Characteristics of Ca^{2+}- and Mg^{2+}-Induced Tension Development in Chemically Skinned Smooth Muscle Fibers

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ABSTRACT Chemically skinned fibers from guinea pig taenia caecum were prepared by saponin treatment to study the smooth muscle contractile system in a state as close to the living state as possible. The skinned fibers showed tension development with an increase of Ca^{2+} in the solution, the threshold tension occurring as 5×10^{-7} M Ca^{2+}. The maximal tension induced with 10^{-4} M Ca^{2+} was as large and rapid as the potassium-induced contracture in the intact fibers. The slope of the pCa tension curve was less steep than that of skeletal muscle fibers and shifted in the direction of lower pCa with an increase of MgATP. The presence of >1 mM Mg^{2+} was required for Ca^{2+}-induced contraction in the skinned fibers as well as for the activation of ATPase and superprecipitation in smooth muscle myosin B. Mg^{2+} above 2 mM caused a slow tension development by itself in the absence of Ca^{2+}. Such a Mg^{2+}-induced tension showed a linear relation to concentrations up to 8 mM in the presence of MgATP. Increase of MgATP concentration revealed a monophasic response without inhibition of Ca^{2+}-induced tension development, unlike the biphasic response in striated muscle. When MgATP was removed from the relaxing solution, the tension developed slowly and slightly, even though the Mg^{2+} concentrations was fixed at 2 mM. These results suggest a substantial difference in the mode of actin-myosin interaction between smooth and skeletal muscle.

INTRODUCTION

Ever since Szent-Györgyi (1949) first described a method of preparing glycerinated skeletal muscle fibers to study muscle contraction while maintaining native structure without the complicated participation of the excitable membrane, much valuable information about the contraction of both skeletal and cardiac muscle has been obtained by this technique. However, only a few studies have been carried out on glycerinated smooth muscle fibers. In such studies, the time-course of tension development induced by the addition of ATP or by the increase of free calcium ions (Ca^{2+}) was extremely slow in comparison with that of the living state (Briggs, 1963; Bozler, 1968; Nasu and Ishida, 1975). From a physiological point of view, it is desirable to study the contractile system in a state as close to the living state as possible. The mechanically skinned skeletal muscle fibers, first introduced by Natori (1954) and subsequently modified by

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Endo (1967), Endo et al. (1970), Hellam and Podolsky (1969), Reuben et al. (1971), and Gordon et al. (1973), seemed to be the most suitable preparation for this purpose. Although this preparation was somewhat difficult, there were some advantages, in that the external solution could enter easily into cells without serious damage to cell organellae, especially to the sarcoplasmic reticulum. However, mechanical skinning of smooth muscle cells is impossible because of their small size. Recently, Ohtsuki et al. (1978) have reported that the treatment of cells with saponin gives rise to holes in the cell membrane, of such a size as to permit the external solution to permeate easily into cells. The saponin treatment is particularly distinguished from other chemical skinning techniques in that it preserves the intracellular structure to the same extent as does the mechanical skinning method. In particular, saponin-treated smooth muscle showed well-preserved arrangement of myofilaments in the sectioned materials fixed after prolonged experiments.[1] The saponin method for preparing chemically skinned skeletal and cardiac muscle fibers has been successfully employed also by Endo (1976) and Kitazawa (1977).

The present paper reports that chemically skinned smooth muscle fibers may be easily prepared by saponin treatment and that much new information on smooth muscle contraction can be obtained from this preparation in various experimental conditions. Research along the same line has been independently pursued by Endo et al. (1977) although their results differ slightly from ours.

METHODS

Myosin B Preparation and Superprecipitation Measurements

Myosin B of smooth muscle was prepared from chicken gizzard according to the routine method of our laboratory (Nonomura and Ebashi, 1974). Superprecipitation was measured by the method of Ebashi (1961). The reaction solution contained 0.1 mg/ml myosin B, 30 mM KCl, 8 mM $MgCl_2$, 20 mM Tris-maleate (pH 6.8), 10^{-5} M Ca^{2+}, and 0.5 mM ATP at 20°C. The change in turbidity of myosin B suspensions was estimated by following the change in absorbance at 660 nm after the addition of ATP. The absorbance measurements were performed using a photoelectric spectrophotometer (Hitachi Ltd., Tokyo).

