Calcium-regulatory Mechanisms

Functional Classification Using Skinned Fibers

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ABSTRACT   The primary purpose of this study was to determine whether various agents (adenosine 3-thiotriphosphate [ATPγS], trifluoperazine [TFP], troponin I, the catalytic subunit of the cyclic adenosine 3′,5′-monophosphate dependent protein kinase [C-subunit], and calmodulin [CaM]) could be used to classify skinned fiber types, and then to determine whether the proposed mechanisms for Ca^{2+} regulation were consistent with the results. Agents (ATPγS, TFP, C-subunit, CaM) expected to alter a light chain kinase–phosphatase system strongly affect the Ca^{2+}-activated tension in skinned gizzard smooth muscle fibers, whereas these agents have no effect on skinned mammalian striated and scallop adductor fibers. Troponin I, which is known to bind strongly to troponin C and CaM, inhibits Ca^{2+} activation of skinned mammalian striated and gizzard fibers but not scallop adductor muscle. The results in different types of skinned fibers are consistent with the proposed mechanisms for Ca^{2+} regulation.

INTRODUCTION

Four Ca^{2+} control mechanisms have been proposed for the regulation of muscle contraction from evidence obtained using biochemical measures of contraction. Two of these mechanisms involve thin-filament or actin-linked regulation. The best characterized of these mechanisms involves the troponin system, which is believed responsible for the Ca^{2+} regulation of mammalian striated muscle (Ebashi et al., 1968). The other mechanism, proposed for smooth muscle, involves the Ca^{2+}-binding protein leiotonin (Mikawa et al., 1977). Two other proposed mechanisms involve myosin linked regulatory systems. The first system described involves regulation by direct Ca^{2+} binding to myosin of the scallop (Szent-Györgyi et al., 1973). The more recently characterized system for smooth muscle regulation evolved from work in several laboratories (Adelstein and Conti, 1975; Cassidy et al., 1979; Chacko et al., 1977; Dabrowska et al., 1977; Hoar et al., 1979; Sobieszek and Small, 1977) and consists of a Ca^{2+}-sensitive light-chain kinase–phosphatase system.

   Experimental evidence suggests that the use of the ATP analogue adenosine 3-thiotriphosphate (ATPγS) (Cassidy et al., 1978; Hoar et al., 1979; Sherry et

al., 1978), phenothiazines (Weiss and Levin, 1978; Kerrick et al., 1980 *a*), catalytic subunit of cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent protein kinase (Adelstein et al., 1978; Kerrick et al., 1981), calmodulin (Dabrowska et al., 1977; Kerrick et al., 1981), and troponin I (TNI) (Greaser et al., 1972; Kerrick et al., 1976; Potter and Gergely, 1975) should affect these regulatory systems differentially. Therefore, the use of these agents in skinned muscle fiber preparations should allow the differentiation of these Ca^{2+} control systems in different muscle types.

The existence of more than one type of Ca^{2+} regulatory system in a single muscle fiber type has resulted in interest regarding the relative importance of these systems in the in vivo regulation of a particular muscle type (Bárány et al., 1980; Lehman and Szent-Györgyi, 1975; Stull and High, 1977; Yagi et al., 1978). The purpose of this study was twofold. Our first goal was to determine whether skinned muscle fibers could be functionally separated on the basis of these agents, and secondly, once having classified these fibers on this basis, to determine whether the regulatory mechanisms proposed for these different fiber types were consistent with the effect of these agents.

METHODS

Chicken gizzard, cat skeletal (adductor magnus and soleus), rabbit cardiac, and scallop (*Chlamys hastata hericia*) adductor muscle were used in this study. Skinned cardiac, skeletal, and gizzard muscle fiber preparations were prepared by gentle homogenation in a skinning solution as previously described (Kerrick and Best, 1974; Kerrick and Krasner, 1975; Hoar et al., 1979). These methods produce single skinned skeletal muscle fibers and bundles of skinned muscle fibers from cardiac and gizzard muscle. Bundles of skinned scallop muscle fibers ~100–200 μm in diameter were prepared by dissection in a relaxing solution. The ends of the fiber preparations, which were ~1–2 mm in length, were inserted into small stainless steel clamps of a tension transducer similar to the one used by Hellam and Podolsky (1969). The skinned fiber preparations were then immersed in a relaxing solution containing 1% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) for 30 min to solubilize any remaining sarcolemma, which facilitated diffusion of the proteins. The fibers were then ready for tension measurements in various test solutions. Similar results to those presented in this paper were also obtained with non-Triton–treated fibers skinned by light homogenation (Hoar et al., 1979; Kerrick and Best, 1974; Kerrick and Krasner, 1975).

