Smooth Muscle 3.8

Learning Objectives

- Compare smooth muscle cell morphology to that of skeletal muscle fibers
- Contrast tonic and phasic smooth muscles
- Distinguish electromechanical coupling from pharmacomechanical coupling
- Define unitary smooth muscle and contrast it from multiunit smooth muscle
- Describe varicosities and their role in smooth muscle stimulation
- List the mechanisms on the sarcolemma for Ca^{2+} entry into the smooth muscle cell
- List the mechanisms on the sarcolemma for Ca^{2+} exit from the smooth muscle cell
- List the mechanisms on the SR for Ca^{2+} exit into the smooth muscle cytoplasm
- List the mechanisms on the SR for Ca^{2+} uptake into the SR from the smooth muscle cytoplasm
- Describe how myosin light chain kinase (MLCK) activates smooth muscle
- Describe how myosin light chain phosphatase (MLCP) inactivates smooth muscle
- Describe what is meant by "Ca^{2+} sensitization"
- Describe how Ca^{2+} activates smooth muscle

SMOOTH MUSCLES SHOW NO CROSS-STRIATIONS

Smooth muscle lines the walls of hollow organs and tubes such as the urinary bladder, gall bladder, uterus, bile ducts, ureters, intestines, and arteries. Contraction of the smooth muscle in tubes either controls the caliber of the tube or propels material through the lumen of the tube. In the hollow organs, contraction of the smooth muscle increases the pressure within the organ, thereby propelling material out of the organ. Smooth muscle cells are small and spindle shaped with no cross-striations. Typically they are only 2–5 μm in diameter and up to about 400 μm long with a single, central nucleus. This contrasts with the very long, multinucleated skeletal muscle fibers.

SMOOTH MUSCLE DEVELOPS TENSION MORE SLOWLY BUT CAN MAINTAIN TENSION FOR A LONG TIME

Skeletal muscle and cardiac muscle develop tension quickly and relax quickly, in accord with their physiological function. Smooth muscle contracts and relaxes much more slowly, but it can sustain contractions for long periods of time without fatiguing, and it can develop as much force as striated muscle. This ability is useful in the **sphincters** of the body. Sphincters are bands of smooth muscle that surround areas that separate adjoining parts of the hollow organs. The sphincters act as valves. Contraction of the band of smooth muscle prevents the movement of material through the sphincter. Examples include the urinary sphincter that controls the movement of urine from the urinary bladder, and the various sphincters in the gastrointestinal tract. These sphincters are usually contracted and open only when necessary.

SMOOTH MUSCLE CAN CONTRACT TONICALLY OR PHASICALLY

Some smooth muscles contract tonically, maintaining force nearly continuously and relaxing only occasionally. Other smooth muscles contract phasically, producing force in phases and alternating between inactive and active states, even in the absence of external stimulation. Still other smooth muscles do not contract at all until they are stimulated. Many smooth muscles are controlled by neurons or other cells that release chemicals that may either stimulate or inhibit the contractile activity of the muscle. Figure 3.8.1 illustrates the force produced by tonically active smooth muscles or phasically active smooth muscles.

SMOOTH MUSCLES EXHIBIT A VARIETY OF ELECTRICAL ACTIVITIES THAT MAY OR MAY NOT BE COUPLED TO FORCE DEVELOPMENT

During relaxation, smooth muscle cells have a resting membrane potential between −40 and −80 mV. The origin of this resting membrane potential is essentially the same as for striated muscle: in the resting state there

DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00032-X

is a large conductance for K^+, with smaller conductances for Na^+ and Cl^-. Some of these resting membrane potentials are unstable, and the membrane potential undergoes cyclic depolarizations and repolarizations. These are called slow waves. In some muscles, these slow waves trigger bursts of action potentials with associated rhythmic contractions. In other smooth muscles, the developed force tracks the membrane potential but there are no action potentials. In still other smooth muscles, the membrane potential is stable and exogenous agents vary the force. The processes linking changes in membrane potential to force development are called excitation–contraction coupling or electromechanical coupling. Pharmacomechanical coupling refers to the process of force development without changes in membrane potential. Figure 3.8.2 illustrates the variety of electrical and mechanical responses of smooth muscles.

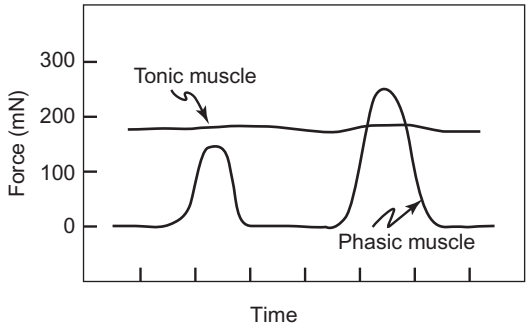

FIGURE 3.8.1 Force developed by tonically active smooth muscle and phasically active smooth muscle. Tonically active muscles develop and maintain force for protracted periods. The degree of tension is their tone. Phasic muscles undergo cycles of contraction and relaxation.

CONTRACTILE FILAMENTS IN SMOOTH MUSCLE CELLS FORM A LATTICE THAT ATTACHES TO THE CELL MEMBRANE

As in skeletal muscle, tension in smooth muscle cells is produced by actin filaments interacting with myosin filaments. Unlike in skeletal muscle, these filaments are not arranged in register but are loosely arranged throughout the cytoplasm. The thin filaments attach to **dense bodies** located throughout the cytoplasm and their ends terminate on myosin filaments in the cytoplasm. These myosin filaments attach to other thin filaments whose ends terminate on the membrane in **attachment plaques**, also sometimes called membrane dense areas. Thus contraction of the myofilaments, in which the actin filaments move along the myosin filaments, draws the dense bodies and attachment plaques closer together. The dense bodies and attachment plaques are believed to be analogous to the Z-disks in skeletal muscle fibers. They are rich in the actin-binding protein, α-actinin, and also contain another protein, vinculin (130,000 Da), which is not found in the Z-disk. Vinculin binds α-actinin and also binds to integral membrane proteins in the attachment plaques. Vinculin appears to anchor the actin filaments to specific membrane adhesion sites (see Figure 3.8.3).

ADJACENT SMOOTH MUSCLE CELLS ARE MECHANICALLY COUPLED AND MAY BE ELECTRICALLY COUPLED

The forces produced by the myofilaments are transmitted to the cell membrane by the attachment plaques on the surface membrane. These forces on the cell membrane are transmitted to adjacent cells through the

