Inositol trisphosphate receptors in smooth muscle cells

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Inositol 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 (SMCs). 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.

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 Ca\textsuperscript{2+}/H\textsuperscript{+} (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 Ca\textsuperscript{2+}-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 developmental 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 ureteric 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), Ca\textsuperscript{2+}-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, NH\textsubscript{2} terminus; C, COOH terminus.
**Table 1. Important features of the three mammalian IP₃R isoforms**

<table>
<thead>
<tr>
<th></th>
<th>IP₃R1</th>
<th>IP₃R2</th>
<th>IP₃R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homology with human IP₃R1</td>
<td>–</td>
<td>77% (242)</td>
<td>72% (123, 242)</td>
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<tr>
<td>Alternative splicing</td>
<td>Yes (163, 241)</td>
<td>None described</td>
<td>None described</td>
</tr>
<tr>
<td>Amino acid number (human)</td>
<td>2,695–2,743 (splice variation-dependent) (69, 163, 241)</td>
<td>2,701 (242)</td>
<td>2,671 (123, 242)</td>
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<tr>
<td>Chromosomal localization (human)</td>
<td>3p25–26 (241)</td>
<td>12p11 (242)</td>
<td>6p21 (242)</td>
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<tr>
<td>Cellular localization in SMCs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central SR</td>
<td>Cerebral artery (1, 2, 254), cultured and noncultured aorta (162, 199, 211), A7r5 cells (224)</td>
<td>Cultured aorta (199, 211)</td>
<td>Cultured aorta (199, 211)</td>
</tr>
<tr>
<td>Peripheral SR</td>
<td>Cerebral artery (1, 2, 254), cultured aorta (199, 211), vas deferens (162), ileum (63)</td>
<td>Cultured aorta (199, 211)</td>
<td>Cultured aorta (199, 211), ureter (26)</td>
</tr>
<tr>
<td>IP₃ affinity</td>
<td>Intermediate [0.27 M]* (217)</td>
<td>Highest (0.10 M)* (217)</td>
<td>Lowest [0.40 M]* (217)</td>
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<tr>
<td>Regulation by Ca²⁺</td>
<td></td>
<td></td>
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<tr>
<td>Activation</td>
<td>Yes [0.17 M]* (216)</td>
<td>Yes [0.15 M]* (216)</td>
<td>Yes [0.06 M]* (216)</td>
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<tr>
<td>Inhibition</td>
<td>Yes [0.37 M]* (216)</td>
<td>Yes [0.16 M]* (216)</td>
<td>Yes [0.17 M]* (216)</td>
</tr>
<tr>
<td>Effect of Ca²⁺ on binding</td>
<td>Decreases (33, 244)</td>
<td>None described</td>
<td>&lt;500 nM increases, &gt;500 nM decreases (33, 244)</td>
</tr>
<tr>
<td>Regulation by ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of binding sites</td>
<td>3–4 (171)</td>
<td>1 (171)</td>
<td>2 (171)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>High [0.13 mM]* (217)</td>
<td>None (217)</td>
<td>Low [2.0 mM]* (217)</td>
</tr>
</tbody>
</table>

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.

**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 aortic SMCs (3). In A7r5 cells, retinoic acid inhibited IP₃R1 gene transcription through a mechanism mediated by activator protein-2, another transcription factor (45). In cultured aortic SMCs, hydrogen peroxide (H₂O₂) stimulated proteosomal degradation of IP₃R1 and IP₃R3, whereas Jak2, a tyrosine kinase, phosphorylated IP₃R1 and prevented proteosomal degradation (129, 227). Transforming growth factor-β (TGF-β) reduced IP₃R1 and IP₃R3 protein in renal arteriolar SMCs, although it was not determined if this was due to protein degradation (138). In A7r5 cells, vasopressin inhibited IP₃R1 gene transcription and also stimulated protein degradation, suggesting that a physiological agonist can modulate SMC IP₃R levels through both of these mechanisms (197). Therefore, regulation of both gene transcription and protein degradation controls IP₃R cellular levels in SMCs.

In SMCs, IP₃Rs locate to the SR in different cellular regions, including central SR around the nucleus and peripheral SR beneath the plasma membrane (Table 1) (2, 26, 63, 162, 199, 211, 224, 254). IP₃R localization to central and/or peripheral SR is isoform and tissue dependent. In cerebral artery SMCs, IP₃R1 distributes to both central and peripheral SR (Fig. 2A) (2, 254). However, IP₃R1 and IP₃R3 located only to peripheral SR in ileal and ureteric SMCs, respectively (26, 63). IP₃Rs on central SR around the nucleus may regulate Ca²⁺-dependent gene expression through signals that do not impact global [Ca²⁺]. Distribution to peripheral SR may position IP₃Rs in close proximity to the plasma membrane, thereby permitting local signaling to membrane proteins, including ion channels (2, 255). Therefore, IP₃R cellular localization in SMCs likely contributes to distinct physiological functions regulated by these proteins.

