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Ion Channels in Vascular Smooth Muscle

Physiology and Pharmacology

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VASCULAR smooth muscle regulation is accomplished by several major regulatory mechanisms, including: (1) neural transmitters (*e.g.*, norepinephrine [NE], adenosine triphosphate [ATP], and nitric oxide [NO]); (2) endothelial factors (*e.g.*, endothelium-derived relaxing factor [EDRF], endothelium-derived hyperpolarizing factor [EDHF], and endothelins); (3) metabolic influences (*e.g.*, oxygen and carbon dioxide tension, *p*H, and temperature); (4) humoral agents (*e.g.*, NE, epinephrine [E], histamine, angiotensin II [Ang II], vasopressin [ADH], vasoactive intestinal peptide [VIP], atrial natriuretic peptide [ANP], and others); and (5) myogenic influences (*e.g.*, intraluminal pressure and stretch).

The pathways that are responsible for the excitation of vascular smooth muscle include: (1) nerve stimulation-induced contractions without change in smooth muscle membrane potential; (2) neurotransmitter-induced smooth muscle action potential generation after nerve stimulation leading to contraction; (3) persistent depolarization of the smooth muscle and muscle contraction caused by neurotransmitters or other vasoactive substances and transmural pressure; and (4) contractions initiated by spontaneous action potentials generated by some smooth muscle cells acting as pacemakers, and the subsequent spread of excitation *via* gap junctions and cell-to-cell conduction.

A number of the above-listed categories may be superimposed on, or occur as a consequence of, previous excitation, such as a steady level of depolarization. Moreover, some smooth muscle cells may not have access to neurally released neurotransmitters, but may be coupled to other cells by a gap junction. Therefore, there is a considerable qualitative and quantitative difference throughout the vascular beds because of the multiplicity of vascular smooth muscle excitations, including the type and density of innervation, presence of pacemaker cells, intraluminal pressure, type and density of receptors, number of low-resistance gap junctions, and type and number of ion channels (fig. 1).

For the most part, two regulatory systems control vascular smooth muscle tone: endothelium and the sympathetic nervous system. Modulation of vascular tone by the sympathetic nervous system reflects the perfusion requirements of the intact organism after input from the peripheral and central baro- and chemoreceptors to the central nervous system pressor centers. The endothelium influences the tone by releasing a number of vasoactive factors in response to a variety of changes in the chemical and physical environment around the specific vascular bed. There are several possible interactions between factors released from neural endings and endothelium, although these interactions depend on the relative distribution of these regulatory systems in a given vessel bed.[1]

The key mechanism in the interaction between the two regulatory systems involves the alteration in the concentration of ionized calcium (Ca^{2+}) in the cytoplasm of the smooth muscle cell. At rest, the intracellular Ca^{2+} concentration in smooth muscle is approximately 0.1 μM, and, during the maximum activation, it ranges from 1 to 10 μM.[2] Depolarization of the vascular smooth muscle membrane by high extracellular K^+ or agonists causes a relatively rapid increase in cytoplasmic Ca^{2+}, which then declines somewhat to a new level during persistent depolarization. The principle source of the Ca^{2+} is likely to be Ca^{2+} influx through the sarcolemma, although Ca^{2+} release from the endoplasmic reticulum is also present. On the other hand, Ca^{2+} mobilization by a number of different li-

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gands may start with a breakdown of membrane phospholipids and the formation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). In vascular smooth muscle, one of the major roles of IP_3 is to release Ca^{2+} from the sarcoplasmic reticulum (SR).

Influx of Ca^{2+} from the extracellular space occurs through the voltage-operated channels (VOC), receptor-operated channels (ROC), and nonselective cation channel (NSCC), and by reversed Na^+/Ca^{2+} exchange mechanism. Additional calcium is released from the mitochondria and sarcoplasmic reticulum (SR; fig. 2). The voltage-gated calcium channels are subdivided into long-lasting (L—), transient (T—), and resting (R—) types of channels. The channels responsible for the release of Ca^{2+} from the SR are ryanodine channels (Ca^{2+}-induced Ca^{2+} release channel, caffeine sensitive) and IP_3 channels (heparin sensitive). Factors contributing to a decrease in intracellular calcium include calcium pumps in the sarcolemma, endoplasmic reticulum, and mitochondria, along with a forward mode of the Na^+/Ca^{2+} exchanger (fig. 2).

