Inositol trisphosphate receptors in smooth muscle cells

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Narayanan D, Adebiyi A, Jaggar JH. Inositol trisphosphate receptors in smooth muscle cells. Am J Physiol Heart Circ Physiol 302: H2190–H2210, 2012. First published March 23, 2012; doi:10.1152/ajpheart.01146.2011.—Inositol 1,4,5-trisphosphate receptors (IP$_3$Rs) are a family of tetrameric intracellular calcium (Ca$^{2+}$) release channels that are located on the sarcoplasmic reticulum (SR) membrane of virtually all mammalian cell types, including smooth muscle cells (SMC). Here, we have reviewed literature investigating IP$_3$R expression, cellular localization, tissue distribution, activity regulation, communication with ion channels and organelles, generation of Ca$^{2+}$ signals, modulation of physiological functions, and alterations in pathologies in SMCs. Three IP$_3$R isoforms have been identified, with relative expression and cellular localization of each contributing to signaling differences in diverse SMC types. Several endogenous ligands, kinases, proteins, and other modulators control SMC IP$_3$R channel activity. SMC IP$_3$Rs communicate with nearby ryanodine-sensitive Ca$^{2+}$ channels and mitochondria to influence SR Ca$^{2+}$ release and reactive oxygen species generation. IP$_3$R-mediated Ca$^{2+}$ release can stimulate plasma membrane-localized channels, including transient receptor potential (TRP) channels and store-operated Ca$^{2+}$ channels. SMC IP$_3$Rs also signal to other proteins via SR Ca$^{2+}$ release-independent mechanisms through physical coupling to TRP channels and local communication with large-conductance Ca$^{2+}$-activated potassium channels. IP$_3$R-mediated Ca$^{2+}$ release generates a wide variety of intracellular Ca$^{2+}$ signals, which vary with respect to frequency, amplitude, spatial, and temporal properties. IP$_3$R signaling controls multiple SMC functions, including contraction, gene expression, migration, and proliferation. IP$_3$R expression and cellular signaling are altered in several SMC diseases, notably asthma, atherosclerosis, diabetes, and hypertension. In summary, IP$_3$R-mediated pathways control diverse SMC physiological functions, with pathological alterations in IP$_3$R signaling contributing to disease.

intracellular calcium (Ca$^{2+}$) signals are produced by both extracellular Ca$^{2+}$ influx and intracellular Ca$^{2+}$ release (22, 29). Ca$^{2+}$ influx can occur through plasma membrane ion channels and transporters, including voltage-dependent L-type Ca$^{2+}$ (Cav1.2) channels, nonselective cation channels, and the Na$^+$/Ca$^{2+}$ exchanger (22, 29). Ca$^{2+}$ can also be released from intracellular stores, such as the sarcoplasmic reticulum (SR)/endoplasmic reticulum (ER) and mitochondria (22, 29). Ca$^{2+}$ signals in smooth muscle cells (SMCs) include Ca$^{2+}$ flashes, oscillations, puffs, ripples, sparklets, sparks, waves, and global intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$\text{_{i}}$) (13, 26, 90, 144, 158, 254). These diverse Ca$^{2+}$ signals regulate physiological functions and are modified in diseases, leading to pathological consequences.

Many plasma membrane receptors couple via heteromeric G$_{q/11}$ proteins to PLC (22, 29). Upon agonist to receptor binding, PLC-catalyzed hydrolysis of phosphatidylinositol 4,5-bisphosphate generates diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$) (21, 57). IP$_3$ binds to and activates SR-localized IP$_3$ receptors (IP$_3$Rs) (21, 57). SMC IP$_3$Rs were first purified from aorta and, following reconstitution into phospholipid vesicles, were identified as Ca$^{2+}$ release channels (34, 52, 134). IP$_3$Rs regulate many cellular processes in SMCs, including contractility, gene expression, migration, and proliferation (2, 60, 149, 156, 234, 237).

The focus of this review is to discuss IP$_3$R expression, cellular localization, channel activity, communication with ion channels and organelles, Ca$^{2+}$ signals generated, physiological functions regulated, and pathological alterations in SMCs. Excellent reviews have summarized knowledge gained from studies of recombinant IP$_3$R channels and those expressed in a wide variety of different cell types (21, 57, 143, 171). Here, IP$_3$R signaling in non-SMC types will only be considered when extrapolation to SMCs is appropriate or when discussion may stimulate future research in SMCs.

IP$_3$R Structure

IP$_3$Rs are tetramers, with each subunit composed of three principal regions: an NH$_2$ terminus, a hydrophobic region comprising six transmembrane domains, and a COOH-terminal tail (Fig. 1) (139, 246). The cytosolic NH$_2$-terminal region is functionally divided into the IP$_3$-binding domain, a suppressor...
domain that inhibits IP₃ binding, and the regulatory domain 
(Fig. 1, A and B) (57, 245). The regulatory domain contains 
binding sites for ATP (Fig. 1A, red squares) and Ca²⁺ (Fig. 1A, 
blue squares) and consensus sequences for phosphorylation 
(Fig. 1A, black squares) (57, 139, 171). A coupling domain 
through which IP₃Rs physically interact with transient receptor 
potential canonical (TRPC) channels is also located in the 
regulatory domain (Fig. 1B) (2, 208). The transmembrane 
domains anchor each IP₃R subunit to the SR membrane (Fig. 1, 
B and C) (139). The luminal loop between transmembrane 
domains 5 and 6 forms the Ca²⁺-permeable pore (Fig. 1B) 
(139). The COOH-terminal tail extends from transmembrane 
domain 6 into the cytosol (Fig. 1, A and B) (139). The 
transmembrane domains and COOH-terminal tail appear to be 
essential for IP₃R tetramerization (141, 188).

**IP₃R Isoforms and Distribution in SMCs of Different Tissues**

To date, three IP₃R isoforms (IP₃R1–3) have been identified, 
each of which is encoded by a different gene (57). Important 
features of mammalian IP₃R isoforms are summarized in 
Table 1. SMCs express all three IP₃R isoforms, with relative 
levels of each determined by the tissue of origin and developmen-
tal stage. IP₃R1 is the predominant isoform expressed in vascular 
SMCs (64, 84, 147, 228, 254, 257). Quantitative PCR performed 
on isolated cerebral artery SMCs indicated that IP₃R1 mRNA was 
the most abundant of the three isoforms, at 82% of total message, 
with much of the residual IP₃R message being IP₃R3 (254). 
Similarly, IP₃R1 was the major isoform detected in basilar and 
mesenteric arteries, whole thoracic aorta, cultured portal vein 
SMCs, and A7r5 cells, an aortic SMC line (64, 84, 147, 211, 228, 
257). Reports indicate that IP₃R isoform expression in vascular 
SMCs shifts during ontogeny and proliferation (210, 211). In 
neonatal (2- to 4-day-old) rats, IP₃R1 protein was lower and 
IP₃R3 protein was higher in SMCs of aorta and portal vein, 
compared with those in juvenile (6 wk-old) rats (210). IP₃R2 and 
IP₃R3 levels were higher in proliferating, cultured aortic SMCs 
than in whole aorta homogenates (211). IP₃R isoform expression 
in nonvascular SMCs exhibits tissue variability. In isolated ure-
teric and myometrial SMCs, all three IP₃R isoforms were detected 
(26, 148). IP₃R3 was the predominant isoform, with IP₃R1 and 
IP₃R2 also present in tracheal SMCs (230). In contrast, cultured 
ureteric and gastric SMCs expressed IP₃R1 and IP₃R3 but not 
IP₃R2 (147, 154). Tissue variability in IP₃R isoform expression 
may contribute to signaling differences in these SMC types.

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Fig. 1. Inositol 1,4,5-trisphosphate receptor (IP₃R) molecular structure. A: schematic representation of an IP₃R subunit depicting important domains and regions. Sites for ATP-binding (black squares), Ca²⁺-binding (blue squares), and phosphorylation (red squares) are indicated. B: single IP₃R subunit illustrating important domains, regions, and pore. C: tetrameric IP₃R channel. SR, sarcoplasmic reticulum; N, NH₂ terminus; C, COOH terminus.
**Cellular Expression and Localization of SMC IP₃Rs**

Cellular levels of IP₃Rs in SMCs are determined by an equilibrium between gene transcription and protein degradation (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227). c-Myb, a transcription factor, binds to the IP₃R1 promoter and stimulates transcription in cultured SMCs (3, 129, 171, 227).

**Table 1. Important features of the three mammalian IP₃R isoforms**

<table>
<thead>
<tr>
<th>IP₃R</th>
<th>Homology with human IP₃R1</th>
<th>Alternative splicing</th>
<th>Amino acid number (human)</th>
<th>Chromosomal localization (human)</th>
<th>Cellular localization in SMCs</th>
<th>IP₃ affinity</th>
<th>Regulation by Ca²⁺</th>
<th>Sensitivity</th>
<th>Number of binding sites</th>
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<tr>
<td>IP₃R1</td>
<td>–</td>
<td>Yes (163, 241)</td>
<td>2,695–2,743 (splice variation-dependent)</td>
<td>3p25–26 (241)</td>
<td>Cerebral artery (1, 2, 254), cultured aorta (199, 211), external aorta (162, 199, 211), A7r5 cells (224)</td>
<td>Intermediate [0.27 µM]⁺ (217)</td>
<td>Yes [0.17 µM]⁺ (216)</td>
<td>High</td>
<td>3–4 (171)</td>
</tr>
<tr>
<td>IP₃R2</td>
<td>–</td>
<td>No described</td>
<td>2,701 (242)</td>
<td>12p11 (242)</td>
<td>Cerebral artery (1, 2, 254), cultured aorta (199, 211), vas deferens (162), ileum (63)</td>
<td>Highest (0.10 µM)⁺ (217)</td>
<td>Yes [0.15 µM]⁺ (216)</td>
<td>None described</td>
<td>None (217)</td>
</tr>
<tr>
<td>IP₃R3</td>
<td>–</td>
<td>None described</td>
<td>2,671 (123, 242)</td>
<td>6p21 (242)</td>
<td>Cultured aorta (199, 211), cultured aorta (199, 211), ureter (26)</td>
<td>Lowest [0.40 µM]⁺ (217)</td>
<td>Yes [0.16 µM]⁺ (216)</td>
<td>Low</td>
<td>2 (171)</td>
</tr>
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IP₃Rs, inositol 1,4,5-trisphosphate receptors; SMCs, smooth muscle cells; SR, sarcoplasmic reticulum. Numbers in parenthesis indicate reference numbers. *EC₅₀ for recombinant IP₃Rs reconstituted into lipid bilayer.

