Nonparallel Isometric Tension Response of Rabbit Soleus Skinned Muscle Fibers to Magnesium Adenosine Triphosphate and Magnesium Inosine Triphosphate

BRIAN KRASNER

From the Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98105. Dr. Krasner's present address is Harvard Medical School, Boston, Massachusetts 02115.

ABSTRACT The isometric tension response of single "skinned" rabbit soleus muscle fibers to MgATP and MgITP in the absence of calcium was studied. [MgATP] or [MgITP] was varied in solutions of ionic strength 0.30 and temperature 20°C. Steady-state tension that developed in MgATP or MgITP solutions was a biphasic bell-shaped function of log [MgATP] or log [MgITP] which increased from zero to maximum tension and then declined again to zero. Analysis of the data showed that, under comparable ionic conditions, percent tension vs. log [MgATP] and percent tension vs. log [MgITP] curves are not parallel. Instead, the percent tension vs. log [MgITP] curve is much broader. Additionally, under comparable ionic conditions maximum tension in MgITP solutions was higher than in MgATP solutions. In addition, in MgATP solutions, pH, [K⁺], and excess ATP were varied. Raising pH from 7 to 8, [K⁺] from 46 mM to 200 mM, or decreasing excess ATP from 2 to 0.5 mM all increased maximum tension. None of these factors, however, influenced the shape or position of the percent tension vs. log [MgATP] curve.

It has long been accepted that MgATP is the energy source for contraction. Evidence has also accumulated indicating that MgATP plays an important role in the regulation of contraction (Weber and Murray, 1973; Inoue and Tonomura, 1974; Godt, 1974; Orentlicher et al., 1977). In an attempt to better understand the dual role of ATP in contraction, several researchers have studied the effects on contraction of replacing ATP in contracting solutions with other nucleotides such as ITP, UTP, and GTP (Weber, 1969; Reuben et al., 1971). Two measures associated with muscle contraction have been used, hydrolysis of nucleotide by myosin and isometric tension development by skinned muscle fibers. An implied assumption in these nucleotide replacement studies is that the muscle will use and react to these nucleotides in an analogous fashion, and that differences in muscle response reflect quantitative but not qualitative differences in the rate processes governing muscle contraction.

From the studies examining nucleotide hydrolysis by rabbit myofibrils and...
regulated actomyosin in the absence of calcium, an important concept has emerged that ATP, ITP, UTP, and GTP not only act in an analogous manner but also in a parallel manner during contraction. Specifically, it has been proposed that the hydrolysis data indicate that all of the nucleotides produce a bell-shape hydrolysis vs. log [MgNTP] curve with activating and inhibiting phases by saturating a single site on the myosin, and that shifts in the curve are accounted for by varying affinities of the nucleotides for that site (Weber, 1969; Weber and Murray, 1973). Nucleotide hydrolysis data in vertebrate myofibrillar preparations do indicate that the nucleotides act in a largely parallel fashion, though the possibility of small but significant deviations from parallel action is not definitely excluded. Additionally, the myofibrillar contractile tension response to different nucleotides is not clearly known.

Reuben et al. (1971), after studying tension in skinned crayfish fibers, made another detailed comparison of contraction with ATP, ITP, UTP, and GTP. But their data certainly did not indicate a parallel response of the fibers to the different nucleotides indicating the role of saturation of a single site. Indeed, they interpreted their data as evidence for two sites. However, crayfish are not vertebrates and the lack of parallelism in this case does not directly contradict the idea of a parallel response of vertebrate muscle fibers to different nucleotides.

Thus, in this study a detailed comparison of tension vs. log [MgNTP] curves for rabbit soleus muscle fibers with ATP and ITP was performed in order to test, using tension as a measure, the idea of a parallel response of vertebrate muscle fibers to different nucleotides. It will be shown that in fact the tension responses are not parallel.