**IP₃R Channel Properties in Vascular SMCs**

Single channel properties of vascular SMC IP₃Rs have been examined using purified aortic IP₃Rs reconstituted into planar lipid bilayers (134). 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 IP₃-mediated Ca²⁺ release in vascular SMCs have been investigated using permeabilized portal vein smooth muscle strips and flash photolysis of caged IP₃ (198). Binding of IP₃ to a single IP₃R subunit, as opposed to all four subunits of the tetramer, was sufficient to induce channel opening (198). IP₃ photolysis elevated [Ca²⁺]i elevation was inversely proportional to [IP₃] (198). The delay between IP₃ photolysis and the [Ca²⁺]i elevation was inversely proportional to [IP₃], with saturating [IP₃] (>4 μM) and submaximal [IP₃] (0.4–0.8 μM) inducing Ca²⁺ release within 10 ms or after 420 ms, respectively (198). Collectively, these studies (134, 198) provided important information regarding biophysical properties of IP₃Rs and the kinetics of IP₃-mediated Ca²⁺ release in vascular SMCs.
**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 Kᵦ of 2–5 nM in SMCs of the aorta, colon, ileum, jejunum, myometrium, and vas deferens (4, 24, 34, 150, 182, 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 square in the pore) (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 IP₃Rs 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 IP₃-induced currents (Fig. 2C) (134). Similarly, in permeabilized portal vein SMCs, ATP dose-dependently increased IP₃-induced Ca²⁺ release with a maximal effect at 0.5 mM ATP (80). ATP also elevates SMC IP₃R Ca²⁺ sensitivity,
PKG phosphorylated SMC IP3Rs at serine 1755 and inhibited IP3R-mediated Ca2+ release in aortic and gastric SMCs (100, 154). FKBP12 phosphorylated IP3Rs via IP3R-associated cGMP kinase substrate (IRAG) in gastric and tracheal SMCs (153, 154, 189, 226). PKG/IRAG-dependent IP3R phosphorylation and subsequent inhibition of IP3R-mediated Ca2+ release relaxed colonic and aortic SMCs (46, 58). These studies suggest that PKG/IRAG regulation of IP3R phosphorylation and Ca2+ release regulates SMC contractility. PKA activation reduced PLC-dependent IP3 generation and decreased the number of binding sites for IP3 on IP3Rs, thereby attenuating IP3R-mediated Ca2+ release in tracheal SMCs (120, 191). Two-dimensional phosphopeptide mapping revealed that PKA phosphorylated SMC IP3Rs at serine 1589 (43). In iris sphincter SMCs, PKA inhibited IP3R-mediated Ca2+ release, resulting in relaxation (48, 204). It has been proposed that PKG is required for PKA-mediated phosphorylation of IP3Rs in SMCs. In gastric SMCs and intact aorta, PKA phosphorylated IP3Rs at two sites: serine 1589, a PKA phosphorylation site, and serine 1755, a PKG-specific site, suggesting that PKA activates PKG to phosphorylate IP3Rs (101, 154). Therefore, PKA-PKG cross talk may contribute to PKA-mediated phosphorylation of SMC IP3Rs.

PKC and tyrosine kinases enhance SMC IP3R signaling through IP3R phosphorylation and by elevating intracellular [IP3] ([IP3]i). PKC and tyrosine kinase phosphorylation sites on SMC IP3Rs are yet to be identified. PKC-mediated IP3R phosphorylation elevated [Ca2+]i 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-γ1 phosphorylation and elevations in [IP3]i and [Ca2+]i, suggesting that tyrosine kinases stimulate IP3 generation in SMCs (126). Similarly, tyrosine kinase inhibitors attenuated agonist-induced elevations in [IP3]i and [Ca2+]i, 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 IP3R-mediated Ca2+ signaling and contraction directly, through phosphorylation and indirectly, by controlling IP3 generation and IP3 binding.