Fiber Preparation

A strip of taenia caecum, freshly isolated from male guinea pig, was kept in Locke's solution of the following composition: 155.2 mM NaCl, 5.3 mM KCl, 3.6 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 2.8 mM glucose, saturated with air, at 20°C. A small bundle of the muscle fibers, ~100–150 μm in width and several millimeters in length, was teased apart using jeweler's forceps under a binocular microscope in a Ca^{2+}-free Locke's solution. The bundle of muscle fibers was set up in a small trough in which test solution was perfused rapidly by jetting from one end and by sucking simultaneously with a water pump from the other end. The two ends of the fibers (1–2 mm in length) were fixed between pieces of Scotch double-stick tape (3M Co., St. Paul, Minn.), and isometric tension was recorded with a strain gauge transducer (U-gage, Shinko Co. Ltd.).

[1] Ohashi, M., and Y. Nonomura. 1978. Manuscript in preparation.

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Experimental Procedure

Isotonic two-thirds K-Locke's solution, in which two-thirds of the sodium was replaced by potassium, was used to induce contracture of the small bundle of muscle fibers before they were chemically skinned. After the potassium-induced contracture was recorded, the solution was changed to a "standard relaxing solution" containing 130 mM KCl, 20 mM Tris-maleate, 5 mM $MgCl_2$, 3.3 mM ATP, and 4 mM EGTA, pH 6.8. Saponin treatment was then carried out by keeping the specimen for 20 min in standard relaxing solution containing 0.1 mg/ml saponin (Merck & Co., Inc., Rahway, N. J.). The specimen was washed with the standard relaxing solution and left for a while until the tension level became constant. Immediately before the application of calcium solution, the specimen was exposed to relaxing solution containing a lower concentration of EGTA (0.5 mM) to make diffusion of the subsequently applied calcium solution easy.

Various Ca^{2+} concentrations were prepared by adding appropriate amounts of $CaCl_2$ to EGTA, keeping the total EGTA concentration fixed at 2 mM; the apparent binding constant of the Ca-EGTA complex was considered to be 10^6 M^{-1}, pH 6.8, at 20°C. This parameter was directly measured in the present experimental solution by a dual-beam photometer using essentially the method of Ogawa (1968). The value of 4×10^3 M^{-1}, calculated from Martell's and Schwarzenbach's results (1956), was used as the binding constant of ATP for Mg^{2+} at pH 6.8.

RESULTS

Effect of Saponin on Smooth Muscle Myosin B

The effect of saponin on the superprecipitation of smooth muscle myosin B was examined before the experiment with saponin-treated fibers. As shown in Fig. 1, 0.1 mg/ml saponin did not have any remarkable effects on superprecipitation. With an increase of saponin concentrations above 0.1 mg/ml, the superprecipi-

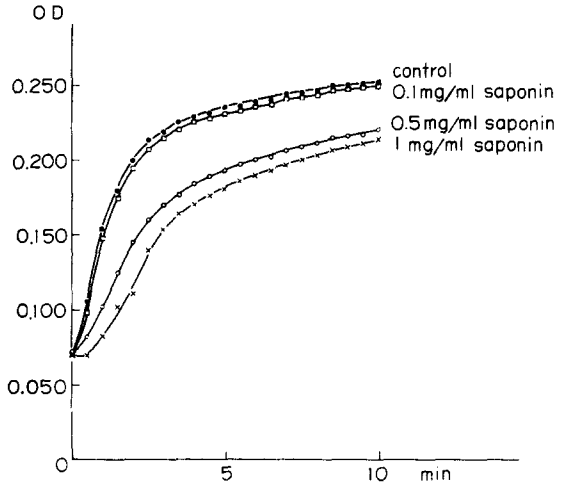

FIGURE 1. Effect of saponin on gizzard myosin B superprecipitation. Myosin B was exposed for 20 min to solutions containing the concentrations of saponin indicated in the figure; the reaction was then started by adding ATP. The ordinate shows the optical density (OD) of myosin B superprecipitation at 660 nm.

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tation was gradually suppressed. However, when myosin B was treated with >0.1 mg/ml of saponin and washed to eliminate saponin, and then prepared for superprecipitation, the turbidity increase was the same as under control conditions without suppression. We could thus conclude that saponin concentrations used in the experiment on tension development had virtually no effect on contractile proteins.