**METHODS**

**Preparation**

Red soleus muscle fibers from 2–4-kg female rabbits were used in this study. Segments of single fibers were dissected from the muscle while the muscle was soaked in a solution of 2 mM MgATP and 7 mM EGTA and no calcium (pCa 8), pH 7, and ionic strength 0.15. This solution has been shown to be effective in relaxing skinned muscle fibers (Hellam and Podolsky, 1969).

The main method for removing the sarcolemma of the muscle fiber was the method of Natori (1954). In this method, the fiber is held by its end and the membrane is peeled with a fine needle. The secondary method was that of grinding pieces of muscle as described in a recent paper by Kerrick and Krasner (1975).

**Force Measurement**

Skinned muscle fibers will contract and generate isometric tension when exposed to solutions containing micromolar concentrations of MgATP or MgITP and "no" calcium (pCa >9). In order to measure the isometric tension produced by the skinned fibers, I mounted the fibers in the forceps tips of a photoelectric force transducer similar to that used by Hellam and Podolsky (1969). The two transducers used in most of the data collection in this study had compliances of 1 and 0.9 mm/g, respectively. The fibers were mounted in the transducer forceps and stretched until all the slack was taken up, which was generally accompanied by a very small amount
of resting tension. Fibers stretched this way had a sarcomere length of 2.2-2.7 μm as measured by either phase-contrast microscopy or laser diffraction.

**Solutions**

Solutions bathing the fiber were changed as described by Kerrick and Donaldson (1972). No calcium was added to any solutions and solutions were designed to minimize [Ca++]}. [MgATP] was the main variable in the ATP solutions, varying from 0.1 to 10 μM. There were three types of ATP solutions. The ionic compositions of these solutions were:

(a) pH 7, [K+] 200 mM, [ATP]t (total concentration of ATP) 0.5 mM, [EDTA]t (total concentration of EDTA) 15 mM, enough imidazole to bring the pH to 7, enough imidazole propionate (~50 mM) to make the ionic strength 0.30, enough potassium propionate (~150 mM) to make [K+] 200 mM, and varying amounts of magnesium to vary [MgATP].

(b) pH 8, [K+] 200 mM, [ATP]t 0.5 mM, [EDTA]t 15 mM, enough Tris to bring the pH to 8, enough Tris propionate (~50 mM) to make the ionic strength 0.30, enough potassium propionate (~150 mM) to make the [K+] 200 mM, and varying amounts of magnesium. In some control experiments [ATP]t was varied.

(c) pH 7, [K+] 46 mM, [ATP]t 0.5 mM, [EDTA]t 15 mM, enough imidazole propionate (~200 mM) to make the ionic strength 0.30, and varying amounts of magnesium. In some control experiments [ATP]t was varied.

[MgITP] was the main variable in ITP solutions, varying from 2 μM to 2 mM. ITP solutions had the final ionic composition: pH 8, [K+] 200 mM, [ITP]t 8 mM, [EGTA]t 8 mM, Tris to bring the pH to 8, tris propionate (~50 mM) to make the ionic strength 0.30, potassium propionate (~150 mM) to make the [K+] 200 mM, and varying amounts of magnesium to vary [MgITP]. In some control experiments [ITP]t was varied.

A computer program similar to that used by Kerrick and Donaldson (1972) was used to determine the amount of constituent stock solutions to be added to a test solution in order to gain the desired ionic composition. Binding constants for EDTA were obtained from Sillen and Martell (1964); Tris’ binding constants were given by Sigma Chemical Co., St. Louis, Mo., and other binding constants came from Kerrick and Donaldson. Also, techniques used by Kerrick and Donaldson were used to determine the accuracy of mixing of solutions, total concentrations of cations in the solutions, total nucleotide concentrations, and the amount of contamination of solutions by other solutions caused by transferring fibers from one solution to the other. The amount of contamination caused by solution transfer was less than 0.3% per transfer.