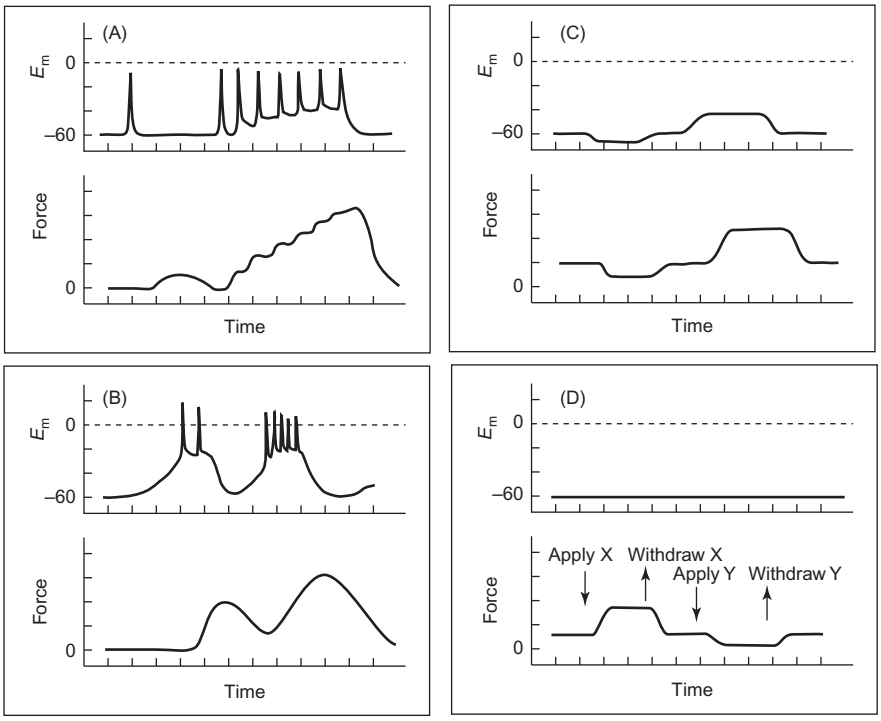

FIGURE 3.8.2 Membrane potential (E_m) and force in different types of smooth muscle. In some smooth muscles (A), pacemaker cells generate action potentials that are transmitted through gap junctions to all of the cells, producing force incremental with the frequency of stimulation. In other smooth muscles (B), slow waves trigger bursts of action potentials with associated force development. In still other muscles (C), the muscle maintains a tone that depends on the membrane potential, but action potentials are not generated. Tissues that show pharmacomechanical coupling (D) have a stable membrane potential that is unaltered by agents that produce force by altering the smooth muscle cytoplasmic $[Ca^{2+}]$ without altering the membrane potential. *Adapted from R.M. Berne and M.N. Levy,* Principles of Physiology, *3rd Ed., Mosby, St. Louis, 2000.*

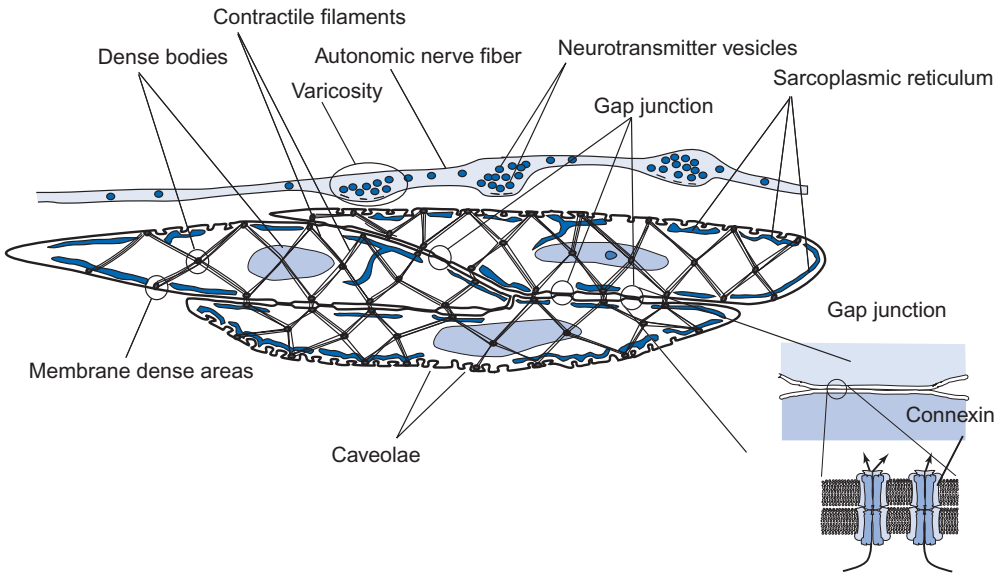

FIGURE 3.8.3 Simplified schematic drawing of the structure of smooth muscle cells. The cells are small with a single, centrally located nucleus. Contractile filaments are anchored in the cytosol at dense bodies, which are the functional analogues of Z-disks in skeletal and cardiac muscles. These form a lattice surrounding the nucleus. The filaments are anchored in the membranes at places called membrane dense areas or attachment plaques. The surface membrane is covered with small invaginations called caveolae. These may serve as local sources of Ca^{2+} for activation of the muscle. The SR provides transient changes in cytosolic [Ca^{2+}], but the extent and activity of the SR in smooth muscle is much less than in the striated muscles. Cells may be coupled with adjacent cells both mechanically through mutual connections to filaments in the extracellular matrix and electrically through gap junctions. At gap junctions, each cell contributes a hemichannel consisting of six connexin molecules in a hexagonal array. Connexins have molecular weights between 25 and 50 kDa and at least 12 different varieties have been identified. Apposition of two hemichannels forms a conducting pathway between the cells so that electrical signals and small-molecular-weight signaling molecules can move between the cells. Smooth muscle is usually innervated but the release of neurotransmitters does not occur in focal regions like the skeletal neuromuscular junction, but instead release occurs from varicosities and the neurotransmitters spread by diffusion throughout the local extracellular fluid.

extracellular matrix. Thus adjacent smooth muscle cells are mechanically coupled. These cells also may be electrically coupled by gap junctions (see Figure 3.8.3). Ions and low-molecular-weight materials pass between the cells, and so cells attached by gap junctions are both electrically and metabolically coupled. Different smooth muscles differ in their degree of coupling. Intestinal smooth muscle cells, for example, are highly coupled so that excitation of one rapidly passes to the others and the cells contract in unison. These muscles are called unitary smooth muscle. Other smooth muscles, such as the vas deferens, lack gap junctions and these muscles are called multiunit smooth muscle, and each cell must be activated individually. Smooth muscles can possess coupling anywhere in the spectrum between these two extremes.

SMOOTH MUSCLE IS CONTROLLED BY INTRINSIC ACTIVITY, NERVES, AND HORMONES

Smooth muscle cells contain a variety of receptors on their surface membrane that respond to chemical signals that are released from nerves or that arrive via the circulation. Many of the chemical signals that arrive via the circulation are hormones. Some smooth muscle cells receive no innervation. Even those that ordinarily receive innervation usually maintain organ function when innervation is interrupted. This is important in transplantation when the vascular tone must be maintained even when the transplanted heart or kidney or liver lacks its usual innervation.

NERVES RELEASE NEUROTRANSMITTERS DIFFUSELY ONTO SMOOTH MUSCLE

There is no neuromuscular junction in smooth muscle as there is in skeletal muscle. Instead, the nerves form varicosities, or bulges in their axons, where vesicles containing neurotransmitter accumulate and release their neurotransmitter in response to action potentials in the axon. The neurotransmitters are released into the extracellular space to diffuse to their receptors on the smooth muscle cell membranes (see Figure 3.8.3).

CONTRACTION IN SMOOTH MUSCLE CELLS IS INITIATED BY INCREASING INTRACELLULAR [CA^{2+}]

Phasic and tonic smooth muscle contractions both involve changes in the cytoplasmic free [Ca^{2+}] from resting levels near 80−140 nM to activating concentrations in the range of 500−700 nM. Figure 3.8.4 shows that phasic contractions typically involve a single Ca^{2+} transient that is followed by force development. Repetitive stimulation can cause summation of both the Ca^{2+} transient and force. In tonically contracting smooth muscle, the force is maintained by the

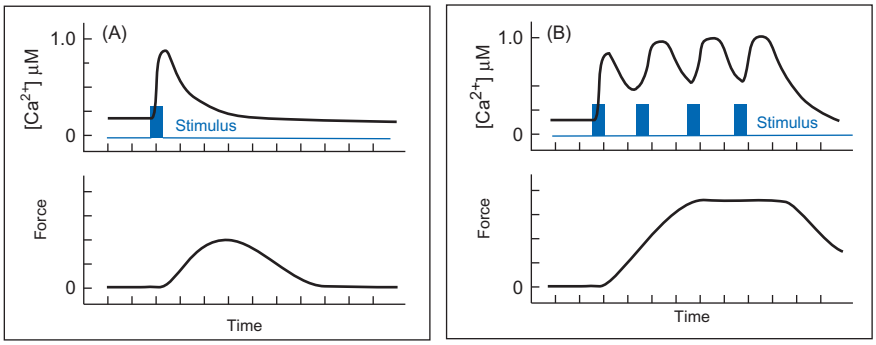

FIGURE 3.8.4 [Ca^{2+}] and force in phasic smooth muscle cells during a single short-lasting stimulation (A) and during repetitive stimulation (B). A single stimulation (blue) results in a transient increase in the cytoplasmic [Ca^{2+}] and subsequent development of force (A). Repetitive stimuli can result in the summation of [Ca^{2+}] transients and greater force (B). *Adapted from R.M. Berne and M.N. Levy,* Principles of Physiology, *3rd Ed, Mosby, St. Louis, 2000.*