Calcium-Induced Tension Development

Increase of Ca^{2+} concentration from the resting state (Ca^{2+} below 10^{-7} M) developed tension which reached a plateau that was maintained during the presence of increased Ca^{2+}. One example of the time-course of tension development due to the increase of Ca^{2+} is shown in Fig. 2. Ca^{2+}-induced tension development was as large and rapid as the potassium-induced contracture observed in intact smooth muscle fibers. The rate of rise in tension and the

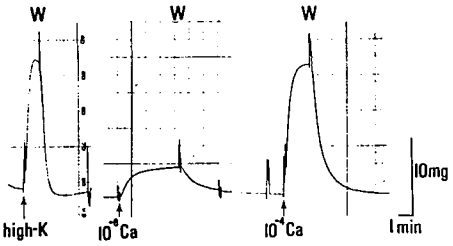

FIGURE 2. Time-course of tension development in intact and skinned fibers in the same preparation. The first record shows K-contracture of the intact muscle induced with two-thirds K^+-Locke solution. The second and third records show Ca^{2+}-induced tension development of the chemically skinned fibers after treatment with 0.1 mg/ml of saponin for 20 min. Notice the rapid and large tension development, particularly in 10^{-4} M Ca^{2+}. The experimental solution was applied as indicated at each arrow. W represents washing with Locke's solution and the relaxing solution.

extent of plateau tension were enhanced with the increase of Ca^{2+} concentrations in solution with 2 mM Mg^{2+} and 3 mM MgATP (Fig. 3). The minimum concentration for Ca^{2+}-induced tension was 5×10^{-7} M Ca^{2+}, and the tension reached a maximum at 10^{-4} M. After washing with standard relaxing solution containing 4 mM EGTA, the tension fell relatively rapidly to the initial level. Half-times of the contraction with high potassium in intact fibers and with 10^{-4} M Ca^{2+} in chemically skinned fibers were 18 and 19 s, respectively, whereas those of relaxation were 18 and 36 s as measured from Fig. 2. Thus, there was little or no difference between the time-courses of contraction; however, the time-course of relaxation of skinned fibers was retarded in comparison with that of intact fibers. The Ca^{2+}-induced tension development could be consistently repeated for several hours in the same preparation unless concentrations of free magnesium ions (Mg^{2+}) were widely and frequently changed. Even with constant Mg^{2+} concentration, the size of the Ca^{2+}-induced tension decreased

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gradually with repetition of contraction. In such a case, Ca^{2+}-induced tension in 10^{-5} M Ca^{2+}, 2 mM Mg^{2+}, and 3 mM MgATP was inserted as a standard tension to obtain a relative value for the correction.

The relationship between plateau tension and pCa in solutions with 2 mM Mg^{2+} and 3 mM MgATP is shown in Fig. 4 by a curve with open circles. A characteristic of this curve is that it is S-shaped, with a gentle slope. This means that tension development in chemically skinned smooth muscle is strongly dependent on Ca^{2+}. Increasing MgATP from 3 to 5 mM while keeping Mg^{2+} constant at 2 mM resulted in a shift to the left of the pCa tension curve (filled circles). Decrease of ionic strength from 130 mM to 50 mM KCl in the experimental solution reduced the Ca^{2+}-induced tension slightly and caused a shift of the pCa tension curve (dashed line with open circles in Fig. 4) to the right. This tendency is in contrast to the effect of ionic strength on actin-myosin interaction observed in contractile proteins prepared from skeletal muscle.

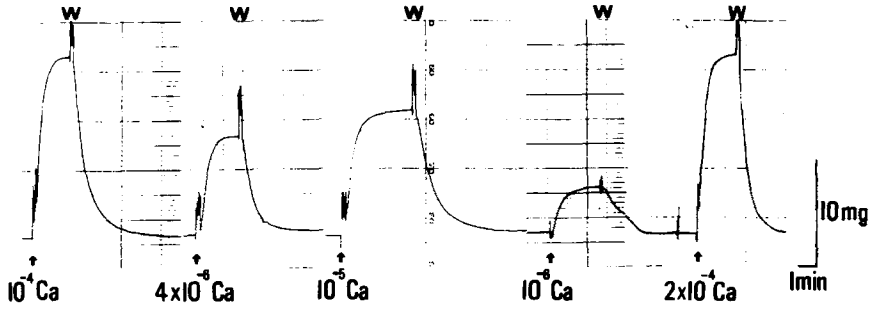

FIGURE 3. Tension development induced by different Ca^{2+} concentrations. A Ca^{2+} solution containing 2 mM Mg^{2+}, 3 mM MgATP, and the indicated concentrations of Ca^{2+} was applied to the skinned fibers at each point indicated. W represents washing with the relaxing solution.

