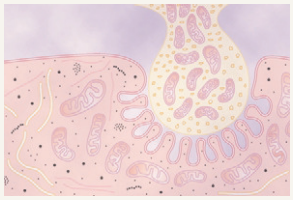
# Excitation of Skeletal Muscle: Neuromuscular Transmission and Excitation-Contraction Coupling



## Transmission of Impulses from Nerve Endings to Skeletal Muscle Fibers: The Neuromuscular Junction

The skeletal muscle fibers are innervated by large, myelinated nerve fibers that originate from large motoneurons in the anterior horns of the spinal cord. As pointed out in Chapter 6, each nerve fiber, after entering the muscle belly, normally branches and stimulates from three to several hundred skeletal muscle fibers. Each nerve ending makes a junction, called the *neuromuscular junction,* with the muscle fiber near its midpoint. The action potential initiated in the muscle fiber by the nerve signal travels in both directions toward the muscle fiber ends. With the exception of about 2 per cent of the muscle fibers, there is only one such junction per muscle fiber.

**Physiologic Anatomy of the Neuromuscular Junction—The Motor End Plate.** Figure 7–1*A* and *B* shows the neuromuscular junction from a large, myelinated nerve fiber to a skeletal muscle fiber. The nerve fiber forms a complex of *branching nerve terminals* that invaginate into the surface of the muscle fiber but lie outside the muscle fiber plasma membrane. The entire structure is called the *motor end plate.* It is covered by one or more Schwann cells that insulate it from the surrounding fluids.

Figure 7–1*C* shows an electron micrographic sketch of the junction between a single axon terminal and the muscle fiber membrane. The invaginated membrane is called the *synaptic gutter* or *synaptic trough,* and the space between the terminal and the fiber membrane is called the *synaptic space* or *synaptic cleft.* This space is 20 to 30 nanometers wide. At the bottom of the gutter are numerous smaller *folds* of the muscle membrane called *subneural clefts,* which greatly increase the surface area at which the synaptic transmitter can act.

In the axon terminal are many mitochondria that supply adenosine triphosphate (ATP), the energy source that is used for synthesis of an excitatory transmitter *acetylcholine.* The acetylcholine in turn excites the muscle fiber membrane. Acetylcholine is synthesized in the cytoplasm of the terminal, but it is absorbed rapidly into many small *synaptic vesicles,* about 300,000 of which are normally in the terminals of a single end plate. In the synaptic space are large quantities of the enzyme *acetylcholinesterase,* which destroys acetylcholine a few milliseconds after it has been released from the synaptic vesicles.

### Secretion of Acetylcholine by the Nerve Terminals

When a nerve impulse reaches the neuromuscular junction, about 125 vesicles of acetylcholine are released from the terminals into the synaptic space. Some of the details of this mechanism can be seen in Figure 7–2, which shows an expanded view of a synaptic space with the neural membrane above and the muscle membrane and its subneural clefts below.