Receptors

The stimulation of different receptors in smooth muscle cells by agents such as norepinephrine, acetylcholine, and serotonin induces the breakdown of polyphosphoinositides.[3] This process initially involves the activation of phospholipase C enzyme, which hydrolyzes phosphatidylinositol 4,5 bisphosphate (PIP_2), leading to the production of IP_3 and 1,2-diacylglycerol (DAG). Both compounds are intracellular second messengers. From the cell membrane, the IP_3 diffuses to the SR and mobilizes intracellular Ca^{2+}. It is generally accepted that IP_3-sensitive and IP_3-insensitive Ca^{2+} stores can coexist in the same smooth muscle cells with the two pools being interdependent.[4] It was proposed that caffeine could deplete a caffeine- (and Ca^{2+}-) sensitive pool, and also indirectly affect the IP_3 pool. The increase in ionized Ca^{2+} appears to be responsible for the phasic component of the smooth muscle contractile response. However, to obtain the maximal and longer contractile response from the smooth muscle, a tonic component is necessary that requires the activation of ROC or VOC.[2] Both types of contractions could be induced, for example, by simultaneous stimulation of α_1 and α_2 adrenoceptors leading to Ca^{2+} release from intracellular stores and Ca^{2+} influx, respectively. It should be pointed out, however, that vascular smooth muscle contraction is generally tonic in nature. The blood vessels, for instance, unlike cardiac and skeletal muscle, can remain in a contracted state for prolonged periods of time.

Most blood vessels have both α_1- and α_2-adrenergic subtypes, and their stimulation by agonists leads to

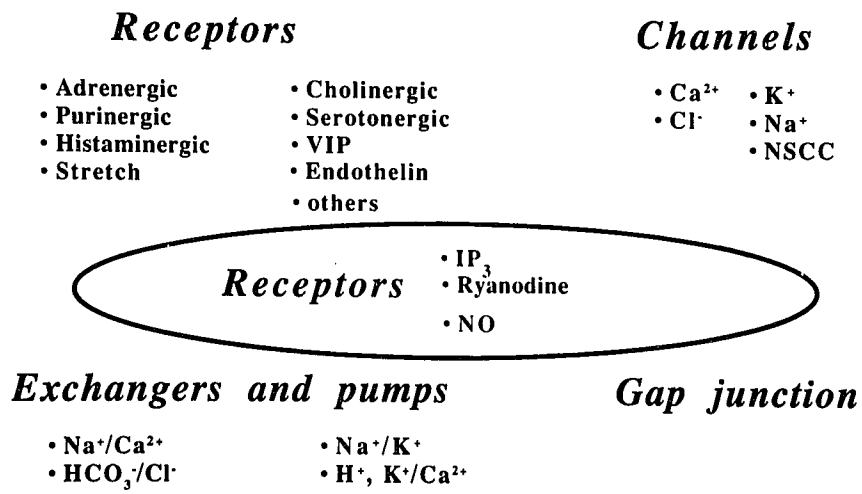

Fig. 1. Vascular smooth muscle cells have both plasma membrane-bound and intracellular receptors. The major sarcolemmal receptors include α-adrenergic, β-adrenergic, and muscarinic cholinergic receptors. Also present are the receptors for ATP, endothelin, serotonin, histamine, angiotensin II, VIP, stretch, and others. Intracellular receptors responsible for the release of Ca^{2+} from the intracellular stores include inositol 1,4,5-triphosphate (IP_3) and ryanodine (Ry) receptors. In addition, nitric oxide (NO) will enhance the activity of cellular guanylyl cyclase. Along with channels for Ca^{2+}, Cl^-, K^+, and Na^+, the sarcolemma of the vascular smooth muscle also contains a nonspecific cation channel (NSCC). The ionic exchangers in the sarcolemma include Na^+/Ca^{2+}, $H_{CO_3}-/Cl^-$, and Na^+/H^+ exchanger; and the ATP-dependent pumps are Na^+/K^+ and Ca^{2+} pumps with H^+ or K^+ as a counterion. Gap junctions are formed between the cells, and are responsible for the ionic exchange between cells and electrotonic coupling.