However, in a smooth muscle actomyosin system, increase of ionic strength does not suppress actin-myosin interaction until KCl is increased to ~0.15 M (Ebashi et al., 1978). Such evidence is consistent with the present results obtained from the chemically skinned fibers.

Magnesium-Induced Tension Development

When the Mg^{2+} concentration in the standard relaxing solution containing 4 mM EGTA was increased while keeping MgATP constant at 3 mM, the tension gradually became elevated in spite of the absence of Ca^{2+} ($<10^{-7}$ M) and reached an equilibrium which depended on the Mg^{2+} concentration (Fig. 5). When Mg^{2+} in the solution was decreased, the tension gradually declined and reached a different equilibrium. Although the degree of Mg^{2+}-induced tension varied from specimen to specimen, the tension induced by Mg^{2+} has an apparent linear relation to the Mg^{2+} concentration as shown in Fig. 6. An increase in the Ca^{2+} concentration at the equilibrium level of Mg^{2+}-induced tension caused further tension development (Fig. 5). We could not decide whether to measure the size

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of Ca^{2+}-induced tension from the equilibrium level of the Mg^{2+}-induced tension or from the basic tension level. Additionally, the size of Mg^{2+}-induced tension varied as demonstrated in Fig. 6, and the reversibility of Mg^{2+}-induced tension with decrease in concentration of Mg^{2+} was not very stable. Inasmuch as the interpretation of the effect of Ca^{2+}-induced tension on the Mg^{2+}-induced tension level was complicated as described above, we could not examine the relation of Ca^{2+}-induced tension to Mg^{2+} concentrations.

On the other hand, we can assume from Fig. 6 that the presence of >1 mM Mg^{2+} is necessary to maintain the basic tension in chemically skinned fibers, corresponding to the resting tension level in intact fibers. In fact, if the specimen was exposed to low concentrations of Mg^{2+} (<0.5 mM) for over 30 min, even in the presence of several mM of MgATP, tension development could not be induced with increase in Ca^{2+} or Mg^{2+}.

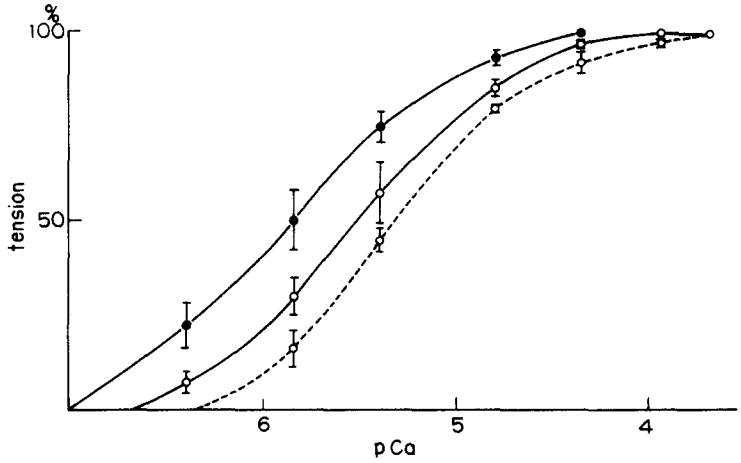

FIGURE 4. Tension curves as a function of pCa under different conditions. Solid lines: the standard solution with 2 mM Mg^{2+} and 3 mM MgATP (open circles) or with 2 mM Mg^{2+} and 5 mM MgATP (filled circles). Dashed line: low ionic strength solution with 50 mM KCl, 20 mM Tris-maleate (pH 6.8), 2 mM Mg^{2+}, and 3 mM MgATP. Each point corresponds to the mean value of five experiments.

Effect of Varying MgATP at Fixed Mg^{2+} Concentration on Tension Development

In the following experiment we examined the effect of changing the MgATP concentration from 1 to 7 mM while keeping the Mg^{2+} fixed at 2 mM. Inasmuch as free ATP concentration increases to around 1 mM with increases in MgATP in the range mentioned above, it would be desirable to determine the effect of free ATP at relatively fixed levels of MgATP and Mg^{2+}. We studied 10^{-5} M Ca^{2+}-induced tension development under two conditions with high concentrations of $MgCl_2$ and ATP (8 mM $MgCl_2$ with 9 mM ATP and 8 mM $MgCl_2$ with 10 mM ATP) in which free ATP concentration changed within the range of 1 mM or more, but MgATP and Mg^{2+} remained at almost constant concentrations. In that the Ca^{2+}-induced tension development under the former condition (8 mM $MgCl_2$ with 9 mM ATP) was similar to that under the latter condition, in spite of

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the changes of free ATP concentration within the range of 1 mM or more, we could ignore the effect of changes of free ATP concentration in the following experiment.