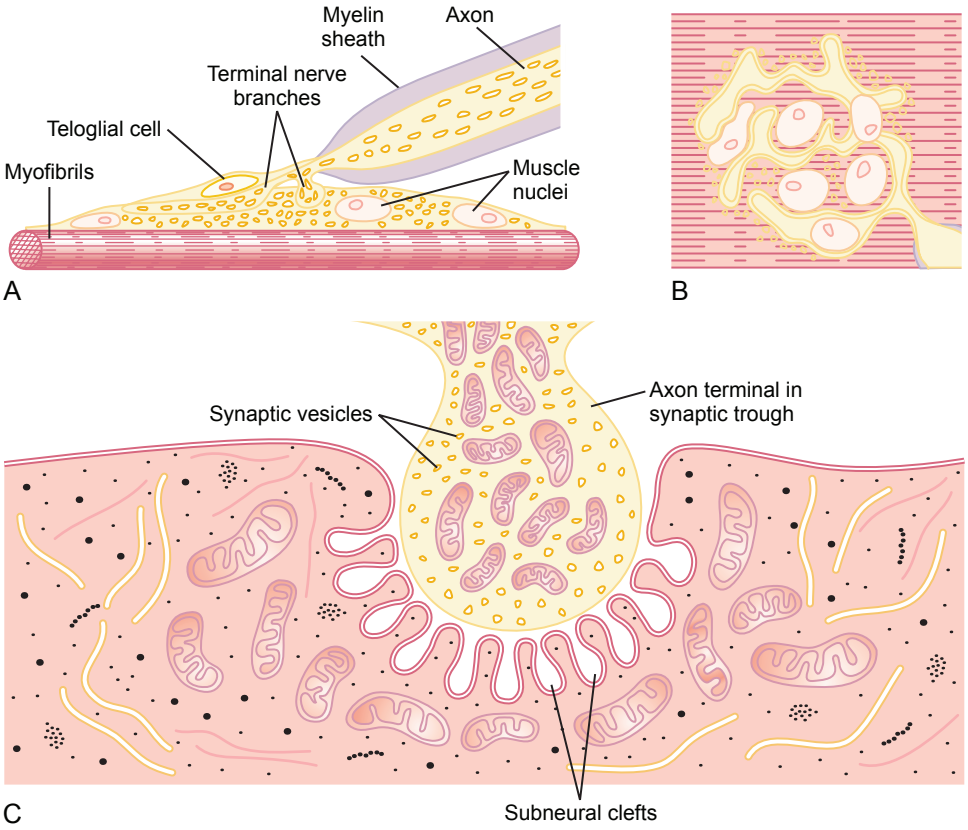



**Figure 7–1**

Different views of the motor end plate. *A,* Longitudinal section through the end plate. *B,* Surface view of the end plate. *C,* Electron micrographic appearance of the contact point between a single axon terminal and the muscle fiber membrane. (Redrawn from Fawcett DW, as modified from Couteaux R, in Bloom W, Fawcett DW: A Textbook of Histology. Philadelphia: WB Saunders, 1986.)

On the inside surface of the neural membrane are linear *dense bars,* shown in cross section in Figure 7–2. To each side of each dense bar are protein particles that penetrate the neural membrane; these are *voltage-gated calcium channels.* When an action potential spreads over the terminal, these channels open and allow calcium ions to diffuse from the synaptic space to the interior of the nerve terminal. The calcium ions, in turn, are believed to exert an attractive influence on the acetylcholine vesicles, drawing them to the neural membrane adjacent to the dense bars. The vesicles then fuse with the neural membrane and empty their acetylcholine into the synaptic space by the process of *exocytosis.*

Although some of the aforementioned details are speculative, it is known that the effective stimulus for causing acetylcholine release from the vesicles is entry of calcium ions and that acetylcholine from the vesicles is then emptied through the neural membrane adjacent to the dense bars.

**Effect of Acetylcholine on the Postsynaptic Muscle Fiber Membrane to Open Ion Channels.** Figure 7–2 also shows many very small *acetylcholine receptors* in the muscle fiber membrane; these are *acetylcholine-gated ion channels,* and they are located almost entirely near the mouths of the subneural clefts lying immediately below the dense bar areas, where the acetylcholine is emptied into the synaptic space.

Each receptor is a protein complex that has a total molecular weight of 275,000. The complex is composed

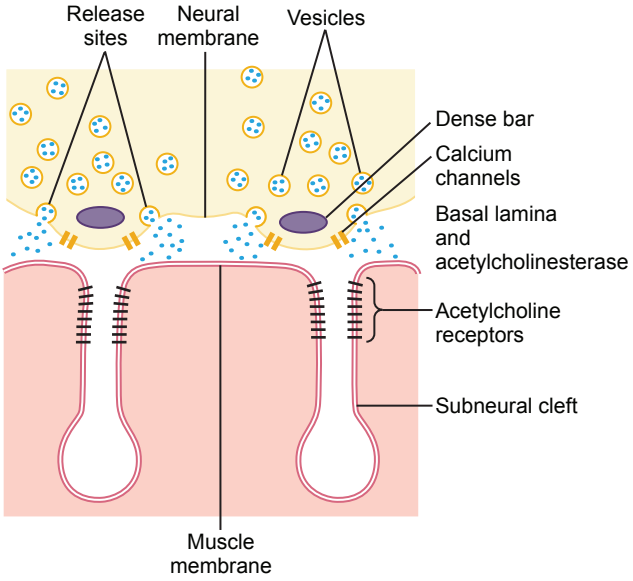



**Figure 7–2**

Release of acetylcholine from synaptic vesicles at the neural membrane of the neuromuscular junction. Note the proximity of the release sites in the neural membrane to the acetylcholine receptors in the muscle membrane, at the mouths of the subneural clefts.