Test solutions contained 70 mM K^+ plus Na^+, 2 mM $MgATP^{2-}$, 1 mM Mg^{2+}, 7 mM etheleneglycol-*bis*-(β-aminoethyl ether)-*N*,*N*′-tetraactetic acid (EGTA), 10^{-8}–$10^{-3.2}$ M Ca^{2+}, propionate as the major anion, and an ATP-regenerating system consisting of 15 mM creatine phosphate and 15 U of creatine phosphokinase/ml. For solutions in which ATPγS was substituted for ATP, no ATP-regenerating system was included, and [K^+] was increased to 85 mM. Ionic strength was adjusted to 0.15 and the pH was maintained at 7.0 with imidazole propionate. Temperature was maintained at 21°C. Relaxing solutions contained 10^{-8} M Ca^{2+}. Maximal contracting solutions are solutions that give a maximal Ca^{2+}-activated tension, such that further increasing the Ca^{2+} concentration does not result in an increase in maximal tension. The concentrations of the various ionic species were determined by solving ionic equilibrium equations using published binding constants (Donaldson and Kerrick, 1975). The sodium added as Na_2ATP and Na_2creatine phosphate (obtained from

Sigma Chemical Co., St. Louis, Mo.) was treated as K^+ in the program. ATPγS was obtained from Boehringer-Mannheim (Indianapolis, Ind.) or prepared enzymatically in this laboratory by the method of Cassidy and Kerrick (1979). Trifluoperazine and chlorpromazine hydrochlorides were gifts of Smith Kline and French Laboratories (Philadelphia, Pa.). Purified beef brain calmodulin (Yagi et al., 1978) and troponin I from rabbit skeletal muscle (Greaser and Gergely, 1973) were prepared as previously described. Catalytic subunits of beef skeletal and cardiac cAMP-dependent protein kinase were gifts from Drs. J. Beavo and E. Krebs (Department of Pharmacology, University of Washington), respectively.

RESULTS

Certain agents, such as the ATP analogue ATPγS and the catalytic subunit of the cyclic AMP-dependent protein kinase, are known to affect thiophosphorylation (Gratecos and Fischer, 1974; Sherry et al., 1978) and phosphorylation levels of proteins (Krebs and Beavo, 1979), respectively. ATPγS is used by enzymes to thiophosphorylate proteins, which are then resistent to dethiophosphorylation by phosphatases. Previous in vitro studies and skinned fiber studies have shown that ATPγS in the presence of Ca^{2+} causes irreversible thiophosphorylation of the myosin light chains and subsequent activation of actomyosin ATPase (Sherry et al., 1978) and tension, even·in the absence of Ca^{2+} (Cassidy et al., 1978; Hoar et al., 1979; Kerrick et al., 1980). The catalytic subunit of cAMP-dependent protein kinase has been shown to phosphorylate many enzymes, including the catalytic subunit of light-chain kinase (Adelstein et al., 1978) and cardiac muscle troponin I (Stull and Buss, 1977). Other in vitro and skinned fiber studies have shown that the catalytic subunit of the cAMP-dependent protein kinase causes phosphorylation of the light-chain kinase itself and a concomitant inhibition of light-chain phosphorylation (Conti and Adelstein, 1980) and Ca^{2+}-activated tension (Kerrick et al., 1981). Therefore, skinned fibers from three muscle types (vertebrate smooth, mammalian striated, and striated scallop) believed to be regulated by three distinct Ca^{2+}-regulatory mechanisms were exposed to these agents in the presence of Ca^{2+}, and their effect on tension was observed (Figs. 1 and 2).

