Characterization of the Effects of Mg\(^{2+}\) on Ca\(^{2+}\)- and Sr\(^{2+}\)-Activated Tension Generation of Skinned Rat Cardiac Fibers

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**ABSTRACT** Submaximum and maximum forces of the cardiac muscle contractile apparatus, activated by Ca\(^{2+}\) or Sr\(^{2+}\), were determined as a function of Mg\(^{2+}\) concentration. Apical left ventricular tissue from Sprague-Dawley rats was broken by homogenization into small bundles of fibers with disrupted sarcolemmas (skinned). Tension generation was activated by and graded according to the concentration of Ca\(^{2+}\) or Sr\(^{2+}\) in solutions bathing the skinned fibers and measured with a photodiode force transducer. Steady-state tensions for various levels of activation at each of four concentrations of Mg\(^{2+}\) (5 \times 10^{-5}, 1 \times 10^{-3}, 5 \times 10^{-3}, and 10 \times 10^{-3} \text{ M}) in the bathing solutions were analyzed. Other bathing solution constituents and parameters mimicked significant normal intracellular conditions while providing adequate buffering of [H\(^{+}\)], [Ca\(^{2+}\)], and [MgATP\(^{−}\)] (magnesium adenosine triphosphate). To assess changes in sensitivity of the mechanical system to activation by Ca\(^{2+}\) (or Sr\(^{2+}\)), each submaximum tension was expressed as a percentage of the given fiber bundle's maximum force generated at saturating [Ca\(^{2+}\)] (or [Sr\(^{2+}\)]) at the same [Mg\(^{2+}\)]. When plotted as saturation curves these data demonstrate that increasing [Mg\(^{2+}\)] depresses Ca\(^{2+}\) sensitivity of the force-generating mechanism. The Ca\(^{2+}\) and Sr\(^{2+}\) sensitivity of the cardiac force-generating apparatus is very similar at every [Mg\(^{2+}\)], indicating that the magnitude of Mg\(^{2+}\) effect is similar for both types of activation. However, absolute maximum tensions at saturating activating cation concentration increased as [Mg\(^{2+}\)] increased; the effect of Mg\(^{2+}\) on maximum force was proportionately the same for Ca\(^{2+}\) and Sr\(^{2+}\) activation. But because saturating [Ca\(^{2+}\)] always resulted in a lower maximum force than saturating [Sr\(^{2+}\)], this site of Ca\(^{2+}\)-Mg\(^{2+}\) interaction appears distinct from the one influencing Ca\(^{2+}\) sensitivity.

**INTRODUCTION**

Mg\(^{2+}\) is present in a millimolar range in cardiac muscle (Polemini and Page, 1973) and depresses the Ca\(^{2+}\)-activated tension generation of “skinned” (sarcolemma removed or disrupted) mammalian cardiac cells (Kerrick and Donaldson, 1975; Fabiato and Fabiato, 1975) and myofibrillar ATPase (Solaro and Shiner, 1976). The action may be important functionally, because total Mg\(^{2+}\) concentra-
tion varies in cardiac muscle (Polemini and Page, 1973). Initial results obtained by Kerrick and Donaldson (1975) indicated that the Ca\(^{2+}\) sensitivity and the effects of a limited change in [Mg\(^{2+}\)] on Ca\(^{2+}\)-activated tension generation are similar for frog skeletal muscle and rat cardiac muscle. However, Best et al. (1977), in studying the effects of variations in [MgATP\(^{2-}\)] on Ca\(^{2+}\)-activated tension generation of rat cardiac muscle, noted an interaction between the effects of MgATP\(^{2-}\), ATP\(^{4-}\), and Mg\(^{2+}\) as well as an effect of Mg\(^{2+}\) on maximum tension generation. Inasmuch as neither of these findings had been observed in frog skeletal muscle (Kerrick and Donaldson, 1972; Donaldson and Kerrick, 1975), there appeared to be some important differences between cardiac and skeletal muscle that could be brought out by larger changes in [Mg\(^{2+}\)]. Furthermore, Solaro and Shiner (1976) have observed that the effects of Mg\(^{2+}\) on myofibrillar ATPase are different for cardiac and skeletal muscle. Clarification of these differences may be useful in identifying the sites of Mg\(^{2+}\) action; although Mg\(^{2+}\)-binding sites on the contractile proteins have been identified (Potter and Gergely, 1975; Potter, 1975, 1977; Bremel and Weber, 1975; Morimoto and Harrington, 1973; Leavis, 1977), their relation to tension generation is not known.