of five subunit proteins, two *alpha* proteins and one each of *beta, delta,* and *gamma* proteins. These protein molecules penetrate all the way through the membrane, lying side by side in a circle to form a tubular channel, illustrated in Figure 7–3. The channel remains constricted, as shown in section A of the figure, until two acetylcholine molecules attach respectively to the two *alpha* subunit proteins. This causes a conformational change that opens the channel, as shown in section B of the figure.

The opened acetylcholine channel has a diameter of about 0.65 nanometer, which is large enough to allow the important positive ions—sodium ($Na^+$), potassium ($K^+$), and calcium ($Ca^{++}$)—to move easily through the opening. Conversely, negative ions, such as chloride ions, do not pass through because of strong negative charges in the mouth of the channel that repel these negative ions.

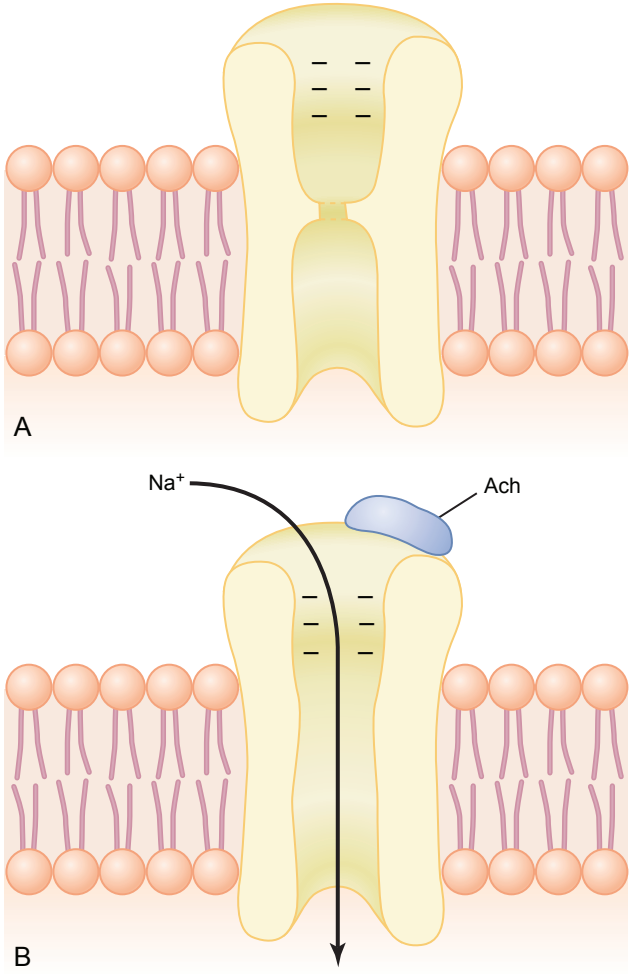



**Figure 7–3**

Acetylcholine channel. *A,* Closed state. *B,* After acetylcholine (Ach) has become attached and a conformational change has opened the channel, allowing sodium ions to enter the muscle fiber and excite contraction. Note the negative charges at the channel mouth that prevent passage of negative ions such as chloride ions.

In practice, far more sodium ions flow through the acetylcholine channels than any other ions, for two reasons. First, there are only two positive ions in large concentration: sodium ions in the extracellular fluid, and potassium ions in the intracellular fluid. Second, the very negative potential on the inside of the muscle membrane, –80 to –90 millivolts, pulls the positively charged sodium ions to the inside of the fiber, while simultaneously preventing efflux of the positively charged potassium ions when they attempt to pass outward.