[NTP]t was in large excess of [MgNTP] to buffer the concentration of the magnesium salt of the nucleotide as will be discussed later. EDTA was used in ATP solutions to buffer magnesium and to insure that [Ca++] was negligible. EGTA rather than EDTA was used in ITP solutions, because at [Mg++] corresponding to [MgITP]s which produced tension, EDTA would be saturated with magnesium and useless for controlling [Mg++] or [Ca++]}. EGTA was used instead, which only controls [Ca++]}. It was possible that the cross-contamination of solutions caused by transferring the fiber from one solution to another could affect the [Mg++] of ITP solutions. Thus, in ITP solutions the [Mg++] was checked before and after the experiment, and only results where there was less than a 10% change in the magnesium concentration were used. The cross-contamination was too small to affect the concentrations of the other constituents in either the ITP or ATP solutions.
Fiber Characteristics

Parameters describing the fibers used in the study of tensions were fiber length, fiber diameter, maximum tension, maximum shortening due to transducer compliance, and maximum tension per cross-sectional area of the fiber. These were studied systematically only at pH 8, using MgATP as substrate, but the figures for fiber length and diameter were similar for all the fibers studied. The average length of the fibers when mounted as described was 1.1 ± 0.1 mm (n = 6). The average diameter of the fibers was 85 ± 6 μm (n = 6). The calculated average maximum shortening, given as a percentage of the starting length, was 2 ± 0.3% (n = 6). The maximum tension per cross-sectional area of the fiber was 0.42 ± 0.04 kg/cm² (n = 6).

Protocol

RELAXATION In this study, fiber tension was relaxed by immersing the fiber in a solution containing 10 mM magnesium pyrophosphate (PPI), 18 mM pyrophosphate in excess of magnesium, 7 mM EGTA, no calcium, and ionic strength 0.30 at pH 7. That this solution was relaxing was determined by comparison with a solution of 2 mM MgATP and 7 mM EGTA and no calcium (pCa 8), the relaxing solution described earlier. The fiber did not develop tension when transferred from the MgATP relaxing solution to the pyrophosphate solution, and the pyrophosphate solution would relax any developed tension in the fiber to the same degree as the MgATP solution. Additionally, when the fiber was stretched 1-2% beyond rest length while immersed in the pyrophosphate solution less than 1 mg tension resulted. The small amount of total ATP or ITP that contaminated the pyrophosphate solution as a result of transferring solutions did not, by itself, have a relaxing effect in various control experiments.

After immersion in the pyrophosphate solutions, fibers were transferred and allowed to soak for 1 min in solution containing no ATP, 15 mM EDTA, ionic strength 0.30, and pH 7 or pH 8 depending on the test solution pH. This soaking was done to remove any magnesium that might be transferred by the fiber. With ITP experiments an additional wash was done in a solution containing no ATP, 7 mM EGTA, ionic strength 0.30, and pH 8 in order to prevent any transfer of EDTA to the ITP solutions. No tension developed in these wash solutions, but if the fiber was stretched 1-2% beyond rest length, more than 10 mg tension resulted indicating that the fiber had entered into some sort of rigor state.

CONTRACTION The fibers were then transferred to the test solutions where tension was generated that reached and maintained a constant level as is shown in Fig. 1. Generally, the fibers were allowed to remain contracted for at least 1 min after tension had reached a constant level before being relaxed again in the pyrophosphate solution in order to insure that steady state had been achieved. However, due to a slower initial rate of rise of tension at pH 8, [K⁺] 200 mM, and small [MgATP]s, it was necessary if the [MgATP] was less than 0.5 μM to let the tension remain constant for at least 3 min, and if the [MgATP] was less than 0.1 μM, to let the tension remain constant for at least 5 min in order to insure that steady state had been achieved. The validity of these criteria were verified by first determining a maximum relative rate of rise of tension at the end of a contraction compared to the initial rate of rise of tension by assuming that even though the tension appeared to remain constant, it had increased by the limit of resolution of the transducer (~0.4 mg), and secondly, dividing this quantity by the duration of the apparent tension plateau. If the maximum possible rate of rise of tension was greater than 5% of the initial rate of rise of tension, the data were discarded. The tension vs. [MgATP] relation determined
using this criterion was virtually the same as that determined using the three and five minute criteria.