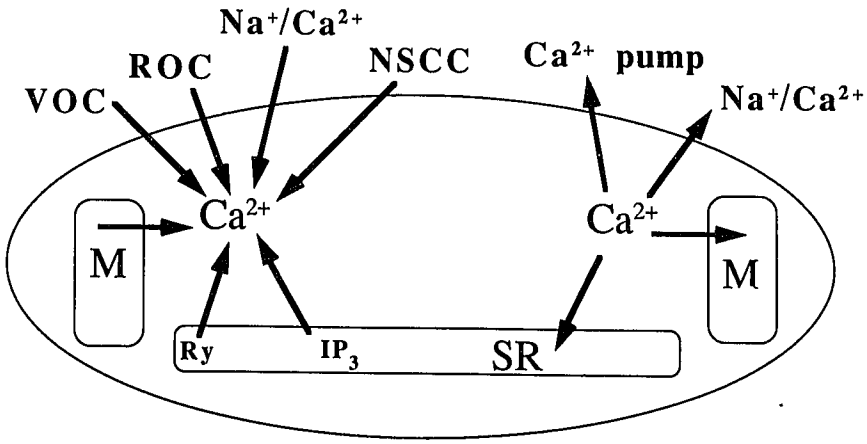

Fig. 2. Diagram depicting different Ca^{2+} pathways in the sarcolemma and sarcoplasmic reticulum (SR) of the vascular smooth muscle cell. Different pathways contributing to an increase in intracellular Ca^{2+} are depicted on the left of this schematic diagram. Influx of Ca^{2+} from the outside of vascular smooth muscle cell occurs *via* voltage-operated channels (VOC), receptor-operated channels (ROC), reversed Na^+/Ca^{2+} exchanger, and nonspecific cation channel (NSCC). Although some Ca^{2+} can be derived from mitochondria (M), most of the Ca^{2+} supplied for contraction from intracellular stores comes from the SR after the stimulation of ryanodine (Ry) or inositol 1,4,5-triphosphate (IP_3) channels. Factors contributing to a decrease in intracellular Ca^{2+} are shown on the right, and include Ca^{2+} pump and forward mode of the Na^+/Ca^{2+} exchanger in sarcolemma. An active reuptake by the mitochondria and the SR also contribute to the cellular sequestration of ionized Ca^{2+}.

smooth muscle contraction. The relative proportion of adrenergic subtypes and their numbers vary considerably between different vascular beds. Norepinephrine, when applied exogenously, either depolarizes the sarcolemma or has no effect on the membrane potential of the smooth muscle cells. Because norepinephrine, with few exceptions (such as cerebral arteries), consistently evokes contraction, depolarization of the membrane is not always a prerequisite for the initiation of contraction after α_1 adrenoceptor stimulation. An α_2 adrenoceptor stimulation (coupled to G protein) activates the membrane-associated Ca^{2+} channel to produce Ca^{2+} influx and cellular depolarization. By stimulating α_1 receptors, norepinephrine activates phosphatidylinositide hydrolysis by phospholipase C, which, in turn, leads to the generation of two second messengers: IP_3 and DAG.[5] After the release of Ca^{2+} from the SR, intracellular Ca^{2+} leads to an increase in chloride conduction and the opening of nonspecific cation channels and subsequent membrane depolarization.[6] Equilibrium potentials for both Na^+ ($E_{Na} = +50$ mV) and Cl^- ($E_{Cl} = -20$ mV) are more positive than the resting membrane potential ($E_m = -40$ to -55 mV).[7] In addition, norepinephrine appears to enhance voltage-dependent Ca^{2+} channel current by increasing the open-state probability (P_o) of single Ca^{2+} channels, but the membrane depolarization induced by activation of Ca^{2+} activated chloride channels is the prerequisite for further stimulation of Ca^{2+} channels in response to norepinephrine. Furthermore, norepinephrine may induce a shift in the relationship between P_o of the voltage-gated Ca^{2+} channel and the membrane potential.[8] At a given potential, more of the voltage-sensitive Ca^{2+} channels are open in the presence of norepinephrine. Therefore, norepinephrine increases intracellular Ca^{2+} concentration *via* release of Ca^{2+} from intracellular stores, stimulation of Ca^{2+} influx through receptor-operated channel, and activation of voltage-dependent Ca^{2+} channels.[2]

β-Adrenergic receptors are also found in most blood vessels, and, when stimulated, they inhibit contraction of the vascular smooth muscle. β_1 and β_2-adrenoreceptor subtypes are coupled to adenylyl cyclase *via* G_s protein. In turn, adenylyl cyclase mediates the conversion of ATP to cAMP that can lead to vasodilation. In some cells, this inhibition is essentially ineffective, because α_2 receptors are coupled to the same adenylyl cyclase *via* G_i protein that inhibits cAMP production. Nevertheless, some vessels only dilate in the presence of NE, most likely because of a specific adrenoceptor population in the sarcolemma.

Stimulation of muscarinic cholinergic receptors on the smooth muscle cells will cause contractions that are likely to be mediated by phosphoinositides. This direct effect will not be seen in most vessels, because ACh will release EDRF from the endothelial cells at the

same time. Although vasodilation will be dominant in most arteries, peripheral veins may dilate only with lower levels of ACh and constrict at higher concentrations.

Both norepinephrine and ATP are released from functional sympathetic nerve terminals. The ATP can act from intra- and extracellular sites on the smooth muscle cells. From intracellular sites, ATP activates voltage-dependent Ca^{2+} channels, and, from extracellular sites, ATP acts on the purinergic-2 (P-2) receptor and, possibly, on the voltage-dependent Ca^{2+} channels. ATP can depolarize the membrane of vascular smooth muscle cells (an exception appears to be coronary artery) by binding to the P-2 receptors that are coupled to Ca^{2+} channel producing contraction.

