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 **547**

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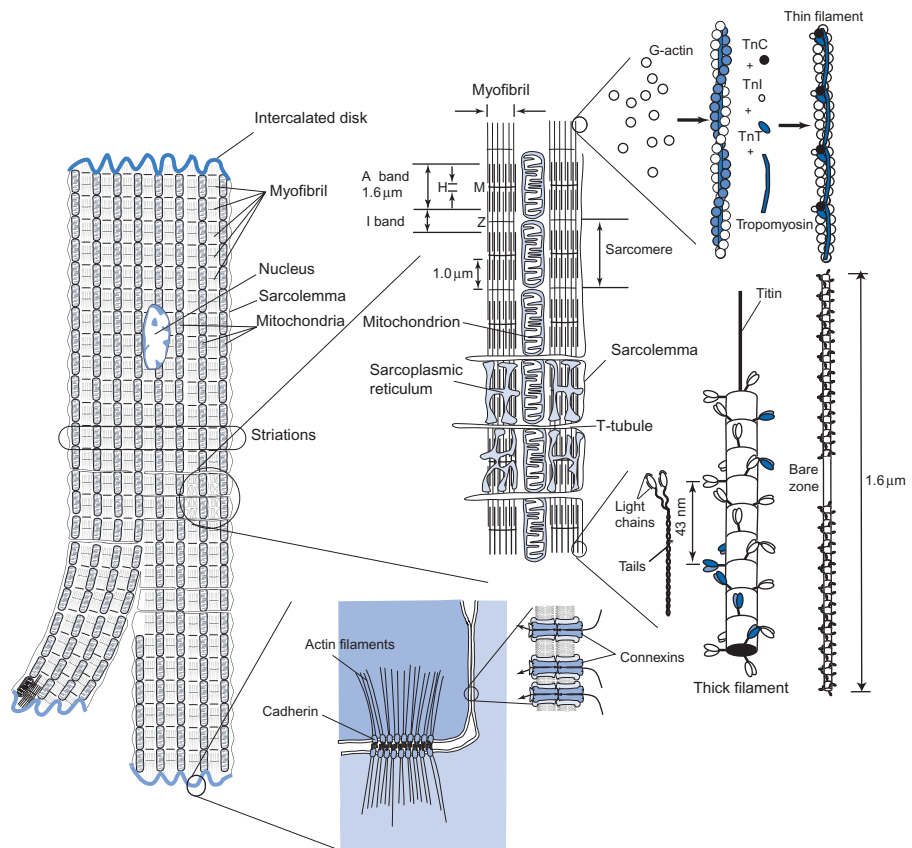
cardiac muscle does not respond to repetitive stimulation. So what regulates cardiac muscle contractility? We will first discuss cardiac muscle structure and excitation−contraction coupling before we attempt an answer.

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 μm 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 μm 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 μm 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 supercoiled 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 μm 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 μm 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 μm 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 α and β 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 troponin complex consisting of three separate proteins resides on the end of each tropomyosin. **Troponin I** inhibits actin−myosin interaction; **troponin C** binds Ca^{2+} ions; and **Troponin T** binds to tropomyosin. The interaction of thick and thin filament is controlled by 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 [Ca^{2+}] CONTROLS ACTOMYOSIN CROSS-BRIDGE CYCLING

