IN the performance of its function as a pump that regulates its output according to the changing requirements of the organism, the heart must be able to modify the characteristics of its contraction within a broad range. The force and rate of development of tension as well as the frequency of contraction can be changed, each by several fold. Since it is generally accepted that all cells in a healthy myocardium contract during each beat of the heart, modulation of contractility must occur at the cellular level, with each myocardial cell capable of various states of contractility. Each parameter of contractility that can be identified as a variable by measurements of the performance of the organ must have a cellular mechanism as the basis for variability.

The pioneering work of Ebashi and his collaborators (Ebashi and Ebashi, 1964; Ebashi and Kodama, 1968; Ebashi and Endo, 1968) and of Weber (1963) showed that skeletal muscle is maintained in a relaxed state by the proteins troponin and tropomyosin, and the contractile mechanism can be activated by calcium ions that overcome the inhibitory effects of these two so-called regulatory proteins on the thin filament. The amount of force that is developed depends on the concentration of ionic calcium. Based on these notions, the primary responsibility for variations in contractility has been attributed to the different concentrations of activator calcium that can be achieved in the cytoplasm following depolarization. In mammalian hearts, it is generally believed that calcium responsible for activating contraction is released from stores in the sarcoplasmic reticulum by membrane depolarization and enters from the extracellular space as inward calcium current during the plateau of the action potential (Winegrad and Shanes, 1962; Reuter, 1973; Morad and Goldman, 1973; Fabiato and Fabiato, 1975). The amount of calcium that enters the cell during the action potential can be changed by altering either the number of calcium channels or the probability of a channel's opening in response to sarcolemmal depolarization (Reuter, 1973; Reuter and Scholz, 1977). These changes are produced by β-adrenergic stimulation and by changes in the configuration of the action potential by adrenergic or cholinergic stimulation. The rate of accumulation of calcium and the total storage capacity of the sarcoplasmic reticulum are increased by phosphorylation of a protein, phospholamban, in the sarcoplasmic reticulum (La Raia and Morkin, 1974; Tada et al., 1975).

All modulation of contractility, however, is not due to variation in the rise in cytoplasmic calcium that is produced by the action potential. It is now abundantly clear that the properties of the contractile proteins themselves change during the physiological regulation of the contraction. Some of these changes have been demonstrated in both intact tissue and in the isolated proteins, whereas others have been observed in either one or the other, but not both. To understand the physiological function of these mechanisms, it is necessary to know both the molecular basis for the change in the properties of the contractile proteins and the conditions under which these changes occur in the intact organ. There are problems inherent in demonstrating each of these. In an intact cell, how does one eliminate the possibility that a change in contractility is due to some component of the contractile mechanism other than the proteins, such as elements of the electromechanical coupling process, including transsarcolemmal calcium current and intracellular calcium release? In the case of the isolated proteins, the problem arises from the need for adequate preservation of the regulatory state that existed in the intact cell throughout the procedures that were necessary to isolate the proteins. Consequently, a reasonable level of understanding of a mechanism that alters the properties of the contractile proteins re-
quires the integration of several different types of information. First, there must be some clearly demonstrable change in the properties of the contraction of the intact cell that is produced by a physiologically relevant mechanism. Second, the changes must be produced in the isolated proteins by physiologically relevant molecules under physiologically relevant conditions. Third, the changes should be shown in some model of a cardiac cell in which the alterations in the proteins can be produced and the properties of the proteins directly probed to eliminate any contribution from modifications of electromechanical coupling. Skinned fibers or some version of skinned fibers offer the best possibility for satisfying this requirement. Fourth, in order to explain the changes in mechanical performance of the intact cell and organ under a controlled set of mechanical conditions, maximum calcium-activated force, stiffness, and either shortening velocity or ATPase activity should be measured [since the last two have a positive correlation (Barany, 1967)]. The contraction can be altered by changes in either the function of individual crossbridges, the number of active crossbridges, or the way in which crossbridges interact with each other. Theoretically, maximum force can be changed by either the number of active crossbridges or the force each generates. Stiffness is a reflection primarily of the number, but also the properties, of individual crossbridges. The rate of ATP splitting, probably the least specific of the three measurements, measures only the product of the number of active crossbridges and the turnover rate of the individual crossbridge. The meaningful understanding of a mechanism that regulates the contractile proteins requires the combination of physiological, biophysical, and biochemical data.