In this study we determined the effects of a 200-fold change in [Mg\(^{2+}\)] on the Ca\(^{2+}\) sensitivity and maximum capability of the tension generating apparatus of mammalian cardiac tissue. We also determined the Mg\(^{2+}\) effects for Sr\(^{2+}\)-activated tension. Sr\(^{2+}\) activation of skinned fiber force generation is analogous to Ca\(^{2+}\) activation, but the Sr\(^{2+}\) bathing solutions would be more accurate, because EGTA buffering of Sr\(^{2+}\) concentration would be much better than that of Ca\(^{2+}\) in the concentration ranges required for activation of force. In addition, a comparison of the effects of Mg\(^{2+}\) on Sr\(^{2+}\)- and Ca\(^{2+}\)-activated tension could potentially yield information related to the number of Mg\(^{2+}\) sites of action. A preliminary report of this work appeared earlier (Donaldson et al., 1976).

METHODS

Preparation

Small bundles of apical cardiac tissue from male rats (Sprague-Dawley, 200 ± 20 g) were "skinned" (sarcolemma mechanically disrupted) by homogenization as described previously (Kerrick and Best, 1974; Best et al., 1977). The adequacy of the skinning procedure in removing diffusion barriers is discussed elsewhere (Best et al., 1977).

Isometric Tension Measurement

Bundles of cardiac fibers, 30-100 μm wide and 1-3 mm long, were mounted by their ends in the forceps of a photodiode force transducer (Donaldson and Kerrick, 1975; Best et al., 1977). The force-voltage relationship of the transducer was linear to well beyond the range of forces measured; inasmuch as maximum shortening of the fibers was <5% of their mounted lengths, isometric tensions were measured.

The mounted fiber bundles were contracted and relaxed sequentially at selected Mg\(^{2+}\) concentrations by immersing them in bathing solutions of varying Ca\(^{2+}\) or Sr\(^{2+}\) concentrations; tension generation was continuously recorded. Base-line tension generation was established as the steady-state voltage output while the fiber bundle was immersed in a relaxing solution, described below. Steady-state (constant for 1-min minimum) deviations
in voltage from base-line were used as measures of tension generation, so that possible transient alterations in ionic composition within the bundles, and thus transient changes in tension generation resulting from, for example, Ca$^{2+}$ uptake and (or) release by structures such as the sarcoplasmic reticulum and mitochondria, were eliminated.

**Bathing Solutions**

Ionic environment of the contractile proteins within the fibers was manipulated by equilibrating the skinned fiber bundles with bathing solutions of variable ionic composition; fiber bundles mounted in the force transducer were transferred between 1-ml baths of solutions. Room temperature was 20 ± 1°C and the bathing solutions were topped with silicone oil to prevent evaporation. The oil also limited transfer of solutions between the baths. Details of procedures for mixing, assaying final composition, and storage of bathing solutions and accuracy of the binding constants used to solve the complex equilibria are as described elsewhere (Donaldson and Kerrick, 1975; Best et al., 1977).

All bathing solutions contained $10^{-7}$ M H$^+$, 70 mM (K$^+$ + Na$^+$), 7 mM total EGTA (ethylene glycol bis-(β-amino-ethyl ether)-N,N'-tetraacetic acid), 4 mM MgATP$^{2-}$, 15 mM CP$^+$ (creatine phosphate) and 15 µ/ml CPK (creatine phosphokinase). Imidazole was used to buffer [H$^+$] and its concentration was varied to maintain ionic strength at 0.15 M. Chloride was the major anion. The encountered variations in imidazole, K$^+$, Na$^+$ concentrations from in vivo levels and between bathing solutions do not affect Ca$^{2+}$-activated force generation of skinned muscle fibers (Kerrick and Best, 1974; Donaldson and Kerrick, 1975; Best et al., 1977).