The kinetics of IP₃-mediated Ca²⁺ release in vascular SMCs have been investigated using permeabilized portal vein smooth muscle strips and flash photolysis of caged IP₃ (198). Reconstituted IP₃Rs were found to be IP₃-gated ion channels, most permeable to Ca²⁺, followed by K⁺ and Cl⁻ (134). Bath application of 0.5 µM IP₃ activated ~32-pS single channel currents after a delay of ~1 min in standard buffer with 50 mM Ca²⁺ as the charge carrier (Fig. 2B) (134). The delayed response between bath application of IP₃ and stimulation of channel currents was suggested to be related to the diffusion of IP₃ in the bath (134). IP₃-induced channel currents were enhanced by ATP (Fig. 2C) and blocked by heparin (134).

The kinetics of IP₃R-mediated Ca²⁺ release in vascular SMCs have been investigated using permeabilized portal vein smooth muscle strips and flash photolysis of caged IP₃ (198). Reconstituted IP₃Rs were found to be IP₃-gated ion channels, most permeable to Ca²⁺, followed by K⁺ and Cl⁻ (134). Bath application of 0.5 µM IP₃ activated ~32-pS single channel currents after a delay of ~1 min in standard buffer with 50 mM Ca²⁺ as the charge carrier (Fig. 2B) (134). The delayed response between bath application of IP₃ and stimulation of channel currents was suggested to be related to the diffusion of IP₃ in the bath (134). IP₃-induced channel currents were enhanced by ATP (Fig. 2C) and blocked by heparin (134).
Cellular Regulators of SMC IP3R Activity

This section will describe ligands, protein kinases, regulatory proteins, and other modulators that influence IP3R activity in SMCs. The reader is referred to earlier reviews (57, 127, 172, 213) where additional details of these and other IP3R regulators derived from reports in non-SMC types are discussed.

Ligands. Ligands that regulate SMC IP3R activity include IP3, cytosolic Ca2+, SR luminal Ca2+, and ATP, with IP3 and cytosolic Ca2+ acting as principal modulators. Regulation of IP3R channel activity by IP3, cytosolic Ca2+ concentration ([Ca2+]c), and ATP is summarized in Table 1.

IP3 is the major stimulus for inducing IP3R-mediated Ca2+ release in SMCs (34, 134, 198). [3H]IP3 binding assays indicated that IP3 bound to IP3Rs with a KD of 2–5 nM in SMCs of the aorta, colon, ileum, jejunum, myometrium, and vas deferens (4, 24, 34, 150, 152, 223, 251). Evidence from studies in non-SMCs suggest that binding of IP3 induces a conformational change in the IP3-binding domain, which is transmitted via the central regulatory domain to the transmembrane domains to elicit channel opening (141, 155, 222). SMC IP3R activity is modulated by an intricate interplay between IP3 and [Ca2+]c (77, 79, 134). SMC IP3R channel activation occurs at [Ca2+]c <300 nM, and released Ca2+ initially stimulates IP3Rs via Ca2+-induced Ca2+ release (CICR) (77, 79). However, an elevation in [Ca2+]c >300 nM inhibits SMC IP3Rs (77, 79). Studies (74, 140) in non-SMCs suggested that for Ca2+ to inhibit IP3Rs, [Ca2+]c >300 nM and CaM, a Ca2+-binding protein, were both required. IP3R activity regulation by [Ca2+]c is controlled by distinct stimulatory and inhibitory Ca2+-binding sites (195, 196). One luminal and seven cytosolic Ca2+-binding sites (Fig. 1A, blue squares) have been identified on IP3Rs, suggesting that Ca2+ binds to and directly influences IP3R activity (195, 196). A [Ca2+]c elevation can also inhibit IP3 binding to SMC IP3Rs, thereby indirectly regulating channel activity (16). Thus [Ca2+]c regulates IP3R activity in SMCs via two simultaneously occurring mechanisms: 1) directly, by binding to stimulatory or inhibitory sites on IP3Rs; and 2) indirectly, by modulating IP3 binding to IP3Rs. Evidence also suggests that IP3 determines if Ca2+ stimulates or inhibits IP3Rs (124). IP3 binding to IP3Rs displaces Ca2+ from inhibitory sites, leading to a reduction in Ca2+-dependent inhibition, and exposes stimulatory Ca2+-binding sites (Fig. 1A, blue squares), thereby permitting Ca2+-mediated activation (124). Therefore, IP3 and [Ca2+]c influence SMC IP3R activity directly, by binding to IP3Rs and indirectly, by modulating effects of each other.

SMC IP3Rs are modulated not only by cytosolic Ca2+ but also by SR luminal Ca2+. In A7r5 cells, an elevation in SR Ca2+ concentration ([Ca2+]SR) enhanced IP3R-mediated Ca2+ release (30). Depletion of SR Ca2+ abolished IP3R-mediated Ca2+ release in SMCs (81, 86, 117, 136, 151). Ca2+ binding to a site located in the luminal loop between transmembrane domains 5 and 6 of IP3Rs (Fig. 1A, blue squares) (195) stimulated cerebellar IP3Rs (23). [Ca2+]SR may also regulate SR Ca2+ release in SMCs by determining the driving force for Ca2+ efflux, which should modulate the amplitude of IP3R-mediated Ca2+ signals. Therefore, [Ca2+]SR may influence SMC IP3R activity through more than one mechanism, although experimental evidence for such regulation is limited.

ATP binds to three sites located in the IP3R regulatory domain (Fig. 1A, black squares). ATP is not required for SMC IP3R activation but enhances IP3 and Ca2+ sensitivity, thereby augmenting channel activity (77, 80, 134). ATP alone did not stimulate aortic IP3Rs reconstituted into planar lipid bilayers but elevated IP3R-induced currents (Fig. 2C) (134). Similarly, in permeabilized portal vein SMCs, ATP dose-dependently increased IP3R-induced Ca2+ release with a maximal effect at 0.5 mM ATP (80). ATP also elevates SMC IP3R Ca2+ sensitivity,
leading to an increase in Ca\textsuperscript{2+} activation, which can augment CICR (77). Thus, in contrast to IP\textsubscript{3}, which activates IP\textsubscript{3}Rs by reducing Ca\textsuperscript{2+}-mediated inhibition, ATP stimulates IP\textsubscript{3}Rs by enhancing Ca\textsuperscript{2+}-dependent activation (57). In summary, IP\textsubscript{3}, Ca\textsuperscript{2+}, and ATP modulate multiple regulatory processes to control and fine tune IP\textsubscript{3}R activity in SMCs.

**Protein kinases.** PKA and PKG inhibit SMC IP\textsubscript{3}Rs via phosphorylation, through regulation of IP\textsubscript{3} binding, and by controlling IP\textsubscript{3} generation. Consensus sequences for PKA- and PKG-mediated IP\textsubscript{3}R phosphorylation are located in the regulatory domain of SMC IP\textsubscript{3}Rs (Fig. 1A, red squares) (43, 100). PKG phosphorylated SMC IP\textsubscript{3}Rs at serine 1755 and inhibited IP\textsubscript{3}-induced Ca\textsuperscript{2+} release in aortic and gastric SMCs (100, 154). PKG phosphorylated IP\textsubscript{3}Rs via IP\textsubscript{3}-associated cGMP kinase substrate (IRAG) in gastric and tracheal SMCs (153, 154, 189, 226). PKG/IRAG-dependent IP\textsubscript{3}R phosphorylation and subsequent inhibition of IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release relaxed colonic and aortic SMCs (46, 58). These studies suggest that PKG/IRAG regulation of IP\textsubscript{3}R phosphorylation and Ca\textsuperscript{2+} release regulates SMC contractility. PKA activation reduced PLC-dependent IP\textsubscript{3} generation and decreased the number of binding sites for IP\textsubscript{3} on IP\textsubscript{3}Rs, thereby attenuating IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release in tracheal SMCs (120, 191). Two-dimensional phosphopeptide mapping revealed that PKA phosphorylated SMC IP\textsubscript{3}Rs at serine 1589 (43). In iris sphincter SMCs, PKA inhibited IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release, resulting in relaxation (48, 204). It has been proposed that PKG is required for PKA-mediated phosphorylation of IP\textsubscript{3}Rs in SMCs. In gastric SMCs and intact aorta, PKA phosphorylated IP\textsubscript{3}Rs at two sites: serine 1589, a PKA phosphorylation site, and serine 1755, a PKG-specific site, suggesting that PKA activates PKG to phosphorylate IP\textsubscript{3}Rs (101, 154). Therefore, PKA-PKG cross talk may contribute to PKA-mediated phosphorylation of SMC IP\textsubscript{3}Rs.

PKC and tyrosine kinases enhance SMC IP\textsubscript{3}R signaling through IP\textsubscript{3}R phosphorylation and by elevating intracellular [IP\textsubscript{3}] ([IP\textsubscript{3}]). PKC and tyrosine kinase phosphorylation sites on SMC IP\textsubscript{3}Rs are yet to be identified. PKC-mediated IP\textsubscript{3}R phosphorylation elevated [Ca\textsuperscript{2+}]; and contracted gallbladder SMCs and A10 cells, an aortic SMC line (240, 248). In cultured aortic SMCs, tyrosine kinase inhibitors reduced ANG II-induced PLC-\gamma1 phosphorylation and elevations in [IP\textsubscript{3}] and [Ca\textsuperscript{2+}], suggesting that tyrosine kinases stimulate IP\textsubscript{3} generation in SMCs (126). Similarly, tyrosine kinase inhibitors attenuated agonist-induced elevations in [IP\textsubscript{3}] and [Ca\textsuperscript{2+}], and contraction in cultured gluteal and pulmonary artery SMCs but not in cultured aortic SMCs (55, 214). Collectively, these studies indicate that protein kinases regulate SMC IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} signaling and contraction directly, through phosphorylation and indirectly, by controlling IP\textsubscript{3} generation and IP\textsubscript{3} binding.