The upper trace of Fig. 1 shows that exposure of smooth muscle (chicken gizzard) in the presence of Ca^{2+} to ATPγS results in maximal activation when the fiber is reexposed to a low-Ca^{2+} solution (pCa 8.0) containing ATP and that further activation cannot be achieved by increasing the $[Ca^{2+}]$. In contrast, the striated mammalian (cat soleus) and scallop adductor muscle skinned fibers (middle and lower traces of Fig. 1) are relaxed, not activated, when immersed in solutions containing low $[Ca^{2+}]$ and ATP after exposure to solutions containing high $[Ca^{2+}]$ and ATPγS. Additionally, these fibers contract in the presence of high $[Ca^{2+}]$ (pCa 3.8) and relax under low $[Ca^{2+}]$ (pCa 8.0) conditions after exposure to ATPγS and thus remain Ca^{2+} sensitive. Cardiac and fast-twitch muscles also showed no irreversible effects. Therefore, smooth muscle can be separated from striated skeletal, cardiac, and scallop adductor muscle on the basis of the response to low $[Ca^{2+}]$ and ATP after pretreatment in high $[Ca^{2+}]$ and ATPγS, since only smooth muscle is irreversibly activated.

Fig. 2 shows that the Ca^{2+}-activated tension of smooth muscle (chicken gizzard) skinned fibers is strongly inhibited by the presence of 10^{-6} M of the catalytic subunit of cAMP-dependent protein kinase. In contrast, Ca^{2+}-activated tensions of striated mammalian (rabbit heart shown in Fig. 2) and scallop adductor skinned fibers are not affected by the presence of the catalytic subunit. No effects on fast- and slow-twitch muscle fibers were observed (data not shown). These results show that skinned smooth muscle fiber preparations can be distinguished by the catalytic subunit from mammalian striated and scallop muscle skinned fibers.
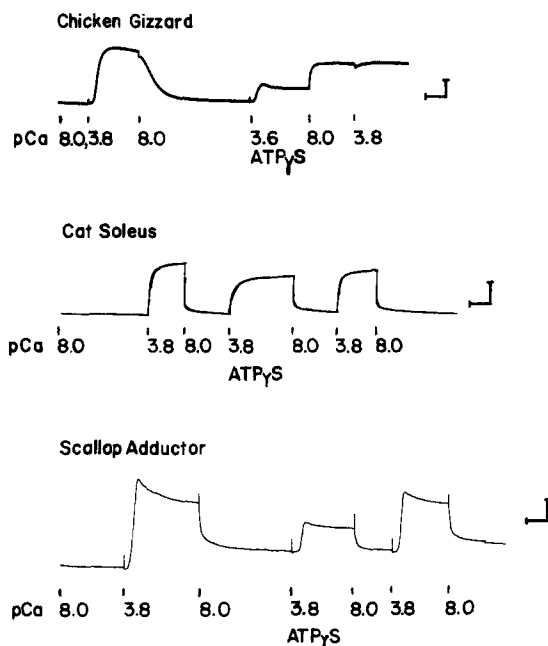

FIGURE 1.   The effect of pretreatment of skinned muscle fibers with ATPγS in high Ca^{2+} concentration (pCa 3.8) on subsequent activation of the fibers. Horizontal calibration bar = 5.0 min in all records. Vertical calibration bar = 8.3 mg in upper, 5.3 mg in middle, and 7.1 mg in lower records. All solutions contained 2 mM Mg ATP, except ATPγS was substituted for ATP where indicated. Vertical marks just above each pCa show the time the fiber was transferred to the indicated solutions.

Calmodulin has been shown to be the Ca^{2+}-binding regulatory subunit of light-chain kinase in both smooth (Dabrowska et al., 1977) and skeletal muscle (Yagi et al., 1978). Additionally, the phenothiazines (trifluoperazine or chlorpromazine) have been shown to bind to calmodulin and to inhibit its biological activity (Weiss and Levin, 1978). We have shown that trifluoperazine (Kerrick et al., 1980) will inhibit Ca^{2+}-activated tension of smooth muscle. Therefore, the use of these two agents, calmodulin and trifluoperazine, should serve to distinguish between calcium-regulatory systems that involve a light-chain kinase-phosphatase system and those that do not.

Fig. 3 shows that the maximum Ca^{2+}-activated tensions of skeletal (cat soleus) and scallop adductor muscle are not affected by 50 μM trifluoperazine, in contrast to that of smooth muscle (chicken gizzard), which is strongly inhibited by 50 μM trifluoperazine. Additionally, the inhibition resulting from the trifluoperazine can be partially overcome by the addition of 5 μM calmodulin in the presence of Ca^{2+}. These data show that trifluoperazine only affects (inhibits) smooth muscle Ca^{2+}-activated tension.

