of the usual packaging of neurotransmitter in synaptic vesicles and release by exocytosis). In addition to serving as a neurotransmitter, NO also functions in signal transduction of guanylyl cyclase in a variety of tissues including vascular smooth muscle (see Chapter 4).

Neuropeptides

There is a long and growing list of neuropeptides that function as neuromodulators, neurohormones, and neurotransmitters (see Table 1-4 for a partial list).

- **Neuromodulators** are substances that act on the presynaptic cell to alter the amount of neurotransmitter released in response to stimulation. Alternatively, a neuromodulator may be cosecreted with a neurotransmitter and alter the response of the postsynaptic cell to the neurotransmitter.
- **Neurohormones,** like other hormones, are released from secretory cells (in these cases, neurons) into the blood to act at a distant site.
- In several instances, **neuropeptides** are copackaged and cosecreted from presynaptic vesicles along with the classical neurotransmitters. For example, vasoactive intestinal peptide (VIP) is stored and secreted with ACh, particularly in neurons of the gastrointestinal tract. Somatostatin, enkephalin, and neurotensin are secreted with norepinephrine. Substance P is secreted with serotonin.

In contrast to classical neurotransmitters, which are synthesized in presynaptic nerve terminals, neuropeptides are synthesized in the nerve cell body. As occurs in all protein synthesis, the cell's DNA is transcribed into specific messenger RNA, which is translated into polypeptides on the ribosomes. Typically, a preliminary polypeptide containing a signal peptide sequence is synthesized first. The signal peptide is removed in the endoplasmic reticulum, and the final peptide is delivered to secretory vesicles. The secretory vesicles are then moved rapidly down the nerve by **axonal transport** to the presynaptic terminal, where they become the synaptic vesicles.

Purines

Adenosine triphosphate (ATP) and adenosine function as neuromodulators in the autonomic and central nervous systems. For example, **ATP** is synthesized in the sympathetic neurons that innervate vascular smooth muscle. It is costored and cosecreted with the "regular" neurotransmitter of these neurons, norepinephrine. When stimulated, the neuron releases both ATP and norepinephrine and both transmitters cause contraction of the smooth muscle; in fact, the ATP-induced contraction precedes the norepinephrine-induced contraction.

SKELETAL MUSCLE

Contraction of skeletal muscle is under voluntary control. Each skeletal muscle cell is innervated by a branch of a motoneuron. Action potentials are propagated along the motoneurons, leading to release of ACh at the neuromuscular junction, depolarization of the motor end plate, and initiation of action potentials in the muscle fiber.

What events, then, elicit contraction of the muscle fiber? These events, occurring between the action potential in the muscle fiber and contraction of the muscle fiber, are called **excitation-contraction coupling.** The mechanisms of excitation-contraction coupling in skeletal muscle and smooth muscle are discussed in this chapter, and the mechanisms of excitation-contraction coupling in cardiac muscle are discussed in Chapter 4.

Muscle Filaments

Each muscle fiber behaves as a single unit, is multinucleate, and contains myofibrils. The myofibrils are surrounded by sarcoplasmic reticulum and are invaginated by transverse tubules (T tubules). Each myofibril contains interdigitating thick and thin filaments, which are arranged longitudinally and cross-sectionally in sarcomeres (Fig. 1-21). The repeating units of sarcomeres account for the unique banding pattern seen in striated muscle (which includes both skeletal and cardiac muscle).

Thick Filaments

The thick filaments comprise a large molecular weight protein called **myosin,** which has six polypeptide chains including one pair of **heavy chains** and two pairs of **light chains** (see Figure 1-21*A*). Most of the heavy-chain myosin has an α-helical structure, in which the two chains coil around each other to form the "**tail**" of the myosin molecule. The four light chains and the N terminus of each heavy chain form two globular "**heads**" on the myosin molecule. These globular heads have an actin-binding site, which is necessary for cross-bridge formation, and a site that binds and hydrolyzes ATP (myosin ATPase).

Thin Filaments

The thin filaments are composed of three proteins: actin, tropomyosin, and troponin (see Fig. 1-21*B*).

Actin is a globular protein and, in this globular form, is called G-actin. In the thin filaments, G-actin is polymerized into two strands that are twisted into an α-helical structure to form filamentous actin, called F-actin. Actin has myosin-binding sites. When the muscle is at rest, the myosin-binding sites are covered by tropomyosin so that actin and myosin cannot interact.

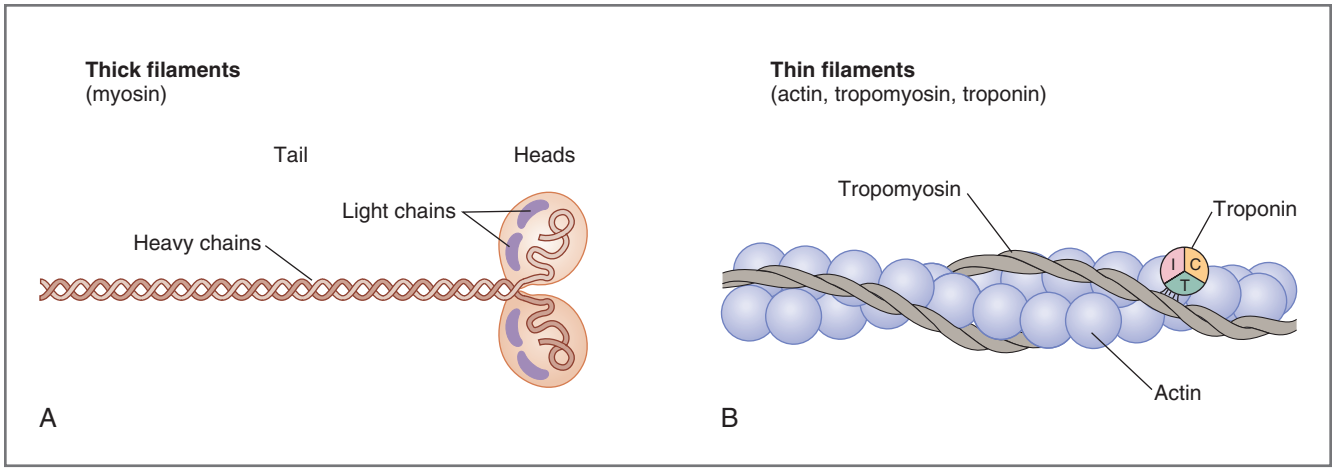


Figure 1–21 Structure of thick (A) and thin (B) filaments of skeletal muscle. Troponin is a complex of three proteins: I, troponin I; T, troponin T; and C, troponin C.