**Regulatory proteins.** Evidence suggests that cytosolic proteins, including the 12-kDa FK506 binding protein (FKB12) and receptor for activated PKC 1 (RACK1) influence IP\textsubscript{3}R channel activity in SMCs (15, 36, 118, 173). FKB12, an intracellular ligand for immunosuppressants FK506 and rapamycin, interacts with and regulates SMC IP\textsubscript{3}Rs (15, 118). FKB12 coimmunoprecipitated with IP\textsubscript{3}R1 in colonic SMCs, suggesting physical interaction (118). FKB12 modulated colonic SMC IP\textsubscript{3}R activity via three effector proteins: calcineurin, FK506, and mammalian target of rapamycin (mTOR) (15, 118). FKB12 potentiated IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release through mTOR and inhibited IP\textsubscript{3}R activity through calcineurin and FK506 in colonic SMCs (15, 118). In contrast, in aorta, FKB12 did not coimmunoprecipitate with IP\textsubscript{3}Rs and IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release was unaffected by FK506 or rapamycin (118, 119). Explanations for these tissue-specific differences in FKB12 regulation of SMC IP\textsubscript{3}Rs are unclear but may be due to variable expression of FKB12 and important regulatory proteins, including calcineurin, FK506, and mTOR.

RACK1 is a scaffolding protein that shuttles PKC to its substrates, thereby facilitating interaction (173). RACK1 coimmunoprecipitated with IP\textsubscript{3}R1 in PC12 cells, a non-SMC line, and elevated [\textsuperscript{3}H]IP\textsubscript{3} binding to cerebellar IP\textsubscript{3}Rs, suggesting that RACK1 interacts with IP\textsubscript{3}Rs and increases IP\textsubscript{3} affinity (173). RACK1 enhanced agonist-induced IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release in A7r5 cells and cultured preglomerular microvascular SMCs, suggesting that RACK1 also stimulates IP\textsubscript{3}R channel activity in SMCs (36, 173). Although evidence suggests functional regulation of IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release by RACK1 in SMCs, whether these effects are due to direct binding to IP\textsubscript{3}Rs or by PKC-dependent pathways has not been resolved.

**Other modulators.** In addition to endogenous ligands, kinases, and regulatory proteins, cellular pH and reactive oxygen species (ROS) also modulate SMC IP\textsubscript{3}Rs. pH influences SMC IP\textsubscript{3}R activity by controlling IP\textsubscript{3} binding and IP\textsubscript{3}-Ca\textsuperscript{2+} sensitivity (38, 78, 152, 215, 251). Elevating pH activated recombinant IP\textsubscript{3}R1 and IP\textsubscript{3}R3 channels, with IP\textsubscript{3}R1 being more sensitive at pH 6.8 and IP\textsubscript{3}R3 at pH 7.5, suggesting that pH regulation is isoform dependent (44). IP\textsubscript{3} binding to SR membranes from aortic, colonic, and tracheal smooth muscle was weak at pH <7 and maximal at pH 8–9 (38, 152, 251). In portal vein and taenia caeci SMCs, increasing pH from 6.7 to 7.0–7.3 reduced the Ca\textsuperscript{2+} concentration required for IP\textsubscript{3}R activation, thereby elevating IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release (78, 215). Evidence suggests that H+ may compete with IP\textsubscript{3} and Ca\textsuperscript{2+} to inhibit their binding to SMC IP\textsubscript{3}Rs, resulting in channel inhibition (215).

ROS regulate SMC IP\textsubscript{3}Rs through processes that include control of [IP\textsubscript{3}], and modulation of IP\textsubscript{3}-IP\textsubscript{3} affinity (31, 112, 183, 201). Superoxide reduced IP\textsubscript{3} hydrolysis in SR vesicles of thoracic aorta, thereby enhancing [IP\textsubscript{3}], and IP\textsubscript{3}-induced Ca\textsuperscript{2+} release (201). Thimerosal, an oxidizing agent, enhanced the affinity of IP\textsubscript{3}R1 for IP\textsubscript{3} and potentiated IP\textsubscript{3}-induced Ca\textsuperscript{2+} release in A7r5 cells (31). Similarly, in SMCs of the pulmonary and systemic vasculature, H\textsubscript{2}O\textsubscript{2} stimulated IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release, suggesting that ROS enhance IP\textsubscript{3}R signaling in SMCs (112, 183). In contrast, in coronary artery SMCs, H\textsubscript{2}O\textsubscript{2}, superoxide, and peroxynitrite inhibited the SR Ca\textsuperscript{2+}-ATPase (SERCA), leading to a reduction in [Ca\textsuperscript{2+}]\textsubscript{SR} that attenuated IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release (53, 65). In gallbladder and mesenteric artery SMCs, superoxide and H\textsubscript{2}O\textsubscript{2} did not alter IP\textsubscript{3}R activity (225, 238). Therefore, ROS regulation of SMC IP\textsubscript{3}Rs is multimodal and may be dependent on tissue of origin and ROS species involved.

In summary, IP\textsubscript{3}, [Ca\textsuperscript{2+}], and ATP are major modulators of IP\textsubscript{3}R activity in SMCs and protein kinases, pH, and ROS regulate IP\textsubscript{3}Rs through phosphorylation, controlling [IP\textsubscript{3}], IP\textsubscript{3} binding to IP\textsubscript{3}Rs, and Ca\textsuperscript{2+} sensitivity of IP\textsubscript{3}Rs.

**IP\textsubscript{3}R Communication with Ion Channels and Mitochondria in SMCs**

SMC IP\textsubscript{3}Rs communicate with several SR- and plasma membrane-localized ion channels and mitochondria to control intracellular signals and influence physiological functions.
Ryanodine-sensitive Ca\textsuperscript{2+} release channels. Ryanodine-sensitive Ca\textsuperscript{2+} release channels (RyRs) are tetrameric proteins that share a number of structural and functional characteristics with IP\textsubscript{3}Rs, including substantial amino acid sequence homology of the pore region and carboxy terminus (59, 142, 247). Analytical cell fractionation studies revealed that RyRs and IP\textsubscript{3}Rs are both SR membrane localized in SMCs (28, 56, 91). There is uncertainty as to whether IP\textsubscript{3}Rs and RyRs release Ca\textsuperscript{2+} from the same or distinct pools in SMCs. IP\textsubscript{3}Rs and RyRs have been suggested to share the same Ca\textsuperscript{2+} pool in SMCs of mesenteric, small pulmonary and renal arteries, portal vein, and small intestine (86, 91, 168, 219, 256). In colonic SMCs, two SR populations were identified: one expressing only RyRs and another with both RyRs and IP\textsubscript{3}Rs (56). Reports have also suggested that RyRs and IP\textsubscript{3}Rs do not share the same Ca\textsuperscript{2+} pool and that each channel can generate distinct Ca\textsuperscript{2+} signals in SMCs of the ureter and mesenteric and large pulmonary arteries (26, 32, 91, 219). Some of this discrepancy may have arisen due to different experimental conditions used to obtain data. Whether IP\textsubscript{3}Rs or RyRs release Ca\textsuperscript{2+} from the same or distinct pools may also depend on anatomical origin and physiological functions of the SMC types studied. Additional data, including that obtained by using identical experimental approaches in different SMC types, will be required to investigate these possibilities.

Ca\textsuperscript{2+} released from an IP\textsubscript{3}R or RyR activates the other channel type via CICR (12, 25, 28, 62, 91, 232). IP\textsubscript{3}R activation stimulated RyR-mediated Ca\textsuperscript{2+} release in SMCs of gallbladder, gastric antrum, portal vein, and renal artery (12, 25, 28, 62, 91, 232). RyR inhibitors also attenuated norepinephrine- and phenylephrine-induced IP\textsubscript{3}R-dependent Ca\textsuperscript{2+} release in portal vein and renal artery SMCs (28, 91). Therefore, IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release activates RyRs in SMCs and, in turn, RyRs also feedback to regulate IP\textsubscript{3}R activity. However, ryanodine, a RyR blocker, and anti-RyR channel antibodies did not alter agonist-induced IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release in pulmonary artery and ureteric SMCs (39, 91), suggesting that IP\textsubscript{3}R-RyR cross talk may not exist in all SMC-containing tissues.

Plasma membrane-localized ion channels. Research over the last decade has demonstrated that IP\textsubscript{3}Rs can regulate the activity of plasma membrane TRPC and large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BK\textsubscript{Ca}) channels through both SR Ca\textsuperscript{2+} release-dependent and -independent mechanisms in SMCs. Evidence suggests that IP\textsubscript{3}R communication with these ion channels is enabled by caveolae, which are plasma membrane microdomains located between the SR and plasma membranes in many cell types, including SMCs (Fig. 3, A and B).

TRPC CHANNELS. IP\textsubscript{3}R activation stimulates TRPC channels directly, through physical coupling and indirectly, through Ca\textsuperscript{2+}-dependent activation and induction of store-operated Ca\textsuperscript{2+} entry (SOCE). Endothelin (ET)-1 and uridine 5’-triphosphate, PLC-coupled receptor agonists, and IP\textsubscript{3}R stimulated physical coupling between the IP\textsubscript{3}R1 NH\textsubscript{2}-terminal TRPC coupling domain and the TRPC3 channel COOH-terminal CaM and IP\textsubscript{3}R binding (CIRB) domain in cerebral artery SMCs (Fig. 3C) (1, 2, 253). In mammals, the TRPC coupling domain sequence (L--E-W-L--W--D) and the CIRB domain sequence (Y--MK-LV-RYY) are conserved among the three IP\textsubscript{3}R isoforms and seven TRPC channels, respectively (208). The IP\textsubscript{3}R coupling domain displaces inhibitory CaM from the TRPC CIRB domain, leading to channel activation (253). Cerebral artery SMC TRPC6 channels also contain a CIRB domain but do not physically couple to IP\textsubscript{3}R1 due to spatial separation of these proteins (2). IP\textsubscript{3}R1-induced TRPC3 channel activation induced a cation current (I\textsubscript{Ca}), resulting in membrane depolarization, Ca\textsubscript{2+}/Ca\textsubscript{1.2} channel activation, and vasoconstriction (Fig. 3C) (237). This SR-Ca\textsuperscript{2+} release-independent mechanism is a major contributor to agonist-induced IP\textsubscript{3}R-mediated global [Ca\textsuperscript{2+}]\textsubscript{i} elevation and vasoconstriction in cerebral arteries (2, 237).