Regulation of the Calcium Sensitivity of the Contractile System

Variation of the calcium requirements for activating contraction (defined as calcium sensitivity) is the best understood regulatory mechanism of the contractile protein. The first indication that calcium sensitivity of cardiac contractile proteins might vary in a physiologically meaningful way came from the work of Bailey and Villar-Pilasi (1971), who showed that exposure of isolated cardiac myofibrils to a cAMP-regulated protein kinase altered the concentration of calcium necessary to increase the Mg-activated or actomyosin ATPase. They observed a decrease in the required concentration of calcium to produce full activation, that is, an increase in calcium sensitivity. Subsequently, Cole and Perry (1975) and Grand et al. (1976) showed that a cAMP-sensitive protein kinase could produce phosphorylation of the inhibitory component of both isolated cardiac and skeletal troponin (TNI), but in the presence of calcium and the complete troponin system, phosphorylation of TNI occurred only in the cardiac contractile protein. A marked difference between the structure of skeletal and cardiac troponin existed in the region of the amino acid that was phosphorylated. England (1975, 1976; Ray and England, 1976) first showed that phosphorylation of TNI was a physiologically meaningful reaction by perfusing isolated hearts with β-adrenergic agonists. After a positive inotropic effect had been observed, the hearts were quickly frozen, and the amount of phosphorylation of the isolated contractile proteins was determined. The degree of phosphorylation of TNI and the concentration of the β-adrenergic agonist in the perfusion medium were related. Although, initially, both the time course and the extent of the increase in contractile force correlated well with the degree of phosphorylation of TNI, the two could be dissociated (England, 1976). Contractility, as judged by the maximum force developed, was not altered by the phosphorylation of TNI, nor was the maximum actin-activated myosin ATPase activity. What changed, however, was the concentration of calcium ions that was necessary to produce a given percentage of maximum ATPase activity. Phosphorylation of TNI approximately doubled the concentration of calcium necessary for a given level of activation. This correlation, the opposite of what had been seen by Bailey and Villar-Pilasi (1971), has been observed in several different laboratories and is now generally accepted (Mope et al., 1980; Holroyde et al. (1979). A decrease in calcium sensitivity during exposure to β-adrenergic agonists has been suggested from experiments with intracellular calcium-sensitive microelectrodes (Marban et al., 1980).

