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TRP Channels in Hypertension

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Summary

Pulmonary and systemic arterial hypertension is associated with profound alterations in Ca^{2+} homeostasis and smooth muscle cell proliferation. A novel class of non-selective cation channels, the transient receptor potential (TRP) channels, have emerged at the forefront of research into hypertensive disease states. TRP channels are identified as molecular correlates for receptor-operated and store-operated cation channels in the vasculature. Over 10 TRP isoforms are identified at the mRNA and protein expression levels in the vasculature. Current research implicates upregulation of specific TRP isoforms to be associated with increased Ca^{2+} influx, characteristic of vasoconstriction and vascular smooth muscle cell proliferation. TRP channels are implicated as Ca^{2+} entry pathways in pulmonary hypertension and essential hypertension. Caveolae have recently emerged as membrane microdomains in which TRP channels may be co-localized with the endoplasmic reticulum in both smooth muscle and endothelial cells. Such enhanced expression and function of TRP channels and their localization in caveolae in pathophysiological hypertensive disease states highlights their importance as potential targets for pharmacological intervention.

Abbreviations

CCE	capacitative Ca^{2+} entry
EC	endothelial cell
NCX	Na^{+} - Ca^{2+} exchanger
PAEC	pulmonary artery endothelial cell
PAH	pulmonary arterial hypertension
PASMC	pulmonary artery smooth muscle cell
ROC	receptor-operated Ca^{2+} channel
SERCA	sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase
SMC	smooth muscle cell
SOC	store-operated Ca^{2+} channel
SR	sarcoplasmic reticulum
TRP	transient receptor potential channel

Pulmonary Arterial Hypertension and Essential Hypertension

In all vascular beds, precise regulation of circulatory hemodynamics is predominantly controlled by the cardiac output and vascular resistance. The pulmonary circulation, being a high flow, low-pressure system, has less than one tenth of the flow resistance observed in the high-pressure systemic vasculature.

Essential hypertension (or primary systemic arterial hypertension) is characterized by a sustained blood pressure over 140/90 mmHg. In the early stages of the disease, cardiac output is increased although total peripheral resistance remains constant; cardiac output drops when the disease is sustained and total peripheral resistance is elevated, with changes in peripheral resistance mainly reflecting the degree of arterial tone. Over 90% of all cases of adult systemic arterial hypertension have no clear cause and are therefore referred to as essential or primary hypertension.

Pulmonary arterial hypertension (PAH) develops when the mean pulmonary artery pressure is sustained at an elevated level ≥ 25 mmHg at rest or 30 mmHg during exercise and is associated with a progressive and sustained increase in pulmonary vascular resistance. Most of the patients with PAH have normal cardiac output, indicating that increased pulmonary vascular resistance is the major cause for the elevated pulmonary artery pressure in these patients. PAH is characterized by progressive pulmonary vasculopathy which, if left untreated, leads to right heart failure with poor prognosis. Increased pulmonary vascular resistance in PAH patients may be caused by sustained pulmonary vasoconstriction and considerable obstruction of the lumen of small arteries caused primarily by excessive proliferation of pulmonary artery smooth muscle cells (PASMC) in the vascular wall.

Based on the most recent classification of pulmonary hypertension, the two predominant

forms of PAH are currently classified as idiopathic and familial PAH. Idiopathic PAH is rare, principally affecting women, and its underlying cause remains undetermined. Familial PAH represents hereditary communication of PAH and is thought to be the cause of 6-10 % of PAH cases. Familial PAH is genetically predicted where successive family generations develop PAH. Pulmonary vascular intimal and medial hypertrophy is the characterizing features of idiopathic and familial PAH.