SMC IP\textsubscript{3}Rs also activate TRP channels through SR Ca\textsuperscript{2+} release-dependent mechanisms (61, 106, 206). ET-1-induced IP\textsubscript{3}R stimulation activated Ca\textsuperscript{2+} influx that was inhibited by TRPC1 knockdown in cultured aortic SMCs (206). IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release also activated Ca\textsuperscript{2+}-sensitive PKC isoforms, leading to PKC-dependent TRPC1 activation in portal vein SMCs (106). These studies suggested that IP\textsubscript{3}R stimulation activates TRPC1 channels via SR Ca\textsuperscript{2+} release, although it was unclear if physical coupling was also involved. In cerebral artery SMCs, SERCA and IP\textsubscript{3}R inhibitors reduced TRPM4-mediated native I\textsubscript{Ca} (61). TRPM4 is Ca\textsuperscript{2+} sensitive and does not contain a CIRB domain (2), indicating that SR-released Ca\textsuperscript{2+} and not direct interaction with IP\textsubscript{3}Rs is likely to regulate TRPM4 activation.

A reduction in SR Ca\textsuperscript{2+} load following IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release may feedback to stimulate SOCE, which exists in some but not all contractile and migratory/proliferative SMC types (5, 88, 97, 159, 185, 237, 239). In addition to Orai and STIM proteins, TRP channels may be a molecular component of SOCE channels (73, 180, 229). TRPC channels, including 1, 5, 6, and 7 contribute to SOCE in SMCs of coronary and mesenteric arteries and portal vein (186, 207). IP\textsubscript{3}R-mediated SR Ca\textsuperscript{2+} depletion activated TRPC4-dependent SOCE in SMCs of inferior vena cava, portal vein, and pulmonary arteries (108, 114, 160, 180).

SMC TRP channels are also activated by IP\textsubscript{3}R-independent mechanisms. TRPC channels, including 1, 3, 4, 5, 6, and 7 were activated by pathways that required PLC, phosphatidylinositol 4,5-bisphosphate, and/or DAG, but not IP\textsubscript{3}R or IP\textsubscript{3}Rs, in SMCs of coronary, ear, and mesenteric arteries, portal vein, and gastric antrum (5, 6, 93, 109, 110, 174, 185). Therefore, in SMCs, TRP channels are activated by PLC-mediated IP\textsubscript{3}R-dependent and -independent pathways. IP\textsubscript{3}R-dependent pathways include physical coupling with TRPC channels, Ca\textsuperscript{2+} release-mediated activation, and SR Ca\textsuperscript{2+} store depletion-induced stimulation.

BK\textsubscript{Ca} CHANNELS. SMC IP\textsubscript{3}Rs communicate with nearby plasma membrane BK\textsubscript{Ca} channels via SR Ca\textsuperscript{2+} release-dependent and -independent mechanisms (255) (Fig. 3D). IP\textsubscript{3}R-mediated SR Ca\textsuperscript{2+} release activated BK\textsubscript{Ca} channels in basilar artery SMCs (95). IP\textsubscript{3}R1 is located in close spatial proximity to, and coinmunoprecipitates with, plasma membrane BK\textsubscript{Ca} channels in cerebral artery SMCs (255). SMC IP\textsubscript{3}R1 activation elevated BK\textsubscript{Ca} channel Ca\textsuperscript{2+} sensitivity, thereby facilitating channel activation at lower Ca\textsuperscript{2+} concentrations in cerebral artery SMCs (255). The IP\textsubscript{3}R-mediated elevation in BK\textsubscript{Ca} channel Ca\textsuperscript{2+} sensitivity would enhance channel activation by SR Ca\textsuperscript{2+} release by local IP\textsubscript{3}Rs (255). IP\textsubscript{3}R-induced BK\textsubscript{Ca} channel activation would oppose TRPC3 channel-mediated membrane depolarization in SMCs and attenuate the IP\textsubscript{3}R-induced global [Ca\textsuperscript{2+}]\textsubscript{i} elevation and vasoconstriction (255).

MACROMOLECULAR COMPLEXES CONTAINING IP\textsubscript{3}R, IP\textsubscript{3}Rs, TRPC3, and BK\textsubscript{Ca} channels appear to be located in macromolecular
complexes that span from the SR membrane to plasma membrane caveolae in SMCs. Caveolae disruption, knockdown of caveolin-1 (cav-1), a caveolae scaffolding protein, and a cav-1 scaffolding domain peptide spatially separated IP3R1 and TRPC3 channels and attenuated IP3-induced ICa\textsubscript{L} activation in cerebral artery SMCs (1, 47, 83). Coimmunoprecipitation and immunofluorescence studies indicated that BKCa channels associate with cav-1 in aortic and cerebral artery SMCs (7, 255). Thus cav-1 likely maintains close spatial proximity of SR IP3R1 channels with both TRPC3 and BKCa channels in SMCs (Fig. 3, C and D) (1, 7, 255). Caveolae may permit localized coupling of IP3R1 to both TRPC3 and BKCa channels to modulate SMC membrane potential and arterial contractility. Therefore, IP3Rs, TRPC3, and BKCa channels appear to be located within the same cav-1-containing macromolecular complex that bridges the SR and plasma membrane in arterial SMCs (1, 255). RyRs generate Ca\textsuperscript{2+}-sparks that activate nearby plasma membrane BKCa channels in SMCs, inducing relaxation (90, 159). Given that SMC RyR channels can be located immediately (~20 nm) beneath the plasma membrane (90), IP3Rs and RyRs may also be contained within the same macromolecular complexes and communicate locally to each other and nearby plasma membrane ion channels. Conceivably, SMC RyRs may communicate with TRP and BKCa channels, thereby regulating membrane potential and contractility. Future research should be directed at exploring cellular localiza-
tion of RyRs and local signaling to IP₃Rs and plasma membrane ion channels in SMCs.

**Mitochondria.** Spatial localization of mitochondria nearby the SR permits local signaling between these two organelles. Given their local proximity, SR Ca²⁺ release can alter local mitochondrial activity and mitochondria can feedback to regulate nearby SR Ca²⁺ channels.

**IP₃R Regulation of Mitochondrial Ca²⁺ Concentration.** In airway and vascular SMCs, mitochondria are located near the SR membrane and IP₃R activation elevates mitochondrial Ca²⁺ concentration ([Ca²⁺]ₘito) (Fig. 3B and 4A) (41, 50, 66, 137, 146, 169, 176, 203). In tracheal SMCs, ~99% of mitochondria were within 30 nm of the SR membrane and ~82% of mitochondria were ensheathed by the SR (Fig. 4A) (41). Such close spatial proximity between mitochondria and SR enables local Ca²⁺ signaling between these two organelles in SMCs (41). The mitochondrial Ca²⁺ uniporter, the major mitochondrial Ca²⁺ uptake pathway, is sensitive to micromolar Ca²⁺ (175, 187). In SMCs, physiological global [Ca²⁺]ᵢ, which is ~100–300 nM, should not modify [Ca²⁺]ₘito (175, 187), a conclusion supported by published data (156). Local high [Ca²⁺]ᵢ elevations generated by nearby Ca²⁺ channels are necessary to elevate SMC [Ca²⁺]ₘito (35, 175). Receptor agonists that elevate IP₃ and activate IP₃Rs increase [Ca²⁺]ₘito to micromolar concentrations in permeabilized A10 cells and in cultured pregglomerular afferent arteriole, cultured and non-cultured aortic, pulmonary artery, and colonic SMCs (50, 66, 137, 146, 169, 176). SMC [Ca²⁺]ₘito measurements in these studies were obtained primarily by using inorganic fluorescent indicators. Recent evidence (156) obtained using a genetically encoded mitochondria-targeting Ca²⁺ indicator (2mt8CG2) indicated that IP₃R-mediated Ca²⁺ waves, but not global [Ca²⁺]ᵢ, elevated [Ca²⁺]ₘito and stimulated mitochondria-derived ROS generation in cerebral artery SMCs (Fig. 4B). This study provided evidence that in arterial SMCs, Ca²⁺ waves may be the IP₃R-mediated Ca²⁺ signal that communicates with...
nearby mitochondria to control [Ca\(^{2+}\)]\(_{\text{mito}}\) and mitochondrial ROS generation. In A10 cells and cultured aortic SMCs, agonist-induced [Ca\(^{2+}\)]\(_{\text{mito}}\) elevations measured using targeted aequorin closely matched the kinetics of [Ca\(^{2+}\)], transients (175, 203). In contrast, in noncultured aortic and pulmonary artery SMCs, agonist- and IP\(_3\)-induced [Ca\(^{2+}\)]\(_{\text{mito}}\) elevations outlasted [Ca\(^{2+}\)], transients by minutes (50, 66). These data suggest that changes in [Ca\(^{2+}\)] and [Ca\(^{2+}\)]\(_{\text{mito}}\) are not always synchronous and that mitochondria may retain Ca\(^{2+}\) in SMCs. The variability in results may also reflect experimental differences in the mitochondrial Ca\(^{2+}\) indicator used, protocol, or effects of cell culture.

