

# The Cellular Basis of Cardiac Contractility

## 5.7

### Learning Objectives

- Describe the general ultrastructural features of cardiomyocytes including intercalated disks, myofibrils, mitochondria, sarcoplasmic reticulum, sarcolemma, nuclei, and T-tubules
- Compare the structure of cardiomyocytes to skeletal muscle fibers
- Describe the components of the thick filaments
- Describe the components of the thin filaments
- Identify A band, I band, H zone, Z line, M line, and sarcomere
- Trace the events in excitation–contraction coupling from the action potential on the SL to activation of the contractile elements
- Describe calcium-induced calcium release
- List the cellular mechanisms responsible for cytosolic calcium homeostasis
- Identify the mechanisms by which cardiac strength of contraction can be varied
- Describe what is meant by the positive staircase and describe its mechanism
- Describe the basis of the inotropic effect of sympathetic stimulation
- Describe how cardiac contractile force is modulated by stretch

### INTERCALATED DISKS ELECTRICALLY COUPLE CARDIOMYOCYTES

The intercalated disks appear dense in histological specimens or under the electron microscope. They contain **gap junctions**, **adherens junction**, and **desmosomes**. Gap junctions (see Chapter 4.2) consist of fields of connexin hexamers that form one-half of a channel linking adjacent cells. The full channel forms when one hemi-channel in the membrane of one cell joins up with a second hemi-channel in the adjacent cell. The two hemi-channels form an aqueous pathway between the two cells. These gap junctions allow passage of current so that adjacent cells are **electrically coupled**, allowing nearly simultaneous electrical activation of all of the cardiomyocytes. The desmosomes rivet cells together like miniature spot welds. They consist of plaques of cadherin molecules, a transmembrane glycoprotein that spans the gap between cells and anchors the desmosome to desmin filaments in the cytoplasm. Thus, the desmosome joins intermediate filaments in one cell to those in its neighbor. In the adherens junction, the extracellular part of cadherin molecules binds those from the adjacent cell. The cadherin molecules link to actin filaments in the cytoplasm through a variety of anchoring proteins.

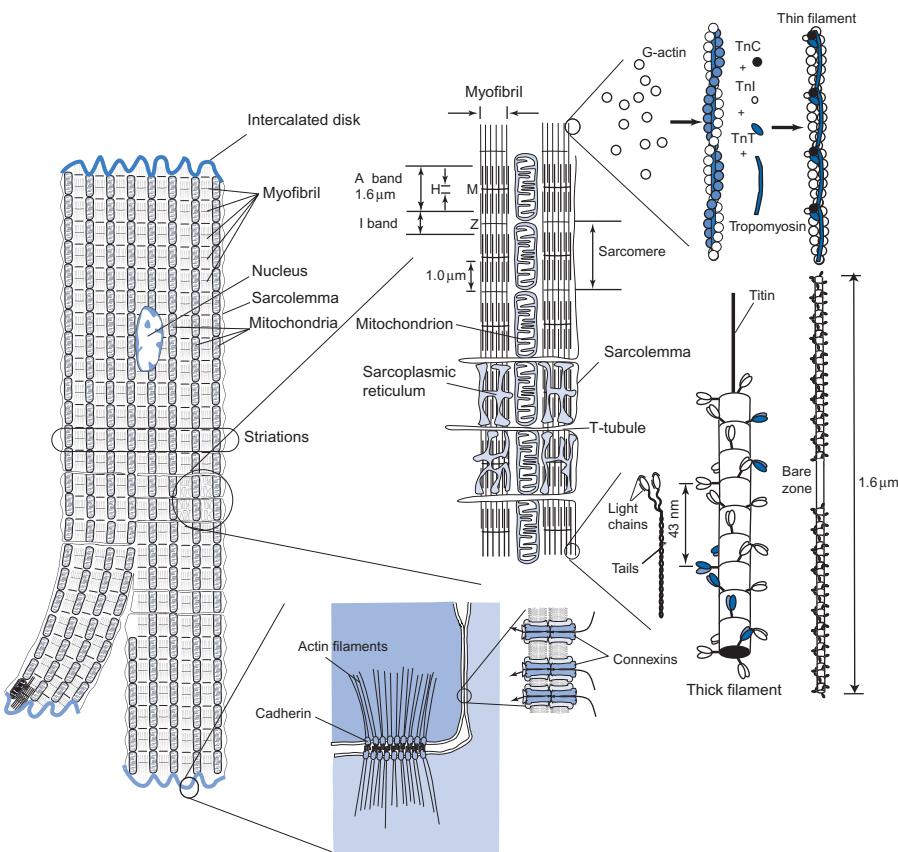
### CARDIAC MUSCLE SHARES MANY STRUCTURAL FEATURES WITH SKELETAL MUSCLE

The main structural features of cardiac muscle reprise those of skeletal muscle, but there are notable differences (see *Figure 5.7.1*). Cardiomyocytes are typically 10–20 µm across and about 50–100 µm long, whereas skeletal muscle fibers are larger in diameter (20–100 µm) and many centimeters long. The cardiomyocytes typically have one or two nuclei, whereas skeletal muscle fibers have many hundreds of nuclei. Muscle fibers themselves do not branch, whereas cardiomyocytes often branch. Because of their different structures, the surface membrane of skeletal muscle fibers generally fuses with tendons, whereas cardiomyocytes form junctions with neighboring cells. These junctions are the **intercalated disks**. Because the heart must contract rhythmically throughout all of life, it is a highly aerobic organ. Accordingly, mitochondria occupy 30–35% of the cellular volume, a greater fraction than any skeletal muscle.

### THE STRENGTH OF CARDIAC MUSCLE CONTRACTION IS NOT REGULATED BY RECRUITMENT OR BY SUMMATION

As in skeletal muscle, electrical activation of cardiomyocytes begins with an action potential that is propagated along the surface of the cell. As discussed in Chapter 5.5, the cardiac action potential differs markedly from the skeletal muscle action potential both in its overall character and in its duration. The cardiac action potential lasts nearly as long as the cardiac muscle contraction, whereas the skeletal muscle action potential is very brief, on the order of a few milliseconds. Because of this, cardiac muscle cannot summate. The force of skeletal muscle force is varied by recruitment of motor units and summation of force by repetitive stimulation. In cardiac muscle, all of the cardiomyocytes are activated for each heart beat because the cells are electrically coupled: there is 100% recruitment all of the time. Because the action potential lasts so long, it is not possible to stimulate the cardiomyocytes for a second time during the heart beat, so