It has been possible to show a direct correlation between the degree of phosphorylation of TNI and the calcium sensitivity of force generation in the same mammalian cardiac cells by first making the sarcolemma permeable to small molecules and ions with EGTA (Mope et al., 1980). These hyperpermeable cells have properties that make them well suited for studies of the contractile proteins. The membrane has been shown to be permeable to calcium, EGTA, ATP, ADP, creatine phosphate, and inorganic phosphate, but at the same time the membrane retains a functional β-receptor, α-receptor, cholinergic receptor, adenylate cyclase, and phosphodiesterase (McClellan and Winegrad, 1978; Mope et al., 1980). The ability to generate force is high, and it remains stable over hours. In these hyperpermeable cells, the concentrations of calcium necessary for production of half maximum tension is closely correlated with the percentage of TNI that has been phosphorylated (Mope et al., 1980). The degree of TNI phosphorylation can be modified by cAMP, cGMP, and by activation of the β-adrenergic system. In this preparation, the range over which calcium sensitivity varies is 5-fold, which is over twice that observed with isolated proteins. Apparently the extent to which some of the properties of the cardiac proteins can be regulated is diminished during the isolation of myofibrils or individual proteins. Regulation of
calcium sensitivity over a 2- to 3-fold range by cyclic nucleotides has been seen in Lubrol-treated pig heart cells (Herzig et al., 1981; Pfitzer et al., 1982). The protein kinase that is responsible for the phosphorylation of TNI is located in the sarcolemma of the myocardial cell (Weisberg et al., 1983; McClellan and Winegrad, 1980). This conclusion is drawn from the results of several studies. When the surface membrane of a mammalian cardiac cell has been either removed by manual dissection or partially removed by exposure to EGTA in the absence of ATP, the calcium sensitivity of the contractile system is maximal and there is little or no phosphorylation of TNI. In these preparations, cAMP cannot induce phosphorylation of TNI, in contrast to cells with an intact sarcolemma, where cAMP with a phosphodiesterase inhibitor produces phosphorylation of TNI and a decrease in calcium sensitivity (Weisberg et al., 1983). These studies indicate that either components of the sarcolemma or its diffusion barrier is required for the phosphorylation. The loss of a necessary soluble cytoplasmic factor, however, is unlikely. cAMP regulation of calcium sensitivity through phosphorylation of TNI disappears when a myocardial cell is treated with a non-ionic detergent that not only removes membrane lipids and phospholipids but also facilitates the loss of soluble cytoplasmic factors. Cyclic nucleotide regulation of calcium sensitivity and TNI phosphorylation is at least partially restored, however, by the addition of mixed phospholipids to the detergent-treated fibers. The loss of a soluble cytoplasmic factor is not primarily responsible for the effects of detergent (McClellan and Winegrad, 1980), but, instead, it appears that some factor located in a lipid-containing organelle, presumably a membrane, is required. Since the sarcomplasmic reticulum remains functional even as regulation of calcium sensitivity is lost in the mechanically skinned fiber, the most likely site for the membrane-bound protein kinase that phosphorylates TNI is the sarcolemma. Isolated cardiac sarcolemma has been shown to contain protein kinase that releases its catalytic subunit in the presence of cAMP (Corbin and Kelley, 1977). This sarcomembrane protein kinase probably occupies a sterically favorable location with respect to membrane-bound adenylate cyclase. Although addition of cAMP alone to a hyperpermeable cell does not decrease calcium sensitivity or phosphorylate TNI, the combination of cAMP with a phosphodiesterase inhibitor does cause phosphorylation and a decrease in calcium sensitivity. The amount of phosphorylation is never as great as that produced by the activation of adenylate cyclase either by a β-agonist or a lipid-soluble material like benzyl alcohol, even in the absence, in the latter case, of any phosphodiesterase inhibition. In other words, cAMP produced by adenylate cyclase in the sarcolemma is much more effective in activating the sarcomembrane protein kinase that produces TNI phosphorylation than cAMP added to either the extracellular or intracellular space (McClellan and Winegrad, 1978).

After cAMP has released the catalytic subunit from the membrane protein kinase, the subunit diffuses to the myofibril where it produces phosphorylation. The catalytic subunit appears to bind to the myofibrils, since removal of sarcolemmal membrane while the cell is stimulated by β-agonist does not totally eliminate the cell's capacity to regulate TNI phosphorylation and calcium sensitivity (Weisberg et al., 1983). If, however, the membrane is removed when the β-adrenergic system has not been activated and the catalytic subunit of sarcomembran protein kinase is presumably still bound to its regulatory subunit in the sarcolemma, there is total loss of regulation of calcium sensitivity. Dephosphorylation of TNI and increase in calcium sensitivity does not occur simply by the withdrawal of the cAMP stimulation of the protein kinase, either in the intact or the hyperpermeable cell (England, 1976; Weisberg et al., 1983). In the hyperpermeable cells, dephosphorylation is due to a cGMP-regulated reaction, presumably catalyzed by a phosphatase (Mope et al., 1980). cGMP with a phosphodiesterase inhibitor decreases TNI phosphorylation, but in the absence of TNI phosphorylation, cGMP has no effect on calcium sensitivity. Dephosphorylation through cGMP may be controlled by the cholinergic system (Horowits and Winegrad, 1983). Although withdrawal of the β-agonist reverses the increased inotropic state of intact cells, it does not reverse TNI phosphorylation (England, 1976). A cholinergic agent does, however, produce dephosphorylation. In hyperpermeable cardiac cells in which the level of calcium sensitivity is low, methacholine increases calcium sensitivity to its maximum, but it has no effect where calcium sensitivity is already maximal. Atropine blocks this effect of the cholinergic agonist. Since cholinergic agents increase the production of cGMP, it seems reasonable that cholinergic agents produce TNI dephosphorylation by stimulating the synthesis of cGMP (George et al., 1970, 1973), and activating a phosphatase. Although the cholinergic agonist may also produce TNI dephosphorylation by inhibiting membrane adenylate cyclase (Jakobs et al., 1979), this probably is not the major mechanism for its effect, since withdrawal of the β-agonist activating adenylate cyclase does not, by itself, reverse TNI phosphorylation.