Tension development induced with 10^{-5} M Ca^{2+} was studied with various concentrations of MgATP. The upper record in Fig. 7 shows various grades of tension development in solutions with 2 mM Mg^{2+}, 130 mM KCl, 20 mM Tris-

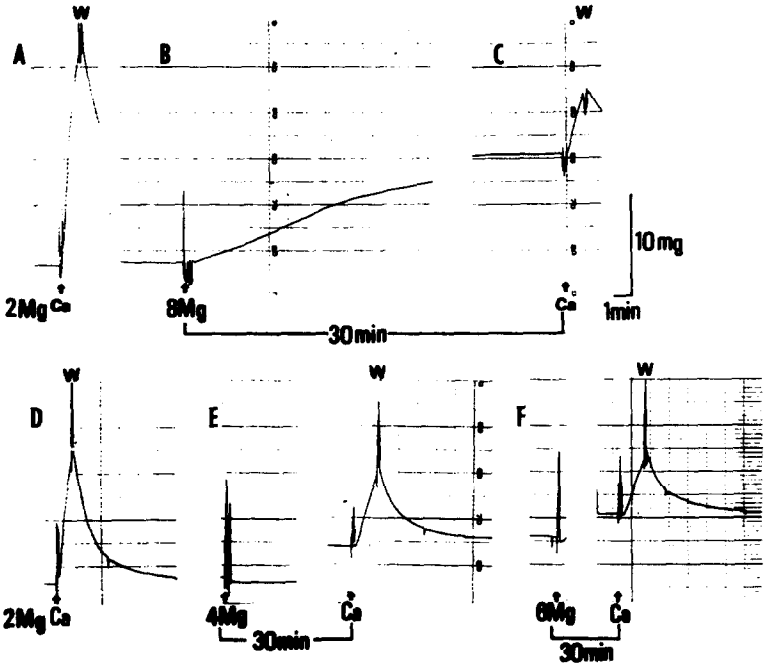

FIGURE 5. Mg^{2+}-induced tension development in the absence of Ca^{2+} and the induction of additional tension by adding Ca^{2+} in the presence of Mg^{2+}-induced tension. Upper record: one series of measurements. (A) Tension induced by 10^{-4} M Ca^{2+} in the standard relaxing solution with 2 mM Mg^{2+}. (B) Tension induced by increasing the concentration of Mg^{2+} from 2 to 8 mM. (C) The effect of adding Ca^{2+} on the steady-state level of tension induced by 8 mM Mg^{2+}. Lower record: another series of measurements. (D) Tension induced by 10^{-4} Ca^{2+}; 2 mM Mg. (E) The effect of adding 10^{-4} M Ca^{2+} after the tension induced by 4 mM Mg^{2+} has reached a steady level. (F) The effect of adding 10^{-4} M Ca^{2+} after the tension induced by 6 mM Mg^{2+} has reached a steady level. W represents washing with the relaxing solution.

maleate, and four different concentrations of MgATP: 1, 3, 5, and 7 mM, respectively. Tension development in Ca^{2+} solutions increased in maximum tension and (or) rate of rise according to increases in the concentration of MgATP at least up to 7 mM. Thus, a relation of plateau tension to increase of MgATP concentration is exhibited as a monophasic response as shown in Fig. 8.

When MgATP was removed from the relaxing solution, a slowly developing and small increase in tension was seen even with Mg^{2+} fixed at 2 mM. After the

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increase in tension induced by removal of MgATP had reached a steady state, an increase in Ca^{2+} did not induce a further increase in tension. If MgATP was then added to the solution, a subsequent increase in Ca^{2+} concentration did induce a further increase in tension, but that increase was markedly reduced as shown in the lower record of Fig. 7.

