher osmolality were compared with the mean of the preceding and subsequent control values by the paired t-test.

For each experimental condition, the following variables were measured: maximum unloaded velocity of shortening, peak velocity of shortening at $L_{\text{max}}$ ($dL/dt$), peak force, peak rate of force development ($dF/dt$), time to peak force, and time to half relaxation. These variables were obtained from three consecutive contractions following a series of stable isotonic beats at $L_{\text{max}}$ (11). A zero load-clamped contraction provided measurement of maximum unloaded velocity of shortening. In the subsequent nonclamped isotonic beat, peak $dL/dt$ was measured at the $L_{\text{max}}$ preload. The third contraction was an isometric beat providing measurement of total force, peak $dF/dt$, time to peak force, and time to half load relaxation. Shortening velocity at both zero load and $L_{\text{max}}$ preload was recorded simultaneously as a function of length and time. All other variables were recorded as a function of time only. All recordings as functions of length and time were displayed simultaneously on one storage display unit (Tektronix 611) and photographed with a hard copy unit (Tektronix 4601). Some typical experimental records of all of these variables have been published previously (10).

Results

Figure 1 illustrates the effects of increasing Ca concentration on all variables. Maximum unloaded velocity of shortening, peak $dL/dt$, peak force, and peak $dF/dt$ were markedly enhanced in a statistically significant manner ($P < 0.01$) when Ca was increased in steps from 1.25 mM to 10 mM. Time to peak force was shortened in a statistically significant way ($P < 0.02$), but time to half relaxation was not altered. These results confirm

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<th>TABLE 1</th>
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<tr>
<td>Basic Characteristics of Thirteen Cat Papillary Muscles Under Control Conditions at $L_{\text{max}}$</td>
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<tr>
<td>$L_{\text{max}}$ (mm)</td>
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<td>7.50 ± 0.55</td>
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Values are means ± SE.
Effects of Ca on contractile performance of cat papillary muscle. Values are means ± SE obtained in thirteen muscles. 

\[ \frac{dF}{dt} = \text{peak rate of force development.} \]

and further extend previous observations of the effects of Ca on papillary muscle (2).

Figures 2 and 3 illustrate the effects of alterations in the osmolality of the external medium by sucrose. In Figure 2, all variables are expressed as a function of Ca concentration for various degrees of osmolality, and in Figure 3 the same variables, except peak \( \frac{dL}{dt} \), are replotted as a function of osmolality for various values of Ca concentration. At Ca concentrations between 1.25 mM and 5.0 mM, maximum unloaded velocity of shortening and peak \( \frac{dL}{dt} \) were augmented in a statistically significant manner (\( P < 0.05 \)) by increasing osmolality from 290 mosmoles to 370 mosmoles. At a Ca concentration of 7.5 mM, these two variables became slightly depressed. On transition to 410 mosmoles, the increase in these variables was present only at Ca concentrations of 2.5 mM or less, and the variables were markedly depressed at higher Ca concentrations. Except for a less marked depression at 410 mosmoles occurring only beyond 5 mM Ca, similar observations were made for peak force. The effects of osmolality on peak force at various Ca concentrations were the result of the interaction of the simultaneously occurring influence of \( \frac{dF}{dt} \) and time to peak force (Fig. 3). The increase in peak force at 1.25 mM Ca when osmolality was augmented was due to a continuously increasing \( \frac{dF}{dt} \) which overcame the slight shortening of time to peak force. At 2.5 mM Ca, the increase in peak total force on transition to 370 mosmoles was also due to the increasing \( \frac{dF}{dt} \) which overcame the shortening of time to peak force. However, at 410 mosmoles force remained unchanged despite a slightly diminished \( \frac{dF}{dt} \) due to prolongation of time to peak force. At 7.5 mM and 10 mM Ca, on increasing osmolality to 370 mosmoles, peak force became slightly depressed in proportion to the falling \( \frac{dF}{dt} \), since the time to peak force barely changed. Peak force was further depressed at 410 mosmoles despite a prolongation of time to peak force. The slight alterations in relaxation shown by values of time to half relaxation were difficult to interpret.