As shown in Figure 7–3*B*, the principal effect of opening the acetylcholine-gated channels is to allow large numbers of sodium ions to pour to the inside of the fiber, carrying with them large numbers of positive charges. This creates a local positive potential change inside the muscle fiber membrane, called the *end plate potential.* In turn, this end plate potential initiates an action potential that spreads along the muscle membrane and thus causes muscle contraction.

**Destruction of the Released Acetylcholine by Acetylcholinesterase.** The acetylcholine, once released into the synaptic space, continues to activate the acetylcholine receptors as long as the acetylcholine persists in the space. However, it is removed rapidly by two means: (1) Most of the acetylcholine is destroyed by the enzyme *acetylcholinesterase,* which is attached mainly to the spongy layer of fine connective tissue that fills the synaptic space between the presynaptic nerve terminal and the postsynaptic muscle membrane. (2) A small amount of acetylcholine diffuses out of the synaptic space and is then no longer available to act on the muscle fiber membrane.

The short time that the acetylcholine remains in the synaptic space—a few milliseconds at most—normally is sufficient to excite the muscle fiber. Then the rapid removal of the acetylcholine prevents continued muscle re-excitation after the muscle fiber has recovered from its initial action potential.

**End Plate Potential and Excitation of the Skeletal Muscle Fiber.** The sudden insurgence of sodium ions into the muscle fiber when the acetylcholine channels open causes the electrical potential inside the fiber at the *local area of the end plate* to increase in the positive direction as much as 50 to 75 millivolts, creating a *local potential* called the *end plate potential.* Recall from Chapter 5 that a sudden increase in nerve membrane potential of more than 20 to 30 millivolts is normally sufficient to initiate more and more sodium channel opening, thus initiating an action potential at the muscle fiber membrane.

Figure 7–4 shows the principle of an end plate potential initiating the action potential. This figure shows three separate end plate potentials. End plate potentials A and C are too weak to elicit an action potential, but they do produce weak local end plate voltage changes, as recorded in the figure. By contrast, end plate potential B is much stronger and causes enough sodium channels to open so that the self-regenerative effect of more and more sodium ions

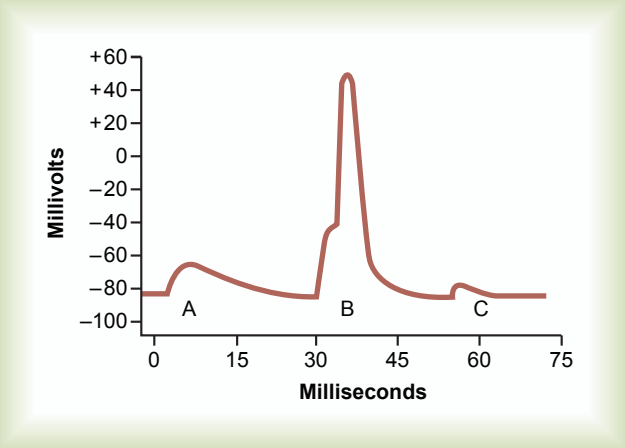



**Figure 7–4**

End plate potentials (in millivolts). *A,* Weakened end plate potential recorded in a curarized muscle, too weak to elicit an action potential. *B,* Normal end plate potential eliciting a muscle action potential. *C,* Weakened end plate potential caused by botulinum toxin that decreases end plate release of acetylcholine, again too weak to elicit a muscle action potential.

flowing to the interior of the fiber initiates an action potential. The weakness of the end plate potential at point A was caused by poisoning of the muscle fiber with *curare,* a drug that blocks the gating action of acetylcholine on the acetylcholine channels by competing for the acetylcholine receptor sites. The weakness of the end plate potential at point C resulted from the effect of *botulinum toxin,* a bacterial poison that decreases the quantity of acetylcholine release by the nerve terminals.

**Safety Factor for Transmission at the Neuromuscular Junction; Fatigue of the Junction.** Ordinarily, each impulse that arrives at the neuromuscular junction causes about three times as much end plate potential as that required to stimulate the muscle fiber. Therefore, the normal neuromuscular junction is said to have a high *safety factor.* However, stimulation of the nerve fiber at rates greater than 100 times per second for several minutes often diminishes the number of acetylcholine vesicles so much that impulses fail to pass into the muscle fiber. This is called *fatigue* of the neuromuscular junction, and it is the same effect that causes fatigue of synapses in the central nervous system when the synapses are overexcited. Under normal functioning conditions, measurable fatigue of the neuromuscular junction occurs rarely, and even then only at the most exhausting levels of muscle activity.