DISCUSSION

In several reports on glycerinated smooth muscle fibers, the time-course of tension development was markedly small and retarded compared with that of the intact living muscle fibers. This may be explained by use of a relatively large-sized muscle bundle which prolongs the diffusion of Ca^{2+} from the bathing

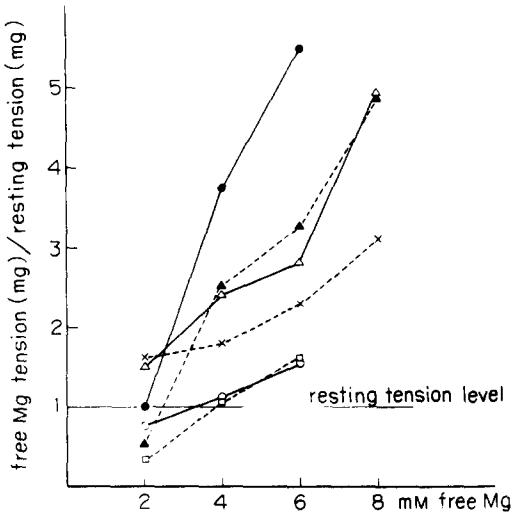

FIGURE 6. Relation between Mg^{2+}-induced tension and concentration of Mg^{2+}. Six independent experiments are shown. Resting tension level: the initial tension level when the intact fibers were set up. Ordinate: the ratio of the tension developed with increase of Mg^{2+} in skinned fibers to the resting tension in intact muscle fibers.

solution to the inside of the bundle. Furthermore, the contractile system may be irreversibly altered by the preservation of muscle fibers in inappropriate conditions. One probable cause for the deterioration of glycerinated fibers could be the lack of Mg^{2+} in glycerol solutions, which would severely damage the structure of myosin filaments. The freshly prepared small bundle of smooth muscle fibers used in our experiment seems to be the most suitable preparation for obtaining a state close to that of the living muscle. In fact, saponin-treated skinned smooth muscle fibers have shown very sensitive responses to Ca^{2+}, Mg^{2+}, and MgATP. The saponin method gave rise to the remarkable advantage that smooth muscle fibers can be easily skinned within a short time; this enabled us to make a direct comparison between the response of intact and skinned fibers in the same preparation.

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The slope of the pCa tension curve in our experiment was more gentle than that of glycerinated and skinned skeletal muscle fibers (Filo et al., 1965; Schädler, 1967; Godt, 1974; Levy et al., 1976; and Kitazawa, 1976). This gentle slope is explained by a difference between smooth muscle and skeletal muscle in the mode of calcium regulation. Filo et al. (1965) showed that there was no significant difference between glycerinated skeletal and smooth muscle fibers in

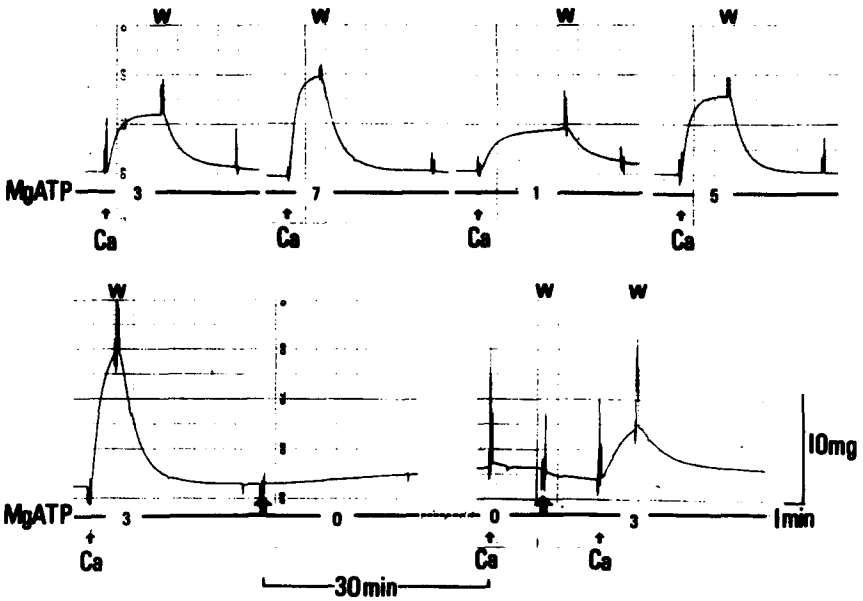

FIGURE 7. Effect on tension development by varying MgATP at fixed Mg^{2+}. Upper record: one series of experiments. Tension development was induced by 10^{-5} M Ca^{2+} (arrow) in various concentrations of MgATP shown as millimolar concentration below the tension records. Concentration of Mg^{2+} was fixed at 2 mM. Lower record: another series of experiments. Tension development was induced by 10^{-4} M Ca^{2+}. Concentration of MgATP is shown as in the upper record. The record at the left shows the slow development of tension after removing MgATP (large arrow) from the standard relaxing solution containing 2 mM Mg^{2+}. The record at the right shows that, if Ca^{2+} is added after the tension induced by removing MgATP has reached a steady level, there is no further increase in tension. Ca^{2+}-induced tension development reappears after adding 3 mM MgATP to the bath, although not fully. In both series of experiments W represents washing with the relaxing solution.