The effect of calmodulin on submaximal Ca^{2+}-activated tension is shown in Fig. 4. Because calmodulin has been shown to be the regulatory Ca^{2+}-binding
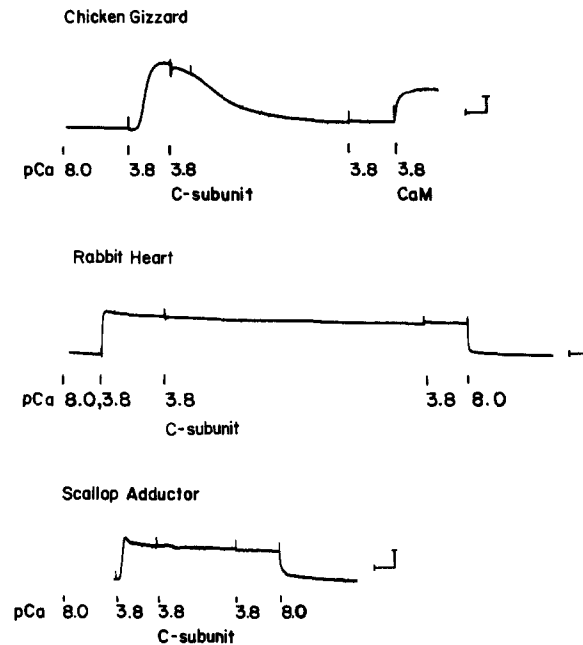
Chicken Gizzard

pCa 8.0   3.8 3.8   3.8 3.8
                C-subunit   CaM

Rabbit Heart

pCa 8.0,3.8   3.8   3.8 8.0
                C-subunit

Scallop Adductor

pCa 8.0 3.8 3.8   3.8 8.0
                C-subunit

FIGURE 2. The effect of 1 μM catalytic subunit of the adenosine 3',5'-monophosphate-dependent protein kinase (*C-subunit*) on maximal Ca^{2+}-activated tension in skinned muscle fibers. Horizontal calibration bar = 5.0 min in all records. Vertical calibration bar = 4.2 mg in upper, 2.5 mg in middle, and 7.1 mg in lower records. Vertical marks just above each pCa show the time the fiber was transferred to the indicated solution. Calmodulin (*CaM*) concentration = 5.0 μM.

subunit of light-chain kinase, which in the presence of Ca^{2+} binds to Ca^{2+} and the 107,000-dalton light-chain kinase to form an active light-chain kinase (Dabrowska et al., 1977), it is reasonable to predict that increasing the concentration of calmodulin would increase the apparent Ca^{2+} sensitivity of skinned fibers controlled by a light-chain kinase–phosphatase system but would have no effect on fibers regulated by other control systems. Fig. 4 shows that addition of 5 μM calmodulin to a smooth muscle (chicken gizzard) in a submaximal Ca^{2+} solution results in near maximal Ca^{2+}-activated tension, since increasing Ca^{2+} concentration results in little increase in tension. In

contrast, addition of calmodulin to skeletal (cat soleus) and scallop adductor skinned fibers results in no significant change in submaximal Ca^{2+}-activated tension. These data show that submaximal Ca^{2+}-activated tension of only smooth muscle is affected by the addition of 5 μM calmodulin to the bathing solutions.

The inhibitory subunit of troponin (troponin I) has been shown in vitro to inhibit actomyosin interaction (Greaser et al., 1972; Potter and Gergely, 1975). Additionally, troponin I has been shown to bind strongly to troponin C (Head
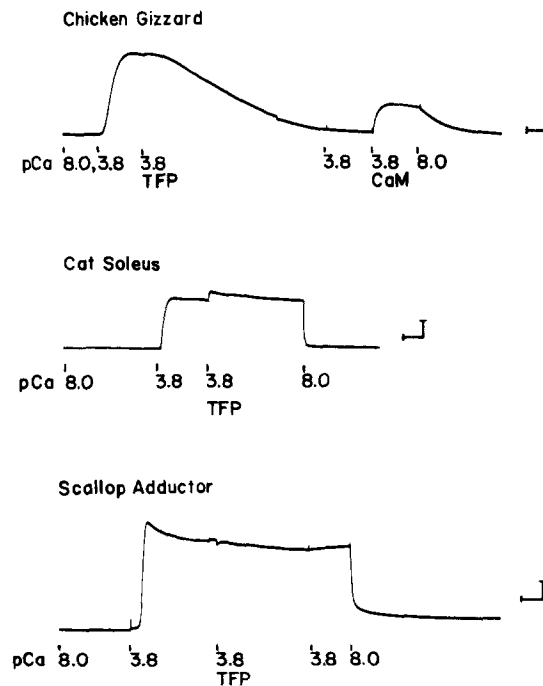