**MITOCHONDRIAL REGULATION OF IP\(_3\)-MEDIATED CA\(^{2+}\) RELEASE.** Mitochondria regulate SMC IP\(_3\)Rs through multiple mechanisms, including buffering local and global [Ca\(^{2+}\)], controlling ATP synthesis and ROS signaling, and by modulating other ion channels that control membrane potential and [Ca\(^{2+}\)], (31, 35, 57, 105, 134, 137, 175, 176, 201–203). Inhibition of mitochondrial Ca\(^{2+}\) uptake and oxidative metabolism attenuated IP\(_3\)-mediated Ca\(^{2+}\) transients in colonic, gallbladder, cultured aortic, and tail artery SMCs (11, 137, 164, 202, 203). These data suggested that mitochondria directly modulate IP\(_3\)R activity and, in turn, influence generation and propagation of Ca\(^{2+}\) signals in SMCs (137, 203). It has been proposed that in SMCs, mitochondrial Ca\(^{2+}\) uptake may decrease [Ca\(^{2+}\)], nearby IP\(_3\)Rs, resulting in a reduction in Ca\(^{2+}\)-dependent inhibition, thereby stimulating further Ca\(^{2+}\) release (137, 203). ATP enhances IP\(_3\)R IP\(_3\) and Ca\(^{2+}\) sensitivity and is required for SERCA activity in SMCs (57, 105, 134). Thus mitochondria may control SMC IP\(_3\)R activity and Ca\(^{2+}\) release indirectly through ATP generation. Mitochondrial ROS and redox potential may also modulate SMC IP\(_3\)R expression and activity, thereby influencing IP\(_3\)R-mediated Ca\(^{2+}\) release (31, 201). In vascular SMCs, mitochondria regulate the activity of several plasma membrane ion channels, including voltage-dependent Ca\(^{2+}\), Ca\(^{2+}\)-activated K\(^+\) (K\(_{\text{Ca}}\)), Ca\(^{2+}\)-activated Cl\(^-\) (Cl\(_{\text{Ca}}\)), and SOCE channels (175). Mitochondrial modulation of these channels will influence membrane potential and also feedback to alter local and global Ca\(^{2+}\) signals, thereby indirectly influencing SMC IP\(_3\)R channel activity. Therefore, mitochondrial regulation of IP\(_3\)Rs in SMCs involves several integrated signaling pathways.

**IP\(_3\)Rs and Intracellular Ca\(^{2+}\) Signals in SMCs**

IP\(_3\)R activation generates a wide variety of Ca\(^{2+}\) signals in SMCs, including flashes, puffs, oscillations, ripples, sparks, waves, and global [Ca\(^{2+}\)], (13, 26, 90, 144, 254). Other Ca\(^{2+}\) channels, including nonspecific cation, RyR, TRP, SOCE, and voltage-dependent Ca\(^{2+}\) channels, also contribute to these events in certain SMC types. The following section will discuss IP\(_3\)-dependent Ca\(^{2+}\) signals and the modulation of these Ca\(^{2+}\) signals by other Ca\(^{2+}\) channels in SMCs. Properties, cell types, contributing Ca\(^{2+}\) channels, and physiological functions of IP\(_3\)-regulated Ca\(^{2+}\) signals in SMCs are summarized in Table 2.

**Ca\(^{2+}\) puffs.** Ca\(^{2+}\) puffs arise from the synchronous opening of ~30 IP\(_3\)R channels distributed within a ~400-nm diameter cluster in Xenopus oocytes (170, 194). Ca\(^{2+}\) puffs correspond to a Ca\(^{2+}\) current of 11–23 pA, with a Ca\(^{2+}\) current of ~0.4 pA per IP\(_3\)R (170, 194). In SMCs, Ca\(^{2+}\) puffs occur due to IP\(_3\)-mediated SR Ca\(^{2+}\) release, with a minor contribution from RyRs. In colonic SMCs, purinergic receptor stimulation elevated Ca\(^{2+}\) puff frequency and amplitude, which was attenuated by both xestospongin C (XeC), an IP\(_3\)R blocker, and ryanodine, indicating involvement of both IP\(_3\)Rs and RyRs (14). However, ryanodine and an anti-RyR antibody did not alter spontaneous and ACH-induced Ca\(^{2+}\) puffs in ureteric SMCs, suggesting that IP\(_3\)R activation alone can also generate these Ca\(^{2+}\) transients (26). Similarly, in guinea pig colonic SMCs, localized photolysis of IP\(_3\)-generated Ca\(^{2+}\) puffs, which were abolished by 2-aminoethoxydiphenyl borate, an IP\(_3\)R inhibitor (164). Thus Ca\(^{2+}\) puffs are stimulated by IP\(_3\)R activation, with RyRs contributing in certain SMC types. Clustering of IP\(_3\)Rs on the SR membrane has been proposed to be essential for Ca\(^{2+}\) puff generation in SMCs (26, 62, 91, 164). Consistent with this conclusion, IP\(_3\)R clustering and Ca\(^{2+}\) puffs were observed in colonic and ureteric SMCs but neither were detected in portal vein and pulmonary artery SMCs (26, 62, 91, 164). The physiological functions of Ca\(^{2+}\) puffs in SMCs have not been identified.

**Ca\(^{2+}\) flashes.** Spontaneous, rapid [Ca\(^{2+}\)], events termed “Ca\(^{2+}\) flashes” were observed during rhythmic phasic contractions of unstimulated gallbladder SMCs (13). Ca\(^{2+}\) flash frequency was reduced by inhibiting voltage-dependent Ca\(^{2+}\) channels, IP\(_3\)Rs, and RyRs, suggesting that all these channels contribute to these Ca\(^{2+}\) signals in SMCs (13). In contrast, in mesenteric artery and urinary bladder SMCs, Ca\(^{2+}\) flashes induced by electrical field stimulation were unaltered by deleting SR Ca\(^{2+}\), indicating that IP\(_3\)Rs and RyRs do not generate these signals in certain SMC types (104, 157). Ca\(^{2+}\) flashes also occurred in ~2% of resting tail artery SMCs but such low occurrence prevented detailed study of the contribution of IP\(_3\)Rs to these events (9).

**Ca\(^{2+}\) oscillations.** Ca\(^{2+}\) oscillations are repetitive, nonpropagating global [Ca\(^{2+}\)], elevations generated by periodic, pulsatile release of SR Ca\(^{2+}\) in SMCs (10, 18, 22, 29, 111). Ca\(^{2+}\) oscillations occur due to cyclical positive and negative feedback of [Ca\(^{2+}\)], on IP\(_3\)R channel activity with contributions from RyRs and TRPC channels (10, 18, 29, 111, 190). In isolated retinal arteriole SMCs, ET-1 increased Ca\(^{2+}\) oscillation frequency and this elevation was inhibited by blockers of IP\(_3\)Rs and RyRs, indicating that IP\(_3\)R-RyR cross talk stimulates Ca\(^{2+}\) oscillations (190, 218). Ca\(^{2+}\) oscillations in A7r5 cells required both IP\(_3\)R1-mediated SR Ca\(^{2+}\) release and TRPC6 as a receptor-operated Ca\(^{2+}\) influx pathway (111). In airway SMCs, IP\(_3\)R inhibition reduced Ca\(^{2+}\) oscillation frequency, leading to relaxation (10, 18). Similarly, in basilar artery SMCs, IP\(_3\)R-mediated Ca\(^{2+}\) oscillations activated Cl\(_{\text{Ca}}\) channels, leading to depolarization, Ca\(^{2+}\) influx, and vasoconstriction (67). These studies suggested that IP\(_3\)-dependent Ca\(^{2+}\) oscillations induce SMC contraction. In contrast, agonist-induced IP\(_3\)-mediated Ca\(^{2+}\) oscillations in mesenteric artery SMCs did not contribute Ca\(^{2+}\) for vasoconstriction (144). Thus the physiological functions of Ca\(^{2+}\) oscillations in some SMC types remain poorly understood.

**Ca\(^{2+}\) ripples.** Ca\(^{2+}\) ripples are spontaneous, low amplitude, propagating, IP\(_3\)-mediated Ca\(^{2+}\) signals that have been observed in unstimulated aortic, mesenteric, and tail artery SMCs (9, 249). Ca\(^{2+}\) ripples occur due to PLC activation and SR Ca\(^{2+}\) release that may be due to IP\(_3\)Rs in tail artery SMCs (9). Future studies would need to be performed using alternative approaches, including selective IP\(_3\)R antagonists and IP\(_3\)R
Table 2. SMC Ca\(^{2+}\) signals regulated directly or indirectly by IP\(_3\)Rs

