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TENSION IN MECHANICALLY DISRUPTED MAMMALIAN CARDIAC CELLS: EFFECTS OF MAGNESIUM ADENOSINE TRIPHOSPHATE

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SUMMARY

1. Maximum and submaximum Ca-activated tension in mechanically disrupted rat ventricular fibres was examined in solutions containing 30 μM, 100 μM and 4 mM-MgATP and either 50 μM or 1 mM ionized Mg.

2. In the absence of added Ca, significant amounts of base-line tension (up to 50% of maximum) develop in solutions containing less than 30 μM-MgATP. This effect is Mg-dependent; more tension is produced with 50 μM-Mg than with 1 mM.

3. Increasing the MgATP concentration shifts the pCa–% maximum tension relationship in the direction of increasing Ca required for activation. At 50 μM-Mg the pCa which produces 50% maximum tension is 5·8, 5·3 and 5·5 for the 30 μM, 100 μM and 4 mM-MgATP solutions. The effect of MgATP on position is relatively independent of the Mg concentration.

4. The steepness of the pCa–% maximum tension curve increases as MgATP is elevated to the millimolar range. The Hill coefficients for the different MgATP curves at 50 μM-Mg are 1·1, 1·3 and 3·0. This change in steepness accounts for the slightly lower Ca concentration needed for half-maximum tension as the MgATP concentration is increased to millimolar levels. Raising the Mg concentration to 1 mM greatly diminishes the effect of MgATP on the slope of the pCa–tension relationship.

5. The maximum tension a fibre bundle can produce decreases as the amount of MgATP is raised from micromolar to millimolar levels. For 50 μM-Mg, maximum tension drops about 35% as MgATP is raised from 30 μM to 4 mM. For any concentration of MgATP, maximum tension is higher at 1 mM-Mg than at 50 μM-Mg.

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6. Existing theories of interaction between myosin heads and the thin filament are sufficient to account for the effects of MgATP on the position of the pCa–tension curves and on maximum tension. The effects on slope are less satisfactorily explained.

INTRODUCTION

Classically, Mg adenosine triphosphate (MgATP) is thought to perform two major functions in muscle. Firstly, it provides the energy for the contractile process, and secondly, it acts as a plasticizing agent which maintains muscle extensibility (Weber & Murray, 1973). MgATP has also been shown to affect, at least indirectly, the activation process in skeletal muscle, that is, the interaction of Ca and the myofibrils that leads to the onset of contractile activity. For example, the relationship between Ca concentration and ATP hydrolysis for isolated myofibrils (Portzehl, Zaoralak & Gaudin, 1969; Weber, 1970) and tension generation in skinned skeletal muscle fibres (Brandt, Reuben & Grundfest, 1972; Godt, 1974) is dependent on the MgATP concentration. If MgATP concentrations are reduced to micromolar levels, tension generation by skinned crayfish muscle fibres (Reuben, Brandt, Berman & Grundfest, 1971), superprecipitation by actomyosin gels (Levy & Ryan, 1965; Weber & Herz, 1963) and myofibrillar ATPase activity (Weber & Herz, 1963; Weber, 1969) are no longer affected by changes in Ca concentration.

In order to determine whether MgATP has similar effects on the activation process in cardiac muscle, we have studied the relationship between Ca concentration and isometric tension in the presence of varying amounts of MgATP in mechanically disrupted rat ventricular fibres. Our results show that MgATP affects not only the position but also the slope of the pCa–% maximum tension curve in cardiac muscle. Further, maximum tension is inversely related to the MgATP concentration. Variations in the free Mg^{2+} ion concentration modified some of the effects of MgATP. These results are compared to the existing data from skeletal muscle, and their agreement with theories of contractile protein interactions are discussed. Preliminary reports of some of the data have appeared (Best & Kerrick, 1975).

METHODS

Preparation of tissue

Bundles of 'skinned' (sarcolemma disrupted) cardiac muscle fibres from male rats (200 ± 20 g) killed by a blow on the head were used. Following rapid removal of the hearts, a 1–3 mm^3 piece of left apical ventricular tissue was homogenized in a relaxing solution (pCa = 8*, see section on solutions for ionic constituents; *pX = $-\log$ [X], where the square brackets denote concentration of the ionic species X). Homogenization was performed with a Thomas Tissue Grinder for less

than 30 sec. After this treatment, much of the tissue was separated into bundles of fibres 1–3 mm long and 10–100 μm wide. The homogenized tissue was kept in a refrigerator when not in use.

The homogenization procedure mechanically disrupted the surface membranes (sarcolemmas) of the cells in the fibre bundles. This was done to remove the diffusion barrier created by the membranes so that the ionic composition of the fluids surrounding the contractile proteins could be accurately controlled. There is ample evidence from previous studies that this procedure does in fact disrupt the surface membranes. In the original description of this method, Fabiato & Fabiato (1972) demonstrated that intracellularly measured tansmembrane potentials of the fibre bundles were generally near zero. Data from their study, the investigation of Kerrick & Best (1974), and this paper clearly indicate that application of buffered Ca^{2+} solutions causes the fibre bundles to contract, suggesting that the sarcolemmas of the cells in the bundles are permeable to the Ca_2EGTA complex. Further, Kerrick & Best (1974) showed that chemical stimuli which would have hyperpolarized or had a negligible effect on the membrane potential of an intact cell could cause tension generation; in contrast stimuli which should have depolarized intact cells (high K^+ for instance) produced none. They interpreted their results as indicating the chemical stimuli acted on intracellular structures, and that if intact cells did exist in the fibre bundles their contribution to the observed tension transients was not significant. Furthermore, the time course of tension development and relaxation and the pCa–tension relationships obtained from studies using the cardiac preparation are directly comparable to data obtained using the conventional skinned frog skeletal muscle preparation (Kerrick & Best, 1974). Finally, electromicrographic studies show disruption of the cell membranes and indicate that extracellular ferritin diffuses freely into the myofibrillar space after homogenization (W. G. L. Kerrick, unpublished).