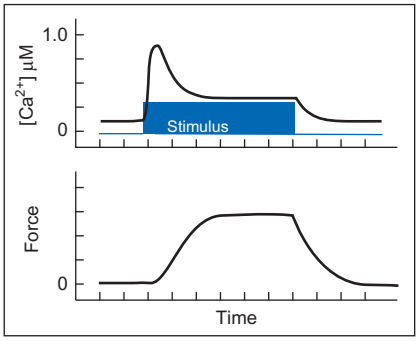

FIGURE 3.8.5 [Ca^{2+}] and force during the stimulation of tonic smooth muscle. The continuous presence of stimulus causes an initial Ca^{2+} spike followed by a continued high cytoplasmic [Ca^{2+}]. The force that is developed depends on the [Ca^{2+}]. However, force can be altered by changing the sensitivity of the force producing system to cytoplasmic [Ca^{2+}]. *Adapted from R.M. Berne and M.N. Levy,* Principles of Physiology, *3rd Ed., Mosby, St. Louis, 2000.*

continued presence of the activating agent. The initial Ca^{2+} transient is followed by a long-lasting increased cytosolic [Ca^{2+}] and long-lasting force (see Figure 3.8.5). When the stimulus is removed, cytosolic [Ca^{2+}] and force return to baseline, unstimulated values.

SMOOTH MUSCLE CYTOSOLIC [CA^{2+}] IS HETEROGENEOUS AND CONTROLLED BY MULTIPLE MECHANISMS

The cytoplasmic [Ca^{2+}] in smooth muscle cells is set by sources and sinks for Ca^{2+} both at the surface membrane and at the sarcoplasmic reticulum (SR). These sources and sinks and their cellular locations are illustrated in Figure 3.8.6. Spatial separation of the sources and sinks allows for spatially distinct regions of cytosolic [Ca^{2+}]. This is further possible because the SR resides immediately underneath the plasma membrane, and so Ca^{2+} influx across the plasma membrane is first conditioned by SR Ca^{2+} transport processes. Either the Ca^{2+} influx is amplified by Ca^{2+}-induced Ca^{2+}-release by RyR, or it is reduced by Ca^{2+} sequestration. Ca^{2+} influx through those channels located in the caveolae, on the other hand, passes directly into the deep cytosol.

SMOOTH MUSCLE [CA^{2+}] CAN BE REGULATED BY CHEMICAL SIGNALS

PHOSPHORYLATION OF PHOSPHOLAMBAN REGULATES SR SERCA ACTIVITY

Phospholamban is a 6-kDa protein that associates reversibly into pentamers. Phospholamban tonically inhibits the SERCA pump by increasing its K_m for Ca^{2+}. Phosphorylation of phospholamban relieves this inhibition, speeding up the SR Ca-ATPase at any given [Ca^{2+}] and therefore lowering the cytoplasmic [Ca^{2+}]. Phosphorylation of phospholamban is controlled by protein kinases that are, in turn, controlled by agonists. Epinephrine, for example, binds to β-receptors on the surfaces of smooth muscle cells, which are coupled to a heterotrimeric G-protein (G_s) that activates adenylyl cyclase. The adenylyl cyclase makes more 3′,5′-cyclic AMP from ATP, and the increased [cAMP] in turn activates protein kinase A (PKA). The G_s mechanism is described in Chapter 2.8. In smooth muscle cells, PKA phosphorylates phospholamban, which helps draw down cytoplasmic [Ca^{2+}] and helps relax smooth muscle that possesses β-adrenergic receptors.

[CA^{2+}] IS REGULATED VIA MEMBRANE POTENTIAL BY ALTERING BK_{CA} (K_{CA}, CALCIUM-ACTIVATED K^+ CHANNEL)

Human airway smooth muscle responds to epinephrine through β_2 receptors, but human airway smooth muscle is unique in that it lacks phospholamban. Instead, PKA activated in these tissues phosphorylates a large-conductance, Ca^{2+}-activated K^+ channel called BK_{Ca}. Its activation increases K^+ efflux from the cell, causing a hyperpolarization and reduction in Ca^{2+} influx through voltage-gated Ca^{2+} channels. BK_{Ca} channels are activated by PKA and PKG, and inhibited by PKC. Thus, it is expected that PKA and PKG would promote relaxation, whereas PKC would contribute to contraction.

THE IP3R OPENS IN RESPONSE TO RECEPTOR BINDING ON THE SURFACE MEMBRANE

A variety of agonists including acetylcholine, norepinephrine, histamine, endothelin, leukotrienes, and