FIGURE 3. The effect of 50 μM trifluoperazine (TFP) on maximal Ca^{2+}-activated tension in skinned muscle fibers. Horizontal calibration bar = 5.0 min in all records. Vertical calibration bar = 4.2 mg in upper, 8.3 mg in middle, and 8.3 mg in lower records. Calmodulin (CaM) concentration where indicated = 5.0 μM. Vertical marks just above each pCa show time the fiber was transferred to the indicated solution.

and Perry, 1974) and to calmodulin (Drabikowski et al., 1977) in the presence of Ca^{2+}. In contrast, no interaction between troponin I and the light chains of myosin have been reported. Previously, we reported the preliminary finding that troponin I inhibited the Ca^{2+}-activated tension of both skeletal and cardiac muscle skinned fibers but not scallop adductor (Kerrick et al., 1976). Therefore, we exposed Ca^{2+}-activated skinned fibers to troponin I to determine whether Ca^{2+}-regulatory systems controlled by either troponin C or calmodulin could be distinguished from those controlled by Ca^{2+} binding to myosin (scallop). Fig. 5 shows that maximal Ca^{2+}-activated tensions of skeletal (cat

adductor) and smooth muscle (chicken gizzard) are strongly inhibited by troponin I and that the readdition of troponin C results in reactivation of the fibers. In contrast, the lower trace of Fig. 5 shows that the maximal Ca^{2+}-activated tension of the scallop is unaffected by addition of troponin I. These results show that the Ca^{2+}-regulatory system responsible for contraction in the scallop can be distinguished from the Ca^{2+}-regulatory systems responsible for the activation of smooth and skeletal muscle. Results similar to those obtained for skeletal muscle were also obtained for cardiac muscle (data not shown).

Table I is a summary of the results presented in this paper. Agents thought
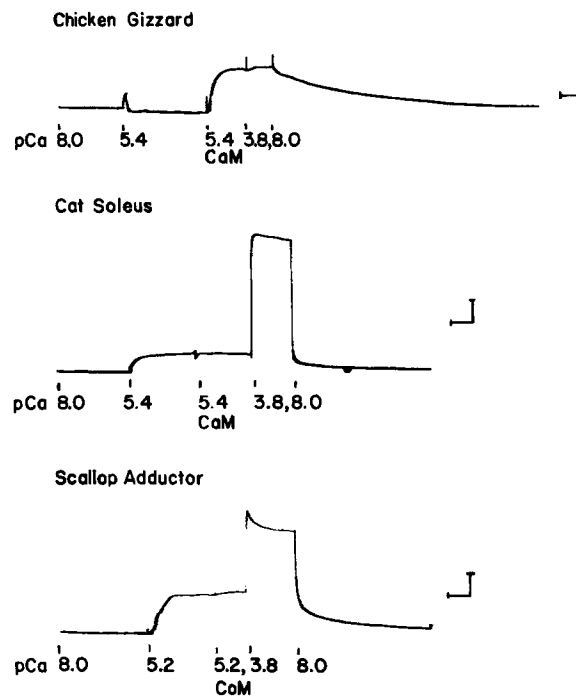
Chicken Gizzard

pCa 8.0   5.4   5.4 3.8,8.0
                   CaM

Cat Soleus

pCa 8.0   5.4   5.4   3.8,8.0
                  CaM

Scallop Adductor

pCa 8.0   5.2   5.2,3.8   8.0
                  CoM

FIGURE 4. The effect of 5 μM calmodulin (*CaM*) on submaximal Ca^{2+}-activated tension in skinned muscle fibers. Horizontal calibration bar = 5.0 min in all records. Vertical calibration bar = 5.0 mg in upper, 2.6 mg in middle, and 2.3 mg in lower records. Vertical marks just above pCa show the time the fiber was transferred to the indicated solution.

to specifically alter a light-chain kinase–phosphatase system strongly affect the Ca^{2+}-activated tension of smooth muscle, whereas these agents have no effect on the other muscle types. Troponin I, which is known to bind strongly to troponin C and calmodulin, inhibits all muscle types tested, with the exception of the scallop striated muscle.