Measurement of isometric tension

The homogenization procedure did not disrupt the longitudinal integrity of the fibre bundles. The bundles were mounted in metal clamps in a photo-electric force transducer similar to the apparatus used by Hellam & Podolsky (1969) and employed in other studies from this laboratory (Kerrick & Donaldson, 1972; Gordon, Godt, Donaldson & Harris, 1973; Kerrick & Best, 1974).

The sensitivity of the transducer was 24 mV/mg. The voltage output was linear up to 1 g, which included the range of forces (10–50 mg) typically measured. The compliance was less than 1 μm/mg. Thus a 2 mm fibre bundle which produced 50 mg force would shorten only a few percent of its length and the contractions can be regarded as isometric. For a maximal contraction, the signal to noise ratio was better than 15:1.

The fibre bundle, which had been mounted slack in the transducer, was adjusted just taut with the micromanipulator. This was accomplished by stretching the fibre until an increase in tension was first noticed. The gain of the recorder was always at least twice the gain used during the course of an experiment while this procedure was being performed. A. M. Gordon & G. Pollack (personal communication) using a laser diffraction technique found that fibre bundles mounted in this manner produced diffraction patterns indicating sarcomere lengths of about 2·2 μm. When fibres broke during the course of an experiment the output of the transducer always fell to within a few millivolts of the base line, indicating that the fibres were not generating tension in the relaxing medium nor were they mounted under significant tension.

Solutions

The bathing solutions were prepared by mixing varying amounts of Na_2H_2ATP, Na_2CP, KCl, K_2H_2EGTA, $MgCl_2$, $CaCl_2$, imidazole and HCl. The amounts of stock reagents to be added for each solution were calculated using a computer programme which has been described elsewhere (Kerrick & Donaldson, 1972; Donaldson & Kerrick, 1975). Briefly, the computer solved the complex equilibria equations resulting from solutions containing ions and chelators using binding constants from the literature.

The desired ionic strength, buffer capacity, free concentrations of K, Ca, Mg, H^+, MgATP, CP (creatine phosphate), and total concentration of EGTA (ethyleneglycol-bis-(β-amino-ethylether)-N,N,N^1,N^1-tetra-acetic acid) were specified. The computer calculated the amount of each of the stock reagents to be added. Solutions were calculated to contain 7 mM-EGTA, 70 mM-K + Na, 15 mM-CP and varying amounts of MgATP, Mg, Ca, and imidazole (pH = 7·0). Ionic strength was maintained at 0·15 M. Creatine phosphokinase (CPK, 15 u./ml.) was added to each solution just before the start of an experiment. The creatine phosphate-creatine phosphokinase regenerating system was used to maintain a constant MgATP concentration. Experiments were performed at $20 \pm 1°$ C.

In an effort to check on the consistency of the chosen binding constants, the computer was programmed to calculate the amount of H^+ needed to bring each solution to pH = 7·0. When the solutions were mixed, the actual and calculated amounts of H^+ were compared. Agreement was always within 5%, which implies that the binding constants chosen were consistent with one another.

Ionic strength was kept constant since it is known to have a significant effect on tension generation in skinned skeletal (Gordon *et al.* 1973). Ionic strength was adjusted by varying the imidazole concentration rather than K concentration since K is known to bind to a site on actin (Martonosi, Molino & Gergely, 1964) and thus might cause variable effects if its concentration changed significantly. Imidazole is reported to have no effect on the ATPase activity of contractile proteins (Murphy & Koss, 1968). There are no significant differences in the imidazole concentrations between different curves relating pCa and tension.

Mg^{2+} is also known to significantly affect isometric tension generation (Donaldson & Kerrick, 1975). To determine whether the effects of Mg interacted with those of MgATP, two Mg concentrations were used: 50 μM and 1 mM. At each Mg concentration three series of solutions of varying Ca concentrations were mixed, one each at 30 μM, 100 μM, and 4 mM-MgATP. In addition, maximally activating Ca solutions were prepared at 2 mM-MgATP.

The mixed solutions and the CP and ATP stocks were kept frozen; other stocks were stored under refrigeration. Creatine phosphokinase was mixed fresh just before addition to the solution troughs.

The accuracy of solution mixing was routinely checked in several ways. Stock KCl, $MgCl_2$ & $CaCl_2$ and HCl solutions were tested for total Cl concentration using a chloridometer. The imidazole stock was checked by titrating a sample to its pKa with HCl of known molarity. Solutions for a particular set of Ca concentrations at a given Mg and MgATP concentration were mixed on at least two separate occasions. Any random mixing error or deterioration of stock solutions (particularly the ATP stock) would thus be apparent when the tensions produced by the solutions prepared at different times were compared.

Protocol

The test solutions were contained in 1 ml. troughs in a spring loaded plastic tray. During the course of an experiment, changes in the bathing solutions were

accomplished by compressing the springs, sliding a different solution filled trough under the fibre bundle, and then raising the tray to its original position. The solutions were covered with a thin layer of silicone oil (Dow Corning 200, 10 cm) to retard evaporation.

Two different protocols were used to collect data (Donaldson & Kerrick, 1975). In the 'stepping' protocol a fibre was placed in a relaxing solution and then transferred to a submaximally activating Ca solution. When a steady-state plateau of tension was developed the fibre was transferred to a solution which had been previously determined to be the maximally activating Ca solution for the particular Mg and MgATP concentrations being studied. Thus the fibre was stepped from one Ca solution to another without being relaxed. The % maximal tension was calculated by dividing the steady-state voltage output of the transducer at the submaximal activating Ca solution by the voltage output of a maximally activating Ca solution and multiplying by 100. It was assumed that tension had reached a plateau when no significant increase was seen for at least 20 sec, as estimated from the tension–time record or, in some experiments, read directly from a digital voltmeter connected in parallel to the recorder. Base-line tension was defined as a straight line joining the relaxation tension of consecutive records.