The steady-state isometric tension that a fiber reached is the tension referred to in this study, and the sequence consisting of transferring the fiber from the washing solution to a test solution allowing it to reach steady-state tension, and finally completely relaxing the fiber by reimmersing it in the pyrophosphate solution is referred to as a contraction. This process is illustrated in Fig. 1. (If, after the fibers had reached steady-state tension, the fibers were allowed rapidly to shorten 2%, tension initially dropped to near zero and then redeveloped to the same level as prior to shortening. This redevelopment of tension indicates that the observed steady state tension probably results from active cycling of cross-bridges.)

To accurately quantify the results, I found it necessary to insert the test contractions between contractions in a standard solution, and initially, to express the results as tension relative to the mean of the bracketing standard tensions. Then, later, since all the contractions had been compared with the same standard, they could be compared directly with each other. The use of the standard was necessary, because as a fiber was contracted, each succeeding contraction with a given solution induced less and less tension as the fiber was contracted. In both ITP and ATP solutions the average reduction of fiber tension per contraction was ~1–3% of the initial standard tension. The total reduction of fiber tension in ITP solutions varied between 46–59% of initial tension and in ATP solutions varied between 0–55% of initial tension. It was thus necessary to correct for this effect to avoid giving solutions tested early an erroneously high effectiveness relative to those tested later. For every fiber, both a standard contraction between test contractions and randomizing the order of testing solutions were used to correct for this effect. Fibers skinned by Natori's method generally showed a less rapid decline in tension, and thus Natori's method was chosen as the main method of skinning the fibers.

**Data Analysis**

All of the results pertaining to tension vs. log [MgNTP] curves were biphasic. In all instances the tension first increased as substrate concentration increased, reached maximum, and then, with further increases in substrate concentration, declined. Tension was thus a biphasic function of substrate concentration.

The idea behind the data analysis is that, if for different substrates relative tension can be plotted vs. log normalized [MgNTP] in such a way that the maximum points of the curves coincide, and if the %T vs. log [MgNTP] curves are parallel, then the normalized curves should superimpose. The analysis of the data, therefore, sought to determine an estimated maximum tension and corresponding substrate concentration.
An estimated curve of relative tension vs. substrate concentration was obtained for each fiber by fitting to the data the empirical function:

\[
\%T(S) = 100 \times \frac{k_1 + S}{k_2 \times S^3 + k_3 \times S^2 + k_4 \times S + k_5},
\]

where \(k_1, k_2, k_3, k_4,\) and \(k_5\) are constants determined by a nonlinear fitting program using a least-squares method (Robertson, 1977) and \(S = [\text{MgNTP}]\). The function (1) produced a smooth curve that closely approximated the data.

An estimated maximum tension (\(T_e\)) and corresponding substrate concentration (\(S_e\)) could be obtained for each fiber from function (1) by using Newton’s method of approximation. Once \(S_e\) and \(T_e\) had been obtained the data could be replotted as 100 × (tension observed)/\(T_e\) (%\(T_e\)) vs. (log \(S - \log S_e\)). Also, an average estimated curve of 100 × (estimated tension)/\(T_e\) vs. (log \(S - \log S_e\)) could be obtained for a condition by averaging the estimated curves for each fiber in a condition. The replot of the observed data for all the fibers and the average estimated curve could then be compared. Then the average estimated curves for different conditions could be compared in order to determine whether the \%\(T\) vs. log [MgNTP] curves were parallel.