A summary of the mechanism for the regulation of calcium sensitivity by phosphorylation of TNI is shown in Figure 1. The major effect of TNI phosphorylation and decrease in calcium sensitivity should be on the time course, rather than the maximum force of contraction of the intact cell (Robertson et al., 1982). Although phosphorylation of TNI increases the concentration of ionic calcium required for maximum activation by as much as 5-fold, it increases the total...
Calcium required for maximum activation by only about 20%. This discrepancy is a consequence of the fact that about 80% of the total calcium necessary for maximum activation is bound by the contractile proteins, and only 20% is required to maintain the concentration in free solution at a level that supports binding by the calcium regulatory site on troponin. The decrease in calcium sensitivity from β-adrenergic activity is more than completely balanced by the increase in inward calcium current during the action potential that also results from increased β-adrenergic activity (Reuter and Scholz, 1977).

Whereas the phosphorylation of TNI has only a relatively small effect on the maximum force developed by the contractile system, it should have a major effect on the kinetics of the contraction—in particular, the rate of relaxation. The reason for the weaker binding of calcium by phosphorylated troponin is the faster rate of release by troponin (Robertson et al., 1982). During relaxation, when cytoplasmic calcium is being removed by the sarcoplasmic reticulum and, possibly, the sarcolemma, the level of activation of the contractile system should decline more rapidly in the presence of phosphorylated than nonphosphorylated troponin. The change in the actual rate of decline of tension may be more complex, since it depends on reactions besides the loss of calcium from troponin. Theoretical calculation of the time course of cytoplasmic calcium, based on intracellular indicator studies and the rates of uptake and release of calcium by the various intracellular organelles, indicates that if tension transients approximately follow the time course of calcium saturation of the calcium-specific site on troponin, the accelerated rate of relaxation during β-adrenergic stimulation could result from the increased rate of release of bound calcium by phosphorylated TNI (Robertson et al., 1982). Modification of the contractile proteins themselves should be an important ingredient in the production of a more rapid relaxation by β-adrenergic stimulation, in addition to the faster uptake of calcium by the sarcoplasmic reticulum.

Calcium sensitivity of the contractile system is also influenced by the length of the muscle fiber. The relation between the concentration of calcium and tension is shifted to lower calcium concentration, that is, calcium sensitivity is increased, as the muscle fiber is stretched to longer sarcomere lengths within the range of 1.9 to 2.5 μm (Hibberd and Jewell, 1982). The shape of the relation changes in contrast to the effect of phosphorylation and dephosphorylation of TNI because the increase in calcium sensitivity at longer sarcomere lengths is greater for submaximal than for maximal activation. Skinned skeletal muscle behaves similarly. For submaximal levels of activation, optimal sarcomere length is about 2.7 μm, but the maximum level of activation is essentially independent of sarcomere length (Endo, 1973).

The mechanism for length dependence of calcium sensitivity is not clear. It cannot be due to changes in the amount of TNI phosphorylated because the phenomenon has been observed in detergent-treated fibers, in which the system for altering TNI phosphorylation is not functional. The length-dependent changes in calcium sensitivity can be observed when calcium has been added after sarcomere length has been increased in the relaxed muscle in which, presumably, there are no interactions between thick and thin filament. It is difficult to see how merely sliding filaments past each other could
change the nature of the calcium-binding site on troponin. In detergent-treated fibers that were used to demonstrate the effect of length on calcium sensitivity, the separation between thick and thin filaments is not changed very much with alteration of length because, in the absence of a sarcolemmal diffusion barrier, the volume of the filament lattice is not maintained constant (Elliott and Matsubara, 1972). Unless there are connections between thin filaments across the center of the sarcomere or interaction between thick and thin filaments at rest, another influence on thin filament conformation that is length dependent, such as from connective tissue (Winegrad, 1980), must exist.