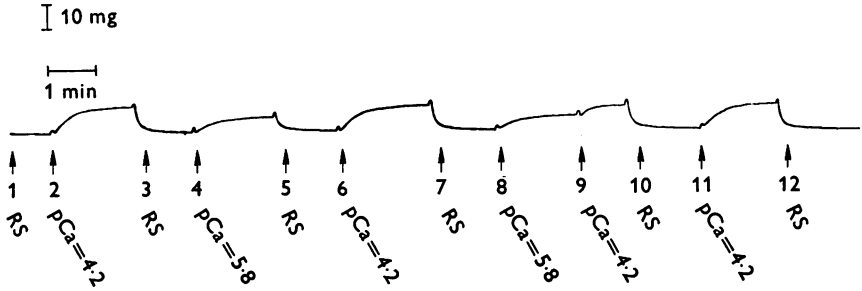

Fig. 1. Sample record of tension tracing. The Figure illustrates the two methods used to determine % maximum tension produced by a submaximally activating Ca solution. Numbered arrows indicate changes in the solution bathing the fibre bundle. All solutions contain 30 μM-MgATP and 50 μM-Mg as well as the indicated amount of free Ca. The relaxing solution (RS) has a pCa of approximately 8. The contractions beginning with arrows 2, 4 and 6 illustrate the bracketing protocol. Contraction of the fibre bundle in a submaximally activating Ca solution (pCa = 5·8, arrow 4) is preceded and followed by contraction in the maximally activating solution (pCa = 4·2, arrows 2 and 6). During the stepping protocol, a fibre bundle is contracted in a submaximal solution (arrow 8) and then in the maximally activating one (arrow 9) without being relaxed. Note that the data obtained from the two methods is very similar and that the maximum tension remains constant with time (compare the first and last contraction).

The fourth contraction in Fig. 1 shows an example of the stepping protocol used to determine the amount of tension produced by a solution containing pCa = 5·8 (50 μM-Mg, 30 μM-MgATP). At arrow 8 the fibre bundle was contracted in the submaximal Ca solution (pCa = 5·8) and when a plateau was reached, contracted again at arrow 9 in the maximally activating solution (pCa = 4·2 for this particular concentration of Mg and MgATP). The bundle was relaxed at arrow 10.

The second protocol used will be termed 'bracketing' since a contraction in a submaximal Ca solution was preceded and followed by one in a maximal Ca solution. This technique is illustrated by the first three contractions in Fig. 1. The fibre bundle was contracted in a maximally activating solution at arrow 2 and allowed to relax at arrow 3. At arrow 4 it was contracted in a submaximally activating solution, and it was then relaxed at arrow 5. The contraction in the maximally activating solution was repeated (arrows 6 and 7). The average of the tensions produced in the maximally activating solutions was used to calculate the percent of maximal tension produced in the bracketed submaximal contraction.

An examination of Fig. 1 reveals that the data obtained from the bracketing and stepping techniques are very similar. In this particular case the % maximal tension produced by the pCa = 5·8 solution was 59% using either protocol. It should also be noted that maximal tension did not decrease substantially with time as can be seen by comparing the first contraction (arrow 2) with the last in the sequence (arrow 11).

Most of the data for the pCa–% maximum tension relationships were collected using the stepping technique, since it required the least time to collect each datum. The effect of varying Mg and MgATP on the maximal tension produced by the fibre bundles was studied using the bracketing technique so that the Mg and MgATP concentration would be constant throughout one contraction–relaxation cycle.

The Ca^{2+} concentration which produced maximum tension was determined for each combination of Mg and MgATP at the beginning of each series of experiments. The pCa–% maximum tension relationships and the maximum tension comparisons for each Mg and MgATP combination were compiled from pooled data taken from experiments using tissue from at least two hearts with at least two fibre bundles used from each heart. The significance of shifts in the pCa–% maximum tension relationships with varying MgATP concentrations was checked by sequentially contracting the same fibre bundle in solutions of identical pCa but different MgATP concentration. Consistent differences in the amount of % maximum tension produced were taken as an indication that the shifts between pCa–% maximal tension curves which were determined in separate experiments on different fibre bundles were real.

Usually fibres could be contracted for many hours with no significant decline in the amount of maximum tension produced. However, tensions did decline occasionally (less than a fifth of the fibres studied) usually after several hours of experimentation. Fibres were discarded if maximum tension dropped below 50% of the initial value. Even when the maximum tension declined, the fibres continued to give values of % maximum tension at a given pCa which were not significantly different from those obtained before the decline. Tensions would sometimes rise to a peak and then decline to a plateau after being placed in a maximally activating Ca solution. Such responses could have been due to Ca-activated Ca release from the sarcoplasmic reticulum (Ford & Podolsky, 1970; Endo, Tanaka & Ogawa, 1970; Kerrick & Best, 1974). To alleviate possible effects of the sarcoplasmic reticulum, all tension measurements were made after a steady state was reached.

Analysis

The pCa *vs.* % maximum tension data were plotted using the following linearized form of the Hill equation (Hill, 1910, 1913):

$$\log_{10} (\%T/(100-\%T)) = n \log_{10} [\mathrm{Ca}] - \log_{10} Q,$$

where $\%T$ is % maximum tension, the square brackets denote concentration, and n and Q are constants. The individual values of % maximum tension produced by

varying Ca concentration and a particular combination of Mg and MgATP were fit by computer with a straight line using a linear regression technique. Values of n and Q thus derived were used to regenerate smooth sigmoid pCa–% tension curves of the form:

$$\%T = ([\mathrm{Ca}]^n/(Q + [\mathrm{Ca}]^n)) \times 100.$$

When the data from this study are plotted linearly, the values of % maximum tension less than 10% or greater than 90% (and their errors) are more heavily weighted than values near 50% (Colquhoun, 1971). To circumvent this problem we have used only % maximum tension data between 10 and 90% when constructing Hill plots to determine n values. As a check on this procedure, some curves were fit using a non-linear least-squares technique. The results obtained did not differ significantly from those obtained with the linear fitting procedure.