## Molecular Biology of Acetylcholine Formation and Release

Because the neuromuscular junction is large enough to be studied easily, it is one of the few synapses of the nervous system for which most of the details of chemical transmission have been worked out. The formation and release of acetylcholine at this junction occur in the following stages:

1. Small vesicles, about 40 nanometers in size, are formed by the Golgi apparatus in the cell body of the motoneuron in the spinal cord. These vesicles are then transported by axoplasm that "streams" through the core of the axon from the central cell body in the spinal cord all the way to the neuromuscular junction at the tips of the peripheral nerve fibers. About 300,000 of these small vesicles collect in the nerve terminals of a single skeletal muscle end plate.
2. Acetylcholine is synthesized in the cytosol of the nerve fiber terminal but is immediately transported through the membranes of the vesicles to their interior, where it is stored in highly concentrated form, about 10,000 molecules of acetylcholine in each vesicle.
3. When an action potential arrives at the nerve terminal, it opens many calcium channels in the membrane of the nerve terminal because this terminal has an abundance of voltage-gated calcium channels. As a result, the calcium ion concentration inside the terminal membrane increases about 100-fold, which in turn increases the rate of fusion of the acetylcholine vesicles with the terminal membrane about 10,000-fold. This fusion makes many of the vesicles rupture, allowing *exocytosis* of acetylcholine into the synaptic space. About 125 vesicles usually rupture with each action potential. Then, after a few milliseconds, the acetylcholine is split by acetylcholinesterase into acetate ion and choline, and the choline is reabsorbed actively into the neural terminal to be reused to form new acetylcholine. This sequence of events occurs within a period of 5 to 10 milliseconds.
4. The number of vesicles available in the nerve ending is sufficient to allow transmission of only a few thousand nerve-to-muscle impulses. Therefore, for continued function of the neuromuscular junction, new vesicles need to be re-formed rapidly. Within a few seconds after each action potential is over, "coated pits" appear in the terminal nerve membrane, caused by contractile proteins in the nerve ending, especially the protein *clathrin,* which is attached to the membrane in the areas of the original vesicles. Within about 20 seconds, the proteins contract and cause the pits to break away to the interior of the membrane, thus forming new vesicles. Within another few seconds, acetylcholine is transported to the interior of these vesicles, and they are then ready for a new cycle of acetylcholine release.

## Drugs That Enhance or Block Transmission at the Neuromuscular Junction

**Drugs That Stimulate the Muscle Fiber by Acetylcholine-Like Action.** Many compounds, including *methacholine, carbachol,* and *nicotine,* have the same effect on the muscle fiber as does acetylcholine. The difference between

these drugs and acetylcholine is that the drugs are not destroyed by cholinesterase or are destroyed so slowly that their action often persists for many minutes to several hours. The drugs work by causing localized areas of depolarization of the muscle fiber membrane at the motor end plate where the acetylcholine receptors are located. Then, every time the muscle fiber recovers from a previous contraction, these depolarized areas, by virtue of leaking ions, initiate a new action potential, thereby causing a state of muscle spasm.

**Drugs That Stimulate the Neuromuscular Junction by Inactivating Acetylcholinesterase.** Three particularly well-known drugs, *neostigmine, physostigmine,* and *diisopropyl fluorophosphate,* inactivate the acetylcholinesterase in the synapses so that it no longer hydrolyzes acetylcholine. Therefore, with each successive nerve impulse, additional acetylcholine accumulates and stimulates the muscle fiber repetitively. This causes *muscle spasm* when even a few nerve impulses reach the muscle. Unfortunately, it also can cause death due to laryngeal spasm, which smothers the person.

Neostigmine and physostigmine combine with acetylcholinesterase to inactivate the acetylcholinesterase for up to several hours, after which these drugs are displaced from the acetylcholinesterase so that the esterase once again becomes active. Conversely, diisopropyl fluorophosphate, which has military potential as a powerful "nerve" gas poison, inactivates acetylcholinesterase for weeks, which makes this a particularly lethal poison.