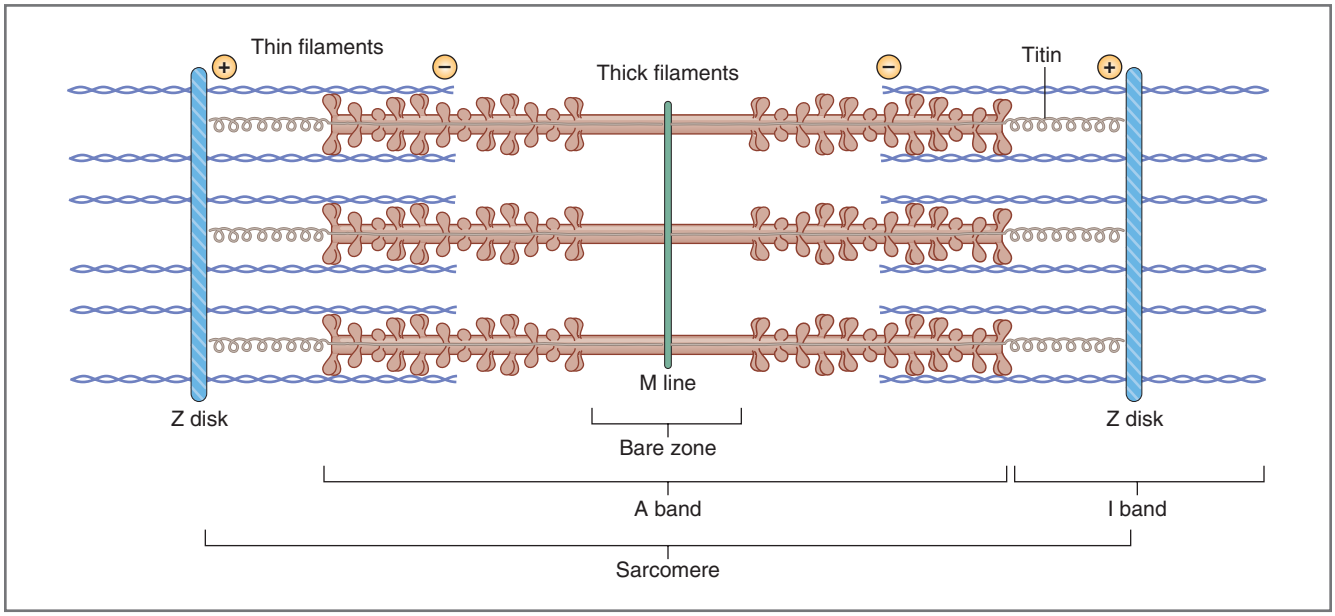


Figure 1–22 Arrangement of thick and thin filaments of skeletal muscle in sarcomeres.

Tropomyosin is a filamentous protein that runs along the groove of each twisted actin filament. At rest, its function is to block the myosin-binding sites on actin. If contraction is to occur, tropomyosin must be moved out of the way so that actin and myosin can interact.

Troponin is a complex of three globular proteins (troponin T, troponin I, and troponin C) located at regular intervals along the tropomyosin filaments. **Troponin T** (T for tropomyosin) attaches the troponin complex to tropomyosin. **Troponin I** (I for inhibition), along with tropomyosin, inhibits the interaction of actin and myosin by covering the myosin-binding site on actin. **Troponin C** (C for Ca^{2+}) is a Ca^{2+}-binding protein that plays a central role in the initiation of contraction. When the intracellular Ca^{2+} concentration increases, Ca^{2+} binds to troponin C, producing a conformational change in the troponin complex. This conformational change moves tropomyosin out of the way, permitting the binding of actin to the myosin heads.

Arrangement of Thick and Thin Filaments in Sarcomeres

The **sarcomere** is the basic contractile unit, and it is delineated by the Z disks. Each sarcomere contains a full A band in the center and one half of two I bands on either side of the A band (Fig. 1-22).

The **A bands** are located in the center of the sarcomere and contain the thick (myosin) filaments, which appear dark when viewed under polarized light. Thick and thin filaments may overlap in the A band; these

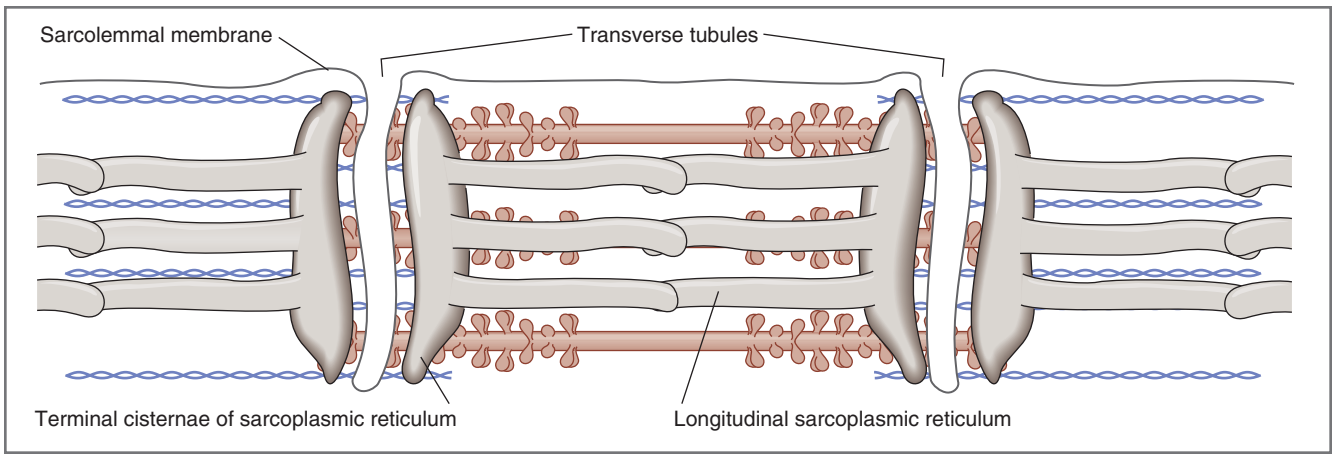


Figure 1–23 Transverse tubules and sarcoplasmic reticulum of skeletal muscle. The transverse tubules are continuous with the sarcolemmal membrane and invaginate deep into the muscle fiber, making contact with terminal cisternae of the sarcoplasmic reticulum.

areas of overlap are potential sites of cross-bridge formation.

The **I bands** are located on either side of the A band and appear light when viewed under polarized light. They contain the thin (actin) filaments, intermediate filamentous proteins, and Z disks. They have no thick filaments.

The **Z disks** are darkly staining structures that run down the middle of each I band, delineating the ends of each sarcomere.

The **bare zone** is located in the center of each sarcomere. There are no thin filaments in the bare zone; thus, there can be no overlap of thick and thin filaments or cross-bridge formation in this region.

The **M line** bisects the bare zone and contains darkly staining proteins that link the central portions of the thick filaments together.

Cytoskeletal Proteins

Cytoskeletal proteins establish the architecture of the myofibrils, ensuring that the thick and thin filaments are aligned correctly and at proper distances with respect to each other.

Transverse cytoskeletal proteins link thick and thin filaments, forming a "scaffold" for the myofibrils and linking sarcomeres of adjacent myofibrils. A system of intermediate filaments holds the myofibrils together, side by side. The entire myofibrillar array is anchored to the cell membrane by an actin-binding protein called **dystrophin.** (In patients with muscular dystrophy, dystrophin is defective or absent.)