RESULTS

Base-line tension

During preliminary experiments, it was determined that significant amounts of base-line tension were developed in solutions of extremely low Ca ion concentration (10^{-8} M) when the MgATP concentration was decreased to below 30 μM. The magnitude of his effect at a particular level of MgATP is dependent on the free Mg concentration. Base-line shifts occur in 20 μM-MgATP at a Mg concentration of 50 μM while no shift is seen at 1 mM-Mg. Large base-line shifts (up to 50% maximum tension) are seen in solutions containing 10 μM-MgATP even in the presence of 1·0 mM-Mg. These results are consistent with the data of Reuben *et al.* (1971) who studied skinned crayfish fibres and found in the absence of added Ca a similar Mg dependent development of tension. We did not investigate this effect in detail, and chose to study 30 μM as our lowest concentration of MgATP, since increases in base-line tension do not occur at this concentration even at low levels of Mg.

Effects of MgATP on the pCa vs. % maximum tension relationship

Changes in the amount of MgATP in the bathing solutions had two effects on the pCa–% maximum tension relationship. Firstly, the steepness of the relationship increases as the MgATP concentration is raised from 100 μM to millimolar levels. This effect is modified by the concentration of Mg present. Secondly, increasing MgATP from 30 to 100 μM shifts the curve to the right along the pCa axis so that more Ca is needed to produce the same % maximum tension.

In order to quantify variations in the slope of the pCa–% maximum tension curves the data were first plotted using a linearized form of the Hill equation (see Methods section). Fig. 2 shows the tension data for 30 and 100 μM-MgATP (50 μM-Mg) replotted in this fashion. The slope and intercepts of these lines were then used to generate the sigmoid curves

seen in Fig. 3. Increases in the slope (n) of linear plots correspond to steeper pCa–% maximum tension curves. The slopes (n) and intercepts (Q) for all the data are listed in Table 1.

The information in Table 1 indicates that at 50 μM-Mg, n is not appreciably different from 1 for either 30 or 100 μM-MgATP. However, when the MgATP is raised to 4 mM the value of n increases dramatically and is

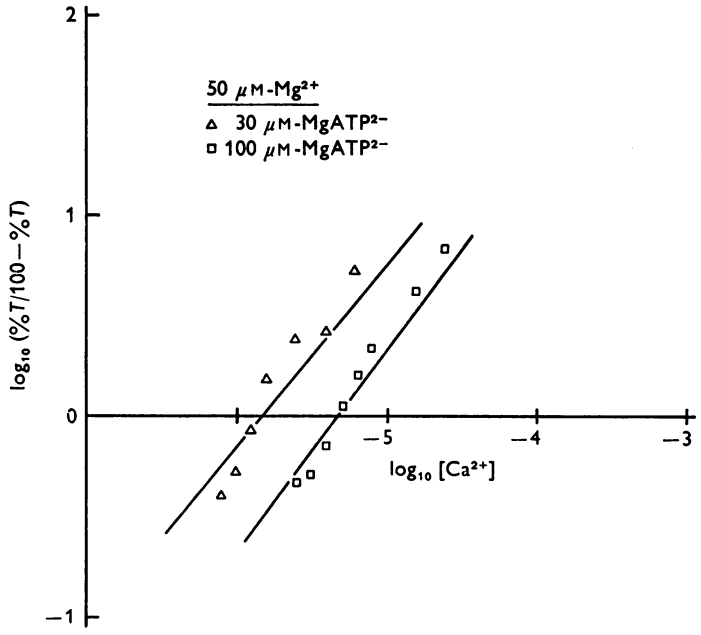

Fig. 2. Hill plots of pCa–% maximum tension data. The lines were fit with a linear least-squares technique applied to the individual data points. Only the averaged data of each value of pCa are shown here. The slopes and intercepts (see Table 1) are used to generate the sigmoid curves seen in Fig. 3*A*.

significantly greater than 1 ($P < 0{\cdot}005$). Correspondingly, the pCa–% tension relationships at 4 mM-MgATP is much steeper than those at 30 and 100 μM (compare Figs. 3 and 4). When the Mg concentration is increased to 1 mM, however, the increase in slope with increasing MgATP is less dramatic (Table 1).

It is also apparent that values of n are generally higher at the low Mg concentration (50 μM) than at high Mg (1 mM). This inverse effect of Mg concentration on slope has also been seen in skinned skeletal fibres (Donaldson & Kerrick, 1975) as well as in heart muscle (Kerrick & Donaldson, 1975).

The effect of raising the MgATP concentration from 30 to 100 μM on the position of the pCa–tension curves in the presence of 50 μM-Mg is shown in Fig. 3*A*. The pCa necessary for 50% activation is 5·8 for the 30 μM curve and 5·3 for the 100 μM curve. This half p-unit shift corresponds to approximately a threefold increase in the Ca concentration needed for half activation at the higher MgATP. Increasing the MgATP concentration further to 4 mM does not produce an additional shift to the right in the curve (Fig. 4*A*) although the steepness of the relationship does increase as described previously. The 50% point for this curve is 5·5. It is clear

TABLE 1. Mid-points, Hill coefficients and constants for pCa–tension curves

	30 μM-MgATP			100 μM-MgATP		
	Mid-point	$n \pm$ *	Q	Mid-point	$n \pm$ *	Q
50 μM-Mg	5·8	1·1 ± 0·1	$10^{-6·4}$	5·3	1·3 ± 0·1	$10^{-6·8}$
1 mM-Mg	5·4	1·2 ± 0·1	$10^{-6·5}$	5·0	1·0 ± 0·1	$10^{-5·2}$

	4 mM-MgATP		
	Mid-point	$n \pm$ *	Q
50 μM-Mg	5·5	3·0 ± 0·1	$10^{-16·5}$
1 mM-Mg	5·1	1·9 ± 0·1	$10^{-9·6}$

* Sample standard deviation of n.