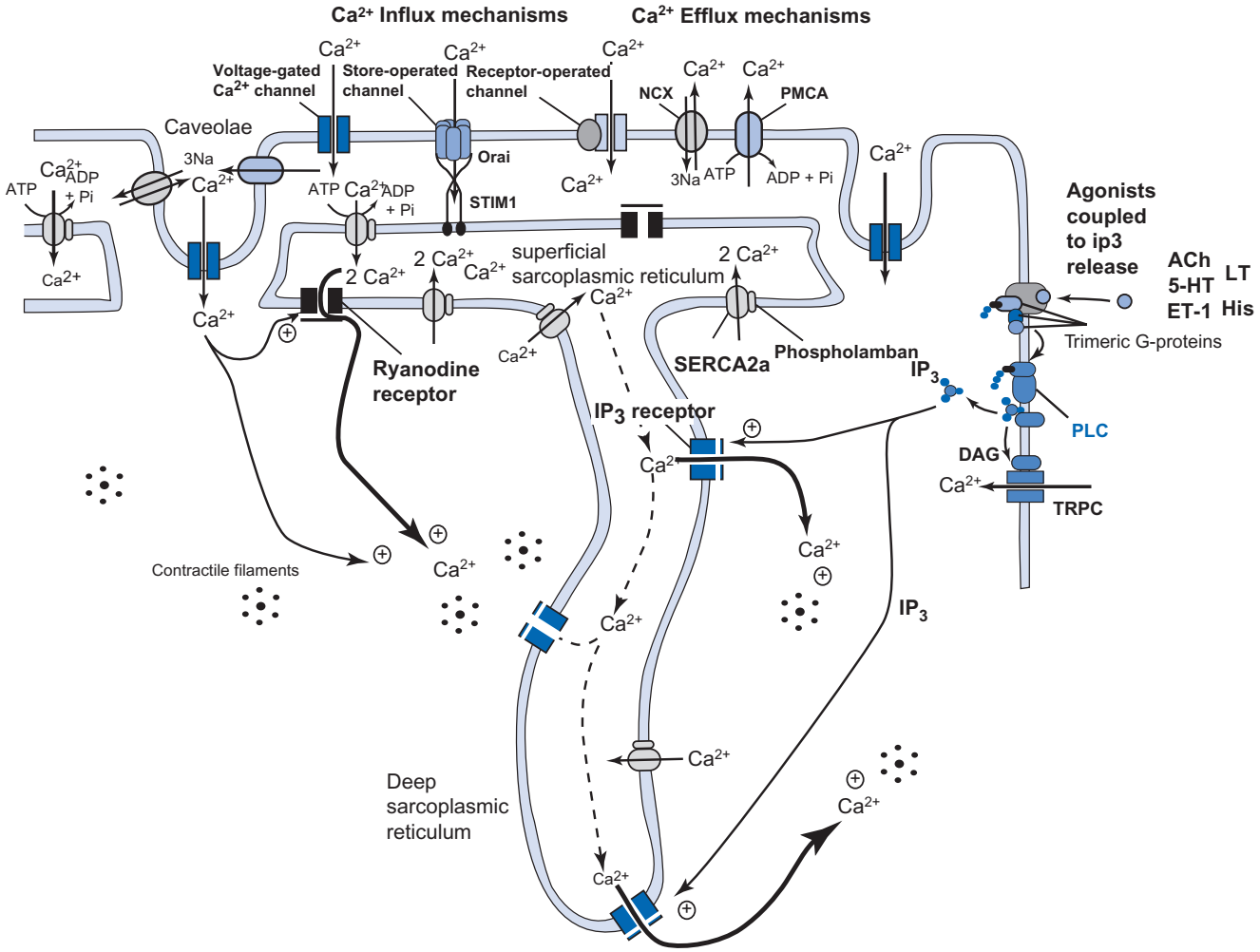

FIGURE 3.8.6 Molecular mechanisms underlying Ca^{2+} homeostasis in smooth muscle cells. Ca^{2+} influx across the plasma membrane is mediated by three separate types of channels: (1) the voltage-gated, L-type Ca^{2+} channel; (2) receptor-operated channels; and (3) store-operated channels that respond to some signal from the SR when it is depleted of Ca^{2+}. Ca^{2+} efflux across the plasma membrane is mediated by (1) the Na^{+}—Ca^{2+} exchanger (NCX), which exchanges 3 Na^{+} for 1 Ca^{2+} and (2) PMCA, which transports 1 Ca^{2+} atom out of the cell per molecule of ATP hydrolyzed. Ca^{2+} influx can occur to a restricted space between the plasma membrane and the SR. In some cases, the Ca^{2+} appears to enter the SR directly without involving active pumping by the SERCA pump on the SR membrane, probably through close apposition of some SR channel with the plasma membrane channels. The store-operated channels use STIM1 to sense SR Ca^{2+} load. With high SR [Ca^{2+}], STIM1 binds Ca^{2+} and disaggregates. In low SR [Ca^{2+}], STIM1 aggregates and interacts with Orai1 to open a Ca^{2+} channel into the restricted space adjacent to the SR. Ca^{2+} that enters the cell can cause additional Ca^{2+} release from ryanodine receptors, RyR, on the SR membrane through a process called Ca^{2+}-induced Ca^{2+}-release. Remaining Ca^{2+} can be taken up into the SR membrane through the SERCA Ca^{2+} pump with a stoichiometry of 2 Ca^{2+} per ATP hydrolyzed. The SERCA pump is regulated by phospholamban, a 6-kDa protein whose tonic inhibition of the pump can be relieved by phosphorylation. The SR is divided into two parts: the part immediately adjacent to the plasma membrane is the superficial SR. Its lumen is continuous with the deep SR. Caveolae are invaginations of the plasma membrane that have concentrations of efflux mechanisms that appear to concentrate Ca^{2+} near the membrane. Ca^{2+} channels there admit Ca^{2+} that can penetrate deeper into the cytosol because there is no SR in the way. IP3 receptors in the deep SR are stimulated by IP3 that is produced by phospholipase C that in turn is activated by agonist binding to a G_q-coupled receptor on the plasma membrane. A variety of agonists can activate Gq-coupled receptors, eventually causing smooth muscle contractions. Examples include acetylcholine (ACh), serotonin (5HT), leukotrienes (LK), histamine (His), and endothelin (ET). The phospholipase C that is activated by these agonists also releases diacylglycerol (DAG) that activates Ca^{2+} influx through a receptor-operated Ca^{2+} channel, TRPC (for transient receptor potential channel). The IP3 receptor releases Ca^{2+} that activates the contractile elements. The spatial separation of sources and sinks of Ca^{2+} produces spatial heterogeneity in the cytosolic [Ca^{2+}].

thromboxane A2 cause release of Ca^{2+} from the SR by indirectly activating inositol triphosphate (IP3) receptors. The IP3 receptors are large-molecular-weight receptors that are related to the ryanodine receptor family. Both consist of tetramers of large molecular weight, both reside in the membrane of the endoplasmic reticulum, and both release Ca^{2+} in their open configuration. The IP3 receptor is gated by binding of IP3. IP3 is a second messenger produced in the cell in response to binding of a first messenger (the agonists listed above) to receptors on the outside of the cell. Agonist binding is coupled to a heterotrimeric G-protein, G_q, which activates yet another enzyme, phospholipase C. Phospholipase C liberates IP3 and diacylglycerol (DAG) from a membrane lipid, phosphatidylinositol 4,5-bisphosphate. This G_q mechanism is described in

Chapter 2.8. A new feature here is the inhibition of BK_{Ca} channels by PKC that tends to depolarize the cell and allow Ca^{2+} entry over the voltage-gated Ca^{2+} channels.

FORCE IN SMOOTH MUSCLE ARISES FROM CA^{2+} ACTIVATION OF ACTIN–MYOSIN INTERACTION

Smooth muscles contain thick and thin filaments, composed predominantly of myosin and actin, respectively. However, their arrangement is quite different from the striated muscles. The filaments are not organized into sarcomeres, and the ratio of thin to thick filaments is closer to 10:1 than it is to the 2:1 ratio found in skeletal and cardiac muscles. The interaction of the actin thin filaments and the myosin in smooth muscle is regulated by cytosolic [Ca^{2+}], but not in the same way as in skeletal and cardiac muscles. Smooth muscle varies its force through recruitment of cross-bridges by phosphorylating the myosin light chains, which is controlled by Ca^{2+}.

MYOSIN LIGHT CHAIN PHOSPHORYLATION REGULATES SMOOTH MUSCLE FORCE

Myosin is a complex of six proteins: two heavy chains of about 200,000 Da each and a total of four myosin light chains. The heavy chains have functionally distinct regions: the long rod-shaped tail imparts rigidity to the myosin; an arm section connects the tail to the globular head region. Each globular head binds actin and contains the site of ATP binding and hydrolysis. The base of each head or neck region binds two light chains. One is called the essential light chain (ELC); the other is the regulatory light chain (RLC or MLC20). As their names imply, regulation of myosin cross-bridge cycling with actin occurs by phosphorylation and dephosphorylation of the RLCs (see Figure 3.8.7).

Myosin light chain kinase (MLCK) phosphorylates serine 19 of the 20,000 Da RLC. This triggers cross-bridge cycling and shortening or force development in smooth muscle. The activity of MLCK depends directly on Ca–**calmodulin**, as shown in Figure 3.8.8. Calmodulin activates MLCK when it binds Ca^{2+}. This is the link between cytosolic [Ca^{2+}] and initiation of force in smooth muscle.

MYOSIN LIGHT CHAIN PHOSPHATASE DEPHOSPHORYLATES THE RLC

Myosin light chain phosphatase (MLCP) catalyzes the dephosphorylation of the RLCs. Dephosphorylating the RLCs of myosin inhibits myosin cross-bridge formation with actin, but dephosphorylating myosin already on actin reduces its off rate, forming the so-called "latch state." The latch state corresponds to the situation where smooth muscle holds tension at low rates of ATP hydrolysis. Although smooth muscle can develop the same force as striated muscle, it does so with much less

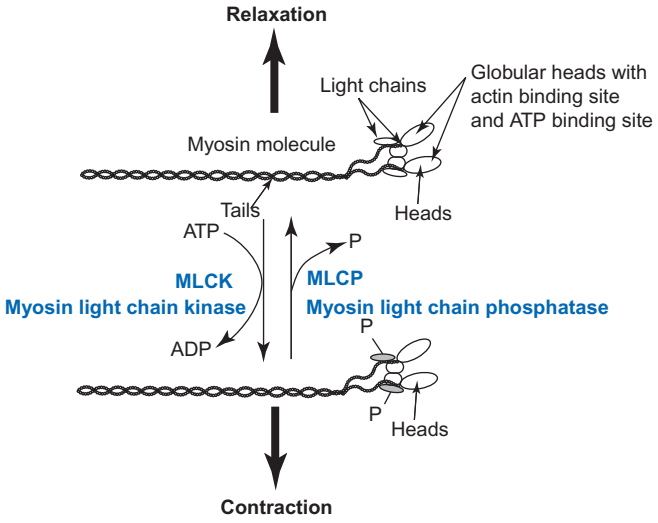

FIGURE 3.8.7 Structure of myosin and regulation of contraction. Each complex consists of two heavy chains and four light chains. The two heavy chains have long tails that form a supercoil. The head groups of the heavy chains contain binding sites for actin and for ATP. This is the "business end" of the heavy chains that is responsible for interacting with actin and hydrolyzing ATP. The RLCs and ELCs bind to the head groups near the neck region. Phosphorylation of the RLCs permits cross-bridge formation and cycling, and thus leads to force development or shortening. The phosphorylation state of the RLCs results from a balance between phosphorylation by MLCK and dephosphorylation by MLCP.

myosin (about one-fifth that of striated muscle) and at much slower rates. The ATP consumption by smooth muscle actomyosin is 1/100–1/500 that of striated muscle, whereas force development is on the order of 1000 times slower in smooth muscle. Figure 3.8.9 shows the effect of MLCP and MLCK on the state of myosin light chain phosphorylation and force development. Thus the state of phosphorylation of the myosin light chains results from a balance between MLCK and MLCP activities (see Figure 3.8.7).