Vasopressin (ADH) triggers IP_3-sensitive release of Ca^{2+} from internal stores,[4] which then propagates throughout the cell as an intracellular Ca^{2+} wave, and activates its own release through Ca^{2+}-induced release of Ca^{2+} by diffusing to remote Ca^{2+} release sites. In addition, vasopressin can block the ATP-sensitive potassium channels (K_{ATP}),[9] leading to depolarization of smooth muscle cell, and subsequent contraction.

The sensitivity of vascular smooth muscle to Ca^{2+} antagonists varies depending on the vascular bed, vessel size, subtype of receptors present, and affinity of receptor-operated channels for the Ca^{2+} blocker. It appears that voltage-operated channels are generally more sensitive to Ca^{2+} antagonists than receptor-operated channels.[10]

While the underlying mechanism of angiotensin II effects on vascular smooth muscle are not fully understood, it appears that angiotensin II contracts the smooth muscle by stimulating Ca^{2+} influx *via* voltage-dependent Ca^{2+} channels.[11,12] This action may be mediated by G protein, and occurs with or without depolarization. In addition, angiotensin II also activates the formation of IP_3 and DAG through a G protein-mediated activation of phospolipase C. In addition to these actions, angiotensin II was shown to block K^+ currents.[13]

Vasoactive intestinal peptide (VIP) has a powerful ability to relax vascular and nonvascular smooth muscle. The VIP binds to a specific membrane receptor and selectively stimulates adenylyl cyclase to promote the accumulation of the cAMP and smooth muscle relaxation.

Endothelin-1 (ET-1) is a powerful vasoactive peptide released by endothelial cells[14] that activates ET-1 receptors in smooth muscle. Stimulation of ET-1 receptors can produce phospholipase C activation, which leads to stimulation of inositol phosphate turnover and the diacylglycerol production. This leads to stimulation of protein kinase C, which induces opening of Ca^{2+} channels. In addition, ET-1 releases Ca^{2+} from the SR through an IP_3 response, which acts as a second messenger in modulating the membrane currents.[15] The elevated intracellular Ca^{2+} is responsible for a sustained activation of Cl^- channel current, which depolarizes the membrane and is responsible for prolonged Ca^{2+} influx, or nonselective cation current and contraction.[16] Therefore, depending on the smooth muscle, ET-1-induced vasoconstriction can be initiated by Ca^{2+} influx across the sarcolemma (both Ca^{2+} selective and nonselective), or by IP_3-triggered release of Ca^{2+} from endoplasmic reticular stores. An additional effect of ET-1 on smooth muscle is increased myofilament Ca^{2+} sensitivity by a G protein-dependent pathway.[17,18] In addition to ET-1, other endothelium-derived peptides have been subsequently described, including ET-2, ET-3, and sarafotoxin.[19]

If the intravascular pressure is elevated, most vessels respond by increasing smooth muscle tone, and, if the pressure is reduced, they vasodilate. The mechanism of the initiation of the myogenic response to increased or decreased transmural pressure or stretch is not known. Membrane potentials of smooth muscle cells measured *in vitro* range between −60 and −75 mV, and those measured *in vivo* are between −40 and −55 mV.[7] When isolated arteries are exposed to physiologic intraluminal pressures, they depolarize (to −40 to −55 mV) and develop tone. There are a number of proposed mechanisms of intravascular pressure or stretch-induced alterations in vascular smooth muscle activation.[20] Some of the mechanisms include: (1) stretch-induced alterations in sarcolemmal properties, and subsequent activation or inhibition of the net inward current, or specific modulation of the signal coupling pathway within the smooth muscle cell; (2) length-induced alterations in contractile protein function; and (3) pressure and/or stretch-induced modulation of endothelial function.

Because the membrane potential of arterial smooth muscle cells *in vivo* falls in the range in which the current through the voltage-operated Ca^{2+} channels is highly voltage dependent, this current is likely to play a significant role in the maintenance of vascular tone. Depolarizations up to −40 mV activate and maintain inward Ca^{2+} current that does not significantly inactivate. It was shown that the P_o of Ca^{2+} channel increases

exponentially with membrane depolarization between −60 and −40 mV.[21] Between these potentials, a membrane hyperpolarization of only 2 mV could decrease P_o and, therefore, Ca^{2+} entry by approximately 30%.