Force generation was elicited in response to externally applied Ca$^{2+}$ or Sr$^{2+}$ at each of four Mg$^{2+}$ concentrations: $5 \times 10^{-5}$, $1 \times 10^{-3}$, $5 \times 10^{-3}$, and $10 \times 10^{-3}$ M. At each [Mg$^{2+}$] two sets of bathing solutions were prepared: (a) a set containing variable concentrations of Ca$^{2+}$ ranging from $pCa = 8$, or no added Ca$^{2+}$, to the [Ca$^{2+}$] required for maximum tension generation and (b) a set containing variable Sr$^{2+}$ concentrations ranging from $pCa = 8$, or no added Sr$^{2+}$, to the [Sr$^{2+}$] required for maximum tension generation. However, Sr$^{2+}$ and Ca$^{2+}$ were never added simultaneously to any solution. At each [Mg$^{2+}$] the relaxing solutions of the Sr$^{2+}$ and Ca$^{2+}$ sets were identical: neither Sr$^{2+}$ nor Ca$^{2+}$ was added, glass-distilled water was the solvent, and both contained an unavoidable, low level of contaminating Ca$^{2+}$.

**Protocol for Data Collection**

Because the fiber bundles varied in diameter and an unknown proportion of each bundle's cross-sectional area did not have longitudinal integrity between the forcep tips, the forces generated were not normalized according to bundle cross-sectional areas. Each steady-state tension was converted to a percentage or proportion of a maximum force for the same fiber bundle. For comparison of maximum force generating capability at a given [Mg$^{2+}$], the same saturating [Ca$^{2+}$] and [Sr$^{2+}$] was used, but this saturating concentration was a function of [Mg$^{2+}$]. Fig. 1 shows maximum Ca$^{2+}$-activated tensions at $1 \times 10^{-3}$ and $10 \times 10^{-3}$ M Mg$^{2+}$, at $5 \times 10^{-3}$ and $10 \times 10^{-3}$ M Mg$^{2+}$, and maximum Ca$^{2+}$- and Sr$^{2+}$-activated tensions at [Mg$^{2+}$] = $10 \times 10^{-3}$ M. To derive the maximum tension ratios, the magnitude of the tension for the second contracture (1 mM Mg$^{2+}$) was divided by the average of the first and third contractures (10 mM Mg$^{2+}$), the tension of the fourth (5 mM Mg$^{2+}$) was divided by the average of those of the third and fifth contractures (10 mM Mg$^{2+}$), and the average of the Sr$^{2+}$-activated tensions were divided by the average tension for the fifth and seventh Ca$^{2+}$-activated tensions.

For subsaturating [Ca$^{2+}$]'s, tensions were converted to percentages of maximum; the

$$pX = -\log_{10}([X^{2+}]),$$ where $X^{2+} = Ca^{2+}$ or Sr$^{2+}$. 


force generated at a given \([\text{Ca}^{2+}]\) (or \([\text{Sr}^{2+}]\)) was divided by the maximum force generated at saturating \([\text{Ca}^{2+}]\) (or \([\text{Sr}^{2+}]\)) at the same \([\text{Mg}^{2+}]\) and multiplied by 100. Fig. 2 shows contractures in the \(\text{Ca}^{2+}\) set of solutions at \([\text{Mg}^{2+}] = 10 \times 10^{-3}\) M. For the first contracture of Fig. 2, the steady-state tension for pCa 3.2 was divided by that at pCa 3.0 and multiplied by 100; similarly, for the second contracture, tension at pCa 4.7 was divided by that at pCa 3.0 and multiplied by 100.

FIGURE 1. Maximum \(\text{Ca}^{2+}\) and \(\text{Sr}^{2+}\) contractures at three \(\text{Mg}^{2+}\) concentrations. Arrows indicate times at which changes in the bathing solutions were made. Changes are indicated below arrows. See text for details. Tension increases upwards, and the abscissa shows time. Fiber bundle diameter = 90 \(\mu\)m.

FIGURE 2. Maximum and submaximum \(\text{Ca}^{2+}\) contractures at \(\text{Mg}^{2+} = 10 \times 10^{-3}\) M. Arrows indicate times at which changes in the bathing solutions were made. Changes are indicated below arrows. See text for details. Tension increases upwards, and the abscissa shows time. Fiber bundle diameter = 100 \(\mu\)m.

As is evident in Figs. 1 and 2, each fiber bundle could be contracted and relaxed many times with little decline in maximum force per contracture. Thus, we could study the effects of a single variable, such as a given change in \([\text{Mg}^{2+}]\), independent of order of contracture or fiber differences by alternating contractures at the two ionic conditions of interest for the same fiber bundle. Data include paired data for individual fiber bundles (a) for a given pCa (or pSr) at different \([\text{Mg}^{2+}]\)'s and (b) for \(\text{Ca}^{2+}\) vs. \(\text{Sr}^{2+}\) at the same \([\text{Mg}^{2+}]\) (see Fig. 1).