Cytoplasmic [Ca^{2+}] controls the inhibitory position of tropomyosin on the thin filament. During diastole, cytoplasmic [Ca^{2+}] is low and tropomyosin inhibits interaction between the thin and thick filaments. During excitation, the sarcoplasmic reticulum (SR) releases Ca^{2+} from its stores and the 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 [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 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 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 corbular 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.
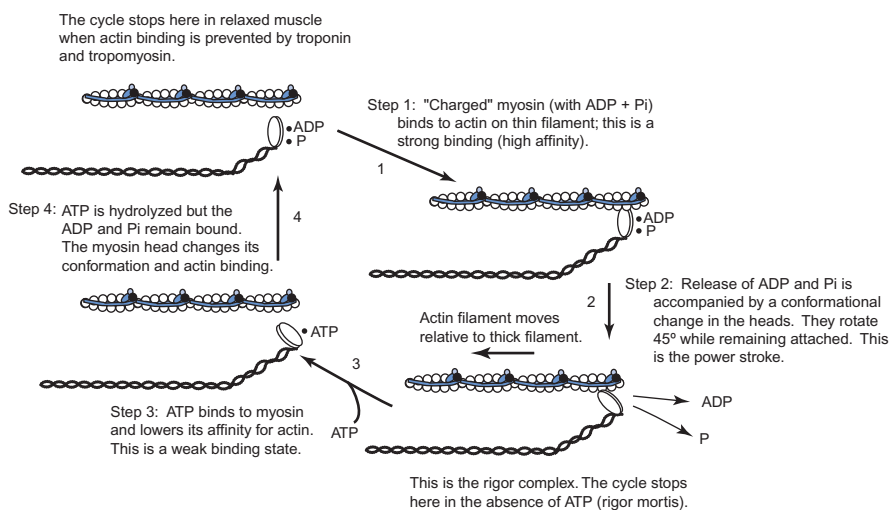

The cycle stops here in relaxed muscle when actin binding is prevented by troponin and tropomyosin.

Step 1: "Charged" myosin (with ADP + Pi) binds to actin on thin filament; this is a strong binding (high affinity).

Step 4: ATP is hydrolyzed but the ADP and Pi remain bound. The myosin head changes its conformation and actin binding.

Step 2: Release of ADP and Pi is accompanied by a conformational change in the heads. They rotate 45° while remaining attached. This is the power stroke.

Actin filament moves relative to thick filament.

Step 3: ATP binds to myosin and lowers its affinity for actin. This is a weak binding state.

This is the rigor complex. The cycle stops here in the absence of ATP (rigor mortis).

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−Pi, but the product ADP and Pi 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−Pi binds to A to form A−M−ADP−Pi. This complex releases first Pi 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 Ca^{2+} CHANNEL

The dihydropyridines are a class of drugs that include nitrendipine, nisoldipine, nifedipine, and BayK 8644. All of these affect the L-type 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 (α_1, α_2, β, γ, and δ), one of which (α_1) forms the Ca^{2+} channel and has the DHPR binding site. The cardiac DHPR appears to lack the γ 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 Ca^{2+} channel, allowing Ca^{2+} to enter the cell.
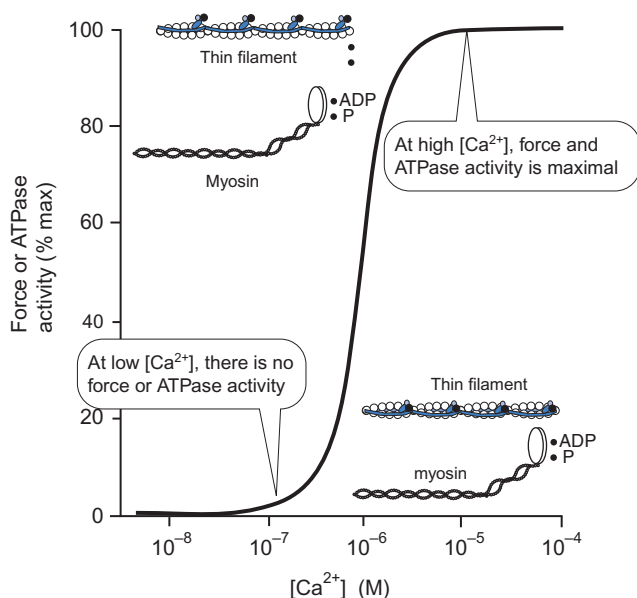

FIGURE 5.7.3 Control of actomyosin cycling and force development by cytoplasmic $[Ca^{2+}]$. No force is developed when cytoplasmic $[Ca^{2+}]$ is less than about 10^{-7} M, and force is maximal when $[Ca^{2+}]$ is about 10^{-6} to 10^{-5} M. The steep dependence of force on $[Ca^{2+}]$ is due to cooperativity.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

Ca^{2+} ENTRY THROUGH THE DHPR TRIGGERS SR Ca^{2+} RELEASE

Release of 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 Ca^{2+} channels in the DHPR that let Ca^{2+} into the cell near the RyR2 receptors on the SR. This Ca^{2+} is insufficient by itself to activate contraction, but it is amplified by causing a further and greater Ca^{2+} release from the SR. This process is shown schematically in Figure 5.7.5.