Exchangers and Pumps

The membrane of vascular smooth muscle also contains Na^+/H^+, $H_{CO_3}-/Cl^-$, and Na^+/Ca^{2+} exchangers, as well as the Na^+/K^+ and Ca^{2+} pump.[22] The Ca^{2+} pump is stimulated by agents that increase cGMP (*e.g.*, ANF, EDRF, and nitrovasodilators).[23] On the other hand, increases in K^+ conductance as a result of activation of any of the K^+ channels will lead to membrane hyperpolarization and inactivation of voltage-operated Ca^{2+} channels, and enhanced Ca^{2+} efflux *via* Na^+/Ca^{2+} exchanger.[24] It was shown that Na^+/Ca^{2+} exchange is dependent on membrane potential, with membrane depolarization decreasing the net Ca^{2+} extrusion by the exchanger.[24]

Channels

Calcium Channels

At least two types of Ca^{2+} currents are present in vascular smooth muscle; L-type (long lasting) and T-type (transient). The T-type current activates and inactivates relatively rapidly at more negative potentials, and its inactivation is monoexponential and slowly voltage dependent. This current is not sensitive to classic Ca^{2+} channel blockers, as compared with the L-type current. The L-type Ca^{2+} channel inactivation is both voltage and Ca^{2+} dependent, and, therefore, biexponential. In addition, the slow Ca^{2+} channel current is abolished when the production of ATP is inhibited.[25] A new type of Ca^{2+} channel, called R-type (resting), was recently described in aortic and renal smooth muscle cells.[26] This type of channel is apparently responsible for the sustained increase of Ca^{2+} during tonic contraction of smooth muscle that occurs during depolarization of the cell membrane in the presence of high levels of extracellular K^+.

Phosphorylation of the Ca^{2+} slow channel protein can be either excitatory (proteinkinase C, PK-C) or inhibitory (cAMP-PK and cGMP-PK). For instance, angiotensin II activates the PL-C *via* a G protein, and PL-C stimulates PI turnover, leading to production of IP_3 and DAG. DAG activates PK-C to phosphorylate a number of proteins, including the Ca^{2+} slow channel, and to stimulate Ca^{2+} influx.[25] As to the inhibitory role of cyclic nucleotides in regulation of intracellular Ca^{2+}, activation of A-cyclase or G-cyclase by agents such as β-adrenergic agonists, prostacycline, or atrial natriuretic factor (ANF), or directly by agents like nitroprusside and EDRF, leads to an increase in cyclic AMP and cyclic GMP and activation of cA-PK and cG-PK. These kinases are responsible for phosphorylation of Ca^{2+} channels, which leads to inhibition of calcium channel opening and phosphorylation of K^+ channels, leading to stimulation of K^+ channel opening. ATP is required for activity of the channel, either by binding to the channel itself or *via* phosphorylation.[25] In addition, the Ca^{2+} influx will be influenced by intracellular Ca^{2+} (increases in cytosolic Ca^{2+} accelerate the inactivation process of the Ca^{2+} channel, as shown in fig. 3) and membrane potential (depolarization will activate receptor-operated channels). Inhibition of Ca^{2+} slow channels, and stimulation of K^+ channels, would inhibit Ca^{2+} influx and lower intracellular Ca^{2+}. In addition to Ca^{2+} and K^+ channels, cA-PK and cG-PK phosphorylate the Na^+/Ca^{2+} exchanger and the sarcolemmal Ca^{2+}-ATPase to stimulate the Ca^{2+} pump, and, therefore, can enhance activities of all three Ca^{2+}-removal systems in vascular smooth muscle, producing vasodilation.[25,27,28] In myocardial cells, however, Ca^{2+} channel phosphorylation by the cAMP-dependent protein kinase leads to an opposite effect, *i.e.*, an increase in Ca^{2+} influx. The effects of phosphorylation by a cGMP-PK are similar in cardiac and smooth muscle cells.

Potassium Channels

In different vascular smooth muscles, a variety of macroscopic K^+ currents have been detected, including: (1) Ca^{2+}-activated K^+ current; (2) delayed rectifier K^+ current; (3) ATP-sensitive K^+ current; (4) inward rectifier K^+ current; and (5) background K^+ current.[26,29,30]

The major outward current in vascular smooth muscle in response to membrane depolarization is carried by the large conductance Ca^{2+}-activated potassium channel (K_{Ca}). The P_o of this channel is relatively low at resting membrane potential, but increases in proportion to membrane depolarization and elevation of intracellular Ca^{2+} concentration.[31,32] An increase in K^+ efflux would cause membrane hyperpolarization, reduce the opening probability of voltage-dependent Ca^{2+} channels, and produce subsequent vascular relaxation.