<table>
<thead>
<tr>
<th>Ca(^{2+}) Signal</th>
<th>Properties</th>
<th>SMC Type</th>
<th>Additional Ca(^{2+}) Channels Involved</th>
<th>Physiological Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{2+}) puff</td>
<td>Frequency: 0.02–0.04 Hz/cell</td>
<td>Colon (14, 164), renal artery (91), ureter (26)</td>
<td>RyR (14, 91, 164)</td>
<td>Unclear</td>
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<tr>
<td></td>
<td>Amplitude*: 1.13–2.02</td>
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<td></td>
<td>Rise time: 57–160 ms</td>
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<td>(t_{1/2}) decay: 107–250 ms</td>
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<td></td>
<td>Spatial spread: 1.89–2.51 (\mu)m</td>
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<tr>
<td>Ca(^{2+}) flash</td>
<td>Frequency: 0.2–0.5 Hz/cell</td>
<td>Gallbladder (13)</td>
<td>RyR, voltage-dependent Ca(^{2+}) (13)</td>
<td>Contraction (13)</td>
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<td></td>
<td>Amplitude*: 1.5–2.6</td>
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<td></td>
<td>Rise time: 0.2–0.8 s</td>
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<td>(t_{1/2}) decay: 0.5–1.5 s</td>
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<tr>
<td>Ca(^{2+}) oscillation</td>
<td>Frequency: 0.07–0.4 Hz/cell</td>
<td>A7r5 cells (111), airway (10, 18), basilar artery (67), mesenteric artery (144), retinal artery (190, 218)</td>
<td>RyR (218), TRPC6 (111)</td>
<td>Contraction (10, 18, 67)</td>
</tr>
<tr>
<td></td>
<td>Amplitude*: 1.1–6.5</td>
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<td>Rise time: 2–6 s</td>
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<td>(t_{1/2}) decay: 3–18 s</td>
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<tr>
<td>Ca(^{2+}) ripple</td>
<td>Frequency: 0.05–0.19 Hz/cell</td>
<td>Aorta (9), mesenteric artery (249), tail artery (9)</td>
<td>Contraction (9, 249)</td>
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<td></td>
<td>Amplitude*: 1.05–1.8</td>
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<td>Rise time: 1–2 s</td>
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<td>(t_{1/2}) decay: 4–6 s</td>
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<td></td>
<td>Velocity(†): −18 (\mu)m/s</td>
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<tr>
<td>Ca(^{2+}) spark</td>
<td>Frequency: 0.07–0.59 Hz/cell</td>
<td>Choroidal and retinal arterioles (190, 218), pulmonary artery (252), trachea (116), vas deferens (233)</td>
<td>RyR (116, 190, 218, 233, 252)</td>
<td>Contraction (190, 218)</td>
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<td>Amplitude*: 1.34–2.05</td>
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<td>Rise time: 40–65 ms</td>
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<td>(t_{1/2}) decay: 30–150 ms</td>
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<td>Spatial spread: 0.52–4.7 (\mu)m</td>
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<tr>
<td>Ca(^{2+}) wave</td>
<td>Frequency: 0.04–1.41 Hz/cell</td>
<td>Cerebral artery (60, 61, 88, 89, 151, 156, 234, 254), choroidal and retinal arteriole (190), cressarteria and arteriole (177, 231), mesenteric arteriole (86, 104, 133, 249), pulmonary artery (76, 219), tail artery (40, 81), airway (17), cecum (70, 82), colon (15, 70, 117, 135, 136), duodenum (26), gallbladder (12), ileum (167), inferior vena cava (42, 107, 108, 184), portal vein (25, 28, 62, 145), trachea (94, 103, 179), ureter (26), urinary bladder (157)</td>
<td>Na(^{+})/Ca(^{2+}) exchanger (107, 108), nonselective cation (42), RyR (12, 17, 25, 28, 62, 70, 81, 88, 94, 145, 151, 179, 184), SOCE (107, 108), voltage-dependent Ca(^{2+}) (12, 42, 60, 70, 88, 107, 108, 179)</td>
<td>Contraction (17, 28, 42, 49, 70, 76, 103, 104, 107, 108, 133, 151, 184, 231, 249), gene expression (60), mitochondrial ROS generation (156), proliferation (234), relaxation (15)</td>
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<tr>
<td></td>
<td>Amplitude*: 1.4–6.9</td>
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<td>Rise time: 0.5–2 s</td>
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<td>(t_{1/2}) decay: 2–10 s</td>
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<td>Spatial spread: 8.8–50 (\mu)m</td>
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<tr>
<td></td>
<td>Velocity(†): 7–126 (\mu)m/s</td>
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</table>

ROS, reactive oxygen species; RyR, ryanodine-sensitive Ca\(^{2+}\) release channels; SOCE, store-operated Ca\(^{2+}\) entry; TRPC, transient receptor potential canonical. *F/F\(_0\), Propagation velocity.

Not only does this provide a comprehensive overview of the role of IP\(_3\)Rs in regulating Ca\(^{2+}\) signals in smooth muscle cells, but it also highlights the complex interplay between these channels and the physiological functions they regulate. This understanding is crucial for advancing our knowledge of vascular regulation and the development of therapeutic strategies for cardiovascular diseases.
sparks have been described in SMCs of a wide variety of tissues, including arteries, arterioles, veins, gallbladder, gastric antrum, intestine, trachea, ureter, and urinary bladder (72, 90, 178, 181, 230). IP$_3$Rs have been reported to contribute to Ca$^{2+}$ sparks, albeit in a small number of SMC types (116, 190, 218, 252). RyR- and IP$_3$R-mediated SR Ca$^{2+}$ release both stimulated Ca$^{2+}$ sparks in SMCs of choroidal and retinal arterioles, pulmonary artery, and trachea (116, 190, 218, 252). In contrast, IP$_3$R blockers reduced spontaneous waves in SMCs, although this may have occurred due to an [Ca$^{2+}$]$_{i}$ elevation (233). Reports (190, 218) in retinal and choroidal arteriole SMCs suggested that IP$_3$R-mediated Ca$^{2+}$ sparks may contribute to global [Ca$^{2+}$]$_{i}$, and contractility. However, whether localized Ca$^{2+}$ sparks directly influence SMC global [Ca$^{2+}$]$_{i}$ is uncertain.

Ca$^{2+}$ waves. Ca$^{2+}$ waves are propagating elevations in [Ca$^{2+}$]$_{i}$, that occur due to SR Ca$^{2+}$ release in vascular and nonvascular SMCs (12, 17, 28, 62, 70, 76, 89, 104, 135, 156, 167). The activation of IP$_3$Rs, RyRs, or both channels can control both the generation and propagation of spontaneous and agonist-induced Ca$^{2+}$ waves in SMCs. Figure 5 illustrates IP$_3$R-mediated Ca$^{2+}$ waves imaged in SMCs of vascular and nonvascular tissues.

Spontaneous Ca$^{2+}$ wave generation was inhibited by ryanodine in SMCs of cecum, colon, gallbladder, cerebral, cremaster and pulmonary arteries, and portal vein, suggesting that RyRs contributed to these events (12, 62, 70, 88, 151, 219, 231). Caffeine stimulated Ca$^{2+}$ waves, supporting the view that RyR activation alone can generate Ca$^{2+}$ waves in SMCs (70, 71, 145, 184). In contrast, IP$_3$R blockers reduced spontaneous Ca$^{2+}$ waves in SMCs of cecum, colon, gallbladder, portal vein, pulmonary, mesenteric, and cremaster feed arteries and arterioles, suggesting that IP$_3$Rs contribute to these signals in these SMC types (12, 15, 62, 70, 219, 231, 249). Basal release of receptor ligands by endothelial cells and circulating receptor agonists may also stimulate G$_q$/PLC-mediated IP$_3$ generation in SMCs, leading to spontaneous IP$_3$R-mediated Ca$^{2+}$ waves (61, 156). However, in cerebral artery SMCs, neither 50% knockdown of IP$_3$R1 or XeC altered baseline Ca$^{2+}$ wave frequency, suggesting that IP$_3$Rs do not contribute to the generation of spontaneous Ca$^{2+}$ waves in all SMC types (254). Thus the contribution of IP$_3$Rs to spontaneous Ca$^{2+}$ waves that occur in the complete absence of agonists in vascular SMCs is unclear. Ligands that bind to PLC-coupled receptors enhanced Ca$^{2+}$ wave generation in several SMC types, including cerebral, mesenteric, pulmonary, and tail arteries, cecum, colon, duodenum, gallbladder, ileum, trachea, inferior vena cava, portal vein, and ureter (12, 26, 28, 42, 49, 76, 82, 103, 104, 135, 167, 254). IP$_3$R inhibitors, anti-IP$_3$R antibodies, and IP$_3$R knockdown attenuated agonist-induced Ca$^{2+}$ waves, indicating that IP$_3$R activation is essential for generation of these Ca$^{2+}$ signals (17, 25, 26, 28, 42, 60, 62, 70, 76, 94, 104, 156, 157, 254). Thapsigargin, ryanodine, and anti-RyR antibodies also inhibited agonist-induced Ca$^{2+}$ waves in certain SMCs, suggesting that RyRs may also contribute to these events (17, 25, 27, 28, 62, 70, 81, 94, 104, 151, 179, 184). Therefore, IP$_3$Rs and RyRs can both influence the generation of spontaneous and agonist-induced Ca$^{2+}$ waves, with relative involvement of each appearing to vary in different SMC types. The contribution of IP$_3$Rs and RyRs to the propagation of Ca$^{2+}$ waves has also been examined in SMCs (28, 62, 135, 184, 231). In colonic SMCs, carbachol and IP$_3$ photolysis stimulated local SR Ca$^{2+}$ release, which transformed into a propagating Ca$^{2+}$ wave only in the presence of cytosolic Ca$^{2+}$.
and a steady [IP$_3$]. (135). This study indicated that IP$_3$R activation alone is sufficient for propagation of these Ca$^{2+}$ signals in SMCs. An alternative concept of Ca$^{2+}$ wave progression suggests that IP$_3$R-mediated Ca$^{2+}$ release initiates a Ca$^{2+}$ wave in SMCs, which then propagates exclusively due to RyR-dependent CICR (28, 62, 184). RyR contribution to Ca$^{2+}$ wave propagation has been examined primarily by using ryanodine to inhibit these channels (28, 62, 184). However, ryanodine may inhibit Ca$^{2+}$ wave propagation by depleting SR Ca$^{2+}$ and not solely by blocking RyRs (81, 86, 117, 136, 151). In contrast, tetracaine, which blocks RyRs without depleting SR Ca$^{2+}$, attenuated spontaneous Ca$^{2+}$ waves in SMCs of cremaster feed arteries, suggesting that RyRs can regulate Ca$^{2+}$ wave propagation in certain SMC types (231). Studies have proposed that Ca$^{2+}$ influx via membrane proteins, including the Na$^+$/Ca$^{2+}$ exchanger, nonselective cation channels, SOCE channels, and voltage-dependent Ca$^{2+}$ channels, also contributes to Ca$^{2+}$ wave propagation and maintenance in certain SMC types (12, 42, 60, 70, 88, 107, 108, 179).