Longitudinal cytoskeletal proteins include two large proteins called titin and nebulin. **Titin,** which is associated with thick filaments, is a large molecular weight protein that extends from the M lines to the Z disks. Part of the titin molecule passes through the thick filament; the rest of the molecule, which is elastic or springlike, is anchored to the Z disk. As the length of the sarcomere changes, so does the elastic portion of the titin molecule. Titin also helps center the thick filaments in the sarcomere. **Nebulin** is associated with thin filaments. A single nebulin molecule extends from one end of the thin filament to the other. Nebulin serves as a "molecular ruler," setting the length of thin filaments during their assembly. **α-Actinin** anchors the thin filaments to the Z disk.

Transverse Tubules and the Sarcoplasmic Reticulum

The **transverse** (**T**) **tubules** are an extensive network of muscle cell membrane (sarcolemmal membrane) that invaginates deep into the muscle fiber. The T tubules are responsible for carrying depolarization from action potentials at the muscle cell surface to the interior of the fiber. The T tubules make contact with the terminal cisternae of the sarcoplasmic reticulum and contain a voltage-sensitive protein called the **dihydropyridine receptor,** named for the drug that inhibits it (Fig. 1-23).

The **sarcoplasmic reticulum** is an internal tubular structure, which is the site of storage and release of Ca^{2+} for excitation-contraction coupling. As previously noted, the terminal cisternae of the sarcoplasmic reticulum make contact with the T tubules in a triad arrangement. The sarcoplasmic reticulum contains a Ca^{2+}-release channel called the **ryanodine receptor** (named for the plant alkaloid that opens this release channel). The significance of the physical relationship between the T tubules (and their dihydropyridine receptor) and the sarcoplasmic reticulum (and its ryanodine receptor) is described in the section on excitation-contraction coupling.

Ca^{2+} is accumulated in the sarcoplasmic reticulum by the action of **Ca^{2+} ATPase** (**SERCA**) in the

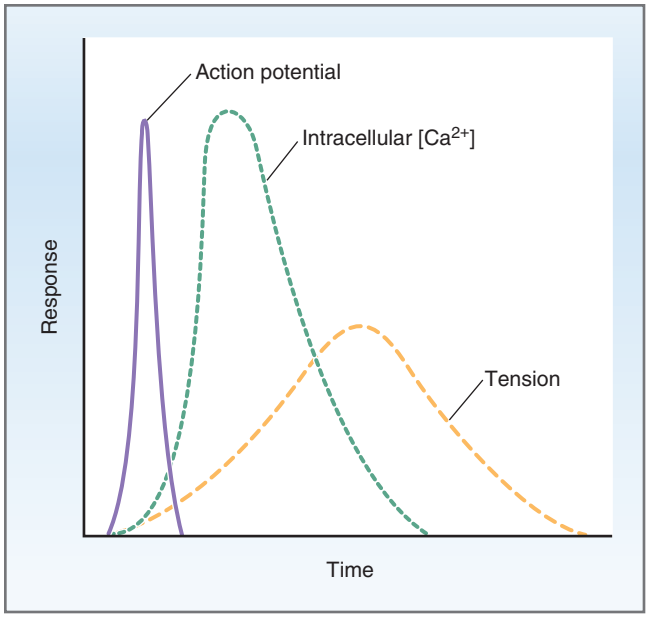


Figure 1–24 Temporal sequence of events in excitation-contraction coupling in skeletal muscle. The muscle action potential precedes a rise in intracellular [Ca^{2+}], which precedes contraction.

sarcoplasmic reticulum membrane. The Ca^{2+} ATPase pumps Ca^{2+} from the ICF of the muscle fiber into the interior of the sarcoplasmic reticulum, keeping the intracellular Ca^{2+} concentration low when the muscle fiber is at rest. Within the sarcoplasmic reticulum, Ca^{2+} is bound to **calsequestrin**, a low-affinity, high-capacity Ca^{2+}-binding protein. Calsequestrin, by binding Ca^{2+}, helps to maintain a low free Ca^{2+} concentration inside the sarcoplasmic reticulum, thereby reducing the work of the Ca^{2+} ATPase pump. Thus, a large quantity of Ca^{2+} can be stored inside the sarcoplasmic reticulum in *bound* form, while the intrasarcoplasmic reticulum *free* Ca^{2+} concentration remains extremely low.

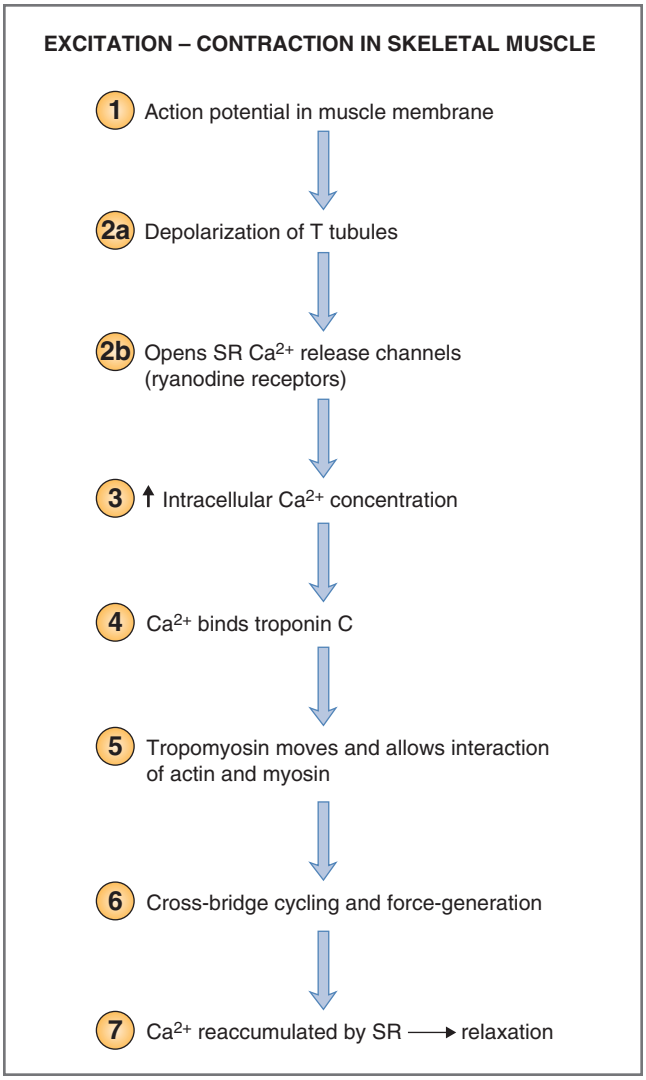


Figure 1–25 Steps in excitation-contraction in skeletal muscle. SR, Sarcoplasmic reticulum; T tubules, transverse tubules. See text for explanation of the circled numbers.

Excitation-Contraction Coupling in Skeletal Muscle

The mechanism that translates the muscle action potential into the production of tension is excitation-contraction coupling. Figure 1-24 shows the temporal relationships between an action potential in the skeletal muscle fiber, the subsequent increase in intracellular free Ca^{2+} concentration (which is released from the sarcoplasmic reticulum), and contraction of the muscle fiber. These temporal relationships are critical in that the action potential always *precedes* the rise in intracellular Ca^{2+} concentration, which always *precedes* contraction.