RESULTS

Maximum Force Generation

Maximum tension data are expressed in two ways reflecting the types of paired data collected. First, for comparisons of either maximum \(\text{Ca}^{2+}\)- or \(\text{Sr}^{2+}\)-activated tensions at various \([\text{Mg}^{2+}]\)'s, forces are expressed as proportions of the corre-
sponding maximum tension at $10 \times 10^{-3}$ M Mg$^{2+}$ for the same fiber bundle (Table 1, rows 1 and 2). Second, at each [Mg$^{2+}$], the Ca$^{2+}$-activated maximum force is also expressed as a proportion of the Sr$^{2+}$-activated maximum force (Table 1, row 3). Thus, the mean proportions for each row of Table 1 were calculated from separate raw data.

As can be seen in Table 1, rows 1 and 2 and the first five contractures in Fig. 1, maximum Ca$^{2+}$-activated force generation is a function of [Mg$^{2+}$]. Maximum force generation was greatest at $10 \times 10^{-3}$ M Mg$^{2+}$ and was decreased similarly for Ca$^{2+}$ and Sr$^{2+}$ as [Mg$^{2+}$] was lowered. The greatest change in maximum tension occurred between $5 \times 10^{-5}$ and $1 \times 10^{-3}$ M Mg$^{2+}$; both the Sr$^{2+}$- and

| [Mg$^{2+}$] | $1 \times 10^{-4}$ M | $5 \times 10^{-4}$ M | $10 \times 10^{-4}$ M |
|-------------|---------------------|---------------------|---------------------|
| $T_{Ca^{2+}}/T_{Mg^{2+}} \times 100$ | 60.3±4.0 | 88.6±2.3 | 91.7±2.3 | 100% |
| $T_{Ca^{2+}}, pCa=4$ | n=4 | n=4 | n=4 | |
| $T_{Sr^{2+}}, pMg=10^{-3}$ | (pCa=4.0) | (pCa=3.6) | (pCa=3.0) | (pCa=3.0) |
| $T_{Sr^{2+}}, pMg=10^{-5}$ | 67.5±2.1 | 80.8±0.5 | 92.5±1.1 | 100% |
| $T_{Sr^{2+}}, pSr=10^{-3}$ | n=7 | n=5 | n=6 | |
| $T_{Sr^{2+}}, pSr=10^{-5}$ | (pSr=4.0) | (pSr=3.6) | (pSr=3.0) | (pSr=3.0) |
| $T_{Ca^{2+}}/T_{Sr^{2+}} \times 100$ | 71.6±3.6 | 91.4±3.6 | 89.8±4.0 | 90.8±1.2 |
| $T_{Ca^{2+}}, pSr=3.0$ | n=5 | n=4 | n=4 | n=7 |

* Sample size and fiber bundle population.

Ca$^{2+}$-activated maximum tensions at $5 \times 10^{-5}$ M Mg$^{2+}$ were significantly less than those at $10 \times 10^{-3}$ M Mg$^{2+}$ ($P's < 0.05$).

The Ca$^{2+}$-activated maximum tension was consistently less than the corresponding Sr$^{2+}$-activated one; the last four contractures in Fig. 1 illustrate this effect at $10 \times 10^{-3}$ M Mg$^{2+}$. The Ca$^{2+}$/Sr$^{2+}$ maximum tension ratio was significantly <1.0 ($P \leq 0.05$) only at $5 \times 10^{-5}$ M Mg$^{2+}$ ($P = 0.005$) and $10 \times 10^{-3}$ M Mg$^{2+}$ ($P = 0.001$) and these two ratios were different from each other ($P < 0.05$); however, we never observed the Ca$^{2+}$-activated tension to be greater than the Sr$^{2+}$-activated tension at any [Mg$^{2+}$]. The data from the first two rows of Table I are not paired Ca$^{2+}$ vs. Sr$^{2+}$ contractures as are the data of row 3.

Ca$^{2+}$ and Sr$^{2+}$ Activation

Mean percentages of maximum tension vs. subsaturating pCa's and pSr's at each [Mg$^{2+}$] are displayed in Figs. 3 and 4. The solid lines of Figs. 3 and 4 represent nonlinear least-squares fittings of the Hill equation:

$$\frac{T}{T_{max}} = \frac{[X^{2+}]^n}{Q + [X^{2+}]^n},$$

where Sr$^{2+}$ or Ca$^{2+}$, and $n$ and $Q$ are derived constants to the weighted (by 1/SEM$^2$) means represented by the symbols. The pX of 50% tension, or
midpoint, for each solid curve is \(-\frac{1}{n}\) log \(Q\). Hill \(n\) and midpoint \(pX\)’s for the solid curves of Figs. 3 and 4 are shown in Table II.