Regulatory proteins. Evidence suggests that cytosolic proteins, including the 12-kDa FK506 binding protein (FKBP12) and receptor for activated PKC 1 (RACK1) influence IP3R channel activity in SMCs (15, 36, 118, 173). FKBP12, an intracellular ligand for immunosuppressants FK506 and rapamycin, interacts with and regulates SMC IP3Rs (15, 118). FKBP12 coimmunoprecipitated with IP3R1 in colonic SMCs, suggesting physical interaction (118). FKBP12 modulated colonic SMC IP3R activity via three effector proteins: calcineurin, FK506, and mammalian target of rapamycin (mTOR) (15, 118). FKBP12 potentiated IP3R-mediated Ca2+ release through mTOR and inhibited IP3R activity through calcineurin and FK506 in colonic SMCs (15, 118). In contrast, in aorta, FKBP12 did not coimmunoprecipitate with IP3Rs and IP3R-mediated Ca2+ release was unaffected by FK506 or rapamycin (118, 119). Explanations for these tissue-specific differences in FKBP12 regulation of SMC IP3Rs are unclear but may be due to variable expression of FKBP12 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 IP3R1 in PC12 cells, a non-SMC line, and elevated [H][H]IP3 binding to cerebellar IP3Rs, suggesting that RACK1 interacts with IP3Rs and increases IP3 affinity (173). RACK1 enhanced agonist-induced IP3R-mediated Ca2+ release in A7r5 cells and cultured preglomerular microvascular SMCs, suggesting that RACK1 also stimulates IP3R channel activity in SMCs (36, 173). Although evidence suggests functional regulation of IP3R-mediated Ca2+ release by RACK1 in SMCs, whether these effects are due to direct binding to IP3Rs 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 IP3Rs. pH influences SMC IP3R activity by controlling IP3 binding and IP3R Ca2+ sensitivity (38, 78, 152, 215, 251). Elevating pH activated recombinant IP3R1 and IP3R3 channels, with IP3R1 being more sensitive at pH 6.8 and IP3R3 at pH 7.5, suggesting that pH regulation is isoform dependent (44). IP3 binding to SR membranes from aortic, colonic, and tracheal smooth muscle was weak at pH <7.3 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 Ca2+ concentration required for IP3R activation, thereby elevating IP3R-mediated Ca2+ release (78, 215). Evidence suggests that H+ may compete with IP3 and Ca2+ to inhibit their binding to SMC IP3Rs, resulting in channel inhibition (215).

ROS regulate SMC IP3Rs through processes that include control of [IP3]i and modulation of IP3R affinity (31, 112, 183, 201). Superoxide reduced IP3 hydrolysis in SR vesicles of thoracic aorta, thereby enhancing [IP3]i and IP3-induced Ca2+ release (201). Thimerosal, an oxidizing agent, enhanced the affinity of IP3R1 for IP3 and potentiated IP3-induced Ca2+ release in A7r5 cells (31). Similarly, in SMCs of the pulmonary and systemic vasculature, H2O2 stimulated IP3R-mediated Ca2+ release, suggesting that ROS enhance IP3R signaling in SMCs (112, 183). In contrast, in coronary artery SMCs, H2O2, superoxide, and peroxynitrite inhibited the SR Ca2+-ATPase (SERCA), leading to a reduction in [Ca2+]SR that attenuated IP3R-mediated Ca2+ release (53, 65). In gallbladder and mesenteric artery SMCs, superoxide and H2O2 did not alter IP3R activity (225, 238). Therefore, ROS regulation of SMC IP3Rs is multimodal and may be dependent on tissue of origin and ROS species involved.

In summary, IP3, [Ca2+]i, and ATP are major modulators of IP3R activity in SMCs and protein kinases, pH, and ROS regulate IP3Rs through phosphorylation, controlling [IP3]i, IP3 binding to IP3Rs, and Ca2+ sensitivity of IP3Rs.

IP3R Communication with Ion Channels and Mitochondria in SMCs

SMC IP3Rs communicate with several SR- and plasma membrane-localized ion channels and mitochondria to control intracellular signals and influence physiological functions.
Ryanodine-sensitive Ca²⁺ release channels. Ryanodine-sensitive Ca²⁺ release channels (RyRs) are tetrameric proteins that share a number of structural and functional characteristics with IP₃Rs, including substantial amino acid sequence homology of the pore region and carboxy terminus (59, 142, 247). Analytical cell fractionation studies revealed that RyRs and IP₃Rs are both SR membrane localized in SMCs (28, 56, 91). There is uncertainty as to whether IP₃Rs and RyRs release Ca²⁺ from the same or distinct pools in SMCs. IP₃Rs and RyRs have been suggested to share the same Ca²⁺ 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 IP₃Rs (56). Reports have suggested that RyRs and IP₃Rs may share the same Ca²⁺ pool and that each channel can generate distinct Ca²⁺ 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 IP₃Rs or RyRs release Ca²⁺ 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.

Ca²⁺ released from an IP₃R or RyR activates the other channel type via CICR (12, 25, 28, 62, 91, 232). IP₃R activation stimulated RyR-mediated Ca²⁺ 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 IP₃R-dependent Ca²⁺ release in portal vein and renal artery SMCs (28, 91). Therefore, IP₃R-mediated Ca²⁺ release activates RyRs in SMCs and, in turn, RyRs also feedback to regulate IP₃R activity. However, Ryanodine, a RyR blocker, and anti-RyR channel antibodies did not alter agonist-induced IP₃R-mediated Ca²⁺ release in pulmonary artery and ureteric SMCs (39, 91), suggesting that IP₃R-RyR cross talk may not exist in all SMC-containing tissues.