Regulation of Maximum Force or Actomyosin ATPase

Regulation of either the maximum calcium-activated force or maximum actin-activated myosin ATPase, a property of the contractile system closely related to the generation of force, has been observed in three different preparations: (1) ventricular cells made hyperpermeable by EGTA (McClellan and Winegrad, 1980; Winegrad et al., 1983a; Winegrad and Weisberg, 1984), (2) freeze-dried sections of quickly frozen heart tissue (Winegrad et al., 1983a), and (3) lightly glutaraldehyde-fixed myofibrils (Franks et al., 1983). The first and second preparations have maintained a high level of filament structure and alignment, although separation between the filaments may have increased (McClellan and Winegrad, 1978). The third preparation uses treatment with a very low concentration of glutaraldehyde to stabilize the filament structure before exposing the myofibril to regulatory molecules. On the other hand, myosin and actomyosin isolated from part of the ventricles of rat hearts fail to show the regulation that can be observed in hyperpermeable fibers prepared from remaining regions of the same rat ventricles (Tucker and Winegrad, unpublished work). The conclusion from these observations is that certain forms of regulation of the cardiac myofibril are fragile. They can be seen only when cellular constituents and structure are retained, and the ability to alter the function of the contractile system diminishes as contractile proteins are isolated and purified. Caution must therefore be exercised in interpreting negative results about physiological regulation when preservation of the filament organization and cell constituents has not been considered and clearly demonstrated.

In hyperpermeable cells prepared from rat ventricular muscle, maximum calcium-activated force can be varied over a range as large as 7-fold by β-adrenergic stimulation or by addition of cAMP to the bathing medium (McClellan and Winegrad, 1980). In order to demonstrate the increase in contractility, a phosphodiesterase inhibitor must be included to prevent rapid hydrolysis by phosphodiesterases that are still active in hyperpermeable cells, and a low concentration of non-ionic detergent must be present to facilitate the release of an active component produced in the presence of the nucleotide by the hyperpermeable cell (McClellan and Winegrad, 1980; Weisberg et al., 1983). In the intact cell, as will be discussed below, the release occurs without the need for detergent. Apparently, the EGTA treatment that produces the hyperpermeable state of the membrane alters intracellular membranes and interferes with the normal physiological release of the active substance during β-adrenergic stimulation.

Regulation of contractility of the contractile proteins by cAMP involves at least two reactions (Fig. 2): the first a phosphorylation reaction that is cAMP dependent and leads to the release of an active

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**FIGURE 2.** Proposed mechanism (right) for the AMP regulation of the maximum calcium-activated force (left) developed by V1 myosin (see text).
factor, and the second an interaction between the active factor and the contractile proteins. In hyperpermeable fibers, the time between exposure to cAMP and detergent has a marked influence on the degree to which contractility is enhanced (Winegrad et al., 1983a). As the interval increases, the increment in contractility becomes smaller, the effect of the exposure to cAMP decaying with a half-time of approximately 4 minutes. If ATPS is used in place of ATP as the phosphate donor during the exposure to cAMP, to slow the rate of dephosphorylation after the removal of cAMP, there is little decline in the increase of contractility, even when the application of detergent has been delayed for as long as 60 minutes. Although the protein that is phosphorylated by the cAMP-regulated protein kinase has not yet been identified, there is no indication of its being one of the contractile proteins (Winegrad et al., 1983a).

The addition of detergent releases a factor, presumably the phosphorylated molecule or a portion of it that has inotropic activity. The evidence for this conclusion comes from studies of cardiac cells in which large holes in the sarcolemma have been produced very quickly (perforated cells) by perfusion of an isolated heart with a solution containing 10 mM EGTA and no ATP (Weisberg et al., 1983). In perforated myocardial cells, soluble cytosolic proteins pass through the sarcolemma and can be collected in the perfusion medium. When a heart has been washed free of the catecholamine released during sacrifice of the animal before its sarcolemma is perforated, the maximum calcium activated force that can be produced by the perforated cells is relatively low, and treatment with cAMP, theophylline, and detergent raises the contractility substantially. If, however, the heart is perfused with β-adrenergic agonist just before perforation of the sarcolemma, maximum calcium-activated force of the cells remains high, close to the enhanced level produced by the first group of hearts after treatment with cAMP, and it can be increased only a small amount by cAMP, theophylline, and detergent. Furthermore, if the volume of the perfusate is kept small, it is possible to show inotropic activity in the perfusate by assay with other hyperpermeable myocardial cells (Lin, McClellan, and Winegrad, unpublished results). Phosphorylation of the contractile proteins themselves is not a requirement for the increase in the contractile state. Some other alteration seems to be involved.