**FIGURE 5.7.1** Gross and fine structure of a ventricular cardiomyocyte. The cell is surrounded by a sarcolemma (SL) which invaginates at regular intervals to form transverse or T-tubules. These bring the excitation of the SL into the interior of the cell. The cell typically has one or two nuclei. The cytoplasm is filled with myofibrils and mitochondria. The myofibrils consist of bundles of contractile filaments. The thin filaments are bundles of filamentous actin that are centered on the Z line. These filaments also contain tropomyosin, and the troponin complex of TnC, TnI, and TnT. The tropomyosin and troponin complex regulate contraction. The thick filaments are centered on the M line and consist of a titin scaffold on which myosin molecules assemble. The thick and thin filaments interdigitate, forming a series of cross-bands as shown. The A band corresponds to the thick filaments, but the H zone in its middle is the part where thin filaments do not overlap the thick filaments. The I band corresponds to the part of the thin filaments that do not overlap the thick filaments. Contraction is controlled by cytosolic  $[Ca^{2+}]$ . The SR is a membranous network that surrounds the myofibril. The SR releases  $Ca^{2+}$  during excitation to trigger contraction. The SR ends contraction by transporting activator  $Ca^{2+}$  back into the SR lumen.



cardiac muscle does not respond to repetitive stimulation. So what regulates cardiac muscle contractility? We will first discuss cardiac muscle structure and excitation–contraction coupling before we attempt an answer.

## CARDIAC MYOFIBRILS HAVE THICK AND THIN FILAMENTS AND FORM THE CROSS-STRIATIONS

Both cardiac and skeletal muscles are **striated**. The striations arise from the regular arrangement of the **myofilaments** which are kept in register all the way across the diameter of the cell. These myofilaments consist of repeating units called **sarcomeres**. Each sarcomere is 1.8–2.0  $\mu m$  long. The bands in each sarcomere are named for their microscopic appearance. The “A” band resides in the middle of the sarcomere and derives its name from the German “anisotropische,” referring to the anisotropic way the proteins in this band handle polarized light. The “I” bands lie adjacent to the A bands on each side. Their name means “isotropische,” referring to their isotropic handling of polarized light. The Z line is a thin partition in the middle of the I band. The sarcomere extends from Z line to Z line. The middle of the A band typically has an “M” line, meaning “mittel” or middle. The M line is in the middle of the “H” zone, from “helles,” meaning “clear.” The contractile proteins in cardiac muscle, like skeletal muscle, are arranged in longitudinal bundles called **myofibrils**. In cardiac muscle, these myofibrils are adjacent to rows of mitochondria. The

myofibrils are typically 1  $\mu m$  across and span the distance from one end of the cell to the other. The myofibrils in turn are made up of contractile proteins that are contained in smaller myofilaments.

The A band contains **thick filaments**, about 15 nm in diameter, composed mainly of **myosin** and **titin**. The I band contains **thin filaments**, 6 nm in diameter. The A band is always about 1.6  $\mu m$  long because the thick filaments are this long. The giant protein titin extends from the M line to the Z line. It contains many binding sites for myosin, which assemble on the titin. Each thick filament contains about 300 myosin molecules. Myosin molecules are about 160 nm long and consist of six proteins. Two myosin heavy chain proteins of about 200,000 Da wind around each other to make the super-coiled tail, and each heavy chain contributes one of the two globular heads. These project away from the coiled tail and enable the myosin molecule to interact with **actin** in the thin filament. Two pairs of **myosin light chains** bind to the globular heads. The myosin assembles on the titin scaffold so that the tails face the M line and the heads are oriented toward the Z line. Thus, the thick filament is polarized. Because of this arrangement, the center of the A band contains a short region, about 0.3  $\mu m$  long, that contains only tails and no heads. This is the **bare zone**.

The thin filaments consist of **filamentous** or **F-actin**, constructed by linking **globular**, or **G-actin**, into two intertwined chains that are about 1.0  $\mu m$  long. G-actin has a molecular weight of about 42,000 Da. About

seven of these make up each half-turn of the F-actin filament, which has a repeat distance of 38.5 nm. The filaments bind to **actinin** at the Z line and extend into the A band in both directions. The I band is only about 0.25  $\mu\text{m}$  wide because the major part of the thin filament interdigitates between the thick filaments in the A band. **Tropomyosin** is a heterodimer made up of  $\alpha$  and  $\beta$  subunits that coil around each other to form a rod-like structure that lays in the groove of the F-actin. Each tropomyosin extends one half-turn, 38.5 nm. A tropomyosin complex consisting of three separate proteins resides on the end of each tropomyosin. **Troponin I** inhibits actin-myosin interaction; **troponin C** binds  $\text{Ca}^{2+}$  ions; and **Troponin T** binds to tropomyosin. The interaction of thick and thin filament is controlled by  $\text{Ca}^{2+}$  binding to TnC as it is in skeletal muscle, except the cardiac TnC is a different isoform from skeletal muscle TnC.

## ACTIN-ACTIVATED MYOSIN ATPase ACTIVITY PRODUCES FORCE AND SHORTENING

The globular head of the myosin heavy chains is an actin-activated ATPase. During rest, the myosin heads cannot interact with the actin filaments because tropomyosin is in the way. When the muscle is activated, tropomyosin shifts out of the way and the globular heads engage the actin filaments, forming cross-bridges between the thick and thin filaments. These cross-bridges cycle between states with high and low affinity for the thin filaments. The cycling requires ATP hydrolysis and results in movement or force development. An abbreviated version of the overall ATPase cycle is shown in Figure 5.7.2.

## CYTOPLASMIC $\text{Ca}^{2+}$ CONTROLS ACTOMYOSIN CROSS-BRIDGE CYCLING

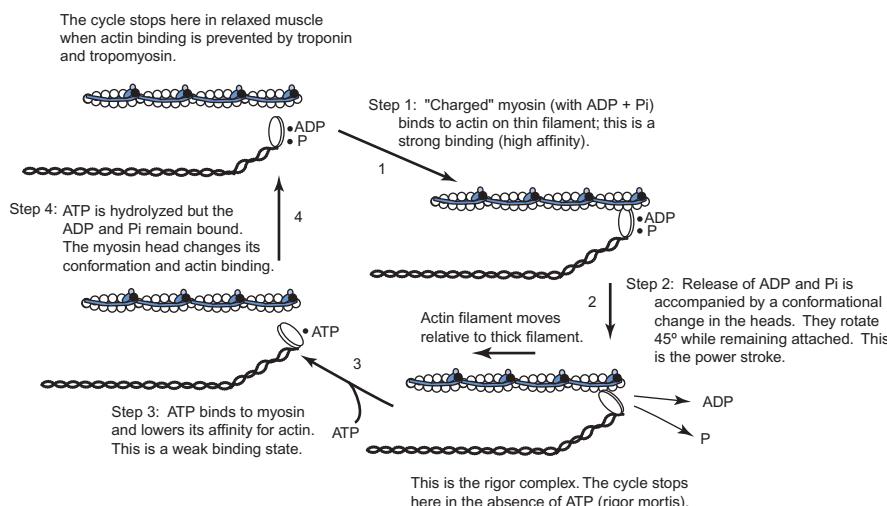
Cytoplasmic  $\text{Ca}^{2+}$  controls the inhibitory position of tropomyosin on the thin filament. During diastole,

cytoplasmic  $\text{[Ca}^{2+}]$  is low and tropomyosin inhibits interaction between the thin and thick filaments. During excitation, the sarcoplasmic reticulum (SR) releases  $\text{Ca}^{2+}$  from its stores and the  $\text{Ca}^{2+}$  binds to TnC, causing a series of conformational changes that remove tropomyosin from its inhibitory position. Cross-bridge cycling then follows and the cardiomyocyte shortens and develops tension. The relation between force and  $\text{[Ca}^{2+}]$  is steep, as shown in Figure 5.7.3.