CA^{2+} SENSITIZATION PRODUCES FORCE AT LOWER [CA^{2+}] LEVELS

MLCP activity alters the phosphorylation level independent of MLCK activity. Thus the activation of MLCP would promote relaxation and the inhibition of MLCP should promote contraction. If MLCP is inhibited without altering MLCK activity, there would be increased contraction at cytosolic [Ca^{2+}] that previously did not cause contractions. This is Ca^{2+} sensitization. The mechanism of α_1 adrenergic Ca^{2+} sensitization involves a G_q-coupled receptor which activates phospholipase C in the surface membrane, causing a release of IP3 and DAG. The DAG stimulates protein kinase C (PKC) that phosphorylates another protein target, called CPI-17. The phosphorylated CPI-17 inhibits MLCP. In this way, α_1 adrenergic stimulation of smooth muscle produces a contraction in part by Ca^{2+} sensitization brought about by inhibition of myosin light chain dephosphorylation and in part by activation of MLCK by increasing [Ca^{2+}] through IP_3-coupled Ca^{2+} release from the SR. The regulation of MLCP is shown in Figure 3.8.10.

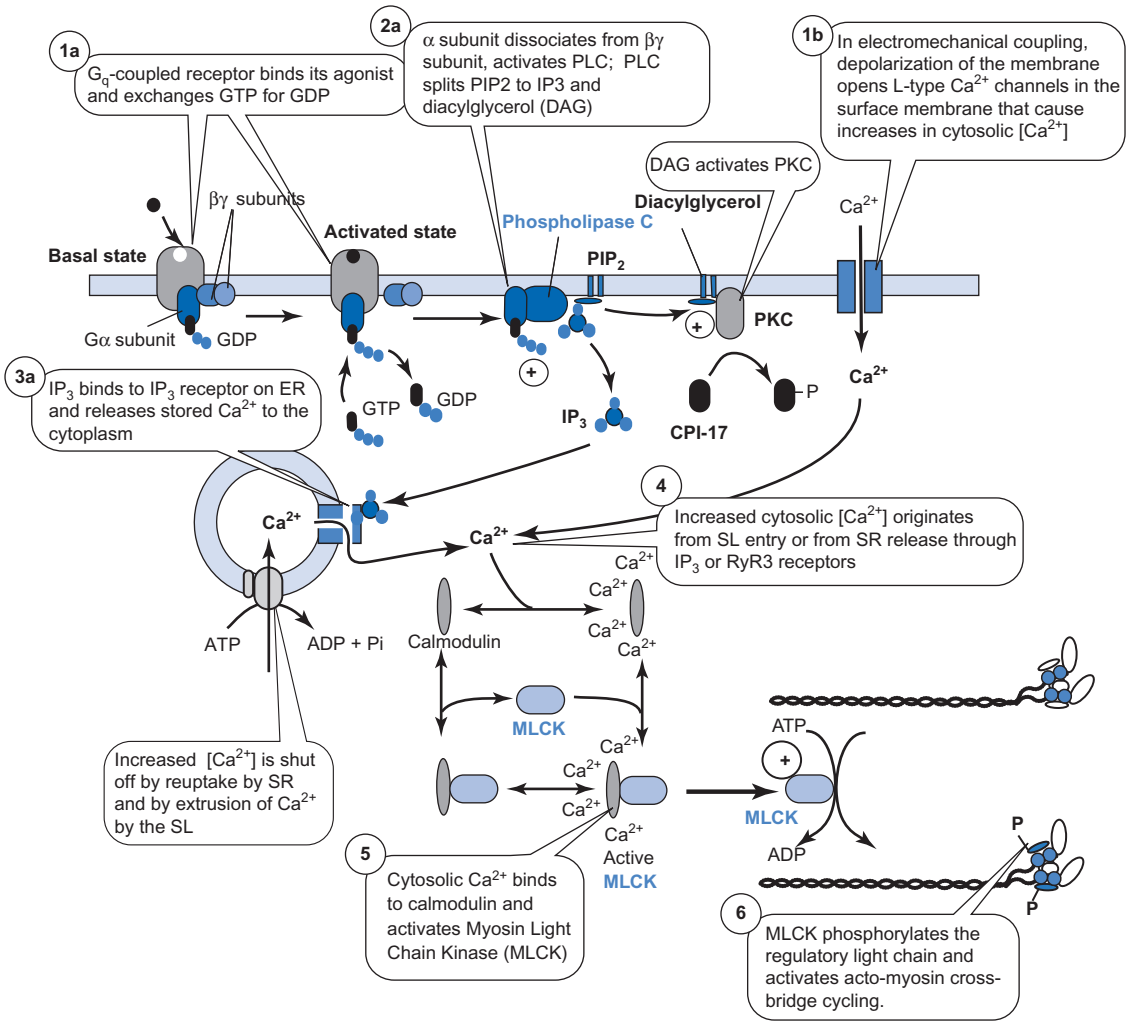

FIGURE 3.8.8 Regulation of cross-bridge cycling by calcium effects on myosin light chain phosphorylation in smooth muscle. When cytoplasmic [Ca^{2+}] rises either through entry from the extracellular fluid over voltage-gated Ca^{2+} channels or activation of G_q-coupled receptors acting through IP3-induced release of Ca^{2+} from intracellular stores, it binds to calmodulin and activates MLCK. MLCK then phosphorylates the RCL of myosin and activates cross-bridge formation and force development. The free [Ca^{2+}] is maintained by all those processes shown in Figure 3.8.6 and mostly omitted here. According to this scheme, removal of Ca^{2+} from the cytosol causes relaxation, either by increased efflux from the cytosol or reduced influx into the cytosol.

A second mode of Ca^{2+} sensitization is through phosphorylation of MLCP by **rho kinase**. Several agonists activate the small monomeric G-protein **RhoA** through binding to heterotrimeric G-protein coupled receptors in the cell membrane. RhoA has GTPase activity. It activates RhoA kinase (ROCK) which in turn phosphorylates the myosin-binding subunit (MYPT1) of MLCP. This phosphorylation inactivates the phosphatase, which thus preserves the phosphorylation of the myosin light chains and maintains force development even in the absence of a sustained Ca^{2+} signal. Figure 3.8.10 also shows the regulation of MLCP by rhoA-GTP.

NITRIC OXIDE INDUCES SMOOTH MUSCLE RELAXATION BY STIMULATING GUANYLATE CYCLASE

Nitric oxide synthases (NOS) from endothelial tissue (eNOS), neural tissue (nNOS), and an inducible, Ca^{2+}-independent nitric oxide synthase called iNOS produce nitric oxide, NO, by converting L-arginine and O_2 to NO and citrulline. The NO diffuses from its site of production to smooth muscle cells where it activates soluble guanylate cyclase to increase cytosolic cyclic guanylyl monophosphate or cGMP. The increased cGMP then is believed to activate a cGMP-dependent protein kinase, PKG, which relaxes smooth muscle by phosphorylating target proteins. The effects of PKG activation are manifold. PKG phosphorylates phospholamban, which brings about relaxation by increasing Ca^{2+} uptake into the SR. PKG also stimulates MLCP activity, thereby reducing the phosphorylation of the myosin light chains, causing a loss of Ca^{2+} sensitivity. PKG also activates a large-conductance K^+ channel (**BK_{Ca}**) that hyperpolarizes the cell and reduces Ca^{2+} entry over voltage-gated Ca^{2+} channels in the surface membrane. The effects of NO on smooth muscle are shown in Figure 3.8.11.