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

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

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

Binding of Ca^{2+} to TnC initiates contraction, and removal of Ca^{2+} from TnC brings about relaxation. Removal of activator 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 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 **s**mooth **e**ndoplasmic **r**eticulum **Ca**-ATPase. The isoform in cardiac SR is **SERCA2a**. This is regulated by phosphorylation of **phospholamban**, a 5-kDa protein in the SR membrane.
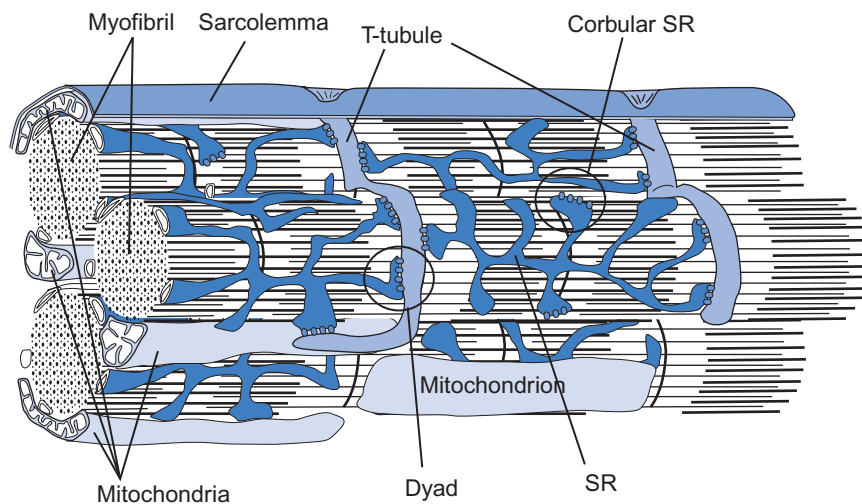