The sensitivity of the response of maximum unloaded velocity of shortening, peak \( \frac{dL}{dt} \), and peak force to alterations in Ca
Ca concentration, the depressive action of increasing osmolality was most pronounced on transition from 370 mosmoles to 410 mosmoles. The unchanged slope of the curves indicates that the sensitivity to alterations in Ca concentration was unaltered regardless of the degree of osmolality except at 410 mosmoles, where both velocity parameters and dF/dt became somewhat less sensitive to Ca alterations as demonstrated by the diminished steepness of the three curves. The apparent unchanged sensitivity of total force to different Ca concentrations at 410 mosmoles can be ascribed to the simultaneously occurring diminished response to Ca of peak dF/dt and time to peak force.

In Figure 5, maximum unloaded velocity of shortening and peak total force in solutions with osmolality increased by sucrose and NaCl have been superimposed. The values with sucrose are the same as those in Figure 3. With NaCl, at any Ca concentration and osmolality, both variables were lower than the corresponding values with sucrose except between 370 mosmoles and 410 mosmoles at 10 mM Ca where maximum unloaded velocity of shortening with both NaCl concentration between 1.25 mM and 5 mM, demonstrated by the slopes of the curves in Figure 2 and the distance between the curves in Figure 3, was hardly influenced by osmolality between 290 mosmoles and 370 mosmoles. At 410 mosmoles, the response of peak force to augmented Ca was essentially unaltered up to 5 mM Ca, whereas the sensitivity of the velocity parameters remained unchanged only on transition from 1.25 mM to 2.5 mM Ca. In the higher Ca range, the sensitivity of all variables to alterations in Ca concentration was markedly diminished by an increase in osmolality.

Figure 4 illustrates the effects of alterations in the osmolality of the external medium by NaCl; the variables considered are the same as those in Figures 1 and 2. With NaCl, at any Ca concentration, maximum unloaded velocity of shortening, peak dL/dt, peak force, and peak dF/dt were progressively depressed by increasing the osmolality. This depression was minimum on transition from 330 mosmoles to 370 mosmoles regardless of the Ca concentration. At any

FIGURE 3
Contractile performance as a function of osmolality established by adding sucrose at various Ca concentrations plotted from the same experimental data used in Figure 2. dF/dt = peak rate of force development.

FIGURE 4
Response of cat papillary muscles (n = 7) to various Ca concentrations at increasing degrees of osmolality established by adding NaCl. Symbols and abbreviations are the same as they are in Figure 2.
Comparison of maximum unloaded shortening velocity and total force in solutions in which either sucrose or NaCl was used to increase osmolality. The data for sucrose are the same as those in Figure 3, and the data for NaCl are taken from Figure 4.

and sucrose coincided. At any Ca concentration, the curve illustrating maximum unloaded velocity of shortening with NaCl between 330 mosmoles and 370 mosmoles tended to become flatter, indicating decreased sensitivity for osmolality changes in this range. These effects, although less marked for total force, were similar to the observations in sucrose-containing solutions.