**RESULTS**

*Curves of Percent Tension vs. log [MgATP]*

The main goal of the experiment was to test the dependence on substrate type of percent tension vs. log [MgNTP] curves. However, as discussed in Methods, ITP and ATP solutions do not have identical ionic compositions, and ITP solutions elicit greater maximal tension. These differences could secondarily explain differences between log [MgATP]- and log [MgITP]-percent tension curves. In order to examine the sensitivity of these curves to ionic conditions or maximal tension, the percent tension vs. log [MgATP] was determined under three ionic conditions differing on pH and [K+]:

1. pH 7, 200 mM [K+], ATP (ATP|total) 0.5 mM
2. pH 8, 200 mM [K+], ATP 0.5 mM
3. pH 7, 46 mM [K+], ATP 0.5 mM

The concentrations of the various ATP and EDTA species vary greatly under these different conditions. Also, at the higher pH, as expected, and unexpectedly at the higher [K+], tension was greater (Robertson, 1977; Donaldson and Hermansen, 1977). Thus, the dependence of the percent tension vs. log [MgATP] curve on ionic conditions and maximum tension was tested. The results showed that there is a characteristic percent tension vs. log [MgATP] curve largely independent of ionic conditions and maximum tension.

The data were obtained and fitted as described in Methods. Fig. 2 shows plots of %\(T_e\) vs. log [MgNTP] - log [MgNTP]e, where for each condition a plot of all the data and the average estimated curve are shown. Fig. 2 A is for ATP-condition (1), Fig. 2 B is for ATP-condition (2), and Fig. 2 C is for ATP-condition (3). Fig. 3 shows all estimated curves on the same graph. As can be seen from Fig. 3, these three curves virtually superimpose for %\(T_e > 50\%\). Additionally, there was little difference in the positions of the curves for the
three ATP conditions. Maximum tension occurred at $-\log [\text{MgATP}]$ 6.0 – 6.2 (see legend of Fig. 2).

**Curve of Percent Tension vs. $\log [\text{MgITP}]$**

The conditions under which the percent tension vs. $\log [\text{MgITP}]$ curve were obtained were $\text{ITP}_t = 8$ mM, $[\text{K}^+] = 200$ mM, pH 8, and ionic strength 0.30. These conditions were chosen so that a direct comparison could be made with the ATP data done under these conditions, since these conditions gave the largest tensions with MgATP. Thus, the possible effects of noise, base-line shifts, and incomplete relaxation were minimized; the variability of tension measurements was reduced (Fig. 2 B compared with Fig 2 C); and the
posibility of measuring differences in the shape of the MgITP and MgATP curves, if there were any, was maximized.

Fig. 2 D shows a plot of %Te vs. log [MgITP] – log [MgITP]e with all of the data and the average estimated curve plotted. Fig. 3 shows the average estimated curve for ITP plotted on the same graph as the average estimated curves for ATP. It is clear from this graph that the ITP curve is much broader than any of the ATP curves, and that the ITP curve does not superimpose on any of the ATP curves.

Maximum Tension

The maximum tensions for the various substrates and conditions were compared in the same fibers with near-maximal concentrations of substrate for each condition. The results are summarized in Table I. The apparent order for effectiveness in inducing tension was ITP (pH 8) greater than ATP (pH 8), greater than ATP (pH 7, 200 mM [K+]), much greater than ATP (pH 7, 45 mM [K+]).

Validity of the Shape of the Curve

Hydrolysis of MgNTP along with a limited rate of diffusion of MgNTP into a fiber can produce a steady-state concentration gradient of [MgNTP] within the fiber. This effect tends to shift the percent tension vs. log [MgNTP] curve to higher concentrations and also to distort the shape of the curve. NTP in excess of MgNTP can be used to effectively eliminate these gradients (Reuben et al., 1971).

If 0.5 mM excess ATP is sufficient excess ATP to prevent significant [MgATP] gradients within a fiber, then reducing the diameter of the fiber or increasing the concentration of excess ATP should have no effect on the shape or position of the percent tension vs. [MgATP] curve. Fig. 4 shows the results of an experiment in which a fiber was cut into two segments, and one of the
segments was tested whole and one was tested split so that the diameter was reduced. Again, the test solutions contained 0.5 mM excess ATP. Reducing the diameter of the fiber did not cause shifts toward small [MgATP]s of the percent tension vs. [MgATP] curve, as would be predicted if 0.5 mM excess