The steps involved in excitation-contraction coupling are described as follows and illustrated in Figure 1-25. (Step 6 is illustrated in Fig. 1-26):

1. **Action potentials** in the muscle cell membrane are propagated to the **T tubules** by the spread of local currents. Thus, the T tubules are continuous with the sarcolemmal membrane and carry the depolarization from the surface to the interior of the muscle fiber.

2a. and b. **Depolarization of the T tubules** causes a critical conformational change in their voltage-sensitive **dihydropyridine receptors.** This conformational change opens Ca^{2+}-release channels (**ryanodine receptors**) on the nearby sarcoplasmic reticulum. (As an aside, although the T tubules' dihydropyridine receptors are L-type voltage-gated Ca^{2+} channels, Ca^{2+} influx into the cell through these channels is *not* required for excitation-contraction coupling in skeletal muscle.)

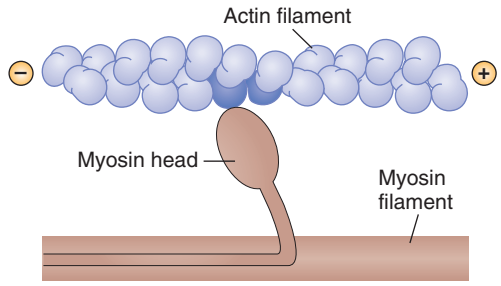
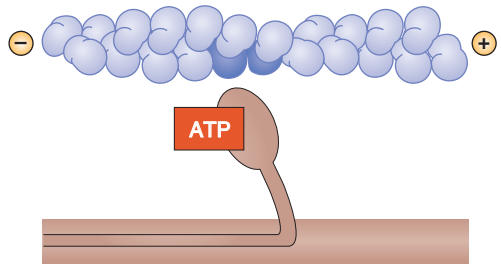
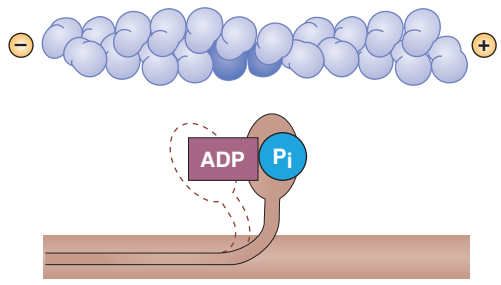
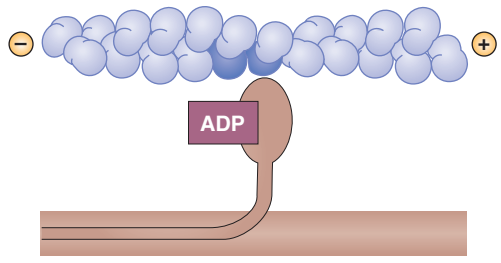
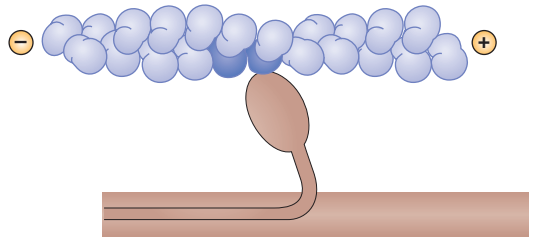
Position of Actin and Myosin During Cross-bridge Cycling	Events	ATP/ADP
	Rigor	No nucleotides bound
	ATP binds to cleft on myosin head Conformational change in myosin Decreased affinity of myosin for actin Myosin released	ATP bound
	Cleft closes around ATP Conformational change Myosin head displaced toward ⊕ end of actin ATP hydrolysis	ATP → ADP + P_i ADP + P_i bound
	Myosin head binds new site on actin Power stroke = force	ADP bound
	ADP released Rigor	No nucleotides bound

Figure 1–26 **Cross-bridge cycle in skeletal muscle.** Mechanism by which myosin "walks" toward the plus end of the actin filament. **A–E**, See the discussion in the text. ADP, Adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate.

3. When these Ca^{2+}-release channels open, Ca^{2+} is released from its storage site in the sarcoplasmic reticulum into the ICF of the muscle fiber, resulting in an **increase in intracellular Ca^{2+} concentration.** At rest, the intracellular free Ca^{2+} concentration is less than 10^{-7} M. After its release from the sarcoplasmic reticulum, intracellular free Ca^{2+} concentration increases to levels between 10^{-7} M and 10^{-6} M.
4. **Ca^{2+} binds to troponin C** on the thin filaments, causing a conformational change in the troponin complex. Troponin C can bind as many as four Ca^{2+} ions per molecule of protein. Because this binding is cooperative, each molecule of bound Ca^{2+} increases the affinity of troponin C for the next Ca^{2+}. Thus, even a small increase in Ca^{2+} concentration increases the likelihood that all of the binding sites will be occupied to produce the necessary conformational change in the troponin complex.
5. The **conformational change in troponin** causes tropomyosin (which was previously blocking the interaction of actin and myosin) to be moved out of the way so that cross-bridge cycling can begin. When tropomyosin is moved away, the myosin-binding sites on actin, previously covered, are exposed.
6. **Cross-bridge cycling.** With Ca^{2+} bound to troponin C and tropomyosin moved out of the way, myosin heads can now bind to actin and form so-called **cross-bridges.** Formation of cross-bridges is associated with hydrolysis of ATP and generation of force.

   The sequence of events in the cross-bridge cycle is shown in Figure 1-26. *A,* At the beginning of the cycle, no ATP is bound to myosin, and myosin is tightly attached to actin in a "rigor" position. In rapidly contracting muscle, this state is brief. However, in the absence of ATP, this state is permanent (i.e., rigor mortis). *B,* The binding of ATP to a cleft on the back of the myosin head produces a conformational change in myosin that decreases its affinity for actin; thus, myosin is released from the original actin-binding site. *C,* The cleft closes around the bound ATP molecule, producing a further conformational change that causes myosin to be displaced toward the plus end of actin. ATP is hydrolyzed to ADP and P_i, which remain attached to myosin. *D,* Myosin binds to a new site on actin (toward the plus end), constituting the force-generating, or power, stroke. Each cross-bridge cycle "walks" the myosin head 10 nanometers (10^{-8} meters) along the actin filament. *E,* ADP is released, and myosin is returned to its original state with no nucleotides bound (*A*). Cross-bridge cycling continues, with myosin "walking" toward the plus end of the actin filament, as long as Ca^{2+} is bound to troponin C.
7. **Relaxation** occurs when Ca^{2+} is reaccumulated in the sarcoplasmic reticulum by the Ca^{2+} ATPase of the sarcoplasmic reticulum membrane (**SERCA**). When the intracellular Ca^{2+} concentration decreases to less than 10^{-7} M, there is insufficient Ca^{2+} for binding to troponin C. When Ca^{2+} is released from troponin C, tropomyosin returns to its resting position, where it blocks the myosin-binding site on actin. As long as the intracellular Ca^{2+} is low, cross-bridge cycling cannot occur and the muscle will relax.