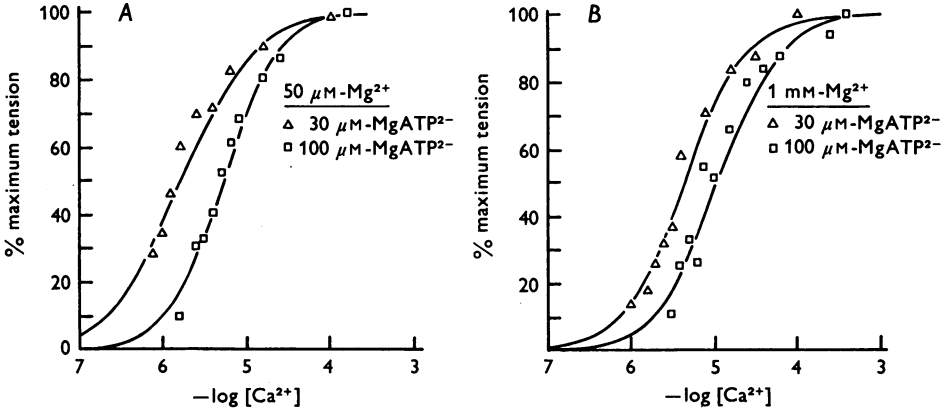

Fig. 3. Ca concentrations–% maximum tension relationship for 30 and 100 μM-MgATP and 50 μM-Mg (panel *A*) or 1 mM-Mg (panel *B*). Sigmoid curves generated with the Hill equation (see text) using individual data points. Plotted points are averages. See Tables 2 and 3 for values of the mean ± S.E. of mean and sample size for each point.

that in the presence of 50 μM-Mg the largest effect of varying MgATP concentration on the position of the pCa–maximum tension relationship occurs between 30 and 100 μM.

Increasing the Mg concentration to 1·0 mM does not qualitatively alter the effects of MgATP on the position of the pCa–% tension curve. Increasing MgATP from 30 to 100 μM in the presence of 1 mM-Mg results in a shift to the right in the curve (Fig. 3*B*) such that the 50 % points change from about 5·4 to 5·0. Further increases in MgATP to millimolar levels have no additional effect (Fig. 4*B*).

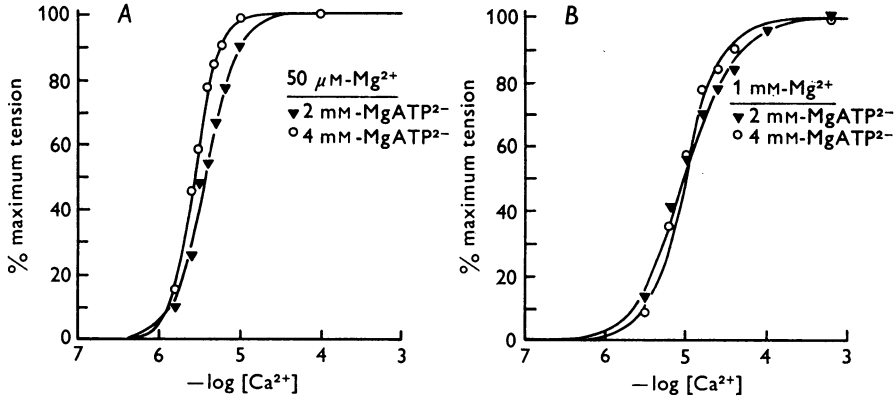

Fig. 4. Ca concentration–% maximum tension relationship for 2 and 4 mM-MgATP and 50 μM or 1 mM-Mg. For details see legend for Fig. 3 and text. Data and curve for 2 mM-MgATP are replotted from Kerrick & Donaldson (1975).

For any given MgATP concentration the pCa–tension curves are shifted to the right (less sensitive to Ca concentration) along the pCa axis as Mg concentration is increased from 50 μM to 1 mM (comparison of Fig. 3*A* and 3*B* with Fig. 4*A* and 4*B*). This shift is consistent with the well documented inverse effect of increasing Mg concentration on Ca-activated tension generation in heart and skeletal muscle fibres (Donaldson & Kerrick, 1975; Kerrick & Donaldson, 1975).

The 2 mM-MgATP curves in Fig. 4*A* and 4*B* are taken from an earlier study from this laboratory (Kerrick & Donaldson, 1975). They are replotted here to show that the effects on slope and position are present at 2 mM-MgATP and are not altered when the MgATP is increased to 4 mM. The means ± S.E. of mean and sample size for the data points in Figs. 3 and 4 are listed in Tables 2 and 3.

TABLE 2. Comparison of % maximum tension at 50 μM-Mg and varying concentrations of MgATP and Ca

pCa	30 μM-MgATP	100 μM-MgATP	4 mM-MgATP
6·1	24 ± 2·6 (7)*	—	—
6·0	29 ± 1·8 (6)	—	—
5·9	47 ± 4·7 (7)	—	—
5·8	61 ± 3·2 (7)	9 ± 0·9 (6)	15 ± 1·9 (12)
5·6	71 ± 1·6 (7)	31 ± 3·2 (7)	45 ± 2·3 (12)
5·5	—	32 ± 2·9 (7)	58 ± 3·4 (12)
5·4	73 ± 1·9 (7)	40 ± 2·7 (7)	77 ± 2·1 (12)
5·3	—	53 ± 2·0 (7)	84 ± 1·1 (12)
5·2	84 ± 3·0 (7)	62 ± 2·0 (9)	90 ± 0·9 (12)
5·1	—	68 ± 1·9 (7)	—
5·0	—	—	98 ± 0·9 (4)
4·8	91 ± 3·6 (7)	81 ± 2·2 (7)	—
4·6	—	87 ± 1·0 (7)	—
4·2	100	—	—
4·0	—	—	100
3·8	—	100	—

* Mean ± S.E. of mean (sample size).