**Drugs That Block Transmission at the Neuromuscular Junction.** A group of drugs known as *curariform drugs* can prevent passage of impulses from the nerve ending into the muscle. For instance, D-tubocurarine blocks the action of acetylcholine on the muscle fiber acetylcholine receptors, thus preventing sufficient increase in permeability of the muscle membrane channels to initiate an action potential.

## Myasthenia Gravis

*Myasthenia gravis,* which occurs in about 1 in every 20,000 persons, causes muscle paralysis because of inability of the neuromuscular junctions to transmit enough signals from the nerve fibers to the muscle fibers. Pathologically, antibodies that attack the acetylcholine-gated sodium ion transport proteins have been demonstrated in the blood of most patients with myasthenia gravis. Therefore, it is believed that myasthenia gravis is an autoimmune disease in which the patients have developed immunity against their own acetylcholine-activated ion channels.

Regardless of the cause, the end plate potentials that occur in the muscle fibers are mostly too weak to stimulate the muscle fibers. If the disease is intense enough, the patient dies of paralysis—in particular, paralysis of the respiratory muscles. The disease usually can be ameliorated for several hours by administering *neostigmine* or some other anticholinesterase drug, which allows larger than normal amounts of acetylcholine to accumulate in the synaptic space. Within minutes, some of these paralyzed people can begin to function almost normally, until a new dose of neostigmine is required a few hours later.

## Muscle Action Potential

Almost everything discussed in Chapter 5 regarding initiation and conduction of action potentials in nerve fibers applies equally to skeletal muscle fibers, except for quantitative differences. Some of the quantitative aspects of muscle potentials are the following:

1. Resting membrane potential: about –80 to –90 millivolts in skeletal fibers—the same as in large myelinated nerve fibers.
2. Duration of action potential: 1 to 5 milliseconds in skeletal muscle—about five times as long as in large myelinated nerves.
3. Velocity of conduction: 3 to 5 m/sec—about 1/13 the velocity of conduction in the large myelinated nerve fibers that excite skeletal muscle.

### Spread of the Action Potential to the Interior of the Muscle Fiber by Way of "Transverse Tubules"

The skeletal muscle fiber is so large that action potentials spreading along its surface membrane cause almost no current flow deep within the fiber. Yet, to cause maximum muscle contraction, current must penetrate deeply into the muscle fiber to the vicinity of the separate myofibrils. This is achieved by transmission of action potentials along *transverse tubules* (T tubules) that penetrate all the way through the muscle fiber from one side of the fiber to the other, as illustrated in Figure 7–5. The T tubule action potentials cause release of calcium ions inside the muscle fiber in the immediate vicinity of the myofibrils, and these calcium ions then cause contraction. This overall process is called *excitation-contraction* coupling.

## Excitation-Contraction Coupling

### Transverse Tubule–Sarcoplasmic Reticulum System

Figure 7–5 shows myofibrils surrounded by the T tubule–sarcoplasmic reticulum system. The T tubules are very small and run transverse to the myofibrils. They begin at the cell membrane and penetrate all the way from one side of the muscle fiber to the opposite side. Not shown in the figure is the fact that these tubules branch among themselves so that they form entire *planes* of T tubules interlacing among all the separate myofibrils. Also, *where the T tubules originate from the cell membrane, they are open to the exterior of the muscle fiber.* Therefore, they communicate with the extracellular fluid surrounding the muscle fiber, and they themselves contain extracellular fluid in their lumens. In other words, the T tubules are actually internal extensions of the cell membrane. Therefore, when an action potential spreads over a muscle fiber