FIGURE 5.7.4 Arrangement of T-tubules and SR in ventricular cardiomyocytes. The SL invaginates to form T-tubules at the level of the Z lines. The SR forms dyad junctions with the T-tubule. Cardiac SR also forms junctions with the peripheral SL and has regions called corbular SR that contain junctional proteins without forming a junction with T-tubule or SL.
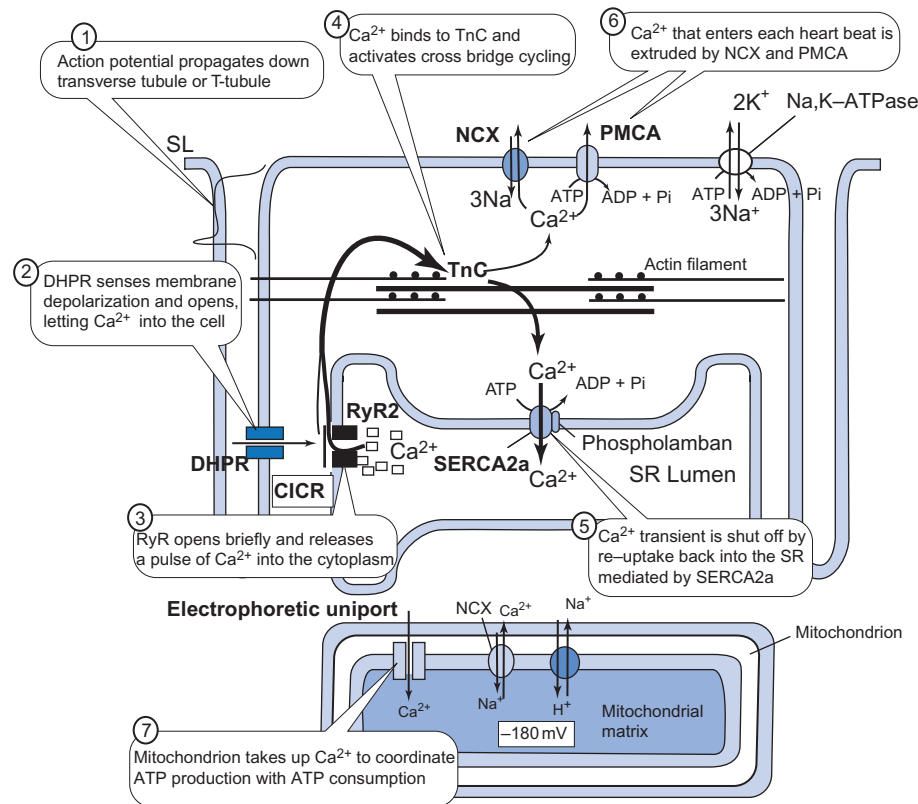
FIGURE 5.7.5 Overall processes involved in Ca^{2+} transport in the ventricular cardiomyocyte. Excitation begins with the cardiac action potential (1) that propagates over the surface of the cell and down into the T-tubules. Both the surface of the cell and T-tubules possess L-type Ca^{2+} channels that open in response to depolarization (2). This lets in a small amount of 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 Ca^{2+} binds to ryanodine receptors (RyR2) on the SR membrane and briefly opens a Ca^{2+} channel in the RyR2 (3). This releases Ca^{2+} from the SR in a process called "calcium-induced calcium release" (CICR). The rapid release of Ca^{2+} transiently raises the cytoplasmic $[Ca^{2+}]$, causing 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 Ca^{2+} from the cytoplasmic 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 (6) that couples the influx of three Na^+ to the exit of one Ca^{2+} ion. A relatively minor component of Ca^{2+} exit is through PMCA. The mitochondria can also take up Ca^{2+} through the electrophoretic uniport, and release it through Na^+-Ca^{2+} exchange (7). The largest source of activator Ca^{2+} is the SR and its greatest sink during relaxation is also the SR.

Ca^{2+} enters each cardiomyocyte with every heart beat. At steady state, the Ca^{2+} that enters with each heart beat must also exit each beat, or else the heart will continue to accumulate Ca^{2+}. The entry of Ca^{2+} is mediated mainly by the L-type Ca^{2+} channels. Most of the efflux of Ca^{2+} from the cell is carried by the Na^+-Ca^{2+} exchanger (NCX) that brings in three Na^+ ions for every Ca^{2+} ion that is extruded from the cell. This is an example of secondary active transport. Because of the changes in $[Ca^{2+}]$ and membrane potential during the action potential, NCX can mediate either Ca^{2+} influx or efflux from the cell. A second efflux mechanism is through the **p**lasma **m**embrane **C**a-ATPase pump, or **PMCA**. This protein has a molecular weight of 138 kDa and is distinct from the SR Ca^{2+} pump. It pumps one 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 Ca^{2+}

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

CALSEQUESTRIN AUGMENTS SR Ca^{2+} UPTAKE AND RELEASE

Pumping Ca^{2+} from the cytoplasm into the SR lumen increases the luminal $[Ca^{2+}]$. The enclosed SR volume is a tiny fraction of the entire cell volume, and so holding all of the activator Ca^{2+} would require high intraluminal $[Ca^{2+}]$ which would slow down pumping because the pump would have to work against a large gradient. **Calsequestrin**, a 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 β_1 receptors that are coupled to a heterotrimeric G_s protein. The α 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 β_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
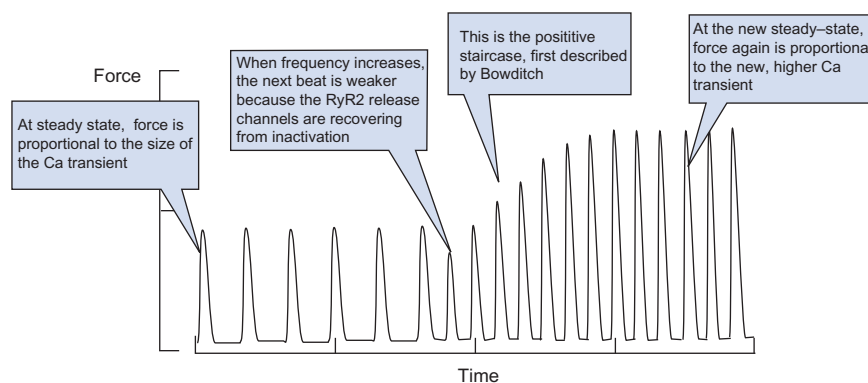