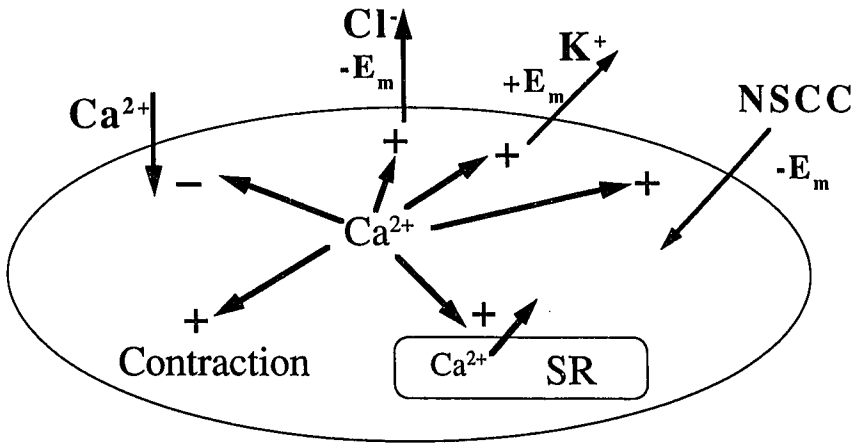

Fig. 3. Schematic diagram of some of the effects of elevated intracellular Ca^{2+}. The Ca^{2+} can release SR Ca^{2+} stores through Ca^{2+}-activated Ca^{2+} release. In addition, Ca^{2+} activates sarcolemmal chloride and nonselective cation channels (NSCC) at negative membrane potentials (E_m), and K^+ channels at more positive membrane potentials. Moreover, cytosolic Ca^{2+} accelerates the inactivation process of the sarcolemmal Ca^{2+} channel.

These channels appear to play a role in a vasodilating feedback pathway that is activated by pressure, intracellular free Ca^{2+}, or membrane depolarization.[33] The open time and frequency of opening of K_{Ca} channels are increased by 5'-GMP, a major metabolite of cGMP. The agents that increase the level of cGMP (ANF, NO, and nitrovasodilators) may, therefore, indirectly stimulate the K_{Ca}. Some of the other modulatory agents for these channels include norepinephrine, histamine, acetylcholine, endothelin, angiotensin II, nitroglycerin, and cGMP.[34] It appears, therefore, that K_{Ca} modulation may be an important regulatory mechanism of smooth muscle function. K_{Ca} current may not contribute to regulation of resting tension, or its role is minor in the presence of normal intracellular Ca^{2+}.[35] Another possible argument for the regulation of resting tension is that a very small number of open K^+ channels is required for maintenance of resting membrane potential, as suggested by Nelson.[21] Moreover, because of their high density per smooth muscle cell, large conductance, and the high input resistance, K_{Ca} channels may also contribute to the resting membrane potential.

The delayed rectifier (also called outwardly rectifying) K^+ current (K_{dr}) is voltage and Ca^{2+} dependent.[7,36,37] This current is activated when the potential across sarcolemma is more positive than −40 mV, and plays a dominant role in membrane repolarization and, to a degree, in maintaining the resting potential of aortic smooth muscle. It can be modulated by second messengers and angiotensin II. The activation of these channels at the physiologic range of membrane potential will lead to membrane hyperpolarization and inactivation of L-type Ca^{2+} channels. On the other hand, high levels of these dilators may lead to cGMP-induced block of L-type Ca^{2+} channels, an effect similar to that produced by isoproterenol.

The ATP-sensitive K^+ channel (K_{ATP}) of smooth muscle is involved in the mechanism of action of many vasodilator substances known as K^+ channel openers (*i.e.*, pinacidil, minoxidil, nicorandil, and chromokalim).[38] These channels are only weakly voltage dependent, and are not influenced by changes in intracellular Ca^{2+}. Hyperpolarization produced by the activation of K_{ATP} channels will effectively inactivate Ca^{2+} channels and decrease Ca^{2+} influx, leading to a lower level of vascular tone. Another characteristic of K_{ATP} channel is that it is inhibited by high intracellular ATP and antidiabetic sulfonil urea compounds, such as glibenclamide and tolbutamine, and it is activated by low levels of cytosolic ATP.[38] If the concentration of ATP is increased inside the membrane, the P_o of K_{ATP} channel is reduced. Several hyperpolarizing vasodilators, such as VIP (vasoactive intestinal polypeptide), calcitonin gene-related peptide (CGRP), and acetylcholine, induce release of EDRF and a hyperpolarizing factor from the endothelium that are blocked by glibenclamide, indicating that these vasodilators may also act by opening K_{ATP} channel.[39] Endothelium-derived hyperpolarizing factor (EDHF) also has properties of K^+ channel openers, although the type of K^+ channel involved is not known.

An inward rectifier K^+ channel is activated by hyperpolarization of the vascular smooth muscle cells, allowing the inward movement of K^+.[7] At membrane potentials positive to equilibrium potentials for potassium (E_K at approximately −90 mV), these channels do allow an outward K^+ current and, therefore, may contribute to the resting membrane potential. These channels will

be closed when the vessel is tonically depolarized during increased sympathetic nerve activity.

The time-independent background K^+ current is sensitive to the external calcium concentration, and may be responsible in setting the level of the resting membrane potential.