## CALCIUM-INDUCED CALCIUM RELEASE COUPLES EXCITATION TO CONTRACTION IN CARDIAC MUSCLE

### THE SR RELEASES STORED $\text{Ca}^{2+}$ TO ACTIVATE CONTRACTION

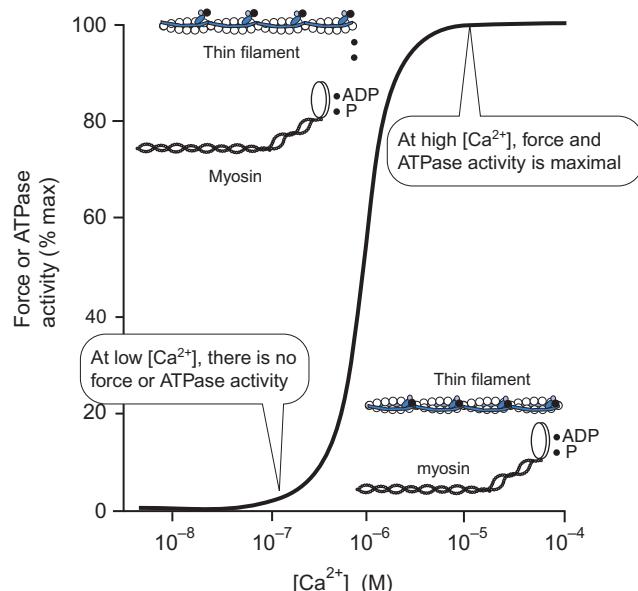
In cardiac muscle, the **transverse** or (T)-tubules penetrate the muscle cell interior at the level of the Z line, so that cardiac muscle has only one T-tubule per sarcomere. The **sarcoplasmic reticulum** or SR membrane is a membranous network that surrounds the myofibrils and makes contact with the T-tubule at junctions called **dyads**. So-called "feet" structures join the junctional SR to the T-tubules. These consist of **ryanodine receptors** (RyR) which form a  $\text{Ca}^{2+}$  channel across the SR membrane. Cardiac muscle also has **peripheral junctional SR** that make contact directly with the sarcolemma (SL), and **extended junctional SR** (also called cisternal SR) that have feet structures while making no direct contact with either SL or T-tubules. Three different ryanodine receptor isoforms exist: RyR1, RyR2, and RyR3. Skeletal muscles express mainly RyR1; heart and brain express RyR2; epithelial tissues, smooth muscle, and brain also express RyR3. At the T-tubule, the RyR closely approaches **dihydropyridine receptors** (DHPRs). A schematic diagram of the approximate anatomic arrangement of the SR in cardiac muscle is shown in Figure 5.7.4.



**FIGURE 5.7.2** The actomyosin cross-bridge cycle. At rest, ATP binds to myosin (M) forming M-ATP. This lowers its affinity for the thin filament actin (A) (3). The ATP is spontaneously hydrolyzed, forming M-ADP-P<sub>i</sub>, but the product ADP and P<sub>i</sub> remains bound (4). This form of myosin has a high affinity for A. At rest, it cannot bind to A because tropomyosin (Tm) prevents the binding. During activation, Tm shifts out of the way and M-ADP-P<sub>i</sub> binds to A to form A-M-ADP-P<sub>i</sub>. This complex releases first P<sub>i</sub> and then ADP, and the myosin head rotates with the attached A filament (A-M) (2). This is the power stroke that moves the thin filament some 5–10 nm past the thick filament. The A-M state is the rigor complex. It forms a stiff attachment of the actin filament to the myosin filament. Binding of ATP to M dissociates the complex so that the cycle can begin again (3). Thus, cycling through the reaction scheme hydrolyzes ATP and causes shortening or force development by the myofibrils.

### THE DHPR SENSES T-TUBULE VOLTAGE AND FORMS A $\text{Ca}^{2+}$ CHANNEL

The dihydropyridines are a class of drugs that include nifedipine, nisoldipine, nifedipine, and BayK 8644. All of these affect the L-type  $\text{Ca}^{2+}$  channel current that is present on ventricular cardiomyocytes. BayK 8644 activates it; the other three inhibit it. The skeletal muscle DHPR consists of five subunits ( $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), one of which ( $\alpha_1$ ) forms the  $\text{Ca}^{2+}$  channel and has the DHPR binding site. The cardiac DHPR appears to lack the  $\gamma$  subunit. This difference probably explains the different mechanisms of excitation–contraction coupling in skeletal and cardiac muscle. The DHPR senses depolarization of the T-tubule membrane and opens its  $\text{Ca}^{2+}$  channel, allowing  $\text{Ca}^{2+}$  to enter the cell.



**FIGURE 5.7.3** Control of actomyosin cycling and force development by cytoplasmic  $[\text{Ca}^{2+}]$ . No force is developed when cytoplasmic  $[\text{Ca}^{2+}]$  is less than about  $10^{-7}$  M, and force is maximal when  $[\text{Ca}^{2+}]$  is about  $10^{-6}$  to  $10^{-5}$  M. The steep dependence of force on  $[\text{Ca}^{2+}]$  is due to cooperativity. Thus, the cytoplasmic  $[\text{Ca}^{2+}]$  is a switch that controls force development and actomyosin cross-bridge cycling.