| Table 1 |
|----------------|
| **Comparison of Near Maximal Tensions Under Various Ionic Conditions** |

| NTP | NTPt | pH | [K⁺] | [MgATP] | Relative Tension ± SE (N) |
|-----|------|----|------|---------|--------------------------|
| ATP | 0.5  | 6.0| 7    | 46      | 1‡                      |
| ATP | 0.5  | 6.0| 7    | 200     | 2.01 ± 0.05 (6)§        |
| ATP | 0.5  | 6.0| 8    | 200     | 3.75 ± 0.16 (7)         |
| ITP | 8.0  | 4.8| 8    | 200     | 5.81 ± 0.45 (7)         |

* pMgNTP = -log [MgNTP].
‡ Average tension in the 0.5 mM total [ATP], pMgATP 6.0, pH 7.0, and 46 mM [K⁺] solution was 0.19 kg/cm² ± 0.02 (n = 13).
§ The comparisons of tension in the pH 8 solutions to tension in the standard pH 7, 46 mM [K⁺] solution were done on different fibers.

Figure 4. Curves of tension vs. pMgATP for intact and split fibers. Shown is data obtained at pH 7, [K⁺] 46 mM for two segments of the same fiber. One segment was left intact and contracted (□). The other segment was split in half, thereby reducing its diameter, and then contracted (■). The same solutions were used for both fiber segments.

ATP were not a sufficient MgATP buffer. Reducing the diameter might even have caused the opposite effect of a shift toward larger [MgATP]s. However, the splitting of the fibers caused additional damage to the fibers and resulted in lower tensions and more rapid deterioration. Thus, the data were more irregular, and the apparent shift toward larger [MgATP]s could have been an artifact.
The experiment of increasing excess ATP was first tried at pH 7 and 46 mM [K⁺] because these were the first conditions used. Tension was determined at seven different pMgATPs (−log [MgATP]) (see Fig. 5) in the presence of the standard 0.5 mM excess ATP, and then in the presence of a larger concentration of excess ATP, 2 mM. Thus, at each pMgATP, tension was induced in the presence of two different concentrations of excess ATP, 0.5 mM and 2 mM. The results for one such fiber are shown in Fig. 5 in which tension relative to the maximum tension (in the 0.5 mM excess ATP solutions) is plotted vs. pMgATP for each of the two concentrations of excess ATP. The data suggest that there was a constant factor by which tension was reduced at each pMgATP as the concentration of excess ATP was raised from 0.5 to 2 mM. On the average, tension fell by nearly a constant factor to 0.65 when excess ATP was increased from 0.5 to 2 mM. Thus, excess ATP was acting to scale the curve, not to shift it or to change its shape. Fig. 6 shows that this result also held when the pH was raised to 8 and [K⁺] to 200 mM. The lack of an effect on position or shape of the curve indicates 0.5 mM excess ATP was sufficient to buffer against the effects of hydrolysis.

Similar arguments apply to the ITP data. If 1 mM excess ITP is sufficient excess ITP to prevent significant [MgITP] gradients within a fiber, then reducing the diameter of the fiber or increasing the concentration of excess ITP should have no effect on the shape or position of the percent tension vs.
[MgITP] curve. Fig. 6 shows the result of an experiment in which tension was determined at three pMgITPs (−log [MgITP]) with two different concentrations of excess ITP, 1 and 8 mM. The results show that increasing the concentration of excess ITP had no significant effect on tension. The lack of effect indicates that 1 mM ITP is sufficient to buffer against the effects of hydrolysis. Since the curve was determined in the presence of 8 mM excess ITP, it is unlikely that the effects of hydrolysis influenced the observed shape of the curve. (Since this result was definite, and since splitting the fiber is a difficult technique that gave more variable results and no additional information in the ATP experiments, I did not do the split fiber test with ITP.)