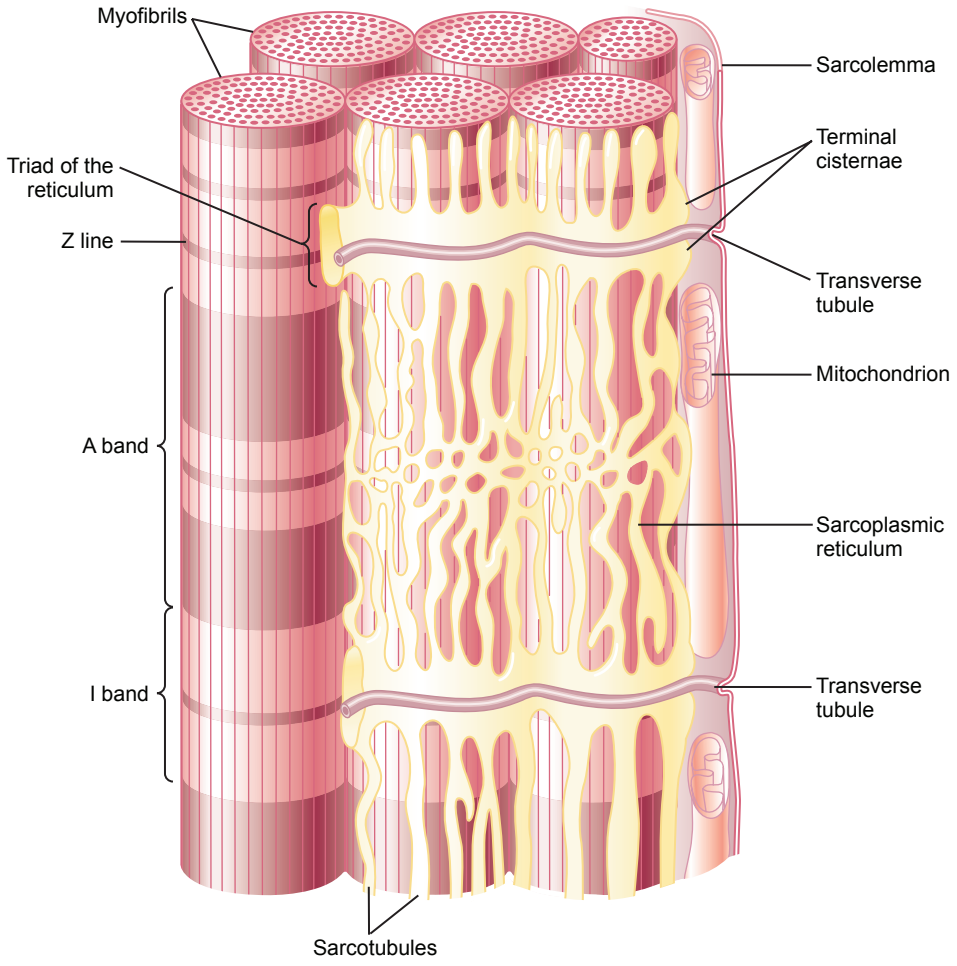



**Figure 7–5**

Transverse (T) tubule–sarcoplasmic reticulum system. Note that the T tubules communicate with the outside of the cell membrane, and deep in the muscle fiber, each T tubule lies adjacent to the ends of longitudinal sarcoplasmic reticulum tubules that surround all sides of the actual myofibrils that contract. This illustration was drawn from frog muscle, which has one T tubule per sarcomere, located at the Z line. A similar arrangement is found in mammalian heart muscle, but mammalian skeletal muscle has two T tubules per sarcomere, located at the A-I band junctions. (Redrawn from Bloom W, Fawcett DW: A Textbook of Histology. Philadelphia: WB Saunders, 1986. Modified after Peachey LD: J Cell Biol 25:209, 1965. Drawn by Sylvia Colard Keene.)

membrane, a potential change also spreads along the T tubules to the deep interior of the muscle fiber. The electrical currents surrounding these T tubules then elicit the muscle contraction.

Figure 7–5 also shows a *sarcoplasmic reticulum,* in yellow. This is composed of two major parts: (1) large chambers called *terminal cisternae* that abut the T tubules, and (2) long longitudinal tubules that surround all surfaces of the actual contracting myofibrils.

## Release of Calcium Ions by the Sarcoplasmic Reticulum

One of the special features of the sarcoplasmic reticulum is that within its vesicular tubules is an excess of calcium ions in high concentration, and many of these ions are released from each vesicle when an action potential occurs in the adjacent T tubule.