pCa tension curves. However, the pCa activity curve of ATPase or the superprecipitation of smooth muscle myosin B demonstrated a gentle slope as did our results in pCa tension (Ebashi et al., 1976). Perhaps Filo's data, derived from glycerinated smooth muscle, did not reflect actual characteristics because of an unsatisfactory preparative procedure.

The glycerinated and skinned skeletal muscle fibers were reported to develop nearly maximal tension at Mg^{2+} concentrations <0.1 mM with appropriate

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concentrations of total ATP, whereas full tnesion development of glycerinated smooth muscle fibers was reported to occur only when $MgCl_2$ concentration was equal to or above the total ATP concentration (Filo et al., 1965). Concerning investigations of contractile proteins, Murphy et al. (1971), Russell (1973), Nonomura and Ebashi (1974), and Ebashi et al. (1975) reported that the activation of ATPase and superprecipitation in smooth muscle myosin B required Mg^{2+} concentrations in the order of millimolar/liter. These observations suggest that the smooth muscle contractile system requires a higher Mg^{2+}

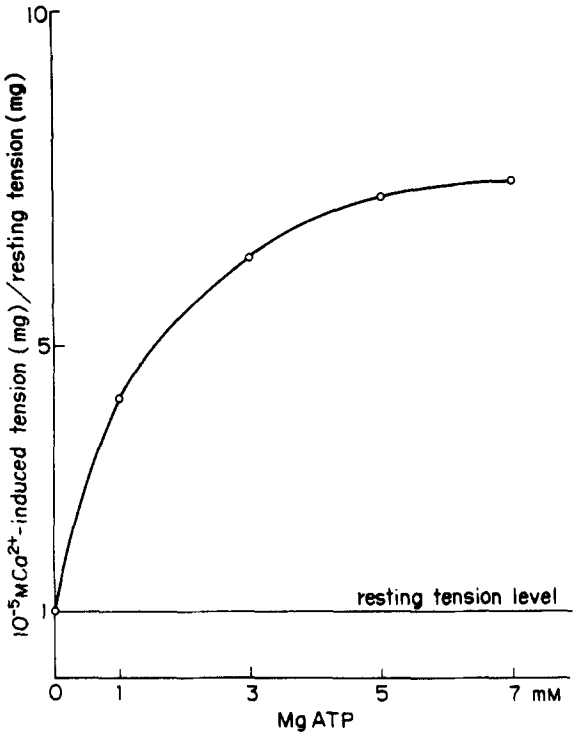

FIGURE 8. Curve of tension developed by 10^{-5} M Ca^{2+} as a function of MgATP concentration. The Mg^{2+} concentration in the solution was fixed at 2 mM. Ordinate: ratio of the Ca^{2+}-induced tension with various concentrations of MgATP (abscissa) to the resting tension in intact muscle fibers.

concentration than does the skeletal muscle contractile system. However, the interpretation is not straightforward because the concentration of MgATP and free ATP ions is changed by adding various amounts of $MgCl_2$. It would be desirable to distinguish between direct effects of Mg^{2+} and the indirect effect of changes in concentration of free ATP ions or MgATP. Russell (1973) concluded that the effects of Mg^{2+} on the ATPase activity of smooth muscle myosin B were not accounted for by lowering the free ATP, but instead by a direct modification of the contractile system by Mg^{2+}. Recently Nonomura et al. (1978)[2] elucidated

[2] Nonomura, Y., T. Kikawa, T. Toyo-oka, and S. Ebashi. 1978. The mode of existence of myosin filament in smooth muscle II. Manuscript submitted for publication.