Gap Junctions

With increasing metabolic demand of the organ (*e.g.*, exercising muscle) a profound ascending vasodilation occurs within seconds.[40] This coordinated increase in blood flow is probably caused by a flow-dependent endothelial cell-mediated relaxation and smooth muscle cell-to-cell conduction. Unlike skeletal muscle, but similar to cardiac muscle, vascular muscle is coupled by gap junctions,[41,42] which provide a cell-to-cell low-resistance conduction pathway for the functional integration. These junctions are membrane structures that contain aqueous (nonselective) channels, linking the cytoplasm of adjacent cells. Each functional channel is formed by two hemichannels and a 12-identical protein subunit called connexins. They are responsible for equilibration of ionic and small molecular pools between the cells, electrotonic coupling, and coordinated vessel responses to different stimuli. Ca^{2+} and, perhaps, IP_3 can diffuse between smooth muscle cells through gap junction channels.[43] A number of agents, including halothane,[44] alcohols, and cGMP, have been shown to decrease junctional conductance in myocardial cells, but an increase is seen in the presence of cAMP. Vascular smooth muscle gap junction conduction is also regulated by second messengers, and it was shown that cAMP is responsible for moderate decreases, and cGMP for no change, in junctional current.[41]

Anesthetics

Although anesthetics are potent vasodilators, the mechanism of their action is not well understood. Inhalational anesthetics cause vasodilation in specific vascular beds, either by a direct depressant action on the vessel, or by an indirect attenuation of vasoconstrictor activity. In the *in vivo* setting, the mechanism for the altered blood flow to a specific organ during anesthesia is likely to involve interaction among endothelium, vascular smooth muscle, arterial pressure, metabolic requirement of the organ, and the autonomic nervous system. For instance, in the intact coronary circulation, the direct effects of volatile anesthetics on arterial muscle are superimposed on their dominant depressant action on the myocardium, *i.e.*, reduced cardiac work and oxygen demand. It appears that isoflurane is a more potent coronary vasodilator than halothane in the isolated perfused heart,[45] and a less potent dilator of isolated coronary arterial segments.[46] In studies on isolated coronary artery rings[44] and isolated tetrodotoxin-arrested rat heart, halothane caused direct dilation of coronary vessels without affecting oxygen consumption or extraction.[47] Recent results have also shown that isoflurane produces potent dose-dependent relaxation of canine middle cerebral arteries *in vitro,* and that this relaxation is endothelium independent.[48]

Halothane and isoflurane were found to increase intracellular cAMP in rat aortic smooth muscle.[49] More recent studies have shown that halothane-induced vasodilation of cerebral vessels is partly mediated by an increase in tissue cGMP levels.[50,51] An increase in the cellular cGMP levels induces vascular smooth muscle relaxation by activating cGMP-dependent protein kinase. As indicated previously, in addition to Ca^{2+} and K^+ channels, cA-PK and cG-PK also phosphorylate the sarcolemmal Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger, and stimulate the Ca^{2+} pump, and, therefore, can enhance activities of all three Ca^{2+} removal systems in vascular smooth muscle, producing vasodilation. It was recently shown that the elevations in cGMP in arterial smooth muscle can also uncouple the stress from myosin phosphorylation.[52] Most tissues have two guanylyl cyclase isoenzymes; a membrane-bound particulate and cytosolic (soluble) enzyme. The soluble enzyme is activated by nitric oxide radicals, and the membrane-bound enzyme is activated by atrial natriuretic factor. An increase in cGMP level induced by halothane results from activation of the particulate, but not soluble, enzyme in canine middle cerebral vessels.[50] Therefore, along with direct modulation of ion channels, some of the effects of halothane on ion channels may be mediated *via* an increase in tissue cGMP or cAMP levels.

Halothane and isoflurane were shown to depress agonist-induced inositol phosphate formation and increases in intracellular Ca^{2+} in isolated coronary vessels.[53] These anesthetics did not have significant effects on contractions evoked by direct activation of protein kinase C, indicating that they acted at the proximal part of the signal transduction pathway (*i.e.*, receptor or G protein).