TABLE 3. Comparison of % maximum tension at 1 mM-Mg and varying concentrations of MgATP and Ca

pCa	30 μM-MgATP	100 μM-MgATP	4 mM-MgATP
6·0	17 ± 3·9 (8)*	—	—
5·8	18 ± 2·9 (8)	—	—
5·7	26 ± 2·6 (8)	—	—
5·6	32 ± 3·1 (8)	—	—
5·5	37 ± 3·6 (7)	11 ± 1·0 (7)	9 ± 3·3 (4)
5·4	58 ± 4·7 (11)	25 ± 2·6 (9)	—
5·3	—	33 ± 3·0 (9)	—
5·2	—	26 ± 1·0 (8)	35 5·1 (11)
5·1	71 ± 3·3 (8)	55 ± 1·0 (11)	—
5·0	—	52 ± 1·4 (5)	57 ± 4·2 (11)
4·8	84 ± 3·2 (5)	66 ± 6·8 (5)	77 ± 2·6 (11)
4·6	—	80 ± 2·1 (7)	84 ± 2·4 (10)
4·5	88 ± 1·6 (8)	—	—
4·4	—	84 ± 2·7 (10)	90 ± 2·5 (2)
4·2	—	88 ± 5·6 (8)	—
4·0	100	—	—
3·8	—	—	100
3·6	—	94 ± 2·2 (4)	—
3·4	—	100	—

* Mean ± S.E. of mean (sample size).

The effect of varying MgATP on maximum tension

Increasing the amount of MgATP in the bathing solution from the micromolar to the millimolar range decreases the maximum tension produced by a fibre bundle. Table 4 shows the averaged data from a number of experiments in which the maximum tension produced by fibres at the specified Mg and MgATP concentration is normalized to the maximum tension produced by the same fibres in a reference solution (50 μM-Mg, 30 μM-MgATP). For 50 μM-Mg the maximal tension drops approximately 15% in a solution containing 100 μM-MgATP as compared to that containing 30 μM-MgATP and decreases further for a total drop of about 35% when the MgATP concentration of the solution is raised to 2 mM. There is no appreciable difference in the maximum tension produced in the 2 mM and 4 mM-MgATP solutions.

TABLE 4. Comparison of maximum tensions at different concentrations of MgATP and Mg*

	30 μM-MgATP	100 μM-MgATP	2 mM-MgATP	4 mM-MgATP
50 μM-Mg	100	85·2 ± 1·7 (10)	65·0 ± 2·4 (5)	62·5 ± 1·1 (5)
1 mM-Mg	109·4 ± 2·5 (9)	86·9 ± 4·5 (9)	79·5 ± 2·1 (6)	75·9 ± 2·2 (6)

* Means of maximum tension ± S.E. of mean (sample size) normalized to maximum tension produced at 50 μM-Mg and 30 μM-MgATP.

A similar pattern is seen for the solutions with 1 mM-Mg. Maximum tension (again expressed as % tension produced in the reference solution) decreases about 25% as the MgATP level is raised to 2 mM. Again, there is no appreciable difference in the maximum tensions produced in the 2 and 4 mM solutions.

It should also be noted that at the higher Mg concentrations (1 mM) the maximum tension at a given concentration of MgATP is increased over that at the lower Mg concentration (50 μM). The calcium concentrations necessary to produce maximum tension for each combination of Mg and MgATP can be found in Tables 2 and 3.

DISCUSSION

The results show three effects of increasing MgATP concentrations from 30 μM to 4 mM on isometric tension generation in cardiac muscle: (1) a decrease in the maximum isometric tension the fibres can develop, (2) a shift in the pCa–% maximum tension relationship in the direction of increasing Ca concentrations required for activation and tension generation, and (3) an increase in the steepness of the pCa–% maximum tension

relationship. The existence and magnitude of these effects is dependent on the free Mg concentration and the range through which the MgATP concentrations are varied. These results differ in some aspects from previous studies on skeletal muscle and place constraints on proposed interpretations of contractile protein interactions.

Maximum tension

The data presented in this paper indicate an inverse relationship between MgATP and maximum tension which saturates when the concentration of MgATP is 2 mM or higher. The data from skeletal muscle on the effects of MgATP on maximum tension are contradictory. Godt (1974) and Brandt *et al.* (1972) found maximum tension to be relatively independent of MgATP concentration in skinned frog and crayfish skeletal fibres at a constant Mg concentration. However, Magid (1974) and Chaplain & Gergs (1974) report results from skinned frog fibres and glycerinated psoas muscle that are similar to our own.

An increase in maximum Ca-activated tension could be explained by either (1) more bridges being made at any instant or (2) more force being produced by each bridge at low MgATP concentrations. Chaplain & Gergs (1974) report X-ray diffraction studies which show an increase in the relative ratio of intensities of 1,1 and 1,0 layer lines in rabbit psoas muscles at maximally activating Ca concentration and low MgATP. Changes in the intensities of these layer lines have been interpreted as resulting from mass transfers from thick to thin filaments (Huxley, 1968). These results support the contention that the number of actomyosin linkages increases at low MgATP levels and thus leads to the increase in maximal force. We know of no data to suggest variation in the force per bridge.

Ca sensitivity and shape of the pCa–tensions relationship

Previous investigators have studied the effects of MgATP on tension generated by skinned skeletal fibres from frog (Godt, 1974) and crayfish (Brandt *et al.* 1972) and on skinned rat cardiac cells (Fabiato & Fabiato, 1975). In addition to differences in species and tissue used, these earlier studies also employed different ionic conditions. However, our data agree qualitatively with the effects of MgATP on the position of the pCa–% maximum tension curve previously reported. Biochemical data suggest a similar activating effect of low MgATP concentrations on the ATPase rate of myofibrils. According to Weber (1970) the Ca concentration necessary for 50% activation of rabbit myofilament ATPase decreases almost one order of magnitude as MgATP is decreased from 2 mM to 20 μM.

In this study, increasing MgATP concentrations to millimolar levels

produced increases in the steepness of the pCa–tension curves that were more dramatic at 50 μM-Mg than at 1 mM-Mg. Contradictory results exist in the literature. Kerrick & Donaldson (1975) report Hill n values for skinned skeletal fibres and mechanically disrupted heart cells contracted in the presence of 2 mM-MgATP and either 2 mM or 50 μM Mg that are similar to those reported here. Furthermore, the data of Godt (1974) & Brandt *et al.* (1972) show little change in the shape of the saturation curves with changes in MgATP in the presence of high Mg concentration. However, Solaro, Wise, Shiner & Briggs (1974) studied tension generation in chemically skinned strips of canine papillary muscle in the presence of millimolar MgATP and Mg and found evidence for cooperative interactions. Biochemical studies on isolated proteins indicate that troponin contains two classes of independent calcium binding sites (Fuchs & Briggs, 1968; Bremel & Weber, 1971; Potter & Gergely, 1975) which do not show cooperative interactions.