Plasma membrane-localized ion channels. Research over the last decade has demonstrated that IP₃Rs can regulate the activity of plasma membrane TRPC and large-conductance Ca²⁺-activated K⁺ (BKCa) channels through both SR Ca²⁺ release-dependent and -independent mechanisms in SMCs. Evidence suggests that IP₃R 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. IP₃R activation stimulates TRPC channels directly, through physical coupling and indirectly, through Ca²⁺-dependent activation and induction of store-operated Ca²⁺ entry (SOCE). Endothelin (ET)-1 and uridine 5′-triphosphate, PLC-coupled receptor agonists, and IP₃ stimulated physical coupling between the IP₃R1 NH2-terminal TRPC coupling domain and the TRPC3 channel COOH-terminal CaM and IP₃R binding (CIRB) domain in cerebral artery SMCs (Fig. 3C) (1, 2, 253). In mammals, the TRPC coupling domain sequence (L.EEVVW.L.W.D) and the CIRB domain sequence (Y.MK.L.V.RYY) are conserved among the three IP₃R isoforms and seven TRPC channels, respectively (208). The IP₃R 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 IP₃R1 due to spatial separation of these proteins (2). IP₃R1-induced TRPC3 channel activation induced a cation current (IC₃), resulting in membrane depolarization, CaV1.2 channel activation, and vasoconstriction (Fig. 3C) (237). This SR-Ca²⁺ release-independent mechanism is a major contributor to agonist-induced IP₃R-mediated global [Ca²⁺], elevation and vasoconstriction in cerebral arteries (2, 237).

SMC IP₃Rs also activate TRP channels through SR Ca²⁺ release-dependent mechanisms (61, 106, 206). ET-1-induced IP₃R stimulation activated Ca²⁺ influx that was inhibited by TRPC1 knockdown in cultured aortic SMCs (206). IP₃R-mediated Ca²⁺ release also activated Ca²⁺-sensitive PKC isoforms, leading to PKC-dependent TRPC1 activation in portal vein SMCs (106). These studies suggested that IP₃R stimulation activates TRPC1 channels via SR Ca²⁺ release, although it was unclear if physical coupling was also involved. In cerebral artery SMCs, SERCA and IP₃R inhibitors reduced TRPM4-mediated native IC₃ (61). TRPM4 is Ca²⁺ sensitive and does not contain a CIRB domain (2), indicating that SR-released Ca²⁺ and not direct interaction with IP₃Rs is likely to regulate TRPM4 activation.

A reduction in SR Ca²⁺ load following IP₃R-mediated Ca²⁺ release may feedback to activate 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). IP₃R-mediated SR Ca²⁺ depletion activated TRPC-dependent SOCE in SMCs of inferior vena cava, portal vein, and pulmonary arteries (108, 114, 160, 180).

SMC TRPC channels are also activated by IP₃R-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 IP₃ or IP₃Rs, in SMCs of coronary, ear, and mesenteric arteries, portal vein, and gastric antrum (5, 6, 93, 109, 110, 174, 185). Therefore, in SMCs, TRPC channels are activated by PLC-mediated IP₃R-dependent and -independent pathways. IP₃R-dependent pathways include physical coupling with TRPC channels, Ca²⁺ release-mediated activation, and SR Ca²⁺ store depletion-induced stimulation.

BKCa CHANNELS. SMC IP₃Rs communicate with nearby plasma membrane BKCa channels via SR Ca²⁺ release-dependent and -independent mechanisms (255) (Fig. 3D). IP₃R-mediated SR Ca²⁺ release activated BKCa channels in basilar artery SMCs (95). IP₃R1 is located in close spatial proximity to, and coimmunoprecipitates with, plasma membrane BKCa channels in cerebral artery SMCs (255). SMC IP₃R1 activation elevated BKCa channel Ca²⁺ sensitivity, thereby facilitating channel activation at lower Ca²⁺ concentrations in cerebral artery SMCs (255). The IP₃R-mediated elevation in BKCa channel Ca²⁺ sensitivity would enhance channel activation by SR Ca²⁺ release by local IP₃Rs (255). IP₃R-induced BKCa channel activation would oppose TRPC3 channel-mediated membrane depolarization in SMCs and attenuate the IP₃-induced global [Ca²⁺] elevation and vasoconstriction (255).
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 IP$_3$R1 and TRPC3 channels and attenuated IP$_3$-induced ICaL 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 IP$_3$R1 channels with both TRPC3 and BKCa channels in SMCs (Fig. 3, C and D) (1, 7, 255). Caveolae may permit localized coupling of IP$_3$R1 to both TRPC3 and BKCa channels to modulate SMC membrane potential and arterial contractility. Therefore, IP$_3$Rs, 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$^{2+}$ 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), IP$_3$Rs 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). 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 $[Ca^{2+}]_{mito}$ and mitochondrial ROS generation. In A10 cells and cultured aortic SMCs, agonist-induced $[Ca^{2+}]_{mito}$ 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 $[Ca^{2+}]_{mito}$ elevations outlasted $[Ca^{2+}]$, transients by minutes (50, 66). These data suggest that changes in $[Ca^{2+}]_{mito}$ and $[Ca^{2+}]_{mito}$ 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, 164, 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 $[Ca^{2+}]_{mito}$, 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 $K^+$ ($K_{Ca}$), $Ca^{2+}$-activated $Cl^-$ ($Cl_{Ca}$), 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 nonselective 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$_3$R-regulated $Ca^{2+}$ signals in SMCs are summarized in Table 2.