The ability of cAMP with theophylline and detergent to increase maximum calcium-activated force in hyperpermeable fibers has been disputed by Kentish and Jewell (1984) because they have been able to produce a maximum increase of only about 50% in their EGTA-treated rat fibers. They attribute the increase in force to the effect of the detergent in removing residual membrane diffusion barriers that impair movement of the calcium EGTA buffer system from the bathing medium into the myofibrils. In support of this conclusion they cite the fact that, in their experiments, the rate of rise of tension in response to an increase in the concentration of calcium is slow—half times are a minute or longer—and detergent shortens the half time. There are some important differences between their preparations and those of McClellan and Winegrad, in which an average of 160% increase in maximum calcium activated force has been produced. In most of the preparations of Kentish and Jewell (1984), maximum force per unit cross-sectional area is low, less than 30% of the values seen by others who used various forms of skinned or permeabilized cardiac fibers. Their claim that this can be explained by the residual membrane barrier to the diffusion of the calcium EGTA buffer is inconsistent with their own data, showing only a small increase in maximum calcium-activated force after treatment with detergent. In the hyperpermeable fibers of McClellan and Winegrad, the half time for the rise of tension after an increase in the concentration of calcium is shorter, 20–30 seconds, and it is not reduced by detergent. Furthermore, EGTA in the bathing solution inhibits both calcium- and caffeine-induced rises in tension (McClellan and Winegrad, 1978), which could happen only if the EGTA in the bathing solution had diffused to the immediate vicinity of the sarcoplasmic reticulum and myofibrils.

Studies of the ATPase activity of actomyosin of quickly frozen hearts show the existence of cAMP-sensitive myofibrillar regulation (Winegrad et al., 1983c; Winegrad and Weisberg, 1984). When contractility of hearts has been altered in situ by endogenous catecholamines released after the administration of either tyramine or 6-OH-dopamine, the myosin ATPase activity of freeze-dried thin sections of the heart is increased. The amplitude and the time course of the change in contractility and ATPase activity correlate well. A similar relation between ATPase in the tissue section and contractility of the heart exists after the perfusion of isolated hearts with β-adrenergic agonist. Both actin activated (actomyosin) and calcium activated (myosin) myosin ATPase activity change, indicating that a modification of myosin is the likely cause of the change in the enzymatic activity of actomyosin. These changes can occur in as short a time as 30 seconds, too fast for changes in the isomeric forms of myosin to occur. There is no detectable rise of ATPase activity of myosin extracted from the same hearts in which ATPase activity of myosin in the frozen sections has been increased. The regulatory state that is sensitive to β-adrenergic stimulation does not survive the procedures used in isolating the protein.

In rat and other small mammalian hearts, there are three isozymes of myosin, which have been labeled V1, V2, and V3, according to the rate at which they migrate in polyacrylamide gel during the electrophoresis in a pyrophosphate medium (Hoh et
The most rapidly migrating isozyme, V1, has the highest calcium-activated and actin-activated ATPase activity, and V3, the most slowly migrating, has the lowest ATPase. V2 is intermediate. The V1 myosin predominates in the young rats but gradually decreases with age. Removal of thyroid function by either propylthiouracil, hypophysectomy, or thyroidectomy produces a total transition to V3 in 10–20 days (Hoh et al., 1977), and thyrotoxicosis is associated with exclusively V1 myosin (Hoh et al., 1977; Martin et al., 1982).

The cAMP-dependent regulatory mechanism can recognize the different isozymes of myosin. β-Adrenergic stimulation of hyperpermeable cardiac cells from animals of different ages and different degrees of thyroid function produces an increase in maximum calcium-activated force that is proportional to the relative concentration of the V1 isozyme of myosin. In cells containing only V3 myosin as a result of hypothyroidism, cAMP with theophylline and detergent does not increase contractility, whereas the increase is largest in young, euthyroid rats, in which over 90% of the myosin is the V1 isomer (Winegrad et al., 1983a). The relation between the response to cAMP and the relative amount of V1 myosin is the same for animals with different degrees of thyroid function and different ages, indicating that other thyroid or age-dependent mechanisms are unlikely to be the basis for the different responses to cAMP. β-Adrenergic stimulation seems to increase the development of force by V1 myosin.