The cross-bridge cycle produces force (tension) at the level of the contractile elements. In order for this force to be transmitted to the muscle surface, the series elastic elements (e.g., titin) must first be stretched out. As a result, there is a delay in transmission of force from the cross-bridges to the muscle surface (see Fig. 1-24). Once cross-bridge cycling has concluded, there is also a delay in the fall of muscle tension; the series elastic elements remain stretched out and thus force transmission to the muscle surface continues after intracellular Ca^{2+} has fallen and cross-bridge cycling has ceased.

Mechanism of Tetanus

A single action potential results in the release of a fixed amount of Ca^{2+} from the sarcoplasmic reticulum, which produces a single twitch. The twitch is terminated (relaxation occurs) when the sarcoplasmic reticulum reaccumulates this Ca^{2+}. However, if the muscle is stimulated repeatedly, there is insufficient time for the sarcoplasmic reticulum to reaccumulate Ca^{2+}, and the intracellular Ca^{2+} concentration never returns to the low levels that exist during relaxation. Instead, the level of intracellular Ca^{2+} concentration remains high, resulting in continued binding of Ca^{2+} to troponin C and continued cross-bridge cycling. In this state, there is a sustained contraction called **tetanus,** rather than just a single twitch.

Length-Tension Relationship

The length-tension relationship in muscle refers to the effect of muscle fiber length on the amount of tension the fiber can develop (Fig. 1-27). The amount of tension is determined for a muscle undergoing an **isometric contraction,** in which the muscle is allowed to develop tension at a preset length (called **preload**) but is not allowed to shorten. (Imagine trying to lift a 500-lb barbell. The tension developed would be great, but no shortening or movement of muscle would occur!) The following measurements of tension can be made as a function of preset length (or preload):

- **Passive tension** is the tension developed by simply stretching a muscle to different lengths. (Think of

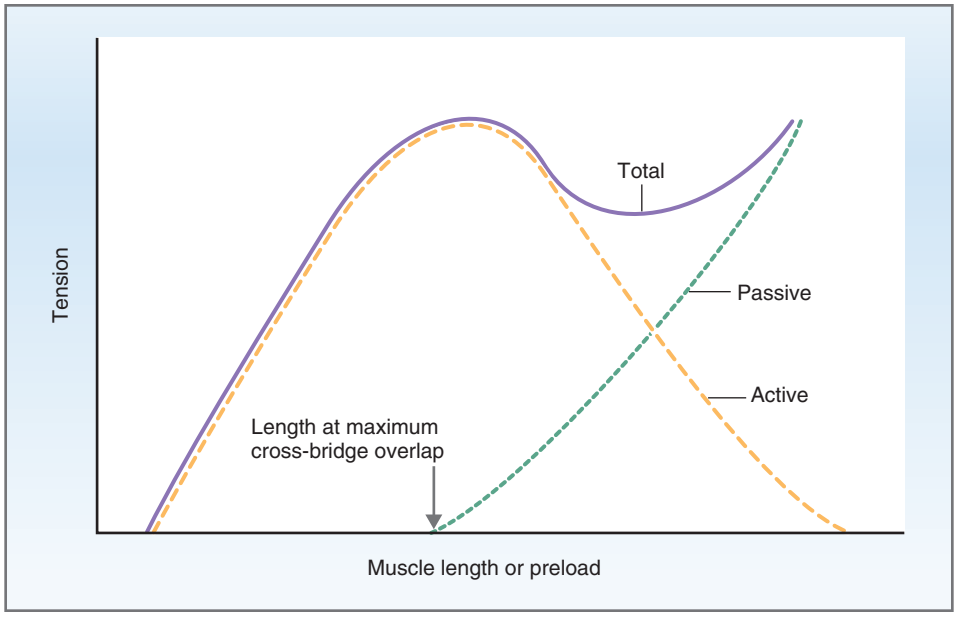


Figure 1–27 Length-tension relationship in skeletal muscle. Maximal active tension occurs at muscle lengths where there is maximal overlap of thick and thin filaments.

the tension produced in a rubber band as it is progressively stretched to longer lengths.)

- **Total tension** is the tension developed when a muscle is stimulated to contract at different preloads. It is the sum of the active tension developed by the cross-bridge cycling in the sarcomeres and the passive tension caused by stretching the muscle.
- **Active tension** is determined by subtracting the passive tension from the total tension. It represents the active force developed during cross-bridge cycling.

The unusual relationship between active tension and muscle length is the **length-tension relationship** and can be explained by the mechanisms involved in the cross-bridge cycle (see Fig. 1-27). The *active tension developed is proportional to the number of cross-bridges that cycle.* Therefore, the active tension is maximal when there is maximal overlap of thick and thin filaments and maximal possible cross-bridges. When the muscle is stretched to longer lengths, the number of possible cross-bridges is reduced and active tension is reduced. Likewise, when muscle length is decreased, the thin filaments collide with each other in the center of the sarcomere, reducing the number of possible cross-bridges and reducing active tension.

Force-Velocity Relationship

The force-velocity relationship, shown in Figure 1-28, describes the velocity of shortening when the force against which the muscle contracts, the **afterload,** is varied (see Fig. 1-28, *left*). In contrast to the length-tension relationship, the force-velocity relationship is determined by allowing the muscle to shorten. The force, rather than the length, is fixed, and therefore, it is called an **isotonic contraction.** The velocity of shortening reflects the **speed of cross-bridge cycling.** As is intuitively obvious, the velocity of shortening will be maximal (V_{max}) when the afterload on the muscle is zero. As the afterload on the muscle increases, the velocity will be decreased because cross-bridges can cycle less rapidly against the higher resistance. As the afterload increases to even higher levels, the velocity of shortening is reduced to zero. (Imagine how quickly you can lift a feather as opposed to a ton of bricks!)

The effect of afterload on the velocity of shortening can be further demonstrated by setting the muscle to a preset length (preload) and then measuring the velocity of shortening at various levels of afterload (see Fig. 1-28, *right*). A "family" of curves is generated, each one representing a different fixed preload. The curves always intersect at V_{max}, the point where afterload is zero and where velocity of shortening is maximal.

SMOOTH MUSCLE

Smooth muscle lacks striations, which distinguishes it from skeletal and cardiac muscle. The striations found in skeletal and cardiac muscle are created by the banding patterns of thick and thin filaments in the sarcomeres. In smooth muscle, there are no striations because the thick and thin filaments, while present, are not organized in sarcomeres.

