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THE EFFECTS OF Mg^{2+} ON SUBMAXIMUM Ca^{2+}-ACTIVATED TENSION IN SKINNED FIBERS OF FROG SKELETAL MUSCLE

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SUMMARY

This study evaluates the effects of free Mg^{2+} concentration on submaximum isometric tension generated by single skinned fibers from *semitendinosus* muscles of the frog. Increases in Mg^{2+} concentration are accompanied by decreases in the submaximum isometric tension generated in bathing solutions which have the same Ca^{2+} concentration and same ionic strength. The effects of changes in $MgATP^{2-}$ and ATP^{4-} concentration were separated from those of Mg^{2+} and were found to be very small in comparison to those of Mg^{2+} concentration. The Mg^{2+} concentrations used in two sets of solutions were 0.3, 1, and 2 mM. In one set of solutions $MgATP^{2-}$ concentration changed from approximately 2 to 12 mM and in the other set of solutions ATP^{4-} concentration changed from approximately 10^{-4} to $1.6 \cdot 10^{-5}$ M. Ionic strength was found to have a significant inverse effect on submaximal isometric tension.

INTRODUCTION

Mg^{2+} is important in muscle contraction but its exact role and mechanism of action have not been defined. In order to generate Ca^{2+}-activated tension, both Mg^{2+} and ATP^{4-} must be present in solutions bathing the contractile proteins[1]. From studies of isolated protein systems it seems that Mg^{2+} also has an inverse effect on standard measures of contraction if it is present in a mM concentration range[2-4]. A mM sarcoplasmic Mg^{2+} concentration range is possible since measurements of total Mg concentration are in this range[5]. Chowrashi and Kaldor[6] in a recent study gave evidence that Mg^{2+} interacts with troponin, but Fuchs *et al.*[7] demonstrated that it is unlikely that Mg^{2+} binds on troponin at the same site as Ca^{2+}. Ebashi and Endo[8] attributed a shift toward pCa's in the pCa–tension curve of skinned fibers to an increase in Mg^{2+} concentration, but they did not determine to what extent this shift might have been caused by simultaneous increases in $MgATP^{2-}$ and decreases in ATP^{4-} concentrations.

METHODS

In this study single twitch fibers from the *semitendinosus* muscles of frogs (*Rana pipiens*) were skinned (their sarcolemma peeled off)[9,10] under silicone oil. They were

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

then mounted between the forceps of a photodiode tension transducer and isometric tension was measured using a method similar to that of Hellam and Podolsky[11]. The compliance of the transducer was 50 mV/μm and the shortening of the fibers was always less than 5% of the mounted length of the fiber.

The fibers were dipped into electrolyte solutions covered with silicone oil. Room temperature was maintained at 20 ± 1 °C. All solutions contained 7 mM ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), 90 mM K^+, ATP and imidazole (pH 7.0). Three Mg^{2+} concentrations in the mM range were used: 0.3, 1, and 2 mM. At each Mg^{2+} concentration solutions were mixed which had pCa values of 9–8 (no Ca^{2+} added), 5.5 and 4.6–4.3 (for maximum tension generation). Solutions with a pCa 5.5 were used because the fibers generated submaximum tension in them at each of the three Mg^{2+} concentrations. Thus changes in the tension at this constant Ca^{2+} concentration reflected changes in the shape or position of the pCa–tension curve.

Experiments were done to separate the effects of changes in $MgATP^{2-}$ and ATP^{4-} concentrations from those of Mg^{2+} concentration by using three different sets of solutions. The first set of solutions was mixed to the given concentrations and the amount of ATP added in this set of solutions was varied so that ATP^{4-} remained at 10^{-4} M and $MgATP^{2-}$ varied from about 2 to 12 mM. In the second and third sets of solutions $MgATP^{2-}$ was 2 mM and ATP^{4-} varied from about 10^{-4} to $1.6 \cdot 10^{-5}$ M.