DISCUSSION

We have shown on the basis of the functional tests that skinned muscle fiber preparations can be divided into the following three types: (*a*) smooth muscle,
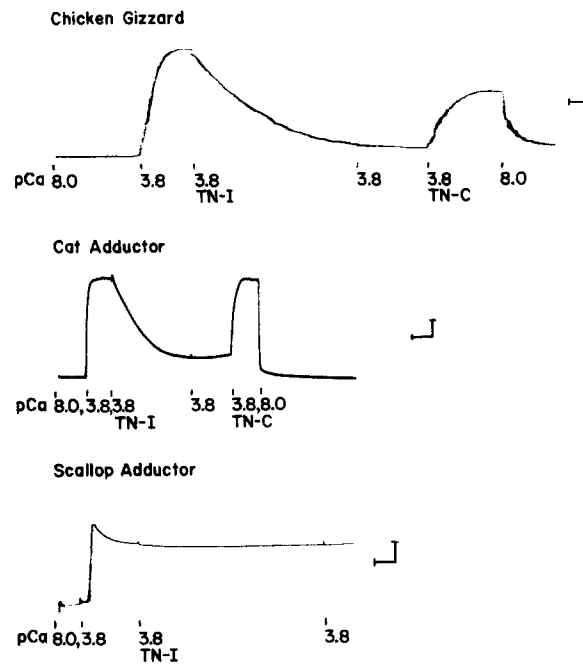
Chicken Gizzard

pCa 8.0   3.8   3.8 TN-I   3.8   3.8 TN-C   8.0

Cat Adductor

pCa 8.0,3.8,3.8 TN-I   3.8   3.8,8.0 TN-C

Scallop Adductor

pCa 8.0,3.8   3.8 TN-I   3.8

FIGURE 5. The effect of 29 μM troponin I (TN-I) on maximal Ca^{2+}-activated tension. Horizontal bar = 5.0 min in all records. Vertical calibration bar = 7.4 mg in upper, 8.3 mg in middle, and 3.0 mg in lower records. Troponin C (TN-C) concentration = 55 μM where indicated. Vertical marks just above pCa show the time the fiber was transferred to the indicated solution.

TABLE I

SUMMARY OF EFFECTS ON Ca^{2+}-ACTIVATED TENSION IN DIFFERENT SKINNED MUSCLE FIBER TYPES

Muscle type	Pretreatment with ATPγS in high [Ca^{2+}]	TFP*	C-subunit‡	CaM§	TNI‖
Cardiac	—	—	—	—	↓
Slow-twitch	—	—	—	—	↓
Fast-twitch	—	—	—	—	↓
Smooth	↑	↓	↓	↑	↓
Scallop	—	—	—	—	—

* Trifluoperazine.
‡ Catalytic subunit of cAMP-dependent protein kinase.
§ Calmodulin.
‖ Troponin I.
—, no effect.
↓, inhibition of Ca^{2+}-activated tension.
↑, Ca^{2+}-insensitive activation with ATPγS or increased Ca^{2+} sensitivity with CaM.

which responds positively to the light-chain kinase–phosphatase tests and is also inhibited by troponin I, (*b*) scallop adductor muscle, which does not respond to troponin I or to tests for a light-chain kinase–phosphatase system, and (*c*) mammalian striated muscle, which does not respond to tests for a light-chain kinase–phosphatase system but is inhibited by troponin I. In general, the results of these functional tests are quite consistent with one of the Ca^{2+}-control systems proposed for the fiber types from in vitro studies.