### $\text{Ca}^{2+}$ ENTRY THROUGH THE DHPR TRIGGERS SR $\text{Ca}^{2+}$ RELEASE

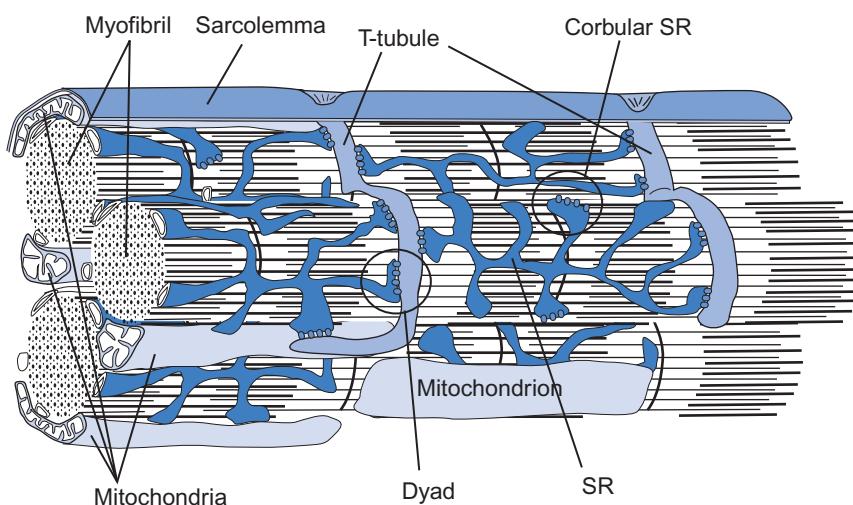
Release of  $\text{Ca}^{2+}$  from the SR in cardiac muscle involves a process called **calcium-induced calcium release**, or CICR. Depolarization of the SL membrane opens voltage-dependent  $\text{Ca}^{2+}$  channels in the DHPR that let  $\text{Ca}^{2+}$  into the cell near the RyR2 receptors on the SR. This  $\text{Ca}^{2+}$  is insufficient by itself to activate contraction, but it is amplified by causing a further and greater  $\text{Ca}^{2+}$  release from the SR. This process is shown schematically in Figure 5.7.5.

### THE SIZE OF THE SL $\text{Ca}^{2+}$ TRIGGER AND THE AMOUNT OF $\text{Ca}^{2+}$ STORED IN THE SR DETERMINE THE SIZE OF THE $\text{Ca}^{2+}$ TRANSIENT

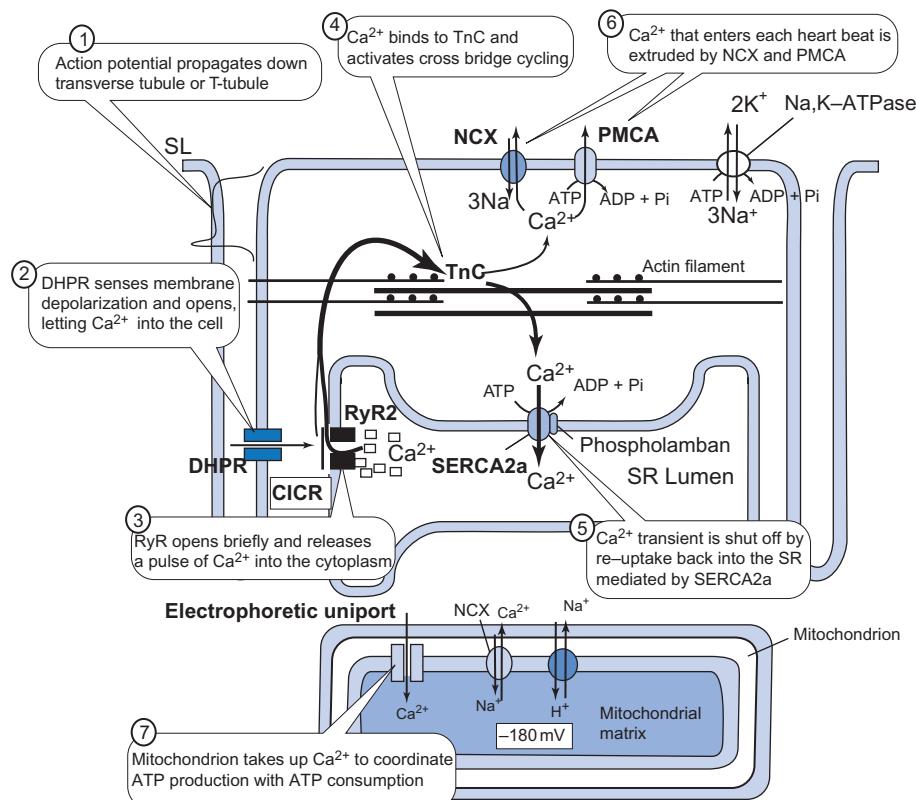
The amount and rate of  $\text{Ca}^{2+}$  that enters the cardiomyocyte upon electrical activation controls the number of RyR2 channels that open. Thus, increasing SL  $\text{Ca}^{2+}$  entry generally increases  $\text{Ca}^{2+}$  release from the SR. Increasing the store of  $\text{Ca}^{2+}$  inside the SR also increases the amount of  $\text{Ca}^{2+}$  that is released.

### REUPTAKE OF $\text{Ca}^{2+}$ BY THE SR AND SL EXTRUSION OF $\text{Ca}^{2+}$ CAUSE RELAXATION

Binding of  $\text{Ca}^{2+}$  to TnC initiates contraction, and removal of  $\text{Ca}^{2+}$  from TnC brings about relaxation. Removal of activator  $\text{Ca}^{2+}$  is accomplished in cardiac muscle by two membranes: the SL and the SR. A **Ca-ATPase pump** on the SR membrane couples movement of two  $\text{Ca}^{2+}$  atoms from the cytoplasm into the SR lumen to the hydrolysis of one ATP molecule. This active transport pump is an isoform of the SERCA pump family, for smooth endoplasmic reticulum Ca-ATPase. The isoform in cardiac SR is SERCA2a. This is regulated by phosphorylation of phospholamban, a 5-kDa protein in the SR membrane.



**FIGURE 5.7.4** Arrangement of T-tubules and SR in ventricular cardiomyocytes. The SL invaginates to form T-tubules at the level of the Z lines. The SR forms dyad junctions with the T-tubule. Cardiac SR also forms junctions with the peripheral SL and has regions called corbulular SR that contain junctional proteins without forming a junction with T-tubule or SL.



**FIGURE 5.7.5** Overall processes involved in  $\text{Ca}^{2+}$  transport in the ventricular cardiomyocyte. Excitation begins with the cardiac action potential (1) that propagates over the surface of the cell and down into the T-tubules. Both the surface of the cell and T-tubules possess L-type  $\text{Ca}^{2+}$  channels that open in response to depolarization (2). This lets in a small amount of  $\text{Ca}^{2+}$  in the vicinity of the junction of the SR with the T-tubule or SL membrane (peripheral junctional SR and extended junctional SR). This trigger  $\text{Ca}^{2+}$  binds to ryanodine receptors (RyR2) on the SR membrane and briefly opens a  $\text{Ca}^{2+}$  channel in the RyR2 (3). This releases  $\text{Ca}^{2+}$  from the SR in a process called “calcium-induced calcium release” (CICR). The rapid release of  $\text{Ca}^{2+}$  transiently raises the cytosolic  $[\text{Ca}^{2+}]$ , causing  $\text{Ca}^{2+}$  to bind to troponin C on the thin filament (4). This binding moves tropomyosin out of its inhibitory position, thereby allowing the thin filament to interact with the thick filament, resulting in force development and actomyosin ATPase activity. The contraction is shut off by the removal of activator  $\text{Ca}^{2+}$  from the cytosolic compartment mainly by reuptake into the SR mediated by the SERCA2a pump in the SR (5), and secondarily by extrusion into the extracellular space by a NCX that couples the influx of three  $\text{Na}^+$  to the exit of one  $\text{Ca}^{2+}$  ion. A relatively minor component of  $\text{Ca}^{2+}$  exit is through PMCA. The mitochondria can also take up  $\text{Ca}^{2+}$  through the electrophoretic uniport, and release it through  $\text{Na}^+-\text{Ca}^{2+}$  exchange (7). The largest source of activator  $\text{Ca}^{2+}$  is the SR and its greatest sink during relaxation is also the SR.

