cluster of ryanodine-sensitive calcium release channels in the sarcoplasmic reticulum (see below). Calcium sparks directly augment intracellular calcium concentration and indirectly increase intracellular calcium concentration by activating chloride channels. In addition, calcium sparks reduce smooth-muscle contractility by activating large-conductance calcium-sensitive K^+ channels, hyperpolarizing the cell membrane and thereby limiting further voltage-dependent increases in intracellular calcium.

Biochemical agonists also increase intracellular calcium concentration, in this case by receptor-dependent activation of phospholipase C with hydrolysis of phosphatidylinositol 4,5-bisphosphate, resulting in the generation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). These membrane lipid derivatives in turn activate protein kinase C and increase intracellular calcium concentration. In addition, IP_3 binds to specific receptors on the sarcoplasmic reticulum membrane to increase calcium efflux from this calcium storage pool into the cytoplasm.

Vascular smooth-muscle cell contraction depends principally on the phosphorylation of myosin light chain, which in the steady state, reflects the balance between the actions of myosin light chain kinase and myosin light chain phosphatase. Calcium activates myosin light chain kinase through the formation of a calcium-calmodulin complex. Phosphorylation of myosin light chain by this kinase augments myosin ATPase activity and enhances contraction. Myosin light chain phosphatase dephosphorylates myosin light chain, reducing myosin ATPase activity and contractile force. Phosphorylation of the myosin-binding subunit (thr695) of myosin light chain phosphatase by Rho kinase inhibits phosphatase activity and induces calcium sensitization of the contractile apparatus. Rho kinase is itself activated by the small GTPase RhoA, which is stimulated by guanosine exchange factors and inhibited by GTPase-activating proteins.

Both cyclic AMP and cyclic GMP relax vascular smooth-muscle cells through complex mechanisms. β agonists, acting through their G-protein-coupled receptors activate adenylyl cyclase to convert ATP to cyclic AMP; NO and atrial natriuretic peptide acting directly and via a G-protein-coupled receptor, respectively, activate guanylyl cyclase to convert GTP to cyclic GMP. These agents in turn activate protein kinase A and protein kinase G, respectively, which inactivate myosin light chain kinase and decrease vascular smooth-muscle cell tone. In addition, protein kinase G can interact directly with the myosin-binding substrate subunit of myosin light chain phosphatase, increasing phosphatase activity and decreasing vascular tone. Finally, several mechanisms drive NO-dependent, protein kinase G–mediated reductions in vascular smooth-muscle cell calcium concentration, including phosphorylation-dependent inactivation of RhoA; decreased IP_3 formation; phosphorylation of the IP_3 receptor–associated cyclic GMP kinase substrate, with subsequent inhibition of IP_3 receptor function; phosphorylation of phospholamban, which increases calcium ATPase activity and sequestration of calcium in the sarcoplasmic reticulum; and protein kinase G–dependent stimulation of plasma membrane calcium ATPase activity, perhaps by activation of the Na^+,K^+-ATPase pump or hyperpolarization of the cell membrane by activation of calcium-dependent K^+ channels.

Control of Vascular Smooth-Muscle Cell Tone The autonomic nervous system and endothelial cells modulate vascular smooth-muscle cells in a tightly regulated manner. Autonomic neurons enter the blood vessel medial layer from the adventitia and modulate vascular smooth-muscle cell tone in response to baroreceptors and chemoreceptors within the aortic arch and carotid bodies and in response to thermoreceptors in the skin. These regulatory components include rapidly acting reflex arcs modulated by central inputs that respond to sensory inputs (olfactory, visual, auditory, and tactile) as well as emotional stimuli. Three classes of nerves mediate autonomic regulation of vascular tone: *sympathetic*, whose principal neurotransmitters are epinephrine and norepinephrine; *parasympathetic*, whose principal neurotransmitter is acetylcholine; and *nonadrenergic/noncholinergic*, which include two subgroups—nitrergic, whose principal neurotransmitter is NO, and peptidergic, whose principal neurotransmitters are substance P, vasoactive intestinal peptide, calcitonin gene-related peptide, and ATP.