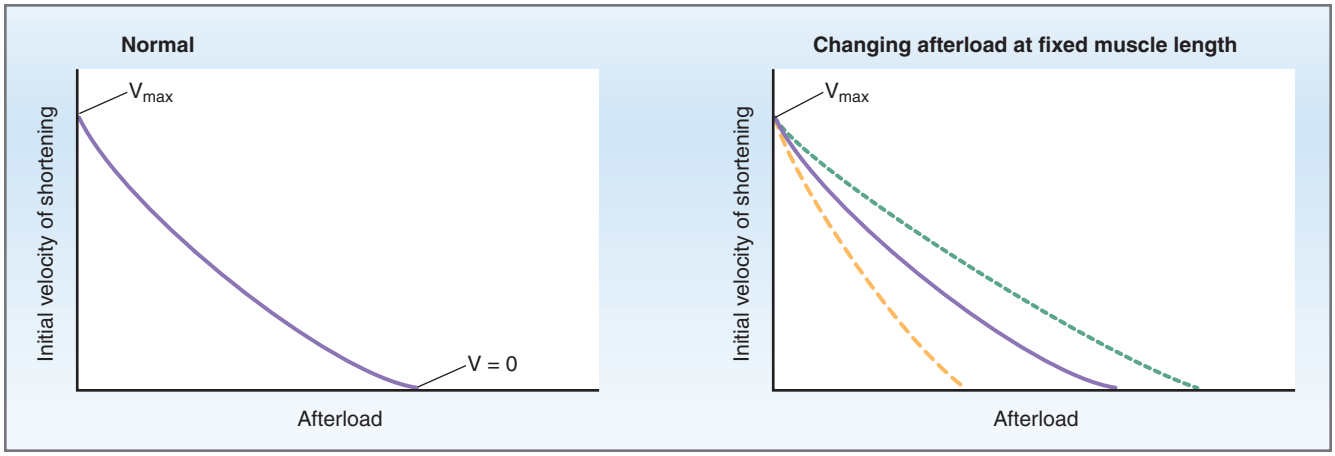


Figure 1–28 Initial velocity of shortening as a function of afterload in skeletal muscle.

Smooth muscle is found in the walls of hollow organs such as the gastrointestinal tract, the bladder, and the uterus, as well as in the vasculature, the ureters, the bronchioles, and the muscles of the eye. The functions of smooth muscle are twofold: to produce motility (e.g., to propel chyme along the gastrointestinal tract or to propel urine along the ureter) and to maintain tension (e.g., smooth muscle in the walls of blood vessels).

Types of Smooth Muscle

Smooth muscles are classified as multiunit or unitary, depending on whether the cells are electrically coupled. Unitary smooth muscle has gap junctions between cells, which allow for the fast spread of electrical activity throughout the organ, followed by a coordinated contraction. Multiunit smooth muscle has little or no coupling between cells. A third type, a combination of unitary and multiunit smooth muscle, is found in vascular smooth muscle.

Unitary Smooth Muscle

Unitary (single unit) smooth muscle is present in the gastrointestinal tract, bladder, uterus, and ureter. The smooth muscle in these organs contracts in a coordinated fashion because the cells are linked by **gap junctions.** Gap junctions are low-resistance pathways for current flow, which permit electrical coupling between cells. For example, action potentials occur simultaneously in the smooth muscle cells of the bladder so that contraction (and emptying) of the entire organ can occur at once.

Unitary smooth muscle is also characterized by spontaneous pacemaker activity, or **slow waves.** The frequency of slow waves sets a characteristic pattern of action potentials within an organ, which then determines the frequency of contractions.

Multiunit Smooth Muscle

Multiunit smooth muscle is present in the iris, in the ciliary muscles of the lens, and in the vas deferens. Each muscle fiber behaves as a separate motor unit (similar to skeletal muscle), and there is little or no coupling between cells. Multiunit smooth muscle cells are densely innervated by postganglionic fibers of the parasympathetic and sympathetic nervous systems, and it is these innervations that regulate function.

Excitation-Contraction Coupling in Smooth Muscle

The mechanism of excitation-contraction coupling in smooth muscle differs from that of skeletal muscle. Recall that in skeletal muscle binding of actin and myosin is permitted when Ca^{2+} binds troponin C. In smooth muscle, however, there is no troponin. Rather, the interaction of actin and myosin is controlled by the binding of Ca^{2+} to another protein, **calmodulin.** In turn, Ca^{2+}-calmodulin regulates myosin-light-chain kinase, which regulates cross-bridge cycling.

Steps in Excitation-Contraction Coupling in Smooth Muscle

The steps involved in excitation-contraction coupling in smooth muscle are illustrated in Figure 1-29 and occur as follows:

1. **Depolarization of smooth muscle** opens voltage-gated Ca^{2+} channels in the sarcolemmal membrane. With these Ca^{2+} channels open, Ca^{2+} flows into the cell down its electrochemical gradient. This influx of Ca^{2+} from the ECF causes an **increase in intracellular Ca^{2+} concentration.** In contrast to skeletal muscle, where action potentials are required to produce contraction, in smooth muscle, subthreshold depolarization (which does not lead to an action

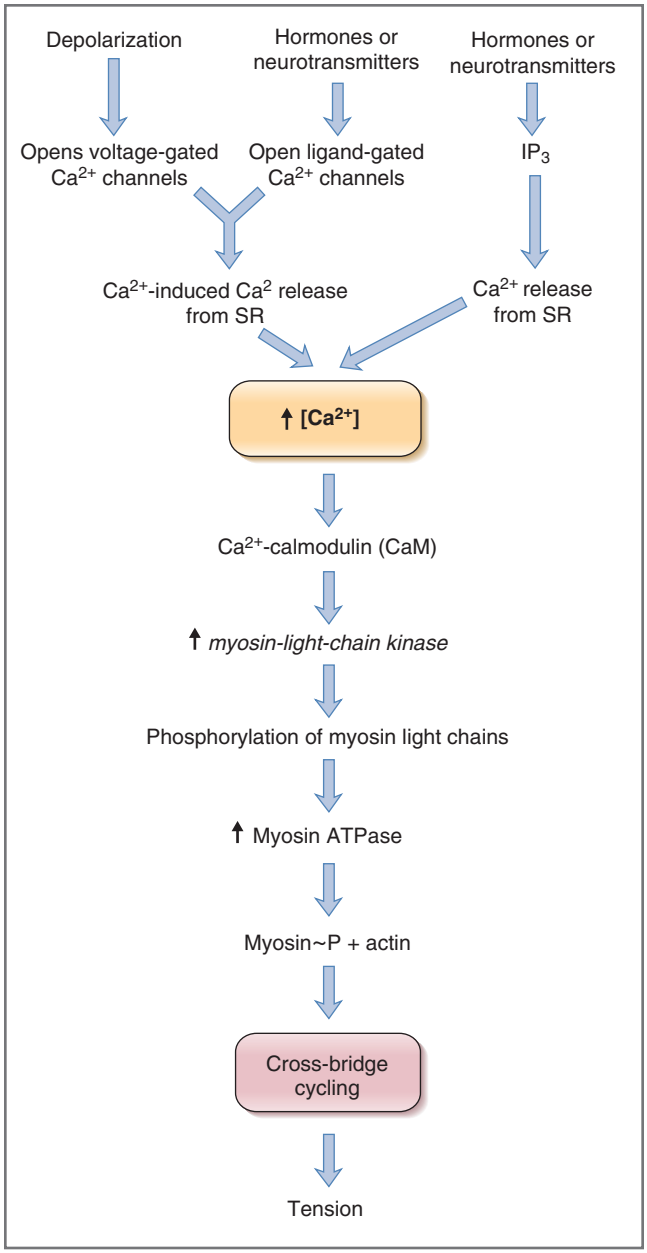


Figure 1–29 The sequence of molecular events in contraction of smooth muscle. ADP, Adenosine diphosphate; ATP, adenosine triphosphate; Myosin~P, phosphorylated myosin; P_i, inorganic phosphate. CaM, calmodulin; ATPase, adenosine triphosphatase; IP_3, inositol 1,4,5 triphosphate; SR, sarcoplasmic reticulum.

potential) can open these voltage-gated Ca^{2+} channels and cause an increase in intracellular Ca^{2+} concentration. *If* the depolarization of the smooth muscle membrane reaches threshold, then **action potentials** *can occur,* causing even greater depolarization and even greater opening of voltage-gated Ca^{2+} channels.