Figure 7–6 shows that the action potential of the T tubule causes current flow into the sarcoplasmic reticular cisternae where they abut the T tubule. This in turn causes rapid opening of large numbers of calcium channels through the membranes of the cisternae as well as their attached longitudinal tubules. These channels remain open for a few milliseconds; during this time, enough calcium ions are released into the sarcoplasm surrounding the myofibrils to cause contraction, as discussed in Chapter 6.

**Calcium Pump for Removing Calcium Ions from the Myofibrillar Fluid After Contraction Occurs.** Once the calcium ions have been released from the sarcoplasmic tubules and have diffused among the myofibrils, muscle contraction continues as long as the calcium ions remain in high concentration. However, a continually active calcium pump located in the walls of the sarcoplasmic reticulum pumps calcium ions away from the myofibrils back into the sarcoplasmic tubules. This pump can concentrate the calcium ions about 10,000-fold inside the tubules. In addition, inside the reticulum is a protein called *calsequestrin* that can bind up to 40 times more calcium.

**Excitatory "Pulse" of Calcium Ions.** The normal resting state concentration (less than $10^{-7}$ molar) of calcium ions in the cytosol that bathes the myofibrils is too little to elicit contraction. Therefore, the troponin-tropomyosin complex keeps the actin filaments inhibited and maintains a relaxed state of the muscle.

Conversely, full excitation of the T tubule and sarcoplasmic reticulum system causes enough release of calcium ions to increase the concentration in the myofibrillar fluid to as high as $2 \times 10^{-4}$ molar

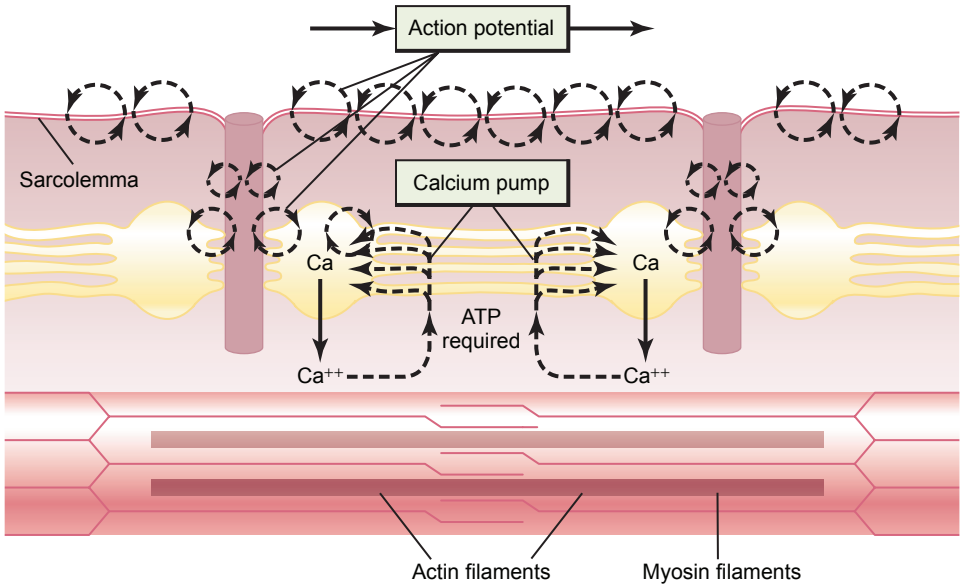



**Figure 7–6**

Excitation-contraction coupling in the muscle, showing (1) an action potential that causes release of calcium ions from the sarcoplasmic reticulum and then (2) reuptake of the calcium ions by a calcium pump.

concentration, a 500-fold increase, which is about 10 times the level required to cause maximum muscle contraction. Immediately thereafter, the calcium pump depletes the calcium ions again. The total duration of this calcium "pulse" in the usual *skeletal muscle* fiber lasts about 1/20 of a second, although it may last several times as long in some fibers and several times less in others. (In heart muscle, the calcium pulse lasts about 1/3 of a second because of the long duration of the cardiac action potential.)

During this calcium pulse, muscle contraction occurs. If the contraction is to continue without interruption for long intervals, a series of calcium pulses must be initiated by a continuous series of repetitive action potentials, as discussed in Chapter 6.

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