The ATP analogue ATPγS has been shown to be used as a substrate by the myosin light-chain kinase of both smooth (Sherry et al., 1978) and skeletal muscle (Perry et al., 1975) to thiophosphorylate the light chains. These phosphorylated light chains are resistant to the action of phosphatases. However, only the thiophosphorylation of the smooth muscle light chains resulted in the irreversible activation of actomyosin ATPase. Concurrently, we showed that exposure of skinned smooth muscle fibers to ATPγS resulted in thiophosphorylation of the light chains and irreversible activation of these fibers (Cassidy et al., 1978; Cassidy et al., 1979; Hoar et al., 1979). The results reported in this paper are consistent with the in vitro studies of thiophosphorylation reported for striated and smooth muscle. Scallop adductor muscle is thought to be regulated by a direct Ca^{2+} binding to myosin (Simmons and Szent-Györgyi, 1978; Lehman and Szent-Györgyi, 1975). No thiophosphorylation has been reported in scallop actomyosin systems. Our results with ATPγS treatment indicate the lack of a light-chain kinase–phosphatase system in the scallop. Although several reports have appeared showing correlations between phosphorylation levels of light chains in skeletal muscle (Stull and High, 1977; Bárány and Bárány, 1977) and the state of activation of those muscles, our results with ATPγS suggest that there is no light-chain kinase–phosphatase system controlling activation of mammalian skeletal muscle fibers. Measurements of the incorporation of ^{35}S from [^{35}S]ATPγS under conditions of high [Ca^{2+}] into the myosin light chains of mammalian straited skinned fibers were made (data not shown), and we could detect no significant incorporation of ^{35}S into the light chains. No such measurements were made on the scallop. In vitro studies show that the regulatory light chains of scallop cannot be phosphorylated (Frearson and Perry, 1975; Kendrick-Jones and Jakes, 1977).

Fig. 1 shows that in the presence of Ca^{2+} and ATPγS, tension can develop. In vitro studies suggest little or no tension should develop under these conditions, since the rate-limiting step of hydrolysis of ATPγS by myosin is the hydrolysis step, which prevents the accumulation of a myosin product complex necessary for actomyosin interactions (Barrington-Leigh et al., 1972; Mannherz et al., 1973). Possibly, this tension is caused by the production of ATP by adenylate kinase from ADP that accumulated from ATPγS hydrolysis. However, this increase in tension could not be prevented by the addition of the adenylate kinase inhibitor diadenosine pentaphosphate (AP_5A), 50 μM, to the solutions, suggesting that ATPγS to a limited extent can be used as a substrate for tension development. It is also possible that ATP is produced from ATPγS by some unknown process.

The phenothiazines (trifluoperazine and chlorpromazine) have been shown to bind with much higher affinity to calmodulin than to troponin C or other proteins and to inhibit the biological function of calmodulin (Weiss and Levin, 1978). Therefore, trifluoperazine or chlorpromazine at the proper concentration should selectively inhibit the light-chain kinase by binding to the Ca^{2+}-regulatory subunit, calmodulin. These drugs would have no effect on troponin C or striated muscle or the regulatory light chains of scallop muscle. Our functional tests agree with the expected results.

The catalytic subunit of the cAMP-dependent protein kinase has been shown to phosphorylate the high molecular weight subunit of light-chain kinase and to inhibit its interaction with the Ca^{2+}-binding regulatory subunit, calmodulin, and, consequently, the activation of the kinase in the presence of Ca^{2+} (Adelstein et al., 1978; Conti and Adelstein, 1980). Others, using crude actomyosin systems from bovine aorta, have shown that the actomyosin ATPase is inhibited by the cAMP-dependent protein kinase in the presence of cAMP and that there is a concomitant increase in phosphorylation of a high molecular weight protein thought to be the light-chain kinase (Silver and DiSalvo, 1979). When the catalytic subunit of protein kinase was added to skinned smooth muscle fibers, the Ca^{2+}-activated tension was inhibited, in agreement with the in vitro results.

In addition, the catalytic subunit of the cAMP-dependent protein kinase preferentially phosphorylates cardiac troponin I (Stull and Buss, 1977). In this state the Ca^{2+} sensitivity of the actomyosin ATPase is decreased, with no effect on the maximal Ca^{2+}-activated ATPase (Holroyde et al., 1979). These in vitro results are consistent with our findings, which show no effect of the catalytic subunit on the maximal Ca^{2+}-activated tension of cardiac or skeletal muscle. No in vitro data concerning the effects of catalytic subunit have been reported for scallop, which supposedly contains only trace amounts of troponinlike proteins. Also, there are no reports of the catalytic subunit of protein kinase affecting the myosin light chains of scallop.