In isolated aortic smooth muscle, halothane was shown to slightly decrease maximum Ca^{2+}-activated

tension development of the contractile proteins, decrease Ca^{2+} accumulation in the SR, and increase Ca^{2+} release from the SR.[54] These decreases in cellular Ca^{2+} are probably responsible for the anesthetic-induced attenuation of norepinephrine-induced oscillation in vascular tension that is associated with intracellular calcium fluxes from the SR.[55]

It was reported that halothane attenuates α_2, but not α_1, adrenoceptor responsiveness in canine saphenous vein.[56] The results indicated that halothane interferes with α_2 excitation-contraction coupling that is dependent on the influx of extracellular Ca^{2+}. In addition, α_1-mediated vasoconstriction caused by the release of intracellular Ca^{2+} was far less sensitive to halothane. Recently, the direct effects of halothane, enflurane, and isoflurane were compared on the tension generated in the mesenteric venous smooth muscle. The results indicate that all three agents attenuate contractile responses to exogenous and endogenous NE, with isoflurane being more potent than halothane or isoflurane.[57]

The α_2-adrenergic agonist, dexmedetomidine, can activate not only α_2 receptors in the central nervous system, but also those in the peripheral vasculature. Current results indicate[58] that dexmedetomidine has a direct vasoconstrictor effect on coronary and cerebral vasculature *in vitro*, and that this effect may be opposed by simultaneous release of endothelium-derived relaxing factor. Nevertheless, the direct stimulation of α_2 adrenoceptors by dexmedetomidine in these vessels resulted in only minimal vasoconstriction.

Recently, the effects of halothane and isoflurane on macroscopic Ca^{2+} and K^+ channel currents were investigated in voltage-clamped vascular muscle cells of the canine coronary artery.[36] At equianesthetic concentrations, halothane was found to be more potent than isoflurane in suppressing both ionic currents in the coronary arterial cells. Moreover, both anesthetics preferentially reduced Ca^{2+} current. Thus, in the coronary artery, which relies on Ca^{2+} entry for vascular muscle activation, the reduced Ca^{2+} influx may represent one of the mechanisms by which volatile anesthetics induce dilation.

The effects of isoflurane on macroscopic long-lasting type Ca^{2+} and K^+ channel currents were also investigated in voltage-clamped single canine middle cerebral artery cells.[37] The results indicate that isoflurane reduced the amplitude of K^+ and Ca^{2+} channel current, and that isoflurane was a more potent blocker of the Ca^{2+} channel current at more negative membrane potentials. A reduction of Ca^{2+} influx would dilate cerebral arteries, which rely predominantly on the influx of external Ca^{2+} for the maintenance of contraction.[59] However, the block of K^+ channel current by isoflurane would favor membrane depolarization, because K^+ conductance is crucial for repolarization of cell membrane potential and cerebral arterial relaxation. Although this membrane depolarization may initially open voltage-dependent Ca^{2+} channels, the effective block of Ca^{2+} influx by isoflurane would probably minimize the contribution of these channels to the cytoplasmic Ca^{2+} concentration. Therefore, the simultaneous depression of Ca^{2+} and K^+ currents by isoflurane may cause electromechanical uncoupling of cerebral vascular smooth muscle. For example, halothane caused membrane depolarization despite simultaneous vascular relaxation, indicating uncoupling between the membrane potential and vascular contractions.[60] These results may be caused by the fact that halothane and isoflurane reduced the amplitude of K^+ and Ca^{2+} currents in the vascular smooth muscle membrane.[36,37] However, the Ca^{2+} current was considerably more sensitive to blockade by volatile anesthetics. In general, contractile mechanisms in cerebral and coronary blood vessels appear to be more dependent on extracellular Ca^{2+} influx than on intracellular stores of Ca^{2+}, and, therefore, modulation of Ca^{2+} influx by volatile anesthetics represents a potential dilator mechanism. In addition, the results of recent studies indicate that volatile anesthetics are more potent direct dilators of cerebral than coronary vascular smooth muscle.[35]

Preliminary studies from our laboratory indicate that halothane at clinically relevant concentrations can produce hyperpolarization of the resting membrane potential in small mesenteric arteries and veins *in vivo*. The inhibition of inward Ca^{2+} current appears to contribute to this hyperpolarization of the resting membrane potential and, therefore, to a significant direct vasodilatory action of the potent volatile anesthetics.

The effects of halothane and isoflurane were recently examined on Ca^{2+}-activated single K^+ channels in single vascular smooth muscle cells isolated from dog cerebral arteries.† Both halothane and isoflurane reversibly decreased the P_o, mean open time, and frequency of opening of a 99-pS K^+ channel. The single-channel amplitude or the slope of the current–voltage relationship

† Eskinder H, Gebremedhin D, Lee JG, Rusch NJ, Kampine JP, Bosnjak ZJ: Halothane and isoflurane decrease the open state probability of K^+ channels in dog cerebral arterial muscle cells (unpublished data).

was not affected by these anesthetics. From these results, it is evident that a depression of Ca^{2+}-activated K^+ channel does not prevent the anesthetic-induced vasodilation of canine cerebral arteries.

In summary, it appears that anesthetics affect a number of sites that are responsible for direct excitation of vascular smooth muscle, and that the individual responses will vary depending on local vascular activity, concentration, and type of anesthetic; mode and specificity of agonist activation; role of endothelium; and other regulatory factors.

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