Increasing \([Mg^{2+}]\) decreases the effect of a given subsaturating \([Ca^{2+}]\) or \([Sr^{2+}]\) in terms of the percentage maximum tension elicited. As a result the \(pCa\) and \(pSr\) tension curves (Figs. 3 and 4) shift in the direction of higher \([X^{2+}]\)’s as \([Mg^{2+}]\) increases. However, no differences are seen between \(Ca^{2+}\) and \(Sr^{2+}\) with respect to the midpoint of the \(Ca^{2+}\) tension and \(Sr^{2+}\) tension curves at any given \([Mg^{2+}]\) (see Table II). The shape of the \(pCa\) and \(Sr\) tension curves is also very similar at each \([Mg^{2+}]\).

All of the \(pX\) tension curves have a Hill \(n\) value > 1.0. The Hill \(n\) values are all approximately 2 ± 0.3, except for the \(pCa\) tension curve of \(5 \times 10^{-5}\) M \(Mg^{2+}\).

Placing greater significance upon the accuracy of the \(Sr^{2+}\)-activated tension data, in that buffering of \(Sr^{2+}\) concentration by EGTA is much better at all \([Mg^{2+}]\)’s than that of \(Ca^{2+}\), the Hill \(n\) values do not appear to change as a function of \([Mg^{2+}]\). The \(pCa\) tension data for \([Mg^{2+}] = 5 \times 10^{-3}\) and \(1 \times 10^{-3}\) M \(Mg^{2+}\) (Fig. 3) were published earlier (Best et al., 1977). In Table II, the Hill \(n\) and midpoint for each of these two curves were derived using weighted means; these values are identical to those previously published where fitting was of all the raw data.

DISCUSSION

Previously published data indicated a similarity in the \(Ca^{2+}\) sensitivity and effects of \(Mg^{2+}\) on tension generation in frog skeletal and rat cardiac muscle (Kerrick and Donaldson, 1975; Fabiato and Fabiato, 1975); the data obtained in this study show some important differences. In our first comparative study of frog skeletal muscle and rat cardiac muscle, \([MgATP^{2-}]\) was 2 mM and only two \([Mg^{2+}]\)’s
were tested: $5 \times 10^{-5}$ and $1 \times 10^{-3}$ M (Kerrick and Donaldson, 1975). We
decided to characterize the effects of Mg$^{2+}$ on cardiac tension generation more
fully using 4 mM MgATP$^{2-}$, in part to provide better buffering of [MgATP$^{2-}$]
within the interior of the bundles of skinned cardiac fibers. The effects of Mg$^{2+}$
on rat cardiac muscle in the present study may be safely compared with those
previously published (Donaldson and Kerrick, 1975) for frog skeletal muscle
even though [MgATP$^{2-}$] was different for the two studies, in that frog skeletal
skinned fiber force generation appears unaffected by equivalent changes in
[MgATP$^{2-}$] (Kerrick and Donaldson, 1972).

![Figure 4. Percentages of maximum tension vs. pSr at four [Mg$^{2+}$]'s. Solid lines
are computer fittings of curves (see text) to means of raw data (symbols). Error
bars show standard errors of the means that are larger than symbol size. Average
$n = 2.8$ fibers.](image)