Ca^{2+} that enters the smooth muscle cells through voltage-gated Ca^{2+} channels releases additional Ca^{2+} from the SR (called **Ca^{2+}-induced Ca^{2+} release**). Thus, the rise in intracellular Ca^{2+} is partly due to Ca^{2+} entry across the sarcolemmal membrane and partly due to Ca^{2+} release from intracellular SR stores.

2. Two additional mechanisms may contribute to the increase in intracellular Ca^{2+} concentration: ligand-gated Ca^{2+} channels and inositol 1,4,5-triphosphate (IP_3)–gated Ca^{2+} release channels. **Ligand-gated Ca^{2+} channels** in the sarcolemmal membrane may be opened by various hormones and neurotransmitters, permitting the entry of *additional* Ca^{2+} from the ECF. **IP_3-gated Ca^{2+} release channels** in the membrane of the sarcoplasmic reticulum may be opened by hormones and neurotransmitters. Either of these mechanisms may augment the rise in intracellular Ca^{2+} concentration caused by depolarization.

3. The rise in intracellular Ca^{2+} concentration causes Ca^{2+} to bind to **calmodulin.** Like troponin C in skeletal muscle, calmodulin binds four ions of Ca^{2+} in a cooperative fashion. The Ca^{2+}-calmodulin complex binds to and activates **myosin-light-chain kinase.**

4. When activated, myosin-light-chain kinase **phosphorylates myosin light chain.** When myosin light chain is phosphorylated, the conformation of the myosin head is altered, greatly increasing its ATPase activity. (In contrast, skeletal muscle myosin ATPase activity is always high.) The increase in myosin ATPase activity allows myosin to bind actin, thus initiating cross-bridge cycling and production of tension. The amount of tension is proportional to the intracellular Ca^{2+} concentration.

5. Ca^{2+}-calmodulin, in addition to the effects on myosin described earlier, also has effects on two thin filament proteins, **calponin** and **caldesmon.** At low levels of intracellular Ca^{2+}, calponin and caldesmon bind actin, inhibiting myosin ATPase and preventing the interaction of actin and myosin. When the intracellular Ca^{2+} increases, the Ca^{2+}-calmodulin complex leads to phosphorylation of calponin and caldesmon, releasing their inhibition of myosin ATPase and facilitating the formation of cross-bridges between actin and myosin.

6. **Relaxation** of smooth muscle occurs when the intracellular Ca^{2+} concentration falls below the level needed to form Ca^{2+}-calmodulin complexes. A fall in intracellular Ca^{2+} concentration can occur by a variety of mechanisms including hyperpolarization (which closes voltage-gated Ca^{2+} channels); direct inhibition of Ca^{2+} channels by ligands such as cyclic AMP and cyclic GMP; inhibition of IP_3 production and decreased release of Ca^{2+} from sarcoplasmic reticulum; and increased Ca^{2+} ATPase activity in sarcoplasmic reticulum. Additionally, relaxation of

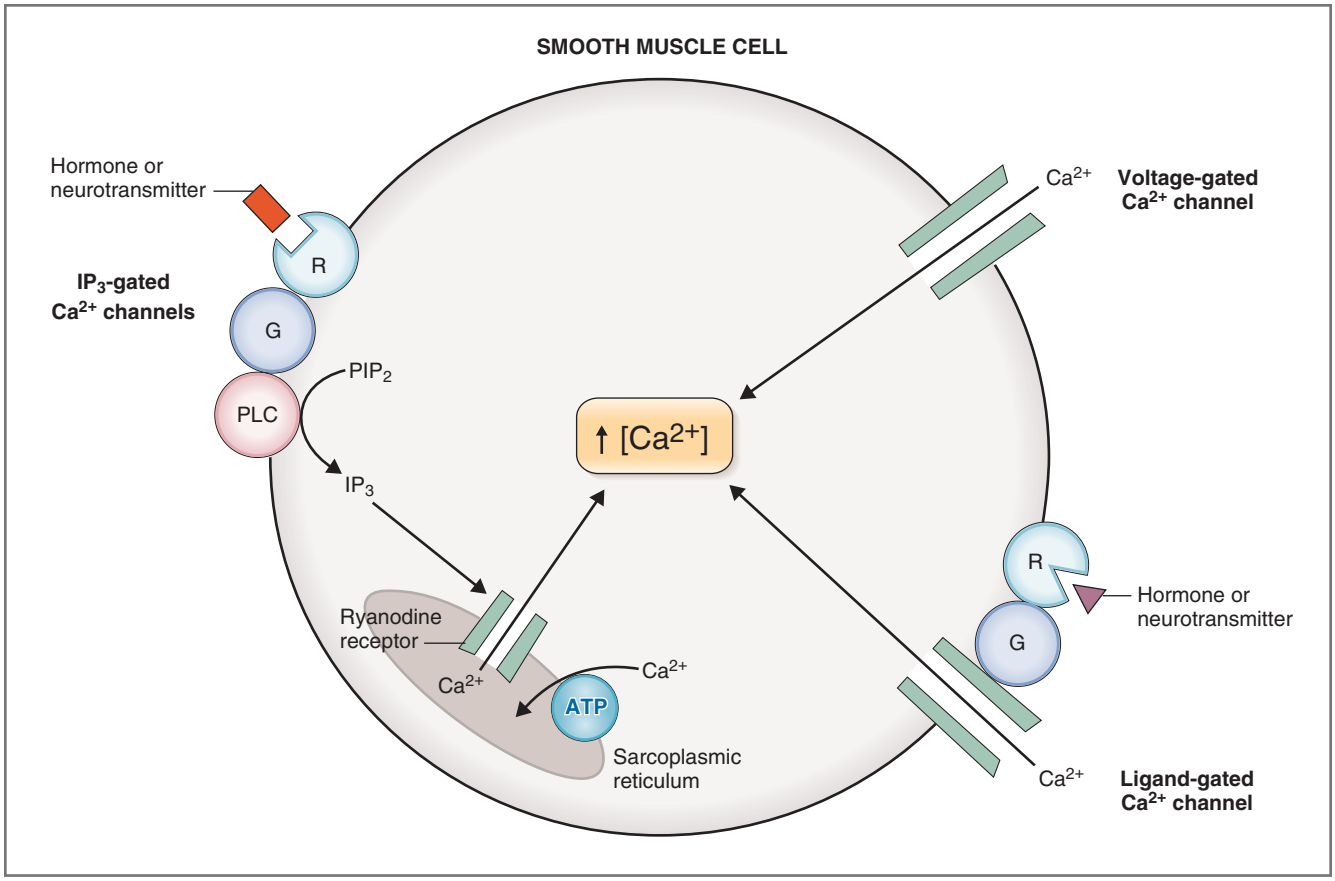


Figure 1–30 Mechanisms for increasing intracellular [Ca^{2+}] in smooth muscle. ATP, Adenosine triphosphate; G, GTP-binding protein (G protein); IP_3, inositol 1,4,5-triphosphate; PIP_2, phosphatidylinositol 4,5-diphosphate; PLC, phospholipase C; R, receptor for hormone or neurotransmitter.

smooth muscle can involve activation of myosin-light-chain phosphatase, which dephosphorylates myosin light chain, leading to inhibition of myosin ATPase.