$Ca^{2+}$ puffs. $Ca^{2+}$ puffs arise from the synchronous opening of $\sim30$ IP$_3$R channels distributed within a $\sim400$-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 $\sim0.4$ 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 $\sim2\%$ 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, non-propagating 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$_3$-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$_{Ca}$ channels, leading to depolarization, $Ca^{2+}$ influx, and vasoconstriction (67). These studies suggested that IP$_3$R-dependent $Ca^{2+}$ oscillations induce SMC contraction. In contrast, agonist-induced IP$_3$R-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$_3$R-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></td>
<td>$t_{1/2}$ decay: 107-250 ms</td>
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<tr>
<td></td>
<td>Spatial spread: 1.89-2.51 μm</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>
</tr>
<tr>
<td></td>
<td>Amplitude*: 1.5-2.6</td>
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<tr>
<td></td>
<td>Rise time: 0.2-0.8 s</td>
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<td></td>
<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></td>
<td>Rise time: 2-6 s</td>
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<tr>
<td></td>
<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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<tr>
<td></td>
<td>Amplitude*: 1.05-1.8</td>
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<td></td>
<td>Rise time: 1-2 s</td>
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<tr>
<td></td>
<td>$t_{1/2}$ decay: 4-6 s</td>
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<tr>
<td></td>
<td>Velocity†: $-18$ μm/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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<tr>
<td></td>
<td>Amplitude*: 1.34-2.05</td>
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<td></td>
<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></td>
<td>Spatial spread: 0.52-4.7 μm</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), choroid arterial (190), cremenartery and arteriole (177, 231), mesenteric artery (86, 104, 133, 249), pulmonary artery (76, 219), tail artery (49, 81), airway (17), cecum (70, 82), colon (15, 70, 117, 135, 136), duodenum (28), 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>
</tr>
<tr>
<td></td>
<td>Amplitude*: 1.4-6.9</td>
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<td></td>
<td>Rise time: 0.5-2 s</td>
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<td></td>
<td>$t_{1/2}$ decay: 2-10 s</td>
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<tr>
<td></td>
<td>Spatial spread: 8.8-50 μm</td>
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<tr>
<td></td>
<td>Velocity†: 7-126 μm/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. *E/F0. †Propagation velocity.

knockdown, to confirm that Ca$^{2+}$ ripples occur due to IP$_3$R-mediated Ca$^{2+}$ release in SMCs. In SMCs of pressurized mesenteric arteries, Ca$^{2+}$ ripples were observed only after the development of myogenic tone (249). Similarly, ANG II receptor antagonists abolished Ca$^{2+}$ ripples and reduced myogenic tone in tail arteries (9). It was concluded that ANG II produced locally within the arterial wall stimulates Ca$^{2+}$ ripples, which regulate myogenic tone (9). Therefore, IP$_3$R-mediated Ca$^{2+}$ ripples may be a Ca$^{2+}$ signal by which the local renin-angiotensin system in the arterial wall contributes to myogenic constriction (9, 249).

Ca$^{2+}$ sparks. Ca$^{2+}$ sparks are localized, intracellular Ca$^{2+}$ transients that occur due to the concerted opening of several spatially localized RyRs (37, 90, 159). RyR-mediated Ca$^{2+}$
 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). IP3Rs have been reported to contribute to Ca2+ sparks, albeit in a small number of SMC types (116, 190, 218, 252). RyR- and IP3R-mediated SR Ca2+ release both stimulated Ca2+ sparks in SMCs of choroidal and retinal arterioles, pulmonary artery, and trachea (116, 190, 218, 252). In contrast, IP3R blockers reduced spontaneous Ca2+ waves in SMCs of choroidal and retinal arterioles, pulmonary artery, and trachea (116, 190, 218, 252). The activation of IP3Rs, RyRs, or both channels can contribute to global Ca2+ waves, leading to spontaneous IP3R-mediated Ca2+ waves (61, 156). However, in cerebral artery SMCs, neither 50% knockdown of IP3R1 or XeC altered baseline Ca2+ wave frequency, suggesting that IP3Rs do not contribute to the generation of spontaneous Ca2+ waves in all SMC types (254). Thus the contribution of IP3Rs to spontaneous Ca2+ waves that occur in the complete absence of agonists in vascular SMCs is unclear. Ligands that bind to PLC-coupled receptors enhanced Ca2+ 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). IP3R inhibitors, anti-IP3R antibodies, and IP3R knockdown attenuated agonist-induced Ca2+ waves, indicating that IP3R activation is essential for generation of these Ca2+ signals (17, 25, 26, 28, 42, 60, 62, 70, 76, 94, 104, 156, 157, 254). Thus the contribution of IP3Rs to spontaneous Ca2+ waves in certain SMCs, suggesting that RyRs may also contribute to these events (17, 25, 27, 28, 62, 70, 94, 104, 151, 179, 184). Therefore, IP3Rs and RyRs can both influence the generation of spontaneous and agonist-induced Ca2+ waves, with relative involvement of each appearing to vary in different SMC types. The contribution of IP3Rs and RyRs to the propagation of Ca2+ waves has also been examined in SMCs (28, 62, 135, 184, 231). In colonic SMCs, carbachol and IP3 photolysis stimulated local SR Ca2+ release, which transformed into a propagating Ca2+ wave only in the presence of cytosolic Ca2+. 