$\text{Ca}^{2+}$  enters each cardiomyocyte with every heart beat. At steady state, the  $\text{Ca}^{2+}$  that enters with each heart beat must also exit each beat, or else the heart will continue to accumulate  $\text{Ca}^{2+}$ . The entry of  $\text{Ca}^{2+}$  is mediated mainly by the L-type  $\text{Ca}^{2+}$  channels. Most of the efflux of  $\text{Ca}^{2+}$  from the cell is carried by the  $\text{Na}^+-\text{Ca}^{2+}$  exchanger (NCX) that brings in three  $\text{Na}^+$  ions for every  $\text{Ca}^{2+}$  ion that is extruded from the cell. This is an example of secondary active transport. Because of the changes in  $[\text{Ca}^{2+}]$  and membrane potential during the action potential, NCX can mediate either  $\text{Ca}^{2+}$  influx or efflux from the cell. A second efflux mechanism is through the plasma membrane Ca-ATPase pump, or PMCA. This protein has a molecular weight of 138 kDa and is distinct from the SR  $\text{Ca}^{2+}$  pump. It pumps one  $\text{Ca}^{2+}$  out of the cell for each ATP hydrolyzed, which differs from the 2:1 stoichiometry of the SR Ca pump. PMCA is another example of primary active transport. It is regulated by phosphorylation by 3',5'-cAMP-activated protein kinase (PKA), and calmodulin-dependent protein kinase (CAM-PK).

## MITOCHONDRIA CAN TAKE UP $\text{Ca}^{2+}$

Mitochondria can take up  $\text{Ca}^{2+}$  through a mechanism called the **electrophoretic uniport** that uses the negative transmembrane potential difference across the inner mitochondrial membrane to drive  $\text{Ca}^{2+}$  movement down its electrochemical gradient. Mitochondria probably take up  $\text{Ca}^{2+}$  mainly to regulate ATP production rather than to participate in beat-to-beat regulation of cytosolic  $[\text{Ca}^{2+}]$ .

## CALSEQUESTRIN AUGMENTS SR $\text{Ca}^{2+}$ UPTAKE AND RELEASE

Pumping  $\text{Ca}^{2+}$  from the cytoplasm into the SR lumen increases the luminal  $[\text{Ca}^{2+}]$ . The enclosed SR volume is a tiny fraction of the entire cell volume, and so holding all of the activator  $\text{Ca}^{2+}$  would require high intraluminal  $[\text{Ca}^{2+}]$  which would slow down pumping because the pump would have to work against a large gradient. Calsequestrin, a  $\text{Ca}^{2+}$ -binding protein inside the SR,

keeps the luminal  $[Ca^{2+}]$  low without sacrificing  $Ca^{2+}$  content. Calsequestrin has many acidic amino acids that bind  $Ca^{2+}$  with low affinity ( $K_D \approx 10^{-3} M$ ). This low affinity allows the SR to store plenty of  $Ca^{2+}$  while still allowing a gradient that favors rapid release of  $Ca^{2+}$  when the RyR2 channels open. Calsequestrin also interacts with RyR2 to promote  $Ca^{2+}$  release.

## WHAT REGULATES CARDIAC CONTRACTILITY?

Skeletal muscle controls force through recruitment of motor units and increased frequency of motor neuron activation. The heart can use neither of these mechanisms because the heart is fully recruited every heart beat and it cannot tetanize. So what regulates cardiac contractility? Muscle force depends on the number of actomyosin cross-bridges. Skeletal muscle SR normally releases enough  $Ca^{2+}$  to fully saturate all of the TnC in the fiber. Cardiac SR typically does *not* raise cytoplasmic  $[Ca^{2+}]$  high enough to saturate TnC. Thus more cross-bridges can be activated by increasing the size of the  $[Ca^{2+}]$  transient or by changing the affinity of the myofilaments for  $Ca^{2+}$ . Accordingly, cardiac contractility can be altered by one of two means:

1. Change the size of the  $[Ca^{2+}]$  transient
2. Change the sensitivity of the myofilaments to a given  $Ca^{2+}$  transient.

The heart uses both of these entirely different mechanisms to vary cardiac contractility.

## THE FORCE GENERALLY INCREASES WITH THE FREQUENCY OF THE HEART BEAT: THE FORCE–FREQUENCY RELATION

In 1871 HG Bowditch observed that increasing the frequency of heart beats causes a gradual increase in the force of contraction. When the frequency is increased from low values, the first beat is usually weaker, but the force of subsequent beats gradually increases until a new steady state is reached. The record of force resembles a staircase, and so the phenomenon is called a **positive staircase** or **Treppe**. A typical record is shown in Figure 5.7.6.

The size of the  $Ca^{2+}$  transients explains the positive staircase or Bowditch effect. Increasing the frequency increases the total influx of  $Ca^{2+}$  across the SL because there are more action potentials. The result is that the SR becomes more loaded with  $Ca^{2+}$  and releases more  $Ca^{2+}$ . The SR gradually loads more because it is exposed to a higher average  $[Ca^{2+}]$  and therefore pumps in more  $Ca^{2+}$ . The increased  $[Ca^{2+}]$  transient recruits more actomyosin cross-bridges to make more force.

The first beat after increasing the frequency is usually weaker because there has been insufficient time for the SR RyR2 channels to recover from the previous excitation. By the next beat, the SR  $Ca^{2+}$  store has increased and so SR  $Ca^{2+}$  release increases.