**Discussion**

The present experiments indicated that the sensitivity of myocardial contractility of intact electrically stimulated cat papillary muscles to Ca concentration changes between 1.25 mM and 5 mM was hardly influenced by osmolality changes over the range between 290 mosmoles and 370 mosmoles. When osmolality was augmented by adding sucrose, at higher nonphysiological Ca concentrations beyond 5 mM, the sensitivity to Ca was markedly diminished over the same range of osmolalities. At a higher osmolality of 410 mosmoles this diminished sensitivity to Ca was obvious in the lower Ca concentration range. To some extent these results are in line with recent observations in isolated skinned skeletal muscle fibers (6), which show that the influence of Ca on $V_{\text{max}}$ at lower ionic strengths results from a variable internal load due to the formation of abnormal cross-bridges, which are also indirectly responsible for the increased resting force (6). In cardiac muscle, an abnormal cross-bridge interaction could appear as an internal load and, hence, as a Ca dependence of $V_{\text{max}}$ with only negligible alterations in the length-tension relations at the $L_{\text{max}}$ preload, since small relative alterations in resting force at $L_{\text{max}}$ preload, which in heart muscle corresponds to the beginning of the steep portion of the length-tension curve, would only appear as negligible length changes. It is also possible that no changes in the resting length-tension relations were observed in the present study because of the quite narrow range of osmolalities tested. An alternative explanation could be that the Ca effects on the contractile proteins of cardiac muscle differ from the
effects in skeletal muscle. Whatever the underlying mechanism it appears that the sensitivity of myocardial contractility to Ca in the lower, and probably physiological, Ca range is independent of osmolality up to the rather high, although frequently encountered clinically, value of 410 mosmole.

In contrast to results in intact skeletal muscles (7, 8) that show a depression of mechanical performance when the osmolality of the external medium is increased, the present study confirms the known potentiating effect of a moderately elevated osmolality in heart muscle at lower Ca concentrations (9, 13, 14). This potentiation could be explained by slight cellular dehydration with a concomitant increase in activating intracellular Ca concentrations either directly (9) or indirectly through a slight increase in intracellular sodium (Na) concentration that enhances Ca influx (15). With respect to both velocity parameters, an additional explanation could be an augmented pliability of the cell membrane (16); as far as force potential is concerned, an increased stiffness of the series elastic component could also be responsible (13, 14, 17). A possible minor additional role played by the adrenergic nervous system has also been suggested (13). In agreement with the depression observed in skeletal muscle (7, 8) are the results obtained at a higher osmolality of 410 mosmole established with sucrose in the higher Ca concentration range, suggesting a similar depressive action of high intracellular ionic strength on the contractile proteins in cardiac and skeletal muscle (18-22), which could possibly be related to a depressive action on actomyosin adenosinetriphosphatase activity (23). However, effects of hypertonicity on excitation-contraction coupling cannot be excluded in skeletal muscle (24, 25).

In contrast to the results obtained with sucrose are two observations obtained when osmolality was increased by the addition of NaCl. First, the presence of a depression instead of a potentiation could be ascribed to the same phenomena mentioned for the higher osmolalities achieved with sucrose and to the known depressive action of a decreased ratio of Ca to Na at any Ca concentration. This finding implies that the latter phenomenon was not entirely overcome by a possible augmentation of intracellular Na due to dehydration or Na permeation with resulting enhanced Ca influx (15). Second, the sensitivity to Ca concentration changes in the Ca concentration range below 5 mM was similar with NaCl and sucrose (Figs. 2 and 4), but it was less affected by osmolality changes over the entire Ca concentration range when osmolality was changed with NaCl. Even the slightly diminished Ca sensitivity at higher Ca concentrations in 410-mosmole solutions was less clearly marked than it was with sucrose. This phenomenon was probably due to an almost symmetrical shift to the right of the Ca response curves at any osmolality with NaCl (Fig. 4) compared with the asymmetrical shift to the left of the corresponding curves with sucrose (Fig. 2). Regardless of the way in which osmolality was changed, by either sucrose or NaCl, the upper limit of contractility was progressively depressed with increasing osmolality, and, hence, increasing intracellular ionic strength, as has been demonstrated in skeletal muscle by Lännergren and Noth (26).

References
8. HOWARTH JY: Behavior of frog muscle in hypertonic solutions. J Physiol (Lond) 144:167-175, 1958
CALCIUM AND OSMOLALITY IN HEART MUSCLE

Contractility in mammalian heart muscle; calcium and osmolality.
M A Goethals, S M Adèle and D L Brutsaert

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