In summary, IP$_3$Rs contribute to Ca$^{2+}$ wave generation and propagation in SMCs. Evidence, obtained in many cases by using nonspecific pharmacological tools, also suggests that RyRs and other Ca$^{2+}$ channels regulate Ca$^{2+}$ wave propagation in some SMC types. Global [Ca$^{2+}$]$_i$, Global [Ca$^{2+}$], is spatially averaged cytosolic [Ca$^{2+}$]. An elevation in global [Ca$^{2+}$], induces contraction, whereas a reduction in global [Ca$^{2+}$], results in relaxation (22, 29, 90). IP$_3$R activation elevates SMC global [Ca$^{2+}$], directly, through SR Ca$^{2+}$ release and indirectly, by stimulating Ca$^{2+}$ influx via plasma membrane-localized ion channels. IP$_3$R-mediated SR Ca$^{2+}$ release elevates global [Ca$^{2+}$], in a wide variety of different SMC types (Table 2). IP$_3$R activation stimulates plasma membrane-localized TRPC1, TRPM4, and voltage-dependent Ca$^{2+}$ channels, leading to Ca$^{2+}$ influx and global [Ca$^{2+}$], elevation in basilar, cerebral artery, and aortic SMCs (61, 67, 206). In cerebral artery SMCs, physical coupling of IP$_3$,IP$_3$R1 to TRPC3 channels results in membrane depolarization and an indirect elevation in global [Ca$^{2+}$], via voltage-dependent Ca$^{2+}$ channel activation (2). In SMCs of aorta, mesenteric, and pulmonary arteries, cremaster arterioles, inferior vena cava, and portal vein, IP$_3$R-mediated SR Ca$^{2+}$ depletion activated SOCE, leading to a global [Ca$^{2+}$] elevation (98, 107, 108, 114, 160, 177, 180, 220, 250). Collectively, these studies indicate that IP$_3$R activation can elevate global [Ca$^{2+}$], directly, through SR Ca$^{2+}$ release and indirectly, through stimulation of plasma membrane ion channel-dependent Ca$^{2+}$ influx in SMCs.

The relative contribution of IP$_3$R-mediated SR Ca$^{2+}$ release-dependent and -independent mechanisms to global [Ca$^{2+}$], elevations was examined in cerebral artery SMCs (237). SR Ca$^{2+}$ depletion reduced IP$_3$-induced global [Ca$^{2+}$], elevation by ~25%, suggesting that the contribution of IP$_3$R-mediated SR Ca$^{2+}$ release to global [Ca$^{2+}$] is minor (237). TRPC3 knockdown reduced IP$_3$-induced global [Ca$^{2+}$], elevation by ~70%, indicating that IP$_3$R physical coupling to TRPC3 channels and the resulting plasma membrane Ca$^{2+}$ influx are responsible for the majority of the global [Ca$^{2+}$], elevation (237). Therefore, IP$_3$R-mediated Ca$^{2+}$ release can make a minor direct contribution to global [Ca$^{2+}$], with IP$_3$R control of membrane potential and Ca$^{2+}$ influx being prominent indirect mechanisms by which IP$_3$Rs influence global [Ca$^{2+}$]. Reports have also suggested that IP$_3$Rs do not regulate global [Ca$^{2+}$], in certain SMC types (88, 144, 157, 254). In mesenteric, cerebral artery, and urinary bladder SMCs, IP$_3$R-mediated Ca$^{2+}$ waves and oscillations did not elevate global [Ca$^{2+}$]. Tissue-specific variability in IP$_3$R modulation of global [Ca$^{2+}$] could be due to multiple factors, including differences in the amplitude of Ca$^{2+}$ signals generated, activation and contribution of other Ca$^{2+}$ channels, and regulation of IP$_3$R activity by tissue-specific proteins. In summary, IP$_3$R activation, sometimes with the involvement of other Ca$^{2+}$ channels, produces a wide variety of intracellular Ca$^{2+}$ signals, which control several physiological functions in SMCs.

**IP$_3$R Regulation of SMC Physiological Functions**

IP$_3$Rs regulate SMC physiological functions, including contractility, gene expression, migration, and proliferation (Table 2). IP$_3$R control of these functions can occur via the modulation of intracellular Ca$^{2+}$ signals and via local communication with ion channels and organelles.

**Contractility.** IP$_3$R-mediated SR Ca$^{2+}$ release stimulates contraction in both vascular and nonvascular SMCs. In gallbladder SMCs, IP$_3$R activation contributed to Ca$^{2+}$ flashes and action potentials that preceded rhythmic contractions of individual smooth muscle bundles (13). IP$_3$R-mediated Ca$^{2+}$ oscillations induced airway and basilar artery contraction (10, 18, 67). Ca$^{2+}$ sparks generated by IP$_3$R and RyR activation contributed to vasoconstriction in retinal and choroidal arteries (190, 218). Reports in mesenteric, pulmonary, and tail arteries, inferior vena cava, and portal vein proposed that IP$_3$R-mediated Ca$^{2+}$ waves directly induce vasoconstriction (28, 42, 49, 76, 86, 104, 107, 108, 133, 184, 249). XeC inhibited agonist-induced contraction in iliac lymph vessels, pulmonary arteries, and small femoral artery branches, indicating that agonists induce vasoconstriction through IP$_3$R activation (87, 99, 121, 128, 235). Similarly, in nonvascular SMCs of ileum, prostate, small intestine, and trachea, IP$_3$R blockers inhibited spontaneous and agonist-induced contraction (54, 103, 122, 167). In airway and intestinal SMCs, membrane depolarization activated IP$_3$Rs and RyRs, which contributed to contraction (17, 70, 96, 115). Collectively, IP$_3$R-mediated Ca$^{2+}$ release regulates spontaneous and agonist-induced contraction of vascular and nonvascular SMCs. IP$_3$R activation and the resulting SR Ca$^{2+}$ store depletion also stimulated SOCE channel-dependent Ca$^{2+}$ influx, which induced contraction of SMCs of aorta, inferior vena cava, cremaster, and pulmonary arteries (107, 108, 160, 177, 220, 250). IP$_3$R activation has also been reported to induce relaxation of certain SMC types (15, 102). In colonic SMCs, IP$_3$R-mediated Ca$^{2+}$ release stimulated K$_{Ca}$ channels, resulting in spontaneous transient outward currents, hyperpolarization, and relaxation (15, 102). However, in urinary bladder SMCs, electrical field stimulation enhanced Ca$^{2+}$ waves, which did not induce contraction (157), suggesting that IP$_3$Rs do not control contractility in certain SMC types.

IP$_3$R regulation of pressure-induced myogenic vasoconstriction has been examined in several vascular beds. U-73122, a PLC inhibitor, dilated pressurized cerebral and ophthalmic arterioles, suggesting that DAG/PKC and/or IP$_3$/IP$_3$Rs contribute to myogenic tone (85, 92, 166, 237). Inhibitors of PLC or IP$_3$Rs attenuated Ca$^{2+}$ waves and dilated pressurized cremaster muscle feed arteries and arterioles, suggesting that IP$_3$R-de-
ependent Ca\(^{2+}\) waves regulate myogenic tone in these vessels (231). Similarly, in pressurized mesenteric and tail arteries, IP\(_3\)R-mediated Ca\(^{2+}\) ripples were proposed to stimulate myogenic constriction (9, 249). SR-dependent Ca\(^{2+}\) waves contributed to myogenic tone at low intravascular pressure in cerebral artery SMCs, although the contribution of IP\(_3\)R release was not determined (151). These studies suggest that IP\(_3\)R-mediated Ca\(^{2+}\) release regulates the myogenic response. In contrast, in pressurized mesenteric and cerebral arteries, IP\(_3\)R-dependent Ca\(^{2+}\) oscillations and waves did not contribute significantly to myogenic vasoconstriction (88, 144, 254). Explanations for these different observations include that the frequency and amplitude of IP\(_3\)R-mediated Ca\(^{2+}\) signals are likely to be important factors that determine functional impact on contractility. In addition, the activation status of other signaling pathways that may enhance the sensitivity of the contractile apparatus to IP\(_3\)R-induced Ca\(^{2+}\) release is also likely to modify functional responses.

IP\(_3\)Rs also control arterial SMC contractility via an SR Ca\(^{2+}\) release-independent mechanism that involves physical coupling to plasma membrane TRPC3 channels (Fig. 3C) (1, 2, 237, 254). In cerebral arteries at physiological intravascular pressure, the majority (~60\%) of IP\(_3\)-induced vasoconstriction occurred through IP\(_3\)R1-TRPC3 physical coupling, with a minor contribution from IP\(_3\)R-mediated SR Ca\(^{2+}\) release (237). At low intravascular pressure, where [Ca\(^{2+}\)]\(_{SR}\) is low and the driving force for cation influx is high, IP\(_3\)R1-TRPC3 physical coupling fully accounted for IP\(_3\)-induced vasoconstriction (237). Thus, in cerebral artery SMCs, IP\(_3\)Rs induce contraction primarily via an SR Ca\(^{2+}\) release-independent mechanism that involves TRPC3 channel activation. In summary, SMC contractility regulation by IP\(_3\)Rs appears to be tissue and stimulus dependent. Contrary to what was previously a prevailing view, IP\(_3\)R-mediated Ca\(^{2+}\) release induces contraction in some, but not all, SMC types, with IP\(_3\)R activation also stimulating contraction via SR Ca\(^{2+}\) release-independent mechanisms.

**Gene expression.** IP\(_3\)R-mediated Ca\(^{2+}\) release can activate transcription factors, thereby regulating gene expression in SMCs (40, 51, 60, 156). In cerebral artery SMCs, IP\(_3\)R-mediated SR Ca\(^{2+}\) release activated NF-kB both directly and indirectly, by stimulating mitochondrial ROS production (Fig. 4B) (156). IP\(_3\)R-dependent NF-kB activation stimulated Cav1.2 channel \(\alpha_{1C}\) subunit gene expression, leading to vasoconstriction (156). NF-kB p105/p50 subunit expression was also upregulated by IP\(_3\)R-mediated SR Ca\(^{2+}\) release via a mitochondrial ROS- and NF-kB-independent pathway (156).

In cerebral artery and cultured aortic SMCs, IP\(_3\)R-dependent SR Ca\(^{2+}\) release stimulated nuclear factor of activated T-cell c3, a transcription factor that can downregulate KCa channel \(\beta1\) subunit and Kv2.1 channel expression (8, 60, 113, 161). IP\(_3\)R-mediated Ca\(^{2+}\) release also activated cAMP response element-binding protein, a Ca\(^{2+}\)-dependent transcription factor, in SMCs of cerebral arteries and the portal vein (40, 51). Therefore, Ca\(^{2+}\) released by IP\(_3\)Rs modulates multiple transcription factors that can control ion channel gene expression in SMCs.