The physiological implications of a mechanism by which the heart can not only alter maximum contractile force but also select the relative activities of different myosin isozymes during a contraction are considerable. Since the maximum velocity of contraction seems to be influenced by the relative amounts of V1 and V3 myosin (Schwartz et al., 1981), the heart, through the cAMP regulatory mechanism, may be able to modulate both maximum force of contraction and maximum velocity of shortening. At least part of the more rapid rise in the rate of development of force during β-adrenergic stimulation could be due to selection of the most rapid myosin isozyme.

Isometric contraction involving the V1 isozyme of myosin requires more energy because crossbridges with the more rapidly cycling V1 split ATP at a faster rate during the maintenance of tension (Alpert and Mulieri, 1982). Consequently, a third parameter of the contraction, the energy cost, may be modulated in addition to maximum force and maximum velocity. The sensitivity of such a mechanism to the metabolic state of the cell, exercised possibly by the degree of coupling of the β-receptor to sarcolemmal adenylate cyclase, would be very useful for the long-term survival of the cell. If energy production was limited, as it might be in cases of coronary artery disease, the contraction would become slower and use less energy, since the maintenance of tension is an important factor in the energy requirements of the actively contracting heart.

Myosin Light Chain Phosphorylation

The contractile protein myosin contains six subunits, two heavy chains with molecular weights of about 200,000 daltons and two different pairs of light chains with molecular weights about 18,000 and 28,000 daltons. Each heavy chain consists of a long rod that is primarily α helical and a globular head that contains both the ATPase and actin-binding sites of the molecule. One light chain from each pair is associated with each heavy chain. The 18,000 dalton light chain, variously called LC2, P-light chain, and regulatory light chain, is important in the ATPase and contractile properties of the myosin molecule (Kendrick-Jones et al., 1976). It contains the calcium-binding site that is responsible for calcium regulation of myosin in many invertebrate striated muscles, and it is the site of phosphorylation that seems to be important in the activation of contraction in smooth muscle (Aksoy et al., 1976; Walsh et al., 1983). The second light chain, known as LC1 or the essential light chain, actually seems not to be essential, inasmuch as it can be removed by alkaline treatment without any as yet detectable loss of ATPase activity.

Mammalian cardiac myofibrils contain highly specific enzymes for the phosphorylation and dephosphorylation of the regulatory light chain (Pires et al., 1974; Perry, 1975; Morgan et al., 1976). Both calcium and calmodulin are required for activity of the kinase. The relative concentrations and activities of the kinase and the phosphatase in cardiac muscle are such as to produce a fine balance between phosphorylation and dephosphorylation (Perry, 1975), so that a small change in the activity of either enzyme could significantly shift the balance between phosphorylation and dephosphorylation. Since the concentration of calcium in the cytosol of cardiac muscle is not absolutely stable—changes occur during the contractile cycle and, possibly, with normal changes in sarcolemmal, sarcoplasmic reticulum, and mitochondrial function—the potential for changes in the degree of phosphorylation during normal physiological function of the heart does exist. As the rates of phosphorylation and dephosphorylation are not fast in terms of the cardiac cycle, changes in the degree of phosphorylation are unlikely to have a significant role in altering the function of the contractile proteins within a single beat.

The effect of phosphorylation of the regulatory light chain on the function of the heart is not clear. In smooth muscle, there is a considerable amount of data to implicate phosphorylation of the light chain in the activation of contraction (Aksoy et al., 1976; Walsh et al., 1983). In skeletal muscle, phosphorylation of the light chain seems to be associated with
the increase in force that develops during maintained activity (Klug et al., 1982; Manning and Stull, 1982). The phosphorylation has been said to decrease the activity of myosin and actomyosin ATPase (Barany et al., 1979; Crow and Kushmerick, 1982), but this has been disputed on the basis of strong experimental evidence (Butler et al., 1982). Studies with isolated myofibrils and glycerol-extracted fibers indicate a decrease in ATPase activity with phosphorylation as long as the contractile system has been treated with a low concentration of glutaraldehyde first, presumably to stabilize the filament lattice (Cooke et al., 1982). Without the use of glutaraldehyde, calcium and calmodulin produce phosphorylation of the light chain, but the ATPase activity is not altered.