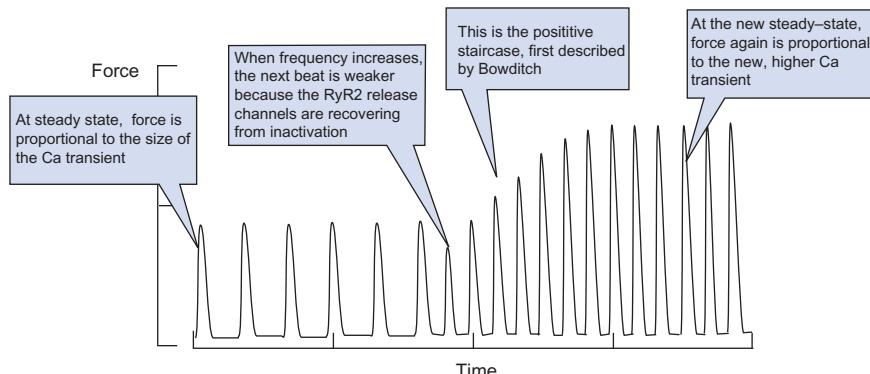
Increasing force with increasing frequency has a limit: if the heart rate becomes too fast, force decreases again, for two reasons: (1) ventricular filling cannot keep pace with the higher contraction frequency, and so there is less volume in the ventricle and less pressure; (2) at high frequencies the action potential duration is shorter and the time for  $Ca^{2+}$  influx shortens. Thus, at very high frequencies the  $Ca^{2+}$  transient becomes smaller again.

## SYMPATHETIC STIMULATION INCREASES FORCE BY INCREASING THE $Ca^{2+}$ TRANSIENT

Noradrenaline released from sympathetic nerves in the heart binds mainly to  $\beta_1$  receptors that are coupled to a heterotrimeric  $G_s$  protein. The  $\alpha$  subunit of this  $G$  protein activates adenylyl cyclase, raising the cytosolic concentration of 3',5'-cyclic AMP, which in turn activates protein kinase A (PKA). PKA phosphorylates a number of proteins within the cell. In the SA node, sympathetic stimulation increases the heart rate (**positive chronotropy**), which by itself also increases the force of contraction through the force–frequency relation. Ventricular myocytes also have  $\beta_1$  receptors that activate PKA to phosphorylate a number of targets, including:

- L-type  $Ca^{2+}$  channel
- Phospholamban (PLB)
- RyR2, the SR  $Ca^{2+}$  release channel
- TnI

Phosphorylation of the L-type  $Ca^{2+}$  channel increases  $I_{Ca}$ , leading to greater SL  $Ca^{2+}$  influx that contributes



**FIGURE 5.7.6** The positive staircase, or Treppe. The curves show pressure developed by the heart at a low frequency and at higher frequencies. When the frequency is increased, the first beat is generally weaker and subsequent beats gradually increase in force, forming a staircase response until a new steady state is reached.

directly to a larger  $\text{Ca}^{2+}$  transient and increased SR  $\text{Ca}^{2+}$  load. It also increases the size of the  $\text{Ca}^{2+}$  trigger to release  $\text{Ca}^{2+}$  from the SR by CICR. All of these effects lead to an increased  $\text{Ca}^{2+}$  transient.

PLB is a 5-kDa protein that forms pentamers in the SR membrane. The dephosphorylated PLB inhibits the SERCA2a Ca-ATPase by reducing its affinity for  $\text{Ca}^{2+}$ . Phosphorylation by PKA removes this inhibition, leading to increased  $\text{Ca}^{2+}$  pumping at submaximal  $[\text{Ca}^{2+}]$ . The result is increased loading of the SR and increased release of  $\text{Ca}^{2+}$  upon excitation. This also increases the rate of relaxation, a phenomenon called the **lusitropic effect**.

There are multiple phosphorylation sites on the RyR2 which are believed to increase the size of the  $\text{Ca}^{2+}$  transient.

TnI is a 32-kDa protein that forms part of the control mechanism for EC coupling. Phosphorylation of TnI by PKA increases the rate of  $\text{Ca}^{2+}$  dissociation from TnC. Since the affinity of TnC is the ratio of the on and off rate constants, increasing  $k_{\text{off}}$  decreases the affinity. This should reduce force development unless the  $\text{Ca}^{2+}$  transient increases enough to overcome the reduced sensitivity of the myofilaments. TnI phosphorylation aids the rate of removal of  $\text{Ca}^{2+}$ , thereby increasing the rate of relaxation and contributing to the lusitropic effect of sympathetic stimulation.

**Figure 5.7.7** illustrates the effect of sympathetic stimulation on parts of the cardiomyocyte that cause its inotropic and lusitropic effects. The chronotropic effects are due to sympathetic stimulation on the SA node as detailed in Chapter 5.5.

### PARASYMPATHETIC STIMULATION OPPOSES SYMPATHETIC EFFECTS (SEE FIGURE 5.7.7)

Parasympathetic stimulation of the heart releases acetylcholine onto M2 receptors which are coupled to a  $G_i$

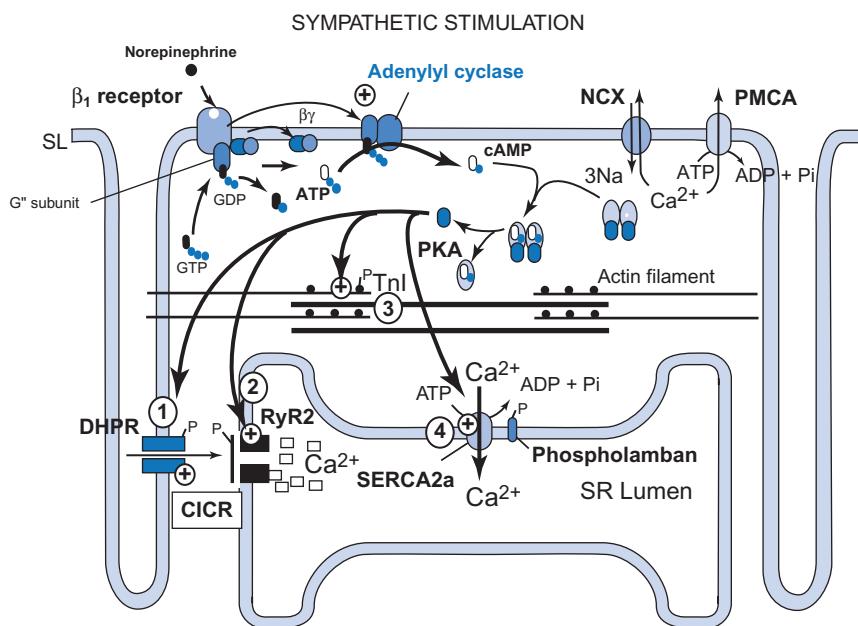
protein that inhibits adenylyl cyclase. Since almost all of the sympathetic effect on the heart is mediated by  $\beta$  receptors linked to  $G_s$ , parasympathetic stimulation should oppose all of the effects of sympathetic stimulation.