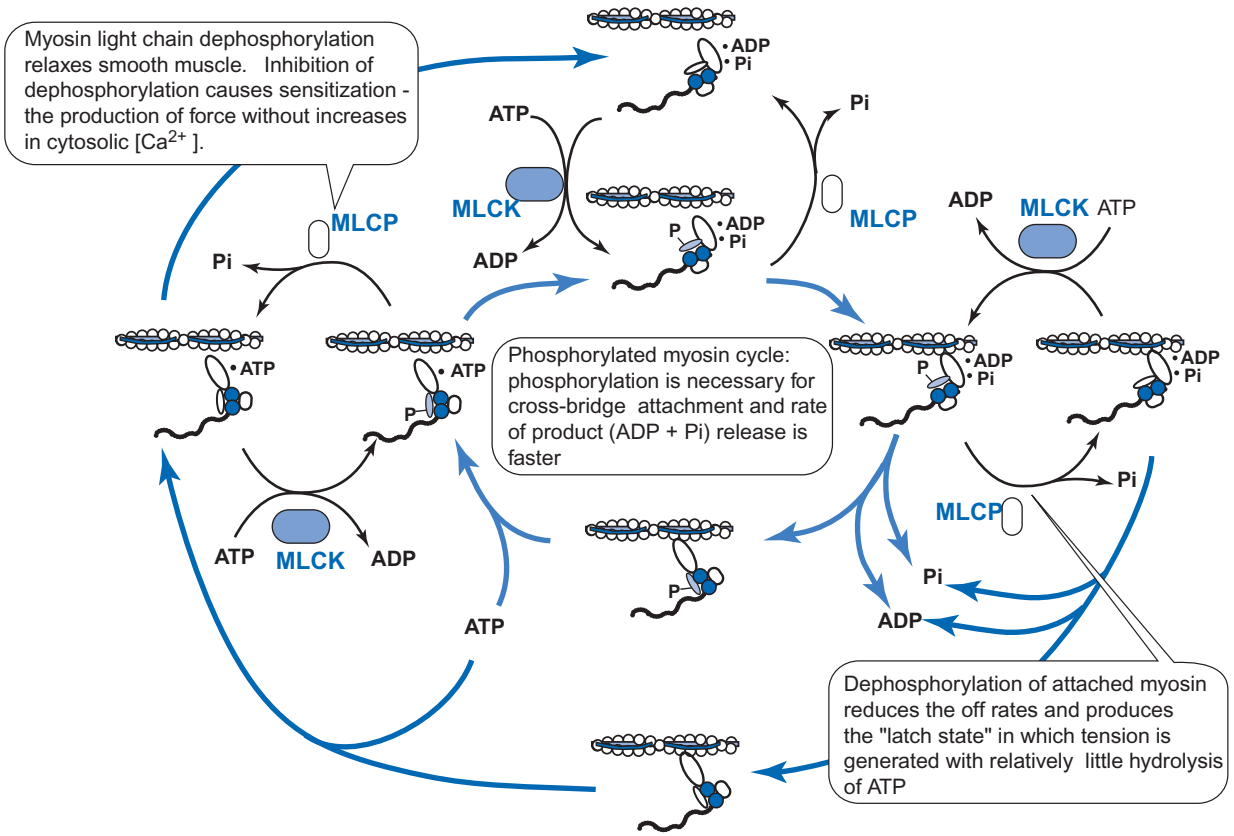

FIGURE 3.8.9 Regulation of cross-bridge cycling by myosin light chain phosphorylation and dephosphorylation in smooth muscle. MLCK phosphorylates the RLCs and activates cross-bridge formation between myosin and actin, initiating the cross-bridge cycling (inner cycle). This cycle is deactivated by dephosphorylation of the light chains by MLCP. MLCP can also dephosphorylate the light chains while myosin is attached to the actin filaments. This produces an alternate cross-bridge cycle (outer cycle) that has slower kinetics. This is the "latch state" that produces force with little expenditure of energy. In effect, it makes the muscle stiff.

ADRENERGIC STIMULATION RELAXES SMOOTH MUSCLES BY REDUCING CYTOSOLIC [CA^{2+}]

Circulating epinephrine or locally released norepinephrine can bind to β-adrenergic, G_S-coupled receptors on some smooth muscle cells. These G_S-coupled receptors activate adenylyl cyclase to increase cytoplasmic concentrations of 3′,5′-cyclic adenosine monophosphate (cAMP). This activates PKA that phosphorylates targets including phospholamban in some smooth muscles. Phosphorylation of phospholamban relieves inhibition of the SERCA2a pump on the SR and increases removal of activator Ca^{2+} from the cytosol, resulting in relaxation. In other tissues, PKA activates BK_{Ca} and relaxes cells by hyperpolarization. These effects are shown in Figure 3.8.11.

SYNOPSIS OF MECHANISMS PROMOTING CONTRACTION OR RELAXATION OF SMOOTH MUSCLE

Figure 3.8.12 illustrates that phosphorylation of the myosin light chains promotes contraction and dephosphorylation promotes relaxation. Contraction is thus controlled by the relative activities of MLCK and MLCP. MLCK is controlled mainly by cytoplasmic [Ca^{2+}], through calmodulin, and the increased [Ca^{2+}] originates either from entry over voltage-dependent Ca^{2+} channels or by release of internal stores. Thus, G_q-coupled receptors cause contraction, and membrane depolarization causes contraction through increases in cytoplasmic [Ca^{2+}], and G_s-coupled receptors and NO cause relaxation by increasing uptake of Ca^{2+} back into the SR through phosphorylation of phospholamban.

Other agonists work by affecting MLCP. $G_{\alpha 12}$-coupled receptors activate Rho kinase that phosphorylates MLCP, inactivating it and thereby promoting phosphorylation of the light chains and contraction. G_q-coupled receptors, besides increasing cytoplasmic [Ca^{2+}] through IP3-induced release of Ca^{2+} from the SR, also activate PKC that phosphorylates CPI-17 that inhibits MLCP, thereby promoting contraction. Other agonists can work through modulation of channels that affect the membrane potential, such as BK_{Ca}. PKA and PKG both activate BK_{Ca}, promoting relaxation, whereas PKC inhibits BK_{Ca}, thereby promoting contraction.

This synopsis shows the general effect of a variety of agonist and mechanisms on a variety of tissues. The mechanisms used vary with the tissue and the species.