**Fig. 5.** IP3R-mediated Ca2+ waves in SMCs of different tissues. Images illustrate Ca2+ waves induced by endothelin-1 (ET-1) in cerebral artery SMCs (A), spontaneously in tail artery SMCs (B), by IP3 photolysis in a colonic SMC (C), and spontaneously in gallbladder SMCs (D). Traces corresponding to changes in Ca2+ over time as determined in regions highlighted by boxes or regions in confocal images are also shown. White boxes in A indicate regions where Ca2+ sparks occurred (not shown). Reproduced with copyright permission (2012): Narayanan et al. (156) from Wolters Kluwer Health (A), Dreja et al. (49) from John Wiley and Sons (B), McCarron et al. (136) from the American Society for Biochemistry and Molecular Biology (C), and Balemba et al. (12) from the American Physiological Society (D).
and a steady $[\text{IP}_3]_i$ (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 propagation 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, nonspecific 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 $[\text{Ca}^{2+}]_i$, Global $[\text{Ca}^{2+}]_i$, is spatially averaged cytosolic $[\text{Ca}^{2+}]_i$. An elevation in global $[\text{Ca}^{2+}]_i$, induces contraction, whereas a reduction in global $[\text{Ca}^{2+}]_i$, results in relaxation (22, 29, 90). IP$_3$R activation elevates SMC global $[\text{Ca}^{2+}]_i$, 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 $[\text{Ca}^{2+}]_i$, in a wide variety of different SMC types (Table 2). IP$_3$R activation stimulated plasma membrane-localized TRPC1, TRPM4, and voltage-dependent Ca$^{2+}$ channels, leading to Ca$^{2+}$ influx and global $[\text{Ca}^{2+}]_i$, elevation in basilar, cerebral artery, and aortic SMCs (61, 67, 206). In cerebral artery SMCs, physical coupling of IP$_3$R1 to TRPC3 channels results in membrane depolarization and an indirect elevation in global $[\text{Ca}^{2+}]_i$, 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 $[\text{Ca}^{2+}]_i$, elevation (98, 107, 108, 114, 160, 177, 180, 220, 250). Collectively, these studies indicate that IP$_3$R activation can elevate global $[\text{Ca}^{2+}]_i$, 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 $[\text{Ca}^{2+}]_i$, elevations was examined in cerebral artery SMCs (237). SR Ca$^{2+}$ depletion reduced IP$_3$-induced global $[\text{Ca}^{2+}]_i$, elevation by $\sim 25\%$, suggesting that the contribution of IP$_3$R-mediated SR Ca$^{2+}$ release to global $[\text{Ca}^{2+}]_i$, is minor (237). TRPC3 knockdown reduced IP$_3$-induced global $[\text{Ca}^{2+}]_i$, elevation by $\sim 70\%$, 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 $[\text{Ca}^{2+}]_i$, elevation (237). Therefore, IP$_3$R-mediated Ca$^{2+}$ release can make a minor direct contribution to global $[\text{Ca}^{2+}]_i$, with IP$_3$R control of membrane potential and Ca$^{2+}$ influx being protein indirect mechanisms by which IP$_3$Rs influence global $[\text{Ca}^{2+}]_i$. Reports have also suggested that IP$_3$Rs do not regulate global $[\text{Ca}^{2+}]_i$, 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 $[\text{Ca}^{2+}]_i$. Tissue-specific variability in IP$_3$R modulation of global $[\text{Ca}^{2+}]_i$, 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 vasconstriction in retinal and choroidal arterioles (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$_3$Rs blocked 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 stimulates 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 KC$_{Ca}$ 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 vasconstriction has been examined in several vascular beds. U-73122, a PLC inhibitor, dilated pressurized cerebral and ophthalmic arteries, 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-
dependent Ca\textsuperscript{2+} waves regulate myogenic tone in these vessels (231). Similarly, in pressurized mesenteric and tail arteries, IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} ripples were proposed to stimulate myogenic constriction (9, 249). SR-dependent Ca\textsuperscript{2+} waves contributed to myogenic tone at low intravascular pressure in cerebral artery SMCs, although the contribution of IP\textsubscript{3}R\textsubscript{2} was not determined (151). These studies suggest that IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release regulates the myogenic response. In contrast, in pressurized mesenteric and cerebral arteries, IP\textsubscript{3}R-dependent Ca\textsuperscript{2+} release contributed to IP\textsubscript{3}-induced vasoconstriction (88, 144, 254). Explanations for these different observations include that the frequency and amplitude of IP\textsubscript{3}R-mediated Ca\textsuperscript{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\textsubscript{3}R-induced Ca\textsuperscript{2+} release is also likely to modify functional responses.