**DISCUSSION**

The earlier reported result of a parallel response of myofibrils to different nucleotides was strong evidence for a simple cooperative model of muscle activation with both contractile response and the regulation of response dependent upon the amount of substrate bound to the myosin hydrolytic site, which amount in turn depended upon fairly simple enzyme kinetics and the $K_m$ of the reaction of the substrate with the myosin site. This model would produce parallel percent contractile response vs. log [MgNTP] curves while models involving a second regulatory site could only produce such parallel response with great difficulty (Weber, 1969). The evidence reported here,
however, shows that the percent contractile response of vertebral muscle fibers to different substrates is not parallel. This evidence does not argue against the cooperative model, in general, but suggests a very simple cooperative model does not adequately describe the fiber data. Instead, general cooperative models, models postulating separate hydrolytic and regulatory sites on myosin and models postulating different functions for the two S-1 heads of myosin have to be considered as possible explanations for this type of data.

Given the complexity of the actomyosin ATPase and proposed cooperative interactions, it was perhaps unrealistic to expect, in general, that percent contractile response or even percent bound substrate would be simply related to the substrates' $K_m$s, as would be the case in a simple enzyme system. In fact, analysis of this type of model shows that percent contractile response generally is a complicated function of several constants (Orentlicher and Gersho, 1977). In this type of system a change in one or two of these constants caused by a change in substrate would not cause a simple shift in the $\% T$ vs. log [MgNTP] curve. However, from Orentlicher and Gersho's presentation of the cooperative model it did not seem that such changes in constants could produce the $\% T$ vs. log [MgITP] curve shown in this study.

Substrate inhibition has been proposed several times to account for the biphasic percent contractile response vs. log [MgNTP] curve (Levy and Ryan, 1967; Reuben et al., 1971). In this model, contractile response is elicited by binding of substrate to the hydrolytic site, while inhibition of the response is caused by binding of substrate to a separate regulatory site. If the affinity of the regulatory and the hydrolytic sites for substrate are nearly the same, the $\% T$ vs. log [MgNTP] curve will be relatively narrow as is the soleus $\% T$ vs. log [MgATP] curve. If the affinity of the regulatory site for substrate is much less than the affinity of the hydrolytic site for substrate, the curve will be relatively broad as is the soleus $\% T$ vs. log [MgITP] curve. In different species the relative affinities of the two sites for different substrates could be different, and the two sites might have more similar affinities for ITP than for ATP and the ITP curve would be narrower, as is the case in crayfish muscle (Reuben et al., 1971). This hypothesis is attractive but the biochemical evidence indicates that there is only one substrate binding site per S-1 molecule (Weber and Murray, 1973).

A related model instead suggests that the two heads of myosin serve different functions, and this functional difference gives rise to a myosin molecule with effectively two different types of sites. Specifically, Tonomura has proposed that of the two S-1 heads of myosin, only one, the working head, can be activated by actin and generate tension. The other head acts as a regulator which, when not bound to substrate, allows the interaction of the working head with regulated actin in the absence of calcium. Depending on the relative $K_m$s of the two heads for substrate, a great variety of tension vs. log [MgNTP] curves can be generated (Inoue and Tonomura, 1976; Shibata-Sekiya and Tonomura, 1976). However, these concepts are still controversial (Taylor, 1972, 1977; Weber and Murray, 1973).
The models discussed, above all, assume that the substrates act in an analogous manner, that if a different \%T results, then there is a different percentage of force-generating bridges. But it may be that, with different substrates or analogues to ATP, different types of force-producing conformations may exist. Thus, the lack of parallel response may not only be due to a nonparallel number of force bridges but also to different types of force bridges.

In summary, the only model which explains almost all of the data is the general cooperative model. Indeed, the data presented in this study are consistent with and support this model. However, part of the explanation for the nonparallel tension response to different substrates may possibly be due to the influence of other factors such as tension and fiber structure on the cooperative interactions of actin and myosin. Fortunately, any such proposed modifications of current models predict different specific relationships between hydrolysis of substrate and tension response. In the future, a combined approach of measuring tension, ATPase, and possibly even amount of bound substrate in a single fiber preparation should begin to decide which of these modifications is justified.

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