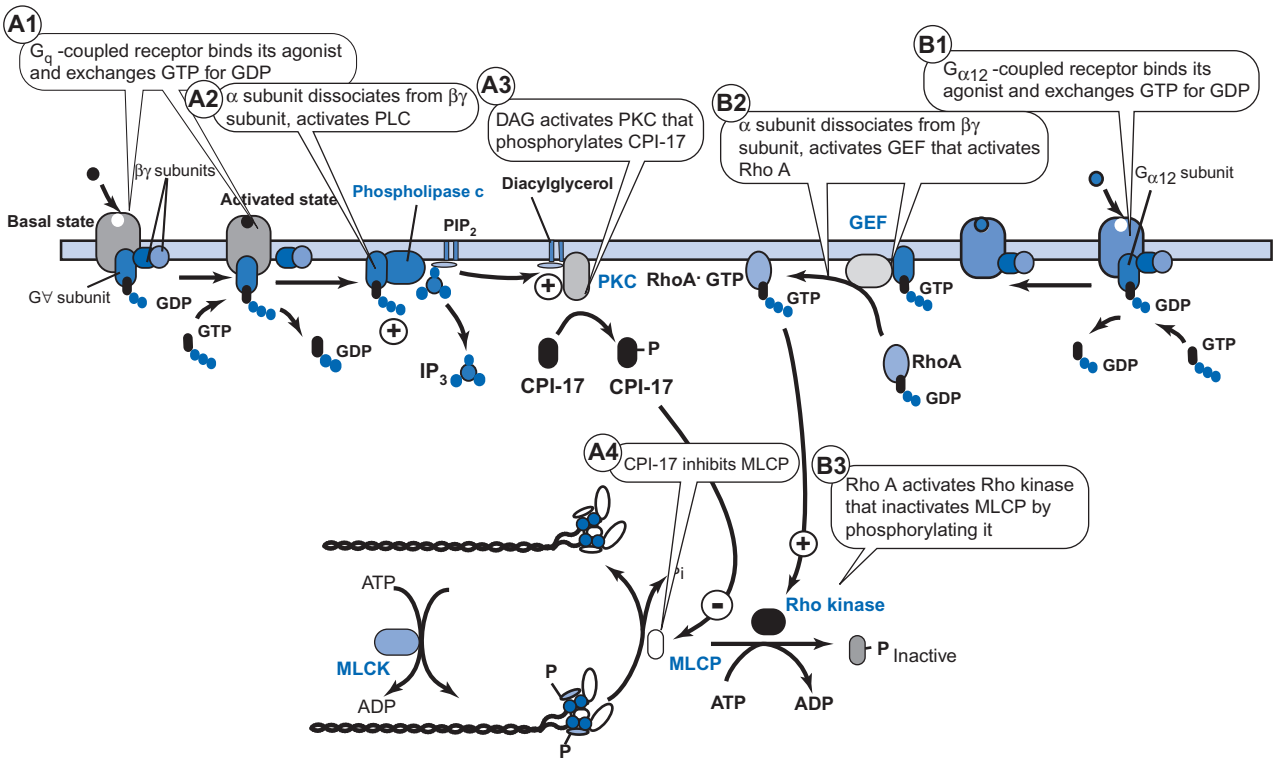

FIGURE 3.8.10 Regulation of MLCP by CPI-17 and Rho kinase. Activation of G_q-coupled receptors (such as adrenergic α_1-receptors) activates phospholipase C which cleaves phosphatidylinositol bisphosphate to form IP3 (which then releases Ca^{2+} from the SR and activates MLCK through calmodulin) and DAG. DAG activates PKC, which phosphorylates target proteins including CPI-17. The phosphorylated form of CPI-17 inhibits myosin light chain phosphates (MLCP), thereby promoting the phosphorylated form of the myosin light chains, which promotes contraction. Other agonists bind to $G_{\alpha 12}$-coupled receptors, whose α-subunit activates a GTP-exchange factor (GEF) to exchange GTP for GDP on a monomeric protein, RhoA. This stimulates Rho kinase, which phosphorylates MLCP, inhibiting it. Thus the activation of the Rho kinase pathway promotes contraction because it inhibits the dephosphorylation of the myosin light chains.

While cAMP-mediated mechanisms may produce relaxation via phosphorylation of phospholamban in one tissue, it may promote relaxation by activation of BK_{Ca} in a different tissue or in the same tissue in a different species. Figure 3.8.12 is meant to illustrate the breadth of the types of effects rather than catalogue the effects in each of the many different types of smooth muscles.

SUMMARY

Smooth muscles line the walls of hollow tubes and organs. They control the caliber of the tubes or move materials by producing a pressure within the hollow organs. They exhibit tremendous variety of physiological responses and mechanisms of actions. Some smooth muscles produce force in association with action potentials, some in response to slow changes in membrane potential, and others produce force completely dissociated from changes in membrane potential. Electromechanical coupling describes the events that link changes in membrane potential to force. Pharmacomechanical coupling refers to the mechanisms that cause force in the absence of changes in membrane potential. Smooth muscles respond to a bewildering list of agonists including acetylcholine, histamine, thromboxane A_2, norepinephrine, endothelin, nitric oxide, and leukotrienes.

Smooth muscle cells are small cells that connect to each other through gap junctions. The actin and myosin filaments form a network within the cell that connects to the cell membrane at attachment plaques. They connect within the cytoplasm at dense bodies. The degree of electrical coupling varies from the extreme of unitary smooth muscles in which all cells are electrically coupled to multiunit smooth muscles in which each cell must be activated separately.

Smooth muscle regulates its force through the phosphorylation state of the RLC of myosin. Ordinarily, smooth muscle contraction begins with increases in cytoplasmic $[Ca^{2+}]$ brought about either by changes in membrane potential or by ligand binding to receptors on the smooth muscle cell membrane. These increase Ca^{2+} influx into the cell by opening voltage-gated Ca^{2+} channels or receptor-operated Ca^{2+} channels on the surface membrane or by releasing Ca^{2+} from the SR. Agonists binding to a G_q protein activate phospholipase C that liberates IP3 from the surface membrane. IP3 then binds to its receptor on the SR and releases Ca^{2+}. Ca^{2+} that enters the cell can also cause Ca^{2+}-induced Ca^{2+}-release from RyR3 receptors on the deep SR.

Increased cytosolic $[Ca^{2+}]$ binds to calmodulin which activates MLCK. This phosphorylates myosin light chains, which activate contraction. Removal of Ca^{2+} by