The SA node and atria receive tonic vagal sympathetic stimulation, so **removal of vagal tone causes a rapid increase in heart rate**. Sympathetic stimulation of heart rate takes longer to produce than removing vagal tone. The negative chronotropic effects of parasympathetic stimulation were explained in Chapter 5.5. They are due to occupancy of M2 receptors and activation of  $I_{K-\text{ACH}}$  that causes a hyperpolarization and a delayed rise in the pacemaker potential.

M2 receptors in the ventricles are less dense than in the atria. During normal activity there is little sympathetic tone to the ventricles. Thus, cAMP levels during normal conditions are low and vagal stimulation or vagal withdrawal does not change this low basal [cAMP]. Vagal stimulation in the presence of sympathetic stimulation would reduce sympathetic inotropy, but otherwise stimulation of M2 receptors has no direct negative inotropic effect.

### CARDIAC GLYCOSIDES INCREASE CARDIAC CONTRACTILITY BY INCREASING THE $\text{Ca}^{2+}$ Transient

The cardiac glycosides are a class of drugs that inhibit the sarcolemmal Na,K-ATPase. Drugs in this class include **ouabain** and **digoxin**. Digoxin is a natural product of a plant called foxglove. It has been used for over two centuries to increase the force of contraction of failing hearts. The SL Na,K-ATPase pumps three  $\text{Na}^+$  ions out of the heart for every two  $\text{K}^+$  that it pumps in, at the expense of ATP hydrolysis. The cardiac glycosides inhibit this pump, which increases the cytoplasmic  $[\text{Na}^+]$ , which decreases the electrochemical gradient for  $\text{Na}^+$  across the SL membrane. This, in turn, slows down the  $\text{Ca}^{2+}$  exit across the SL that relies mainly on the energy of the Na<sup>+</sup>

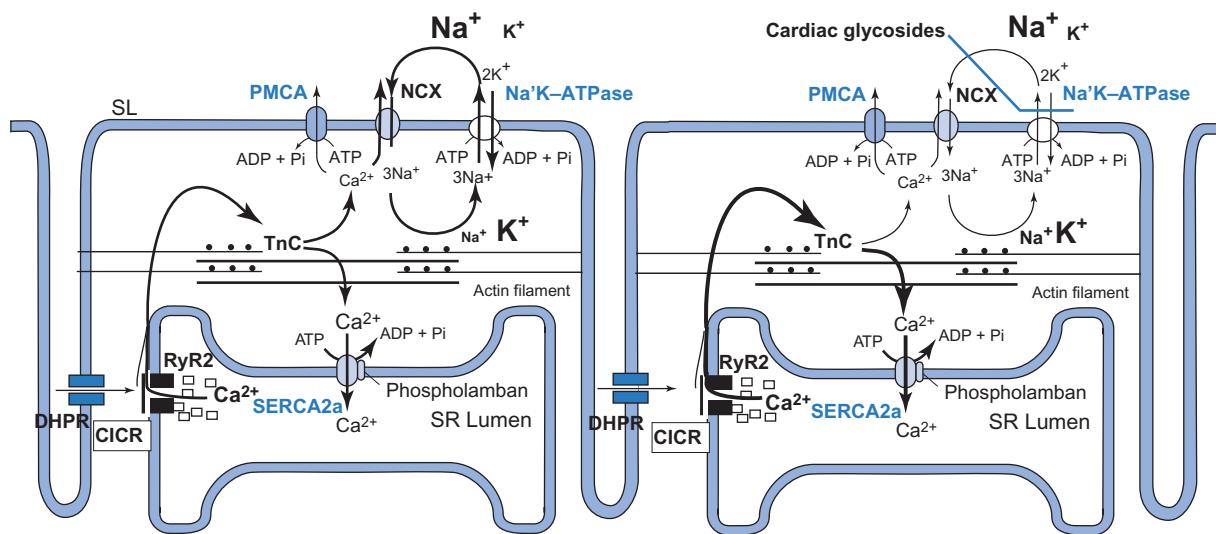


**FIGURE 5.7.7** Autonomic effects on cardiac contractility. Sympathetic stimulation activates adenylyl cyclase that increases the [cAMP] in the cell and activates protein kinase A (PKA). PKA phosphorylates many target proteins, including (1) the L-type  $\text{Ca}^{2+}$  channel; (2) RyR2; (3) TnI; and (4) phospholamban. These effects all increase the  $\text{Ca}^{2+}$  transient either by increasing influx across the SL or by increasing the SR load or by directly increasing SR  $\text{Ca}^{2+}$  release. Phosphorylation of TnI reduces myofilament  $\text{Ca}^{2+}$  sensitivity by increasing the rate of  $\text{Ca}^{2+}$  dissociation. This increases the rate of relaxation of the cardiomyocyte. Parasympathetic stimulation opposes sympathetic effects but does not in itself produce negative inotropy.

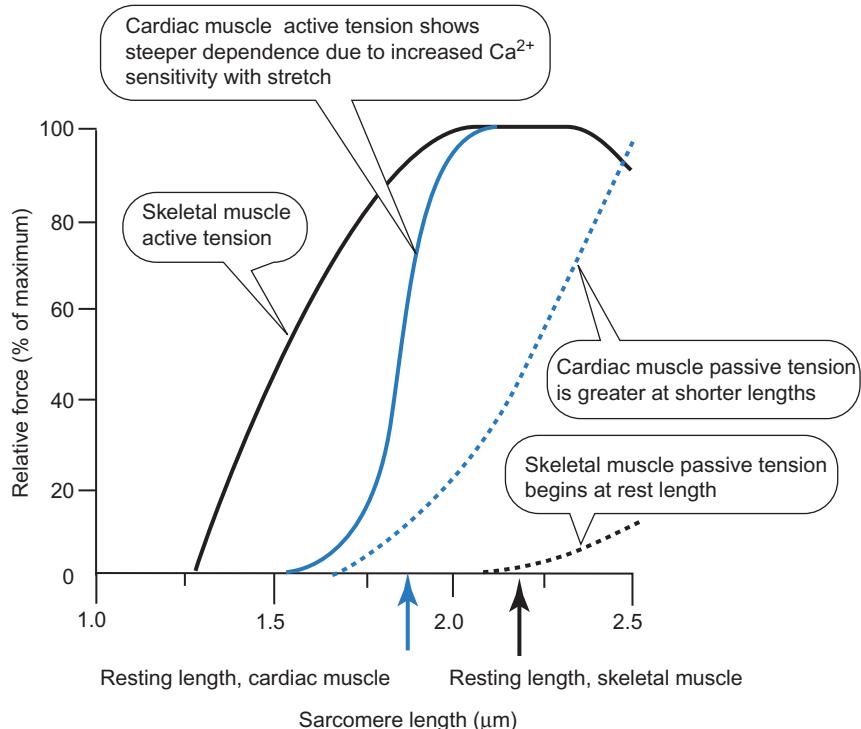
gradient to move  $\text{Ca}^{2+}$  out of the cell. As a result, efflux of  $\text{Ca}^{2+}$  across the SL is reduced and over several beats the SR competes more effectively for  $\text{Ca}^{2+}$  that enters the cell. The SR loads to higher levels and therefore releases more  $\text{Ca}^{2+}$  during excitation. In this way, the  $[\text{Ca}^{2+}]$  transients increase and force development increases. Drugs that increase the force of contraction of the heart have a **positive inotropic effect** and are said to exhibit positive inotropy. Thus, the cardiac glycosides are positively inotropic (see Figure 5.7.8).