FIGURE 5.7.6 The positive staircase, or Treppe. The curves show pressure developed by the heart at a low frequency and at higher frequencies. When the frequency is increased, the first beat is generally weaker and subsequent beats gradually increase in force, forming a staircase response until a new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

Force

At steady state, force is proportional to the size of the Ca transient

When frequency increases, the next beat is weaker because the RyR2 release channels are recovering from inactivation

This is the posititive staircase, first described by Bowditch

At the new steady–state, force again is proportional to the new, higher Ca transient

Time

directly to a larger Ca^{2+} transient and increased SR Ca^{2+} load. It also increases the size of the Ca^{2+} trigger to release Ca^{2+} from the SR by CICR. All of these effects lead to an increased 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 Ca^{2+}. Phosphorylation by PKA removes this inhibition, leading to increased Ca^{2+} pumping at submaximal $[Ca^{2+}]$. The result is increased loading of the SR and increased release of 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 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 Ca^{2+} dissociation from TnC. Since the affinity of TnC is the ratio of the on and off rate constants, increasing k_{off} decreases the affinity. This should reduce force development unless the Ca^{2+} transient increases enough to overcome the reduced sensitivity of the myofilaments. TnI phosphorylation aids the rate of removal of 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 β 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-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 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 Na^+ ions out of the heart for every two K^+ that it pumps in, at the expense of ATP hydrolysis. The cardiac glycosides inhibit this pump, which increases the cytoplasmic $[Na^+]$, which decreases the electrochemical gradient for Na^+ across the SL membrane. This, in turn, slows down the Ca^{2+} exit across the SL that relies mainly on the energy of the Na^+
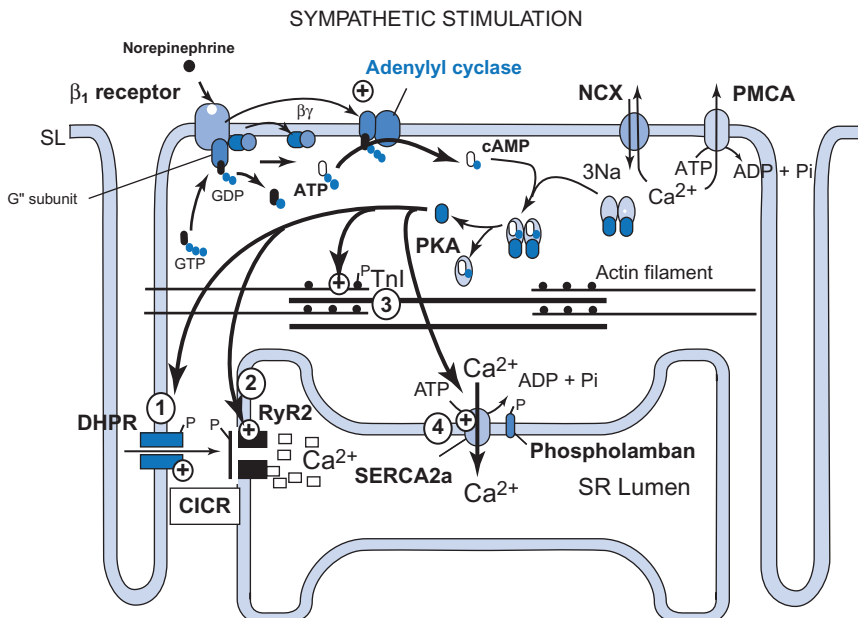