Mechanisms That Increase Intracellular Ca^{2+} Concentration in Smooth Muscle

Depolarization of smooth muscle opens sarcolemmal voltage-gated Ca^{2+} channels and Ca^{2+} enters the cell from ECF. As already noted, this is only *one* source of Ca^{2+} for contraction. Ca^{2+} also can enter the cell through ligand-gated channels in the sarcolemmal membrane, or it can be released from the sarcoplasmic reticulum by second messenger (IP_3)-gated mechanisms (Fig. 1-30). (In contrast, recall that in skeletal muscle the rise in intracellular Ca^{2+} concentration is caused exclusively by release from the sarcoplasmic reticulum—Ca^{2+} does not enter the cell from the ECF.) The three mechanisms involved in Ca^{2+} entry in smooth muscle are described as follows:

- **Voltage-gated Ca^{2+} channels** are sarcolemmal Ca^{2+} channels that open when the cell membrane potential depolarizes. Thus, action potentials in the smooth muscle cell membrane cause voltage-gated Ca^{2+} channels to open, allowing Ca^{2+} to flow into the cell down its electrochemical potential gradient.
- **Ligand-gated Ca^{2+} channels** also are present in the sarcolemmal membrane. They are not regulated by changes in membrane potential, but by receptor-mediated events. Various hormones and neurotransmitters interact with specific receptors in the sarcolemmal membrane, which are coupled via a GTP-binding protein (G protein) to the Ca^{2+} channels. When the channel is open, Ca^{2+} flows into the cell down its electrochemical gradient. (See Chapters 2 and 9 for further discussion of G proteins.)
- **IP_3-gated Ca^{2+} channels** are present in the sarcoplasmic reticulum membrane. The process begins at the cell membrane, but the source of the Ca^{2+} is the sarcoplasmic reticulum rather than the ECF. Hormones or neurotransmitters interact with specific receptors on the sarcolemmal membrane (e.g., norepinephrine with α_1 receptors). These receptors are coupled, via a G protein, to phospholipase C (PLC). **Phospholipase C** catalyzes the hydrolysis of

phosphatidylinositol 4,5-diphosphate (PIP_2) to IP_3 and diacylglycerol (DAG). **IP_3** then diffuses to the sarcoplasmic reticulum, where it opens Ca^{2+} release channels (similar to the mechanism of the ryanodine receptor in skeletal muscle). When these Ca^{2+} channels are open, Ca^{2+} flows from its storage site in the sarcoplasmic reticulum into the ICF. (See Chapter 9 for discussion of IP_3-mediated hormone action.)

Ca^{2+}-Independent Changes in Smooth Muscle Contraction

In addition to the contractile mechanisms in smooth muscle that depend on changes in intracellular Ca^{2+} concentration, the degree of contraction also can be regulated by Ca^{2+}-independent mechanisms. For example, in the presence of a constant level of intracellular Ca^{2+}, if there is activation of myosin-light-chain kinase, more cross-bridges will cycle and more tension will be produced (**Ca^{2+}-sensitization**); conversely, if there is activation of myosin-light-chain phosphatase, fewer cross-bridges will cycle and less tension will be produced (**Ca^{2+}-desensitization**).

SUMMARY

- Water, a major component of the body, is distributed among two major compartments, ICF and ECF. ECF is further distributed among the plasma and the interstitial fluid. The differences in composition of ICF and ECF are created and maintained by transport proteins in the cell membranes.

- Transport may be either passive or active. If transport occurs down an electrochemical gradient, it is passive and does not consume energy. If transport occurs against an electrochemical gradient, it is active. The energy for active transport may be primary (using ATP) or secondary (using energy from the Na^+ gradient). Osmosis occurs when an impermeable solute creates an osmotic pressure difference across a membrane, which drives water flow.

- Ion channels provide routes for charged solutes to move across cell membranes. The conductance of ion channels is controlled by gates, which are regulated by voltage or by ligands. Diffusion of a permeable ion down a concentration gradient creates a diffusion potential, which, at electrochemical equilibrium, is calculated by the Nernst equation. When several ions are permeable, each attempts to drive the membrane toward its equilibrium potential. Ions with the highest permeabilities make the greatest contribution to the resting membrane potential.

- Action potentials in nerve and muscle consist of rapid depolarization (upstroke), followed by repolarization caused by the opening and closing of ion channels. Action potentials are propagated down nerve and muscle fibers by the spread of local currents, with the speed of conduction depending on the tissue's cable properties. Conduction velocity is increased by increasing fiber size and by myelination.

- Synapses between cells may be electrical or, more commonly, chemical. The prototype of the chemical synapse is the neuromuscular junction, which uses ACh as a neurotransmitter. ACh is released from presynaptic nerve terminals and diffuses across the synapse to cause depolarization of the motor end plate. Neurotransmitters at other synapses may be either excitatory (causing depolarization) or inhibitory (causing hyperpolarization).

- In muscle, action potentials precede contraction. The mechanisms that translate the action potential into contraction are called excitation-contraction coupling. In both skeletal and smooth muscle, Ca^{2+} plays a central role in the coupling.

- In skeletal muscle, the action potential is carried to the cell interior by the T tubules, where depolarization releases Ca^{2+} from terminal cisternae of the nearby sarcoplasmic reticulum. Ca^{2+} then binds to troponin C on the thin filaments, causing a conformational change, which removes the inhibition of myosin-binding sites. When actin and myosin bind, cross-bridge cycling begins, producing tension.

- In smooth muscle, Ca^{2+} enters the cell during the action potential via voltage-gated Ca^{2+} channels. Ca^{2+} then binds to calmodulin, and the Ca^{2+}-calmodulin complex activates myosin-light-chain kinase, which phosphorylates myosin. Myosin ~ P can bind actin, form cross-bridges, and generate tension. Other sources of intracellular Ca^{2+} in smooth muscle are ligand-gated Ca^{2+} channels in the sarcolemmal membrane and IP_3-gated Ca^{2+} channels in the sarcoplasmic reticulum membrane.

Challenge Yourself

Answer each question with a word, phrase, sentence, or numerical solution. When a list of possible answers is supplied with the question, one, more than one, or none of the choices may be correct. Correct answers are provided at the end of the book.

1 *Solution A contains 100 mM NaCl, Solution B contains 10 mM NaCl, and the membrane separating them is permeable to Cl^- but not Na^+. What is the orientation of the potential difference that will be established across the membrane?*