Each of these neurotransmitters acts through specific receptors on the vascular smooth-muscle cell to modulate intracellular calcium and, consequently, contractile tone. Norepinephrine activates α receptors, and epinephrine activates α and β receptors (adrenergic receptors); in most blood vessels, norepinephrine activates postjunctional α_1 receptors in large arteries and α_2 receptors in small arteries and arterioles, leading to vasoconstriction. Most blood vessels express β_2-adrenergic receptors on their vascular smooth-muscle cells and respond to β agonists by cyclic AMP–dependent relaxation. Acetylcholine released from parasympathetic neurons binds to muscarinic receptors (of which there are five subtypes, M_{1-5}) on vascular smooth-muscle cells to yield vasorelaxation. In addition, NO stimulates presynaptic neurons to release acetylcholine, which can stimulate the release of NO from the endothelium. Nitrergic neurons release NO produced by neuronal NO synthase, which causes vascular smooth-muscle cell relaxation via the cyclic GMP–dependent and –independent mechanisms described above. The peptidergic neurotransmitters all potently vasodilate, acting either directly or through endothelium-dependent NO release to decrease vascular smooth-muscle cell tone. **For the detailed molecular physiology of the autonomic nervous system, see Chap. 454.**

The endothelium modulates vascular smooth-muscle tone by the direct release of several effectors, including NO, prostacyclin, hydrogen sulfide, and endothelium-derived hyperpolarizing factor, all of which cause vasorelaxation, and endothelin, which causes vasoconstriction. The release of these endothelial effectors of vascular smooth-muscle cell tone is stimulated by mechanical (shear stress, cyclic strain, etc.) and biochemical mediators (purinergic agonists, muscarinic agonists, peptidergic agonists), with the biochemical mediators acting through endothelial receptors specific to each class. In addition to these local paracrine modulators of vascular smooth-muscle cell tone, circulating mediators can affect tone, including norepinephrine and epinephrine, vasopressin, angiotensin II, bradykinin, and the natriuretic peptides (atrial natriuretic peptide [ANP], brain natriuretic peptide [BNP], C-type natriuretic peptide [CNP], and dendroaspis natriuretic peptide [DNP]), as discussed above.

VASCULAR REGENERATION

Growth of new blood vessels can occur in response to conditions such as chronic hypoxemia and tissue ischemia. Growth factors, including vascular endothelial growth factor (VEGF) and forms of fibroblast growth factor (FGF), activate a signaling cascade that stimulates endothelial proliferation and tube formation, defined as *angiogenesis*. Guidance molecules, including members of the semaphorin family of secreted peptides, direct blood vessel patterning by attracting or repelling nascent endothelial tubes. The development of collateral vascular networks in the ischemic myocardium, an example of angiogenesis, can result from selective activation of endothelial progenitor cells, which may reside in the blood vessel wall or home to the ischemic tissue from the bone marrow. True arteriogenesis, or the development of a new blood vessel that includes all three cell layers, normally does not occur in the cardiovascular system of adult mammals. The molecular mechanisms and progenitor cells that can recapitulate blood vessel development de novo are under rapidly advancing study **(Chaps. 88, 89e, and 90e)**.

VASCULAR PHARMACOGENOMICS

The last decade has witnessed considerable progress in efforts to define the genetic differences underlying individual variations in vascular pharmacologic responses. Many investigators have focused on receptors and enzymes associated with neurohumoral modulation of vascular function as well as hepatic enzymes that metabolize drugs that affect vascular tone. The genetic polymorphisms thus far associated with differences in vascular response often (but not invariably) relate to functional differences in the activity or expression of the receptor or enzyme of interest. Some of these