FIGURE 5.7.7 Autonomic effects on cardiac contractility. Sympathetic stimulation activates adenylyl cyclase that increases the [cAMP] in the cell and activates protein kinase A (PKA). PKA phosphorylates many target proteins, including (1) the L-type Ca^{2+} channel; (2) RyR2; (3) TnI; and (4) phospholamban. These effects all increase the Ca^{2+} transient either by increasing influx across the SL or by increasing the SR load or by directly increasing SR Ca^{2+} release. Phosphorylation of TnI reduces myofilament Ca^{2+} sensitivity by increasing the rate of 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 Ca^{2+} out of the cell. As a result, efflux of Ca^{2+} across the SL is reduced and over several beats the SR competes more effectively for Ca^{2+} that enters the cell. The SR loads to higher levels and therefore releases more Ca^{2+} during excitation. In this way, the $[Ca^{2+}]$ 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
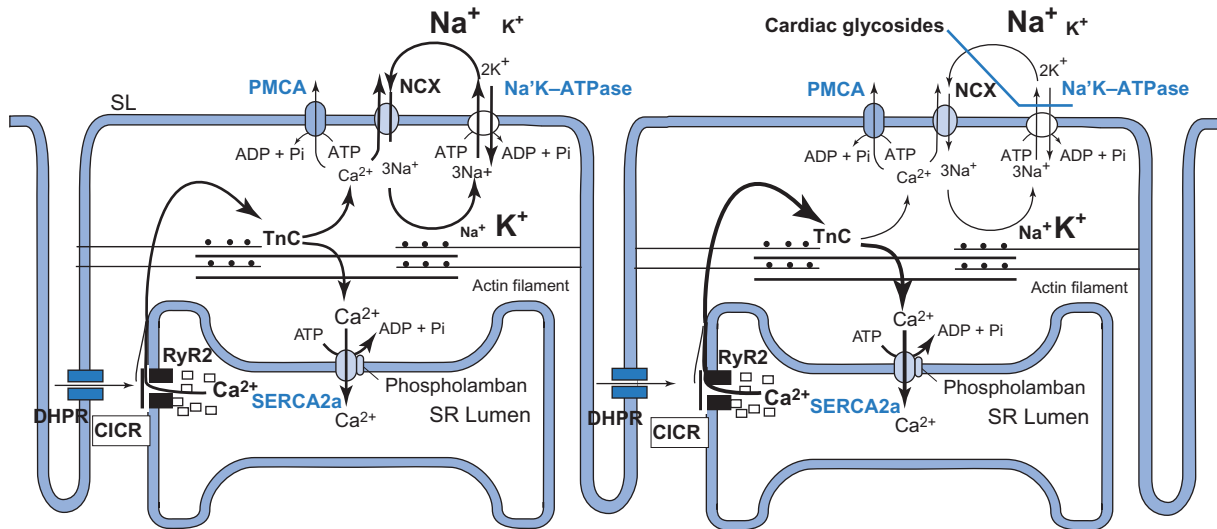