**Migration and proliferation.** Vascular SMCs proliferate and migrate during vasculogenesis and in response to injury and are associated with elevated IP\(_3\)R expression and Ca\(^{2+}\) release (3, 20, 149, 205, 228, 234). Proliferation correlated with an elevation in IP\(_3\)R1 expression in aortic and carotid artery SMCs and upregulation of all three IP\(_3\)R isoforms in mesenteric artery SMCs (3, 20). Consistent with these observations, Ca\(^{2+}\) wave frequency was elevated in proliferating cerebral artery SMCs (234). PLC and IP\(_3\)R antagonists attenuated Ca\(^{2+}\) waves and proliferation in cerebral artery SMCs, indicating that IP\(_3\)R-mediated Ca\(^{2+}\) release stimulates proliferation (234). Similarly, in cultured aortic SMCs, IP\(_3\)R-dependent intercellular Ca\(^{2+}\) waves promoted proliferation and migration (149). An elevation in IP\(_3\)R-mediated Ca\(^{2+}\) release also stimulated proliferation of cultured pregglomerular microvascular SMCs (36). XeC inhibited pulsatile pressure-induced aortic SMC migration, and IP\(_3\)R1 knockdown reduced A7r5 cell proliferation (205, 228). Taken together, these findings provide strong evidence that IP\(_3\)R activation stimulates SMC migration and proliferation.

**IP\(_3\)Rs and SMC Pathologies**

**Vascular SMC diseases.** Vascular SMC IP\(_3\)R expression, [IP\(_3\)], and Ca\(^{2+}\) signaling are altered in several diseases, including hypertension, atherosclerosis, and diabetes-related vascular complications (19, 125, 130, 138, 192, 193, 236). Genetic hypertension in rats is associated with elevations in both IP\(_3\)R1 and IP\(_3\)R IP\(_3\)-binding affinity in SMCs. Basal and phenylephrine-induced [IP\(_3\)]\(_i\) were both higher in cultured aortic SMCs of spontaneously hypertensive rats than Wistar-Kyoto controls (236). A [\(^{3}\)H]IP\(_3\) binding assay indicated that the IP\(_3\)-binding capacity of IP\(_3\)Rs was significantly higher in aortic SMCs of spontaneously hypertensive rats compared with Wistar-Kyoto rats (19). Although myogenic tone and global [Ca\(^{2+}\)]\(_i\) are higher in vascular SMCs of hypertensive rats, compared with normotensive controls (92), altered vascular SMC IP\(_3\)R expression and Ca\(^{2+}\) signaling have not been reported.

During the pathogenesis of atherosclerosis, vascular SMCs are exposed to oxidized LDL, which stimulates plaque formation (130). In aortic SMCs exposed to oxidized LDL and in atherosclerotic aorta, IP\(_3\)R1 protein and IP\(_3\)R-dependent Ca\(^{2+}\) release were reduced (125, 130). SERCA2b expression was also downregulated in atherosclerotic aorta, suggesting that an [Ca\(^{2+}\)]\(_i\) drop may also contribute to the impaired IP\(_3\)R-mediated Ca\(^{2+}\) release in atherosclerosis (125, 130).

Diabetes-related vascular complications are associated with a reduction in IP\(_3\)R expression and Ca\(^{2+}\) release in SMCs of aorta and glomerular arterioles (138, 192, 193). In renal glomerular arteriolar SMCs of diabetic rats, IP\(_3\)R protein was lower than in nondiabetic controls (138). Protein expression of all three IP\(_3\)R isoforms and IP\(_3\)R-mediated [Ca\(^{2+}\)]\(_i\) signals were attenuated in aortic SMCs of genetic and inducible animal models of diabetes (192). High glucose reduced IP\(_3\)R protein in A7r5 cells, suggesting that a diabetes-induced reduction in vascular SMC IP\(_3\)R levels may be due to direct effects of hyperglycemia (192). IP\(_3\)R1 and IP\(_3\)R-mediated [Ca\(^{2+}\)]\(_i\) elevations were reduced, and TGF-\(\beta\) levels were elevated in diabetic rat aorta (193). Intraperitoneal administration of an anti-TGF-\(\beta\) antibody partly restored IP\(_3\)R1 expression and IP\(_3\)R-mediated [Ca\(^{2+}\)]\(_i\) elevations in aortic and glomerular arteriolar SMCs and prevented the development of glomerular hypertrophy (138, 193). These reports suggested that TGF-\(\beta\)-induced suppression of IP\(_3\)R expression underlies vascular dysfunction, leading to diabetic glomerular hypertrophy. Collectively, these studies indicate that an alteration in IP\(_3\)R...
expression and IP₃R-mediated Ca²⁺ signaling in vascular SMCs contributes to the pathogenesis of vascular diseases.

Asthma. Asthma is associated with enhanced SMC [IP₃]ᵢ, IP₃R signaling, and contractility, leading to airway hyperresponsiveness and remodeling (132, 209). In Fisher rats, an asthmatic animal model, IP₃-5-phosphatase expression and activity were both reduced, leading to elevations in [IP₃]ᵢ and IP₃R-mediated Ca²⁺ release in airway SMCs (209). This enhanced IP₃-induced Ca²⁺ release may underlie airway SMC hyperresponsiveness in asthma (209). 2-Aminoethoxydiphenyl borate inhibited acidic pH-induced remodeling of airway SMCs, suggesting that IP₃Rs may also regulate extracellular matrix formation and airway remodeling in asthma (132).

Pathologies associated with IP₃R gene deletion and mutation. Studies performed in IP₃R knockout (IP₃R⁻⁻⁻) mice have provided valuable information regarding pathologies associated with these proteins, although limited information is available regarding SMC dysfunction. IP₃R1⁻⁻⁻ mice are rarely born alive, indicating that IP₃R1 is crucial for embryonic development (131). Animals that survive exhibit severe neurological pathologies, including ataxia and epilepsy (131). IP₃R1⁻⁻⁻ mice displayed increased susceptibility to glucose intolerance, insulin resistance, and diet-induced diabetes (243). A report (200) that examined SMC function in IP₃R1⁻⁻⁻ mice determined that gastric SMCs exhibited irregular bursts of spike potentials, resulting in reduced contractility.

Population analysis studies have identified human diseases caused by IP₃R gene mutations. Similar to observations in knockout mice, there is a strong correlation between IP₃R gene mutations and neurological disease. A heterozygous deletion and a mutation in the IP₃R1 gene have been identified in patients with spinocerebellar ataxia and tremors (68, 221). SMC pathologies have not been reported that are associated with deletions or mutations in IP₃R genes. Recent studies (75, 165) reported that single nucleotide polymorphisms in genes encoding IP₃R3 and IP₃ 3-kinase C, which phosphorylates IP₃ to IP₄, predisposed children with Kawasaki disease, a pediatric systemic vascular autoimmune disease, to coronary artery aneurysms.

Summary

Since the discovery of IP₃Rs more than 20 years ago, research has identified these proteins as SR-located ion channels that are expressed in virtually all cell types. In SMCs, IP₃R

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**Fig. 6. IP₃R signaling in SMCs.** Illustrated are the following: pathways controlling IP₃R cellular expression (A), regulators of IP₃R channel activity (B), ion channels and organelles that communicate with IP₃Rs (C), Ca²⁺ signals generated by IP₃R-mediated Ca²⁺ release (D), and physiological functions modulated by IP₃Rs (E). AP-2, activator protein 2; RyR, ryanodine-sensitive Ca²⁺ release channels; CREB, cAMP response element-binding protein; NFATc3, nuclear factor of activated T-cell c3; RACK, receptor for activated PKC 1; FKBP12, 12-kDa FK506 binding protein.
signaling pathways and mechanisms involved are diverse (Fig. 6). IP₃R isoforms, cellular expression, and localization influence channel functions (Fig. 6A). Several endogenous ligands, kinases, and proteins regulate SMC IP₃R activity (Fig. 6B). IP₃R signaling involves communication with cellular organelles, including mitochondria, RyRs, and plasma membrane ion channels in SMCs (Fig. 6C). IP₃R-mediated cellular pathways include both SR Ca²⁺ release-dependent and -independent mechanisms regulated through physical coupling to ion channels and control of SOCE. IP₃R-mediated Ca²⁺ release generates a wide variety of local and global Ca²⁺ signals in SMCs (Fig. 6D). IP₃R-dependent cellular mechanisms control SMC physiological functions, including contraction, gene expression, migration, and proliferation (Fig. 6E). Diseases of SMC-containing tissues are associated with altered IP₃R expression and channel activity. Thus IP₃Rs control SMC physiological functions and pathological alterations in IP₃R signaling contribute to disease.

Future Directions

This review has summarized studies investigating IP₃R expression, cellular localization, activity, Ca²⁺ signals generated, physiological functions regulated, and associated pathologies in SMCs. There are still major questions that remain regarding IP₃R functionality in SMCs. Studies are necessary to better determine mechanisms that regulate IP₃R gene transcription and protein expression in SMCs. IP₃R signaling in non-SMCs is modulated by a wide variety of proteins, including calmodulin, Ca²⁺-binding protein 1, Ca²⁺- and integrin-binding protein 1, chromogranins, ERp44, IP₃R-binding protein released with IP₃, and Gβγ (57, 212). Modulation of IP₃R signaling by these proteins should be examined in SMCs. Studies should also be aimed at determining other cellular organelles and ion channels that interact with IP₃Rs in SMCs. A better understanding of the contribution of RyRs and other Ca²⁺ channels to IP₃R-mediated Ca²⁺ signals in SMCs is also needed. Research is required to elucidate mechanisms that underlie tissuespecific variability in IP₃R signaling in SMCs. Studies would benefit from the availability of novel, selective, high-affinity, membrane-permeant IP₃R antagonists to investigate IP₃R signaling, physiological functions, and pathological alterations. SMC-specific IP₃R animal models, including those that induce knockout, express IP₃R mutations associated with human diseases, or alter known IP₃R coupling domains, would also provide the capability to investigate the contribution of these proteins to physiological functions and pathological alterations both in vivo and in vitro. SMC dysfunctions in IP₃R knockout mice also need to be examined. Screening of humans to identify potential IP₃R mutations associated with SMC diseases is also necessary.

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