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

**Gene expression.** IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release can activate transcription factors, thereby regulating gene expression in SMCs (40, 51, 60, 156). In cerebral artery SMCs, IP\textsubscript{3}R-mediated SR Ca\textsuperscript{2+} release activated NF-kB both directly and indirectly, by stimulating mitochondrial ROS production (Fig. 4B) (156). IP\textsubscript{3}R-dependent NF-kB activation stimulated Cav\textsubscript{1.2} channel \(\alpha\textsubscript{1C}\) subunit gene expression, leading to vasoconstriction (156). NF-kB p105/p50 subunit expression was also upregulated by IP\textsubscript{3}R-mediated SR Ca\textsuperscript{2+} release via a mitochondrial ROS- and NF-kB-independent pathway (156). In cerebral artery and cultured aortic SMCs, IP\textsubscript{3}R-dependent SR Ca\textsuperscript{2+} release stimulated nuclear factor of activated T-cell c3, a transcription factor that can downregulate K\textsubscript{Ca} channel \(\beta1\) subunit and KV\textsubscript{2.1} channel expression (8, 60, 113, 161). IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release also activated cAMP response element-binding protein, a Ca\textsuperscript{2+}-dependent transcription factor, in SMCs of cerebral arteries and the portal vein (40, 51). Therefore, Ca\textsuperscript{2+} released by IP\textsubscript{3}R\textsubscript{2}s 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\textsubscript{3}R expression and Ca\textsuperscript{2+} release (3, 20, 149, 205, 228, 234). Proliferation correlated with an elevation in IP\textsubscript{3}R1 expression in aortic and carotid artery SMCs and upregulation of all three IP\textsubscript{3}R isoforms in mesenteric artery SMCs (3, 20). Consistent with these observations, Ca\textsuperscript{2+} wave frequency was elevated in proliferating cerebral artery SMCs (234). PLC and IP\textsubscript{3}R antagonists attenuated Ca\textsuperscript{2+} waves and proliferation in cerebral artery SMCs, indicating that IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release stimulates proliferation (234). Similarly, in cultured aortic SMCs, IP\textsubscript{3}R-dependent intercellular Ca\textsuperscript{2+} waves promoted proliferation and migration (149). An elevation in IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release also stimulated proliferation of cultured preglomerular microvascular SMCs (36). XeC inhibited pulsatile pressure-induced aortic SMC migration, and IP\textsubscript{3}R1 knockdown reduced A7r5 cell proliferation (205, 228). Taken together, these findings provide strong evidence that IP\textsubscript{3}R activation stimulates SMC migration and proliferation.

**IP\textsubscript{3}Rs and SMC Pathologies**

**Vascular SMC diseases.** Vascular SMC IP\textsubscript{3}R expression, [IP\textsubscript{3}], and Ca\textsuperscript{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\textsubscript{3}R\textsubscript{1} and IP\textsubscript{3}R IP\textsubscript{3}R-binding affinity in SMCs. Basal and phenylephrine-induced [IP\textsubscript{3}], were both higher in cultured aortic SMCs of spontaneously hypertensive rats than Wistar-Kyoto controls (236). A \(^{[3]H}\)IP\textsubscript{3} binding assay indicated that the IP\textsubscript{3}-binding capacity of IP\textsubscript{3}Rs was significantly higher in aortic SMCs of spontaneously hypertensive rats compared with Wistar-Kyoto rats (19). Although myogenic tone and global [Ca\textsuperscript{2+}], are higher in vascular SMCs of hypertensive rats, compared with normotensive controls (92), altered vascular SMC IP\textsubscript{3}R expression and Ca\textsuperscript{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\textsubscript{3}R1 protein and IP\textsubscript{3}R-dependent Ca\textsuperscript{2+} release were reduced (125, 130). SERCA2b expression was also downregulated in atherosclerotic aorta, suggesting that an [Ca\textsuperscript{2+}]\textsubscript{SR} reduction may also contribute to the impaired IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} release in atherosclerosis (125, 130).