Bremel & Weber (1972) have suggested an explanation for the activating effect of low MgATP concentration. They present evidence that the formation of rigour bridges (actomyosin linkages free of bound nucleotides) affects the inhibitory properties of troponin molecules. They suggest that the presence of rigour bridges 'turns on' adjacent actin molecules in a cooperative manner making them receptive to S-1 binding just as if Ca were bound to troponin. Further, the presence of rigour bridges at low MgATP levels can affect the affinity of the Ca binding sites on troponin. Specifically, when the number of rigour complexes reaches a critical level, the Ca binding affinity of the two low affinity sites (which when complexed with Ca causes activation of the system) is raised. Bremel & Weber report that this effect is mediated through co-operative effects involving the tropomyosin molecule. These results offer a plausible explanation for the shift in the pCa–% maximum tension relationship reported in this study (Godt, 1974; Fabiato & Fabiato, 1975). At low MgATP levels (30 μM), rigour bridges could cause the transformation of low affinity sites to high affinity ones. Thus less calcium would be needed for activation and the pCa–tension relationship would be shifted to the left.

Could a scheme involving actomyosin linkages also explain the change in steepness seen at millimolar MgATP concentrations? It has been suggested (Weber & Murray, 1973) that when the affinity of the low affinity Ca binding sites is raised and becomes comparable to the two high affinity sites also found on troponin the system changes from a second power to a fourth power switch. If this were true, one would expect the saturation curves at low MgATP to be steeper than those at high MgATP. Such a scheme is not consistent with our data which show an increase in steepness at high MgATP.

Assuming that the % maximum tension is an indication of the fraction of activating Ca^{2+} binding sites on troponin saturated with ligand, several possible binding schemes can be modelled and used to predict the shape of the pCa–% maximum tension relationship. We have used the Hill equation since simpler binding schemes which assumed one and two classes of independent sites did not adequately describe all the data. Changes in the Hill n value (Hill $n > 2$ assuming two activating sites, Weber & Murray, 1973) may indicate variations in the interaction (co-operativity) of non-independent Ca^{2+} binding sites assuming the number of sites is constant. However, it is not possible to establish unique mechanisms for cooperative effects from saturation curves (Koshland, Nemethy & Filmer 1966). If the assumption that tension is a precise measure of Ca^{2+} binding to activating sites on troponin is incorrect, then the increase in steepness noted may result from elements in the system other than the Ca^{2+} binding sites on troponin. It should be noted that raising the Mg concentration from 50 μM to 1 mM has only a slight effect on the change in position of the pCa–tension curves but noticeably diminishes the change in slope caused by varying MgATP concentration. This indicates that the two effects result from different mechanisms, which suggests the involvement of elements in the system other than troponin.

Another possible site of action of MgATP is interaction with Ca binding to the myosin molecule. Myosin is known to bind MgATP (Weber & Murray, 1973) and recent studies suggest the existence of a Ca binding site on myosin which has physiological importance. Ca sensitivity in certain invertebrate muscles is found on myosin (Lehman & Szent-Györgyi, 1975) and recently Bremel (1974) has shown a calcium activation system on myosin in vertebrate smooth muscle. Huxley (1972) suggested that calcium might cause movements of myosin heads during activation in skeletal muscle. This conclusion was based on X-ray diffraction studies at long sarcomere lengths which showed movement of heads in the presence of Ca and no filament overlap. Biochemical evidence suggests that vertebrate skeletal muscle myosin binds calcium (Morimoto & Harrington, 1974; Bremel & Weber, 1975). This binding takes place at physiological levels of Ca and Mg and affects the physical characteristics of the myosin molecule. The possibility exists, then, that Ca binding to myosin may play a physiological role in skeletal muscle activation. If this is the case, some effects of varying MgATP levels on Ca-activated tension generation could arise from the interaction of MgATP and Ca binding sites on the thick filaments.

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REFERENCES

BEST, P. M. & KERRICK, W. G. L. (1975). Effects of magnesium adenosine triphosphate on submaximal calcium activated tension in skinned cardiac cells. *Biophys. J.* **15**, 153*a*.

BRANDT, P. W., REUBEN, J. P. & GRUNDFEST, H. (1972). Regulation of tension in the skinned crayfish muscle fiber. II. Role of calcium. *J. gen. Physiol.* **59**, 305–317.

BREMEL, R. D. (1974). Myosin linked calcium regulation in vertebrate smooth muscle. *Nature, New Biol.* **252**, 405–407.

BREMEL, R. & WEBER, A. (1971). The role of myosin in relaxation and activation of contraction. *Biophys. J.* **11**, 237*a*.

BREMEL, R. D. & WEBER, A. (1972). Cooperation within actin filaments in vertebrate skeletal muscle. *Nature, New Biol.* **238**, 97–101.

BREMEL, R. D. & WEBER, A. (1975). Calcium binding to rabbit skeletal myosin under physiological conditions. *Biochim. biophys. Acta* **376**, 366–374.

CHAPLAIN, R. A. & GERGS, U. (1974). Calcium and ATP dependent changes in myosin mass distribution of glycerinated rabbit psoas muscle. *Biochem. biophys. Res. Commun.* **61**, 467.

COLQUHOUN, D. (1971). *Lectures on Biostatistics.* Oxford: Clarendon.

DONALDSON, S. K. B. & KERRICK, W. G. L. (1975). Characterization of the effects of Mg^{2+} on Ca^{2+} and Sr^{2+}-activated tension generation of skinned skeletal muscle fibers. *J. gen. Physiol.* **66**, 427–444.