**Maximum Tension Enhancement**

The finding that maximum Ca$^{2+}$-activated tension is a function of Mg$^{2+}$ is new
and in contrast to the data for frog skeletal muscle and prior data for skinned
rat cardiac fibers (Fabiato and Fabiato, 1975). Inasmuch as Fabiato and Fabiato
use single skinned cardiac cells rather than bundles of fibers, as in this study, we
were concerned that perhaps our result was due to diffusion limitations in the
preparation. It would be expected that any membranous diffusion barriers
would be leakiest at low [Mg$^{2+}$]'s (Winegrad, 1971) and, therefore, diffusion
limitation might be greatest at high [Mg$^{2+}$]'s. However, our results are the
opposite of what would be expected from inadequate skinning; increased
maximum tensions were associated with decreased Ca$^{2+}$ sensitivity rather than
the enhancement of both as is seen with reduced [MgATP$^{2-}$]'s (Best et al.,
1977). The negative results of Fabiato and Fabiato (1975) are puzzling, but are
perhaps related in part to the differences in protocols used for data collection.
It appears that they did not make self-paired comparisons of maximum force by
alternating successive maximum contractures at two [Mg$^{2+}$]'s to obtain a maxi-
The finding that the maximum tension with Sr²⁺ activation was always greater than with Ca²⁺ activation was very surprising. This result cannot be due to errors in binding constants used in solving the complex equilibria of the bathing solutions, in that saturating [Ca²⁺]’s and [Sr²⁺]’s were used. In a prior study of frog skeletal muscle (Donaldson and Kerrick, 1975) we noted that Sr²⁺-activated maximum tension was always less than for Ca²⁺; because higher concentrations of Sr²⁺ were required for saturation, the depression might have been due to an excess of activating cation, which consistently causes inhibition. In the present study the saturating [Ca²⁺]’s and [Sr²⁺]’s were identical at each [Mg²⁺] and the maximum force ratios for Ca²⁺ or Sr²⁺ changed in a similar fashion as [Mg²⁺] increased. All of these data might be accounted for if there were an inhibitory effect of Ca²⁺ and Sr²⁺ (at high concentrations) with Sr²⁺ having a lesser effect than Ca²⁺ and both being counteracted by Mg²⁺. Alternatively, Mg²⁺ might have a direct stimulating effect via a site that binds Ca²⁺ and Sr²⁺ with differential affinity.

These effects are discussed as [Mg²⁺] effects for simplicity; it should be remembered that, inasmuch as [MgATP²⁻] was held constant, [ATP⁴⁻] decreased as [Mg²⁺] increased. There is evidence of an interaction of MgATP²⁻, ATP⁴⁻, and Mg²⁺ in effects on maximum tension (Best et al., 1977).

**Depressant Effect on Ca²⁺ Sensitivity**

As in frog skeletal muscle (Donaldson and Kerrick, 1975), increasing [Mg²⁺] caused a shift of the pCa and pSr tension curves in the direction of higher activating divalent cation concentrations. The pCa and pSr tension curves were closer together than they had been for skeletal muscle, as is consistent with

| Table I I |
| Hill Coefficients and Midpoints of Tension Curves |

| [Mg²⁺] | 5×10⁻⁹ M | 1×10⁻⁹ M | 5×10⁻⁹ M | 10×10⁻⁹ M |
|--------|----------|----------|----------|-----------|
| Hill n* |          |          |          |           |
| Ca²⁺   | 2.92     | 1.89     | 1.86     | 1.62      |
| Sr²⁺   | 1.84     | 2.12     | 2.33     | 1.82      |
| [Mg²⁺] |          |          |          |           |
| pCa    | 5.56     | 5.06     | 4.59     | 4.37      |
| pSr    | 5.56     | 5.10     | 4.80     | 4.41      |

\[
\frac{T}{T_{max}} = \frac{[Ca^{2+}]^{n\ast}}{Q + [Ca^{2+}]^{n\ast}}\]

\[\ast = (1/n)\log_{10} Q.\]
affinity of troponin binding sites for Sr\(^{2+}\) relative to Ca\(^{2+}\) for the two muscle types (Ebashi et al., 1968), and did not change in steepness or relative position despite increases in [Mg\(^{2+}\)] up to 10 × 10\(^{-8}\) M. Thus, Sr\(^{2+}\) behaves as an analog of Ca\(^{2+}\) in activating a given percentage of maximum tension at every [Mg\(^{2+}\)].

However, in contrast to frog skeletal muscle (Kerrick and Donaldson, 1975) cardiac muscle did not show a decrease in the Hill n value to 1.0 with increasing [Mg\(^{2+}\)] at saturating [MgATP\(^{2-}\)] despite the essentially identical ranges of activating Ca\(^{2+}\) concentrations for the two muscle types at each [Mg\(^{2+}\)]. Furthermore, the steepness of the pSr tension curves did not appear to change at all for cardiac muscle as a function of [Mg\(^{2+}\)]. It is possible that error(s) in binding constant(s) for the ionic equilibria could result in an erroneous change in relative steepness of the pCa and pSr tension curves. But this type of error would not explain a smaller change of steepness of the pCa or pSr tension curves for cardiac muscle as compared to those for skeletal muscle. Therefore [Mg\(^{2+}\)] appears to have a lesser or no effect on the steepness of the activating divalent cation-tension relationships for cardiac muscle as compared with skeletal muscle; [MgATP\(^{2-}\)] and (or) [ATP\(^{4-}\)] appear to be the most important determinants of the steepness of the pX tension curve in cardiac muscle (Best et al., 1977).