The minimum concentration of imidazole used was 0.036 M since it has been demonstrated that the $CaEGTA^{2-}$-binding constant is dependent upon imidazole concentration in a range below 0.02 M imidazole[12]. In the first set of solutions, with constant ATP^{4-} concentration, the ionic strength of the solutions as calculated without imidazole increased by 0.03 as Mg^{2+} and $MgATP^{2-}$ concentrations increased. Therefore it was necessary in the first set of solutions to decrease imidazole concentrations by less than one-half (0.144 to 0.090 M) as Mg^{2+} concentration increased from 0.3 to 2.0 mM in order to achieve a constant ionic strength of 0.20. A slightly lower ionic strength might have been used if only this particular study were being considered. The ionic strength value of 0.20 was selected in order that this data could be compared with data from related studies now in progress. The second set of solutions with constant $MgATP^{2-}$ concentration an ionic strength of 0.20, contained 0.144 M imidazole in every solution. The third set of solutions differed from the second set only in imidazole concentration which was 0.036 M in every solution.

It was decided to adjust the ionic strength by varying imidazole (pH 7.0) rather than KCl concentration for two reasons: (1) K^+ is known to bind to a site on actin[13] and thus varying K^+ concentration is likely to cause variable effects on the binding of Mg^{2+} and Ca^{2+} to their respective sites and (2) imidazole does not appear to have any effect on the ATPase activity of the contractile proteins[14].

The ionic equilibria for each solution and the ionic strength were calculated by digital computer using binding constants from the literature as shown in Table I. For each solution the desired ionic concentration of K^+, Mg^{2+}, Ca^{2+}, H^+, and $MgATP^{2-}$ and the total concentration of K_2EGTA (EGTA titrated to pH 8.0 with KOH) were specified and the computer calculated the total amount of $MgCl_2$, $CaCl_2$, KCl, and Na_2ATP to be added. The small amount of Na^+ added as Na_2ATP was treated as added K^+ because Na^+ and K^+ have a similar binding constant for ATP^{4-} (ref. 15). Following the addition of the calculated amounts of these salts to double-distilled water, the pH of the solution was measured and titrated with HCl to pH 7.0. To insure

TABLE I

BINDING CONSTANTS USED IN THE COMPUTER PROGRAM

Definition of binding constant	*Value* (M^{-1})	*Reference*
$\frac{[CaATP^{2-}]}{[Ca^{2+}]\,[ATP^{4-}]}$	$2.5 \cdot 10^4$	A value chosen between that of O'Sullivan and Perrin (1964)[15] and Taguikhan and Martell (1962)[17] and corrected for K^+ binding using data from Botts *et al.* (1965)[18]
$\frac{[CaHATP^{-}]}{[Ca^{2+}]\,[HATP^{3-}]}$	$3 \cdot 10^2$	Same
$\frac{[CaEGTA^{2-}]}{[Ca^{2+}]\,[EGTA^{4-}]}$	$2.6 \cdot 10^{10}$	Unpublished observation of Robert E. Godt done on similar solutions in the laboratory of F. N. Briggs
$\frac{[CaHEGTA^{-}]}{[Ca^{2+}]\,[HEGTA^{3-}]}$	$2.1 \cdot 10^5$	Schwartzenbach and Senn (unpublished) in Sillen and Martell (1964)[19]
$\frac{[MgATP^{2-}]}{[Mg^{2+}]\,[ATP^{4-}]}$	$6 \cdot 10^4$	Value selected between that of Watanabe *et al.* (1963)[20] and that of O'Sullivan and Perrin (1964)[15] with temperature correction from Burton (1959)[21]
$\frac{[MgHATP^{-}]}{[Mg^{2+}]\,[HATP^{3-}]}$	$6 \cdot 10^2$	Same as $\frac{[CaATP^{2-}]}{[Ca^{2+}]\,[ATP^{4-}]}$
$\frac{[MgEGTA^{2-}]}{[Mg^{2+}]\,[EGTA^{4-}]}$	$1.62 \cdot 10^5$	Schwartzenbach *et al.* (1957) [22]
$\frac{[MgHEGTA^{-}]}{[Mg^{2+}]\,[HEGTA^{3-}]}$	$2.3 \cdot 10^3$	Same as $\frac{[CaHEGTA^{-}]}{[Ca^{2+}]\,[HEGTA^{3-}]}$
$\frac{[KATP^{3-}]}{[K^{+}]\,[ATP^{4-}]}$	8	Botts *et al.* (1965)[18]
$\frac{[HATP^{3-}]}{[H^{+}]\,[ATP^{4-}]}$	$8.9 \cdot 10^6$	Smith and Alberty (1956)[23]
$\frac{[H_2ATP^{2-}]}{[H^{+}]\,[HATP^{3-}]}$	$1.1 \cdot 10^4$	Martell and Schwartzenbach (1956)[24]
$\frac{[HEGTA^{3-}]}{[H^{+}]\,[EGTA^{4-}]}$	$2.7 \cdot 10^9$	Chaberek and Martell (1959)[25]
$\frac{[H_2EGTA^{2-}]}{[H^{+}]\,[HEGTA^{3-}]}$	$7.1 \cdot 10^8$	
$\frac{[H_3EGTA^{-}]}{[H^{+}]\,[H_2EGTA^{2-}]}$	480	