## CARDIAC CONTRACTILE FORCE IS POWERFULLY MODULATED BY STRETCH

The force developed by skeletal muscle has two components: a passive force caused by elastic elements in the muscle and an active force that is the additional force a muscle develops when it is stimulated. The active force in skeletal muscle has a biphasic dependence on muscle length that corresponds to the degree of overlap of the thick and thin filaments. The reduction in developed



**FIGURE 5.7.8** Basis of positive inotropy of cardiac glycosides. Left panel:  $\text{Ca}^{2+}$  that enters every heart beat is removed mainly by the NCX that uses the energy of the  $\text{Na}^+$  gradient to extrude  $\text{Ca}^{2+}$ . This energy gradient is maintained by the  $\text{Na}^+,\text{K}^+$ -ATPase. Right panel: Cardiac glycosides inhibit this pump, which alters the  $\text{Na}^+$  gradient so that less  $\text{Ca}^{2+}$  is removed by the NCX. Instead, the  $\text{Ca}^{2+}$  is taken up by the SR, which increases its load of  $\text{Ca}^{2+}$ , which increases the  $\text{Ca}^{2+}$  released upon excitation, creating a larger  $\text{Ca}^{2+}$  transient and more force.



**FIGURE 5.7.9** Comparison of part of the length-tension curve for skeletal muscle and cardiac muscle. As skeletal muscle is stretched from very short lengths, its tension increases because excessive overlap of myofilaments is removed. As cardiac muscle is stretched from very short lengths, its tension increases from removing interfering myofilament overlap and from increasing the number of active cross-bridges by increasing the sensitivity of the myofilaments to  $\text{Ca}^{2+}$ .

force at short muscle lengths when overlap is maximal is caused by interference of the filaments when sarcomere length is less than twice the length of the thin filament.

In skeletal muscle, the length–tension curve is not terribly important because the rest length of the muscle lies near the optimum of the length–tension curve and the fractional shortening or lengthening of skeletal muscle does not deviate too far from this optimum. The active force of cardiac muscle also depends on the overlap of thick and thin filaments, but it must avoid going over the top of the length–tension curve. **If the heart overfills, the active force would decrease and there would be no way to empty the heart!** This is accomplished by a steep rise in the passive force when stretched beyond 2.2–2.3  $\mu\text{m}$ . Normally the heart muscle is never stretched to these long sarcomere lengths because the pressure during ventricular filling is not great enough to stretch it that far. Thus, **the heart normally operates only on the ascending limb of the length–tension curve** (see [Figure 5.7.9](#)).

The length–tension curve in skeletal muscle is typically observed under normal conditions of 100% recruitment and 100% saturation of TnC. Cardiac muscle does not normally saturate its TnC with its smaller  $\text{Ca}^{2+}$  transient. Stretching the muscle at short lengths increases its sensitivity to  $\text{Ca}^{2+}$  so that **stretching increases the fraction of active cross-bridges at any given  $[\text{Ca}^{2+}]$** . In cardiac muscle, the length–tension curve at a given  $[\text{Ca}^{2+}]$  has two components: the alteration of force due to overlap of filaments and the alteration of force due to changes in the number of active cross-bridges.

## SUMMARY

Unlike skeletal muscle, all of the cardiomyocytes are electrically activated every heart beat, and so there is maximum recruitment at every beat. The action potential is nearly as long as the contraction, and therefore there is also no tetany or summation in cardiac muscle. Cardiac muscle regulates its strength of contraction two ways: (1) by regulating the size of the  $\text{Ca}^{2+}$  transient; (2) by regulating the sensitivity of the myofilaments.

The size of the  $\text{Ca}^{2+}$  transient can be experimentally altered in a variety of ways, including: (1) changing extracellular  $[\text{Ca}^{2+}]$ ; (2) altering  $\text{Na}^+-\text{Ca}^{2+}$  exchange across the SL by varying the  $\text{Na}^+$  electrochemical potential difference by changing extracellular  $[\text{Na}^+]$  or by inhibiting the  $\text{Na}^+, \text{K}^+$ -ATPase with cardiac glycosides; (3) by increasing the heart rate, which increases the time-averaged  $\text{Ca}^{2+}$  entry; (4) by regulatory phosphorylation of a

number of transport proteins dealing with the  $\text{Ca}^{2+}$  transient, including the L-type  $\text{Ca}^{2+}$  channel, phospholamban, SERCA2a, and RyR2. In physiological conditions, alterations of the heart rate and sympathetic/parasympathetic stimulation are the main mechanisms for the cellular regulation of cardiac contractility.

Cardiac muscle normally operates on the ascending limb of the length–tension curve because there is significant passive forces at short muscle length in the heart, and the forces that fill the ventricles are not large enough to stretch the heart beyond the ascending limb. Stretch of cardiac muscle at short sarcomere lengths markedly increases force by removing interfering overlap of the myofilaments and by increasing the  $\text{Ca}^{2+}$  sensitivity of the myofilaments. Thus, the degree of filling of the heart strongly influences its force of contraction.

## REVIEW QUESTIONS

- How are cardiomyocytes electrically coupled?
- How does excitation–contraction coupling differ in cardiac muscle compared to skeletal muscle?
- Force generation in cardiac muscle depends on the interaction of the thin filament with the thick filaments. What is the main constituent of the thin filament? Thick filament? What prevents their interaction at rest? What controls activation of the interaction of thick and thin filaments?
- What is the main source of activator  $\text{Ca}^{2+}$ ? How is it released? What determines how much  $\text{Ca}^{2+}$  is released?
- How is the activator  $\text{Ca}^{2+}$  removed to bring about relaxation? Name three transport mechanisms for  $\text{Ca}^{2+}$  removal from the cardiomyocyte cytoplasm. Which is the main mechanism?
- What would happen to the  $\text{Ca}^{2+}$  transient following sympathetic stimulation? How are the transport mechanisms affected?
- What would happen to the  $\text{Ca}^{2+}$  transient following inhibition of the  $\text{Na}, \text{K}$ -ATPase? What mechanisms are affected?
- What is the effect of increasing frequency on the force of heart muscle contraction? Why does this happen?
- What does stretch do to the force of cardiac contractility?
- Why does the heart operate only on the rising part of the length–tension curve?