ENDO, M., TANAKA, M. & OGAWA, Y. (1970). Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibers. *Nature, Lond.* **228**, 34–36.

FABIATO, A. & FABIATO, F. (1972). Excitation-contraction coupling of isolated cardiac fibers with disrupted or closed sarcolemmas. *Circulation Res.* **21**, 293–307.

FABIATO, A. & FABIATO, F. (1975). Effect of magnesium on contractile activation of skinned cardiac cells. *J. Physiol.* **249**, 497–517.

FORD, L. E. & PODOLSKY, R. J. (1970). Regenerative calcium release within muscle cells. *Science, N.Y.* **167**, 58–59.

FUCHS, F. & BRIGGS, F. N. (1968). The site of calcium binding in relation to the activation of myofibrillar contraction. *J. gen. Physiol.* **51**, 655–676.

GODT, R. (1974). Calcium-activated tension of skinned muscle fibers of the frog. Dependence on magnesium adenosine triphosphate concentration. *J. gen. Physiol.* **63**, 722–739.

GORDON, A. M., GODT, R. E., DONALDSON, S. K. B. & HARRIS, C. E. (1973). Tension in skinned frog muscle fibers in solutions of varying ionic strength and neutral composition. *J. gen. Physicl.* **62**, 550–574.

HELLAM, D. C. & PODOLSKY, R. J. (1969). Force measurements in skinned muscle fibres. *J. Physiol.* **200**, 807–819.

HILL, A. V. (1910). The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol.* **40**, 4–7*P*.

HILL, A. V. (1913). The combinations of haemoglobin with oxygen and with carbon monoxide. I. *Biochem. J.* **7**, 471.

HUXLEY, H. E. (1968). Structural difference between resting and rigor muscle; evidence from intensity changes in the low angle equatorial X-ray diagram. *J. molec. Biol.* **37**, 507–520.

HUXLEY, H. E. (1972). Molecular basis of contraction in cross striated muscle. In *The Structure and Function of Muscle*, vol. 1, ed. BOURNE, G. H. New York: Aeademic Press.

KERRICK, W. G. L. & BEST, P. M. (1974). Calcium ion release in mechanically disrupted heart cells. *Science, N.Y.* **183**, 435–437.

KERRICK, W. G. L. & DONALDSON, S. K. B. (1972). The effects of Mg^{2+} on submaximal Ca^{2+}-activated tension in skinned fibers of frog skeletal muscle. *Biochim. biophys. Acta* **275**, 117–122.

KERRICK, W. G. L. & DONALDSON, S. K. B. (1975). Comparative effects of Ca^{2+} and Mg^{2+} on tension generation in the fibers of skinned frog skeletal muscle and mechanically disrupted rat heart muscle. *Pflügers Arch. ges. Physiol.* **358**, 195–201.

KOSHLAND, D., NEMETHY, G. & FILMER, D. (1966). Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry, N.Y.* **5**, 365–385.

LEHMAN, W. & SZENT-GYÖRGYI, A. G. (1975). Regulation of muscular contraction. Distribution of actin control and myosin control in the animal kingdom. *J. gen. Physiol.* **66**, 1–30.

LEVY, H. M. & RYAN, E. M. (1965). Evidence that calcium activates the contraction of actomyosin by overcoming substrate inhibition. *Nature, Lond.* **205**, 703–705.

MAGID, A. D. (1974). The Relationship of MgATP to Force Generation in Striated Muscle. Ph.D. Dissertation, University of Washington.

MARTONOSI, A., MOLINO, C. M. & GERGELY, J. (1964). The binding of divalent cations to actin. *J. biol. Chem.* **239**, 1057–1064.

MARUYAMA, K. & WEBER, A. (1972). Binding of adenosine triphosphate to myofibrils during contraction and relaxation. *Biochemistry, N.Y.* **11**, 2990–2998.

MORIMOTO, K. & HARRINGTON, W. (1974). Evidence for structural changes in vertebrate thick filaments induced by Ca^{2+}. *J. molec. Biol.* **88**, 693.

MURPHY, R. A. & KOSS, P. G. (1968). Hydrogen ion buffers and enzymatic activity: myosin B. adenosinetriphosphatase. *Archs Biochem. Biophys.* **128**, 236–242.

PORTZEHL, H., ZAORALEK, P. & GAUDIN, J. (1969). The activity by Ca^{2+} of the ATPase of extracted muscle fibrils with variation of ionic strength, pH, and concentration of MgATP. *Biochim. biophys. Acta* **189**, 440–448.

POTTER, J. D. & GERGELY, J. (1975). The calcium and magnesium binding sites on troponin and their role in the regulation of myofibrillar ATPase. *J. biol. Chem.* **250**, 4628–4633.

REUBEN, J. P., BRANDT, P. W., BERMAN, M. & GRUNDFEST, H. (1971). Regulation of tension in the skinned crayfish muscle fiber. I. Contraction and relaxation in the absence of Ca (pCa > 9). *J. gen. Physiol.* **57**, 385–407.

SOLARO, R. J., WISE, R. M., SHINER, J. S. & BRIGGS, F. N. (1974). Calcium requirements for cardiac myofibrillar activation. *Circulation Res.* **34**, 525–530.

WEBER, A. (1969). Parallel response of myofibrillar contraction and relaxation to four different nucleoside triphosphates. *J. gen. Physiol.* **53**, 781–791.

WEBER, A. (1970). The dependence of relaxation on the saturation of myosin with adenosine triphosphate. In *The Physiology and Biochemistry of Muscle as a Food*, vol. 2, ed. BRISKEY, E. J., CASSENS, R. G. & MARSH, B. B. Madison, Wisconsin: University of Wisconsin Press.

WEBER, A. & HERZ, R. (1963). Requirement for calcium in the syneresis of myofibrils. *Biochem. biophys. Res. Commun.* **6**, 364.

WEBER, A. & MURRAY, J. M. (1973). Molecular control mechanisms in muscle contraction. *Physiol. Rev.* **53**, 612–673.