accuracy of mixing the total Cl and ATP concentrations of the final solutions were measured.

For each set of solutions and at each Mg^{2+} concentration the fiber was allowed (1) to equilibrate in a solution with pCa 8 to 9, (2) to generate a steady level of tension in either a solution with pCa 5.5 or 4.3–4.6, and (3) then to relax in the original solution. The change in output of the transducer from the baseline was used as the measure of tension generated. The maximum isometric tension for each fiber was determined in solutions with pCa 4.3–4.6 at each Mg^{2+} concentration. The Ca^{2+} concentration required for maximum tension generation varied slightly but the maximum tension for each fiber appeared to be the same in all solutions within each set. The percentage of maximum tension is that obtained by dividing tension generated at pCa 5.5 by the maximum tension developed at the same Mg^{2+}, $MgATP^{2-}$ and ATP^{4-} concentrations and then multiplying by 100. The maximum tension used in this calculation was obtained by interpolating between the maximum tensions generated before and after the tension generated at pCa 5.5. The fibers generated an initial maximum tension of at least 1 kg/cm^2 cross-sectional area and were discarded when maximum tension declined to 50% of the initial value.

RESULTS AND DISCUSSION

Fig. 1 shows the results in the three sets of solutions. Each point represents the mean of the percentage of maximum tensions the fiber generated at pCa 5.5 at that Mg^{2+} concentration. The upper and middle curves are from two sets of solutions differing in ionic strength, third and second sets of solutions, respectively. In these two curves $MgATP^{2-}$ remained at 2 mM and ATP^{4-} concentration decreased as Mg^{2+} concentration increased. Under these conditions the percentage of maximum tension generated at pCa 5.5 decreased as Mg^{2+} concentration increased. The increase in ionic strength from 0.14 to 0.20 reduced the tensions without a marked change in their relative values. The middle and lower curves are the results of experiments done in two different sets of solutions (second and first sets, respectively) of the same ionic strength. The lowest curve shows the results using solutions with constant ATP^{4-} concentration in which $MgATP^{2-}$ and Mg^{2+} concentration both increased. The solutions with 0.3 mM Mg^{2+} were identical in ionic composition for the middle and lower curves with ionic strength at 0.20. The percentage of maximum tension with pCa 5.5 for the lower points decreased with increasing Mg^{2+} concentration in the same manner as the upper points. For the lower and middle curves there is no statistically significant difference ($P=0.4$) between the means of the two points at 1 mM Mg^{2+} and $P=0.01$ for the two means at 2 mM Mg^{2+}.