As yet, however, no physiologically relevant mechanisms making use of these effects have been identified in cardiac muscle. Several laboratories have looked for physiological conditions under which a change in the phosphorylation of light chain of myosin might be associated with a modification of contractile behavior. Most of the interest has been in the degree of phosphorylation of the regulatory light chain as the inotropic state of the tissue is altered by stimulation with β-adrenergic agonists. When the tissues have been maintained under relatively physiological conditions, no significant increase in light chain phosphorylation in response to β-adrenergic stimulation has been observed (Holroyde et al., 1979; Stull, 1980). Without β-adrenergic stimulation, the relative amount of phosphorylation remains about 40–45% of maximum. Phosphorylation of the regulatory light chain is also insensitive to changes in inotropic state produced by variation of the extracellular concentration of calcium. Withdrawal of calcium from the bathing or perfusion medium causes an almost total disappearance of contractility within 30 seconds without any significant change in phosphorylation of the regulatory light chain, although, over a 30-minute period of perfusion with calcium-free solution, the degree of phosphorylation of the P-light chain drops reversibly by 40–50% (Stull, 1980).

Using a protease specific for the 18,000 dalton light chain of dog cardiac myosin that can be isolated from the hearts of dystrophic hamsters Bhan et al. (1978) and Malhotra et al. (1979) have studied the influence of the 18,000 dalton light chain on the enzymatic activity of myocardial myosin. Removal of the light chain increased the actin-activated ATPase of cardiac myosin by about 300% without altering the calcium-activated ATPase. At the same time, the calcium sensitivity of the actin-activated ATPase was unchanged, but the optimum concentration for the substrate MgATP rose 5-fold. Reduction of the isolated light chains restored the original lower level of ATPase activity of cardiac myosin (Bhan et al., 1981). If, however, each mole of light chain was phosphorylated with 2 moles of PO₄⁻ before readdition to myosin, the binding of the regulatory light chain to myosin did not reduce the actin-activated ATPase activity to its original level. The same increase in actin-activated myosin ATPase with removal of LC2 occurs in thyrotoxic hearts (Kuo and Banerjee, 1982). Actomyosin threads prepared from LC2-deficient skeletal myosin, on the other hand, have the same ability to generate force and to shorten as actomyosin containing normal myosin (Srivastava et al., 1980).

Although the regulatory light chain from heart muscle can replace the regulatory light chain on molluscian myosin and preserve the calcium triggering mechanism on myosin in the calcium-sensitive activation of invertebrate muscle (Frearson and Perry, 1975; Kendrick-Jones, 1974), there is no evidence for the existence of a myosin-based calcium trigger in heart muscles.

Two highly specific enzymes that control a specific reaction involving a contractile protein are unlikely to exist without an important function. So far, most of the work has focused on a role in the modulation of contractile function. Other possible functions of the phosphorylation of myosin light chain might be in the regulation of turnover of myosin or in protection from the effects of proteolytic enzymes such as the neutral calcium-activated protease. There is at present too little information in this area to support even speculation.

**Summary**

Modulation of the functional properties of the contractile proteins of mammalian heart muscle plays a significant role in the response of the heart to β-adrenergic stimulation. The most well understood modification is a change in the concentration of calcium ions that is required to activate the contractile system. By means of a cAMP-sensitive phosphorylation of the inhibitory subunit of troponin (TNI), the threshold concentration for activation can be increased as much as 5-fold without changing the maximum calcium-activated force. The protein kinase involved in this regulation is located in the sarcolemma. Cholinergic stimulation causes a dephosphorylation of TNI by a cGMP-sensitive phosphatase. The concentration of calcium ions required to activate contraction also decreases as muscle length increases. This response of the contractile proteins does not involve phosphorylation of TNI.

Regulation of the maximum calcium-activated force can take place by a cAMP-sensitive reaction involving a different protein kinase that is located inside the cell. This mechanism involves at least two sequential reactions, one a cAMP-controlled phosphorylation of a protein bound to an intracellular membrane to release an active factor, and the second, an interaction between the active factor and the contractile proteins to enhance the capacity for generating force in the presence of calcium.

Phosphorylation of the light chain of myosin is
produced by a calmodulin-regulated kinase. The light chain of myosin is partially phosphorylated in the intact heart, but β-adrenergic stimulation of the heart does not increase the decrease of phosphorylation in parallel with the increase in contractility.

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Winegrad / Contractile Proteins

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