Diabetes-related vascular complications are associated with a reduction in IP\textsubscript{3}R expression and Ca\textsuperscript{2+} release in SMCs of aorta and glomerular arterioles (138, 192, 193). In renal glomerular arteriolar SMCs of diabetic rats, IP\textsubscript{3}R protein was lower than in nondiabetic controls (138). Protein expression of all three IP\textsubscript{3}R isoforms and IP\textsubscript{3}R-mediated [Ca\textsuperscript{2+}]\textsubscript{i} signals were attenuated in aortic SMCs of genetic and inducible animal models of diabetes (192). High glucose reduced IP\textsubscript{3}R protein in A7r5 cells, suggesting that a diabetes-induced reduction in vascular SMC IP\textsubscript{3}R levels may be due to direct effects of hyperglycemia (192). IP\textsubscript{3}R1 protein and IP\textsubscript{3}R-mediated [Ca\textsuperscript{2+}]\textsubscript{i} elevations were reduced, and TGF-\(\beta_2\) levels were elevated in diabetic rat aorta (193). Intraperitoneal administration of an anti-TGF-\(\beta\) antibody partly restored IP\textsubscript{3}R expression and IP\textsubscript{3}R-mediated [Ca\textsuperscript{2+}]\textsubscript{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\textsubscript{3}R expression underlies vascular dysfunction, leading to diabetic glomerular hypertrophy. Collectively, these studies indicate that an alteration in IP\textsubscript{3}R...
expression and IP₃-R-mediated Ca²⁺ signaling in vascular SMCs contributes to the pathogenesis of vascular diseases.

Asthma. Asthma is associated with enhanced SMC [IP₃]i, IP₃-R signaling, and contractility, leading to airway hyperre sponsiveness and remodeling (132, 209). In Fisher rats, an asthmatic animal model, IP₃₅-phosphatase expression and activity were both reduced, leading to elevations in [IP₃]i and IP₃-R-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 IP₃-Rs may also regulate extracellular matrix formation and airway remodeling in asthma (132).

Pathologies associated with IP₃-R gene deletion and mutation. Studies performed in IP₃-R knockout (IP₃₁⁻/⁻) mice have provided valuable information regarding pathologies associated with these proteins, although limited information is available regarding SMC dysfunction. IP₃₁⁻/⁻ mice are rarely born alive, indicating that IP₃₁ is crucial for embryonic development (131). Animals that survive exhibit severe neurological pathologies, including ataxia and epilepsy (131). IP₃₁⁻/⁻ mice displayed increased susceptibility to glucose intolerance, insulin resistance, and diet-induced diabetes (243). A report (200) that examined SMC function in IP₃₁⁻/⁻ mice determined that gastric SMCs exhibited irregular bursts of spike potentials, resulting in reduced contractility.

Population analysis studies have identified human diseases caused by IP₃-R gene mutations. Similar to observations in knockout mice, there is a strong correlation between IP₃-R gene mutations and neurological disease. A heterozygous deletion and a mutation in the IP₃₁ 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 IP₃-R genes. Recent studies (75, 165) reported that single nucleotide polymorphisms in genes encoding IP₃-R3 and IP₃₃-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 IP₃-Rs 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, IP₃-Rs regulate Ca²⁺ homeostasis and contractility.
signaling pathways and mechanisms involved are diverse (Fig. 6). IP3, R isoforms, cellular localization, and localization influence channel functions (Fig. 6A). Several endogenous ligands, kinases, and proteins regulate SMC IP3, R activity (Fig. 6B). IP3, R signaling involves communication with cellular organelles, including mitochondria, RyRs, and plasma membrane ion channels in SMCs (Fig. 6C). IP3, R-mediated cellular pathways include both SR Ca2+ release-dependent and -independent mechanisms regulated through physical coupling to ion channels and control of SOCE. IP3, R-mediated Ca2+ release generates a wide variety of local and global Ca2+ signals in SMCs (Fig. 6D). IP3, R-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 IP3, R expression and channel activity. Thus IP3, R controls SMC physiological functions and pathological alterations in IP3, R signaling contribute to disease.

Future Directions

This review has summarized studies investigating IP3, R expression, cellular localization, activity, Ca2+ signals generated, physiological functions regulated, and associated pathologies in SMCs. There are still major questions that remain regarding IP3, R functionality in SMCs. Studies are necessary to better determine mechanisms that regulate IP3, R gene transcription and protein expression in SMCs. IP3, R 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, IP3, R-binding protein released with IP3, and Gβγ (57, 212). Modulation of IP3, R 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 IP3, Rs in SMCs. A better understanding of the contribution of RyRs and other Ca2+ channels to IP3, R-mediated Ca2+ signals in SMCs is also needed. Research is required to elucidate mechanisms that underlie tissue-specific variability in IP3, R signaling in SMCs. Studies would benefit from the availability of novel, selective, high-affinity, membrane-permeant IP3, R antagonists to investigate IP3, R signaling, physiological functions, and pathological alterations. SMC-specific IP3, R animal models, including those that induce knockout, express IP3, R mutations associated with human diseases, or alter known IP3, R 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 IP3, R knockout mice also need to be examined. Screening of humans to identify potential IP3, R mutations associated with SMC diseases is also necessary.

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