Calmodulin is the Ca^{2+}-binding regulatory subunit of light-chain kinase and, on the basis of its isolation properties (Cheung, 1980), is thought to be a soluble or, at best, a loosely bound protein in vivo. Therefore, one would expect that increasing the calmodulin concentration in the smooth muscle skinned fiber would result in an apparent increase in the Ca^{2+} sensitivity if the calmodulin concentration in the skinned fiber were less than maximal. In contrast, one would not expect an effect of calmodulin on the Ca^{2+}-control systems of mammalian striated or scallop adductor muscle, since these systems should contain their full complement of tightly bound Ca^{2+}-regulatory proteins, i.e., troponin and the regulatory myosin light chain. Our functional tests agree with in vitro studies showing that calmodulin increases the Ca^{2+} sensitivity in smooth muscle but has no effect on mammalian striated and scallop adductor muscle.

Troponin I has been shown in many studies to inhibit the interactions of actomyosin ATPase, even in the absence of tropomyosin (Weber and Murray, 1973). Troponin I has been shown to strongly interact with troponin C in the presence of Ca^{2+} (Head and Perry, 1974). Calmodulin (the Ca^{2+}-binding

subunit of light-chain kinase) has been shown to strongly interact with troponin I in the presence of Ca^{2+} (Drabikowski et al., 1977). However, no interaction of troponin I with the scallop muscle light chains has been reported. Therefore, troponin I, as predicted from actomyosin ATPase studies, would inhibit the tension in mammalian striated muscle and possibly also in smooth muscle by interfering with the action of calmodulin. The results of tests reported in this study, inhibition of vertebrate smooth and mammalian striated muscle fibers by TNI, are consistent with the in vitro data from these purified protein systems.

In vitro studies suggest that binding of TNI to the tropomyosin-thin filament complex would inhibit muscle contraction (Eaton et al., 1975). This assumption does not seem valid for these experiments, since the scallop is not inhibited by TNI. Also, thiophosphorylation of the myosin light chain of smooth muscle causes irreversible activation of the fiber, and subsequent TNI treatment has no effect (data not shown). Because TNI binding to scallop or smooth muscle thin filaments was not measured, we cannot make any definitive statements regarding why TNI inhibition did not occur in scallop and ATPγS-treated gizzard skinned fibers. Sodium dodecyl sulfate gels of proteins from skinned mammalian striated muscle fibers treated with TNI do not show removal of TNC from the fibers (data not shown). After inhibition of Ca^{2+}-activated tension of mammalian striated and smooth muscle, the fibers can be partially recovered by the readdition of TNC (Fig. 5) or calmodulin (data not shown), suggesting that it is the TNI binding to some inhibitory site, possibly to TNC or calmodulin, which is responsible for the inactivation of the fibers.

A light-chain kinase–phosphatase system, it is generally agreed, is responsible for the activation of smooth muscle (Cassidy et al., 1979; Chacko et al., 1977; Hoar et al., 1979; Sherry et al., 1978; Sobieszek and Small, 1977). Others, (Mikawa et al., 1977) however, disagree. The results from our study tend to rule out the latter, since all the results for a light-chain kinase–phosphatase system in smooth muscle were positive. Although a light-chain kinase–phosphatase system can be found in striated muscle (Pires and Perry, 1977), and shown to function in vivo (Bárány and Bárány, 1977; Stull and High, 1977), no reported evidence for its affecting the Ca^{2+} activation properties of striated muscle was found (Stull et al., 1980). Our recent experiments show that although troponinlike proteins can be found in trace amounts in scallop (Goldberg and Lehman, 1978), troponin I, which would be expected to affect these proteins, had no effect on the Ca^{2+} activation of scallop muscle. In conclusion, the hypotheses that a light-chain kinase–phosphatase system is responsible for the Ca^{2+} regulation of smooth muscle, that troponin is responsible for the regulation of mammalian striated muscle, and that the regulatory light chains of myosin are responsible for the Ca^{2+} control of scallop adductor muscle are consistent with our functional tests. In addition, there is no evidence for a mixed regulatory system in any of the skinned fiber types examined.

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