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the effects of Mg^{2+} on the smooth muscle contractile system, where several millimolar of Mg^{2+} were necessary for the formation of myosin filaments as observed by negatively stained images in the electron microscope. This problem will be discussed in another paper on ultrastructural changes of the smooth muscle contractile system produced by changing Mg^{2+} concentrations and using skinned smooth muscle fibers.[1]

When Mg^{2+} concentration was kept below 0.5 mM for a while, no contraction in the skinned smooth muscle fibers could be obtained by increasing Ca^{2+}. The skinned smooth muscle fibers thus showed a Mg^{2+} requirement for contraction; however, the concentration of Mg^{2+} was less than that for activation of ATPase and superprecipitation of the myosin B. Increase of Mg^{2+} concentrations above 2 mM in the standard relaxing solution caused tension development in the specimen, even though 4 mM EGTA and 3 mM MgATP were present. This Mg^{2+}-induced tension development without Ca^{2+} is a noteworthy phenomenon in chemically skinned smooth muscle fibers, and recently Gordon[3] has studied the same phenomenon using another type of chemically skinned smooth muscle fibers. Such an unanticipated tension development may be related to the Mg^{2+} requirement for formation of myosin filaments as mentioned above. In fact, when smooth muscle mysoin was extracted with mysoin-extracting solution from the skinned muscle fibers and skeletal muscle myosin was added to reconstitute the muscle fibers, Mg^{2+}-induced tension development was lost while Ca^{2+}-induced tension development was recovered.[4] Although we could not study the influence of Mg^{2+} to the pCa tension curve for Mg^{2+}-induced tension, it does not seem to be as simple in skinned smooth muscle fibers as that reported in skinned skeletal and cardiac muscle fibers (Kerrick and Donaldson, 1972; Fabiato and Fabiato, 1975). In any case, varying the Mg^{2+} concentration in skinned smooth muscle fibers seems to complicate the situation, as it may be accompanied by some structural changes.

Although total magnesium in smooth muscle was suggested to be in the range of several millimolar by Sparrow (1969) using the guinea pig taenia coli, the Mg^{2+} concentration may be practically much lower because protein molecules, including contractile proteins in the cell, have many Mg^{2+} binding sites. If the Mg^{2+} concentration under which Mg^{2+}-induced tension equals the resting tension in intact fibers reflects a physiological state, Mg^{2+} concentration in living muscle seems to be in the range of ~1–2 mM according to the results shown in Fig. 6. It should be noted that the Mg^{2+} concentration in the experimental solution was calculated using an apparent binding constant of ATP for Mg^{2+} in this paper, but recently a much lower Mg^{2+} concentration was obtained by computation with apparent stability constants of all components in the solution, so that actual Mg^{2+} concentration may be lower than the value used in this paper. This problem will be discussed in detail elsewhere.[1]

ATPase activity in the presence of Ca^{2+} has been observed to have a biphasic function of MgATP concentration in the skeletal muscle actomyosin system (Bremel and Weber, 1972). ATPase activity increases with increase in MgATP up to ~0.1 mM, and above that concentration the ATPase activity is suppressed,

[3] Gordon, A. R. Personal communication.

[4] Saida, K., and Y. Nonomura. Manuscript in preparation.

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that is, with the appearance of substrate inhibition. In smooth muscle myosin B, Bremel (1974), Ebashi et al. (1975), and Sobieszek and Small (1975) reported that the ATPase activity and superprecipitation responded in a monophasic manner. In the present study, a monophasic response in the rate of rise in tension and the extent of plateau tension induced with Ca^{2+} was observed in at least up to 7 mM concentrations of MgATP. Furthermore, the increase in MgATP shifted the pCa tension curve to lower Ca^{2+} as shown in Fig. 4, unlike in skinned skeletal muscle fibers (Godt, 1974). This figure shows that increase of MgATP concentration from 3 to 5 mM increases the size of tension at the point of 10^{-5} M Ca^{2+}. This result is in good agreement with a monophasic response to increase of MgATP as shown in Figs. 7 and 8.

Although the ATPase activity in smooth muscle shows smaller values, less than one-tenth of that in skeletal muscle, high concentrations of MgATP can not cause the inhibition of Ca^{2+}-induced tension development, nor the dissociation of the actomyosin system, unlike the effects seen in skeletal muscle. This observation suggests a substantial difference in the mode of actin-myosin interaction between smooth muscle and skeletal muscle as well as evidence of contractile proteins in smooth muscle (Ebashi et al., 1978). Further studies combining a biochemical approach using skinned smooth muscle fibers may bring about remarkable progress in understanding the contraction mechanism in smooth muscle.

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