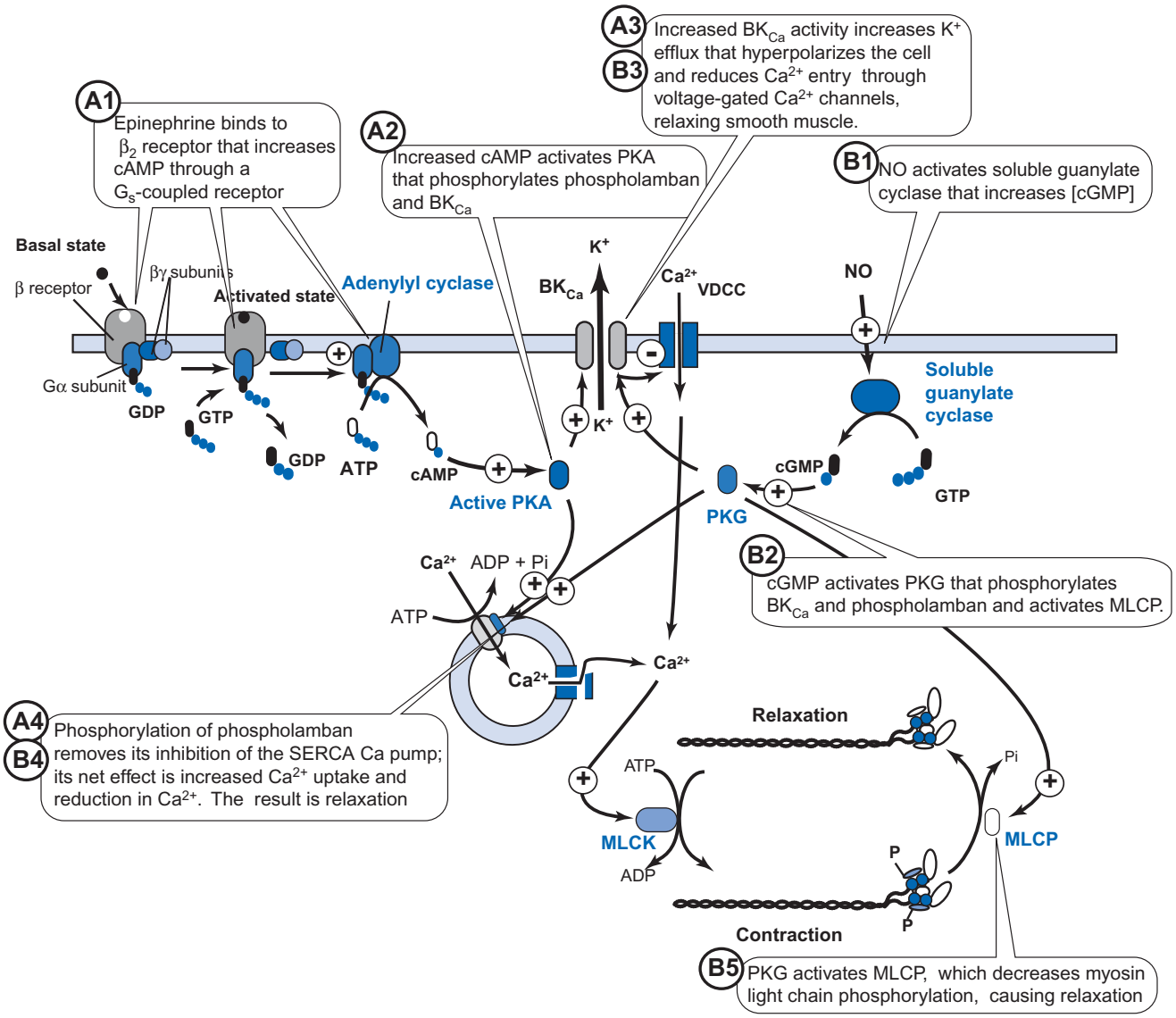

FIGURE 3.8.11 Mechanisms of relaxation by epinephrine and NO. Beta$_2$ agonists activate adenylyl cyclase that increases cAMP and activates PKA. PKA activates BK_{Ca}, a large-conductance channel for K^+ whose activation hyperpolarizes the cell and reduces Ca^{2+} entry over voltage-gated Ca^{2+} channels. PK also phosphorylates phospholamban that stimulates Ca^{2+} uptake by the SR, thereby promoting relaxation. Nitric oxide, NO, produced by other cells enters the smooth muscle cell and activates a soluble guanylate cyclase, which increases cGMP and activates PKG. PKG activates BK_{Ca} to promote relaxation, phosphorylates phospholamban that aids in relaxation and also activates myosin light chain phosphatase (MLCP) that aids in relaxation by dephosphorylating the myosin regulatory light chain.

reuptake by the SR or by efflux from the cell causes relaxation by deactivating MLCK. Beta adrenergic stimulation relaxes smooth muscle by increasing Ca^{2+} uptake into the SR. It does this through a G-protein coupled increase in cAMP that stimulates PKA that in turn phosphorylates phospholamban (PLB). Phosphorylation of PLB removes its inhibition of the SERCA2a Ca-ATPase on the SR membrane. In other tissues, PKA activates a large conductance for K^+ that hyperpolarizes the membrane and reduces cytosolic [Ca^{2+}] by reducing Ca^{2+} influx. Nitric oxide also works through this mechanism except it stimulates a guanylyl cyclase that increases cGMP that activates PKG that phosphorylates PLB and actrivates BK_{Ca}.

The phosphorylation state of the myosin light chains is also controlled by MLCP. Activation of MLCP brings about relaxation. Inhibition of MLCP keeps the light chains phosphorylated. The activity of MLCP is controlled by receptors that activate PLC and by receptors that activate Rho kinase and by nitric oxide, NO. Activation of PLC releases DAG that activates PKC. PKC phosphorylates a protein, CPI-17, which activates its inhibition of MLCP, which prolongs the phosphorylated state of the myosin light chains. Activation of Rho kinase releases a GTP-exchange factor (GEF) that activates a monomeric G-protein, RhoA, which in turn activates RhoA kinase. RhoA kinase phosphorylates MLCP, inactivating it. Thus RhoA also promotes phosphorylation of the myosin light chains. Nitric oxide produced by neighboring cells activates a soluble guanylate cyclase that produces cGMP. This activates PKG which

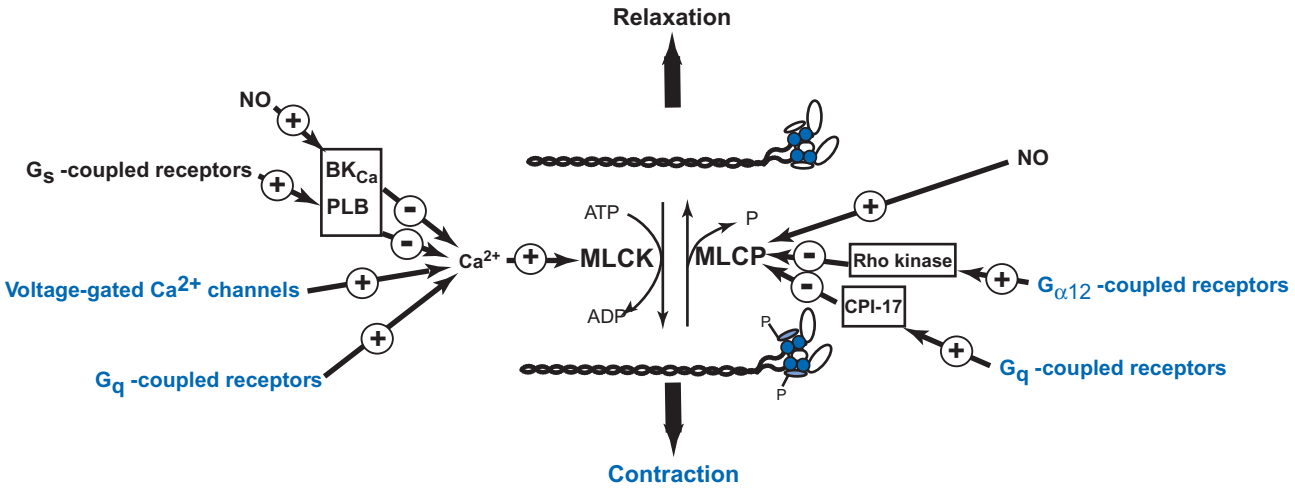

FIGURE 3.8.12 Synopsis of effects on smooth muscle contraction and relaxation. Receptors that promote contraction are shown in light blue; those that promote relaxation are shown in black. Activation of voltage-gated Ca^{2+} channels raises cytoplasmic [Ca^{2+}], activating MLCK and promoting contraction; G_q-coupled receptors increase cytoplasmic [Ca^{2+}] through IP_3-induced release of Ca^{2+} from SR stores, thereby activating MLCK and promoting contraction. G_q also activates PKC that phosphorylates CPI-17 that inhibits MLCP, thereby promoting contraction. $G_{\alpha 12}$ mechanisms activate rho kinase that phosphorylates MLCP, inactivating it and promoting contraction. NO activates a soluble guanylate cyclase that increases cytoplasmic cGMP, activating protein kinase G (PKG) that in some tissues phosphorylates phospholamban (PLB) and increases Ca^{2+} uptake by the SR, reducing cytoplasmic [Ca^{2+}] and promoting relaxation. In other tissues PKG activates BK_{Ca} (a large-conductance channel for K^+) that increases outward K^+ current, making the membrane potential more negative and reducing Ca^{2+} entry through voltage-gated Ca^{2+} channels. In some tissues, G_s-coupled receptors activate adenylyl cyclase that increases cytoplasmic cAMP, activating PKA that similarly phosphorylates phospholamban and promotes relaxation. In other tissues, it activates BK_{Ca} and promotes relaxation. In some tissues, NO activates PKG that activates MLCP, thereby promoting relaxation.

phosphorylates MLCP at a different location from RhoA kinase, and activates it. Thus NO promotes dephosphorylation of the myosin light chains, causing relaxation.

REVIEW QUESTIONS

1. What is a tonic smooth muscle? What is a phasic smooth muscle?
2. What is electromechanical coupling? What is pharmacomechanical coupling?
3. What are slow waves?
4. How are actin filaments anchored at membranes? In the cytosol?
5. What are unitary smooth muscles? Multiunit smooth muscles?
6. How does Ca^{2+} enter the cytosol? How does it exit the cytosol?
7. How does Ca^{2+} initiate smooth muscle contraction? What does MLCK do? What does MLCP do?
8. What effect does phosphorylation of phospholamban have on smooth muscle contraction?
9. What is sensitization? What makes sensitization?
10. What is the latch state?
11. What effect does nitric oxide (NO) have on smooth muscle? What mechanism does it use?
12. How do G_q-coupled receptors promote contraction? How do G_s-coupled receptors promote relaxation?