The similarity of the decline in submaximum tension with increasing Mg^{2+} concentration for the two lower curves suggests that, of the three variables, Mg^{2+} concentration has the greatest effect on submaximum tension in this particular range of concentrations. The small but statistically significant difference between the means of the points at 2 mM Mg^{2+} indicates that in this range the increase in $MgATP^{2-}$ concentration and the decrease in ATP^{4-} concentration may begin to affect the submaximum tension.

There is a slight possibility that the necessary variations in imidazole concentration within the first set of solutions (lower curve, Fig. 1) might account, at least in

part, for the similarity of the means at 1.0 and 2.0 mM Mg^{2+} in the middle and lower curves. However, this seems unlikely in view of the finding that the maximum tension generated was the same in solutions with 0.090 to 0.144 M imidazole.

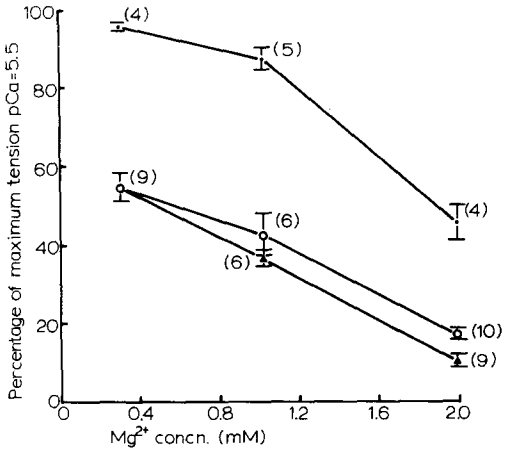

Fig. 1. The effects of Mg^{2+} concentration on the percentage of maximum isometric tension generated by skinned muscle fibers at a constant Ca^{2+} concentration. The abscissa shows Mg^{2+} concentration in mM. The ordinate shows tension at pCa 5.5/maximum isometric tension (pCa 4.3–4.6) multiplied by 100. Each point is a mean and the bars represent standard errors of the mean. The number of observations for each mean is in parentheses. In the upper (●—●) and middle (○—○) curves $MgATP^{2-}$ remained at 2 mM and ATP^{4-} varied from 10^{-4} to $1.6 \cdot 10^{-5}$ M as Mg^{2+} increased from 0.3 to 2 mM. But the upper curve was done at an ionic strength of 0.14 and the middle curve at an ionic strength of 0.20. The lower curve (△—△) was also done at an ionic strength of 0.20 but in this experiment ATP^{4-} remained at 10^{-4} M while $MgATP^{2-}$ varied from 2 to 12 mM as Mg^{2+} concentration increased. All solutions contained 7 mM EGTA, 90 mM K^+, ATP and imidazole (pH 7.0).

The finding that Mg^{2+} in a mM range of concentration has a large effect on submaximum Ca^{2+}-activated tension is consistent with other data from the literature. Using other preparations, Ebashi and Endo[8] and Portzehl *et al.*[2] found that the pCa–tension and pCa–ATPase relationships, respectively, were shifted in the direction of increased Ca^{2+} concentration when Mg^{2+} concentration increased in the mM range along with changes in ATP^{4-} and $MgATP^{2-}$ concentrations. In this same concentration range Mg^{2+} has been shown to bind to a system of proteins which, because of contamination, probably contained troponin, tropomyosin, F-actin and G-actin[13]. Assuming that tension is a direct measure of the amount of Ca^{2+} bound to troponin, calculations from our data show that a simple competition of Mg^{2+} and Ca^{2+} for the same site is unlikely. This is consistent with the finding that the low affinity site where Mg^{2+} binds does not appear to be the Ca^{2+} binding site on troponin, which activates contraction[7]. But it is possible that Mg^{2+} binding to a low affinity site, separate from that for Ca^{2+}, causes an allosteric change in the Ca^{2+} binding site on troponin. Perhaps the Mg^{2+} binding results in a decreased affinity of the troponin site for Ca^{2+}. This type of allosteric mechanism is supported by the finding that increasing Mg^{2+} to the mM range was associated with decreased binding of Ca^{2+} to rabbit myofibrils at a given Ca^{2+} concentration[16].

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