FIGURE 5.7.8 Basis of positive inotropy of cardiac glycosides. Left panel: Ca^{2+} that enters every heart beat is removed mainly by the NCX that uses the energy of the Na^+ gradient to extrude Ca^{2+}. This energy gradient is maintained by the Na^+,K^+-ATPase. Right panel: Cardiac glycosides inhibit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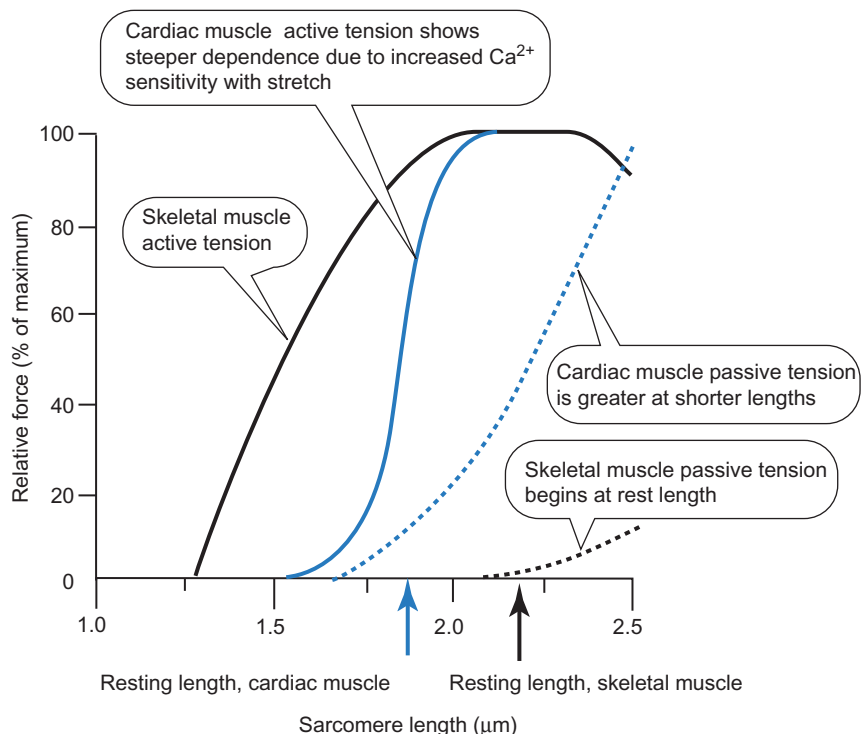

FIGURE 5.7.9 Comparison of part of the length—tension curve for skeletal muscle and cardiac muscle. As skeletal muscle is stretched from very short lengths, its tension increases because excessive overlap of myofilaments is removed. As cardiac muscle is stretched from very short lengths, its tension increases from removing interfering myofilament overlap and from increasing the number of active cross-bridges by increasing the sensitivity of the myofilaments to 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 μm. 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 Ca^{2+} transient. Stretching the muscle at short lengths increases its sensitivity to Ca^{2+} so that **stretching increases the fraction of active cross-bridges at any given $[Ca^{2+}]$**. In cardiac muscle, the length−tension curve at a given $[Ca^{2+}]$ 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 Ca^{2+} transient; (2) by regulating the sensitivity of the myofilaments.

The size of the Ca^{2+} transient can be experimentally altered in a variety of ways, including: (1) changing extracellular $[Ca^{2+}]$; (2) altering $Na^+−Ca^{2+}$ exchange across the SL by varying the Na^+ electrochemical potential difference by changing extracellular $[Na^+]$ or by inhibiting the Na^+,K^+-ATPase with cardiac glycosides; (3) by increasing the heart rate, which increases the time-averaged Ca^{2+} entry; (4) by regulatory phosphorylation of a number of transport proteins dealing with the Ca^{2+} transient, including the L-type 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 Ca^{2+} sensitivity of the myofilaments. Thus, the degree of filling of the heart strongly influences its force of contraction.

REVIEW QUESTIONS

1. How are cardiomyocytes electrically coupled?
2. How does excitation−contraction coupling differ in cardiac muscle compared to skeletal muscle?
3. 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?
4. What is the main source of activator Ca^{2+}? How is it released? What determines how much Ca^{2+} is released?
5. How is the activator Ca^{2+} removed to bring about relaxation? Name three transport mechanisms for Ca^{2+} removal from the cardiomyocyte cytoplasm. Which is the main mechanism?
6. What would happen to the Ca^{2+} transient following sympathetic stimulation? How are the transport mechanisms affected?
7. What would happen to the Ca^{2+} transient following inhibition of the Na,K-ATPase? What mechanisms are affected?
8. What is the effect of increasing frequency on the force of heart muscle contraction? Why does this happen?
9. What does stretch do to the force of cardiac contractility?
10. Why does the heart operate only on the rising part of the length−tension curve?