In that Mg\(^{2+}\) did not influence the steepness and thus the shape of the tension curves, the change in midpoint of the pCa and pSr tension curves is potentially due to an effect of Mg\(^{2+}\) on apparent affinity of the contractile system to activating cation. The midpoint concentration \((Q')^{1/n}\) for each curve can be represented as the following function of [Mg\(^{2+}\)]:

\[
(Q')^{1/n} = \frac{Q^{1/n}([\text{Mg}^{2+}] + Z_{\text{Mg}}^{2+})}{Z_{\text{Mg}}^{1+}},
\]

where \(Q'\) and \(n'\) are the values of \(Q\) and \(n\) at the specified [Mg\(^{2+}\)], \(Q\) and \(n\) are the respective values at 5 × 10\(^{-5}\) M Mg\(^{2+}\), and \(Z_{\text{Mg}}^{2+}\) has the values listed in Table III. For previously published data for Mg\(^{2+}\) = 1 × 10\(^{-3}\) M and 30 \(\mu\)M MgATP\(^{2-}\) (Best et al., 1977) \(Z_{\text{Mg}}^{2+}\) = 10\(^{-51}\) M. Thus, \(Z_{\text{Mg}}^{2+}\) appears to be a constant and the above relationship of [Mg\(^{2+}\)] and \((Q')^{1/n}\) holds over a broad range of [MgATP\(^{2-}\)]s (and thus [ATP\(^{4-}\)]s). Even though \(Z_{\text{Mg}}^{2+}\) is very similar for every curve it cannot be interpreted as having meaning in relation to a true binding (dissociation) constant for Mg\(^{2+}\), nor can \(Q^{1/n}\) be interpreted as a binding

| Divalent cation | \(10^{-4}\) M | \(5 \times 10^{-4}\) M | \(10 \times 10^{-4}\) M |
|----------------|------------|----------------|----------------|
| Ca\(^{2+}\)   | 10\(^{-3.3}\) M | 10\(^{-3.1}\) M | 10\(^{-3.3}\) M |
| Sr\(^{2+}\)   | 10\(^{-3.3}\) M | 10\(^{-3.0}\) M | 10\(^{-3.3}\) M |

See text for definition.
constant for Ca\textsuperscript{2+} or Sr\textsuperscript{2+}, without knowing more about the number and properties of the actual binding sites for Mg\textsuperscript{2+} and Ca\textsuperscript{2+}. Modeling of the system is not useful at this point because too many assumptions related to exact number of binding sites and the translation between ion binding and force generation are required. However, the observed relationship of midpoint concentration of the pCa and pSr tension curves to [Mg\textsuperscript{2+}] should be useful in setting a constraint on any proposed models.

In summary, we have noted a depressant effect of Mg\textsuperscript{2+} on Ca\textsuperscript{2+} sensitivity and a Mg\textsuperscript{2+}-dependent enhancement of maximum Ca\textsuperscript{2+}-activated tension generation of cardiac muscle. The maximum tension effect appears unique to cardiac muscle. The two parameters of the tension generating system that are affected by Mg\textsuperscript{2+} appear to represent separate sites or mechanisms of Ca\textsuperscript{2+}-Mg\textsuperscript{2+} interaction, because at any [Mg\textsuperscript{2+}] the Ca\textsuperscript{2+} and Sr\textsuperscript{2+} sensitivities of the system appear identical while the maximum force response to saturating [Ca\textsuperscript{2+}] is less than that to an equal and saturating [Sr\textsuperscript{2+}]. The functional significance of the effects of Mg\textsuperscript{2+} remains speculative, but they may be important in modulation and alteration of cardiac contraction.

We thank Laura Bolles, Robin Coby, Sylvia Lucas, and Barry Hill for their excellent technical assistance and M. Barnhart and A. Olson for typing of the manuscript.

This investigation was supported by grants HL-17375, HL-13517, and RR-00374 from National Institutes of Health. Dr. Best was a post-doctoral Fellow of the Washington State Heart Association.

Received for publication 15 November 1976.

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