The pulmonary circulation is essential to the maintenance of sufficient circulating O₂ levels. When alveolar O₂ levels are compromised or partial pressure of O₂ is lower than 60 mmHg, it responds uniquely by constricting pulmonary arteries and diverting blood flow to the well-ventilated areas in the lung to ensure maximal oxygenation of the venous blood by adequately matching ventilation with perfusion. The alveolar hypoxia-mediated vasoconstrictive phenomenon is known as hypoxic pulmonary vasoconstriction. Vasoconstriction may also be triggered by a number of receptor-activated mechanisms involving endothelin-1 (ET-1) acting on ET_A and ET_B receptors [1], angiotensin II acting on AT₁ receptors [2], and endoperoxides diffusing from endothelial cells acting on smooth muscle cell (SMC) TP-receptors [3]. Sustained pulmonary vasoconstriction is often accompanied by vascular remodelling, i.e., the muscularization of smaller arteries and arterioles due to SMC proliferation and migration. In severe forms of pulmonary hypertension (such as idiopathic and familial PAH), pulmonary artery remodelling is extensive resulting from intimal fibrosis and medial hypertrophy. Such occlusion of the pulmonary arterioles is associated with the formation of plexiform lesions resultant of the proliferation of endothelial cells, migration and proliferation of SMC, and accumulation of circulating cells (including macrophages and endothelial progenitor cells).

In both pulmonary and systemic circulation, the blood flow and intraluminal pressure are

mainly regulated by changes in vessel diameter (or radius). The contractility and proliferation of smooth muscle is reliant upon increases in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). The fundamental systems coordinating changes in $[\text{Ca}^{2+}]_i$ are: *a*) Ca^{2+} entry via voltage-dependent Ca^{2+} channels, receptor-operated cation channels (ROC), and/or store-operated channels (SOC); *b*) Ca^{2+} release and sequestration from and into the sarcoplasmic (SR) or endoplasmic (ER) reticulum; *c*) Ca^{2+} extrusion to the extracellular space by Ca^{2+} - Mg^{2+} ATPase pumps; *d*) outward transportation of Ca^{2+} by the forward mode of Na^+ - Ca^{2+} exchangers (NCX) and inward transportation of Ca^{2+} by the reverse mode of NCX; *e*) mitochondrial Ca^{2+} release and sequestration; and *f*) release and sequestration from and into other intracellular Ca^{2+} stores (e.g., lysosomes).

The precise molecular entity of ROC and SOC, in particular, remained indefinable until recently. It is now believed that transient receptor potential channels (TRPs) participate in the formation of functional ROC and SOC in the vasculature. TRP channels have emerged at the forefront of research in the physiological and pathophysiological regulation of the vasculature having a wide tissue distribution and diversity of functions. This review examines the current state of knowledge of TRP expression and function in the vasculature, and the potentially pivotal pathophysiological changes in the expression of TRP channels associated with both pulmonary and essential hypertension. Both essential and pulmonary hypertension can lead to cardiac hypertrophy, which itself is also highly dependent on the expression and function of TRP-encoded cation channels. The role of TRPs in cardiac function is not discussed in the review, but readers are invited to peruse related articles for more information [4-8].

Transient Receptor Potential channels (TRP)

TRP channels belong to the superfamily of cation channels formed by tetramers of six transmembrane domains subunits which enclose a pore near the C-terminal end [9] (Fig. 1). Unlike voltage-gated ion (Ca^{2+} and K^{+}) channels, TRP subunits do not possess a voltage-sensing moiety, making their activity insensitive to changes in membrane potential. TRP channels therefore function as voltage-independent, non-selective cation channels which are permeable to Na^{+} , K^{+} , Cs^{+} , Li^{+} , Ca^{2+} , and Mg^{2+} [10]. TRP subunits are split into several subfamilies according to their activation stimuli and the presence of regulatory domains on the cytosolic N- and C-termini (Fig. 1). Canonical TRP (TRPC) is activated by G protein-coupled receptors and receptor tyrosine kinases linked to phosphoinositide hydrolysis via phospholipase C (PLC) activation [11, 12] (Fig. 2). A variety of chemical and physical stimuli including capsaicin, lipids, acid, heat, shear stress, and hypoosmolality can activate vanilloid receptor related TRP (TRPV). Melastatin related TRP (TRPM) [11] are either constitutively active or activated in response to increased $[\text{Ca}^{2+}]_i$, oxidative stress, or exposure to the cold. Less distinctly related families associated with specific genetic disorders, include the polycystins (TRPP), mucolipidins (TRPML), mechanoreceptor potential C (TRPN), and ankyrin (TRPA) [11] TRP subfamilies.

Expression Pattern and Regulation of TRPs in the Vasculature

In vascular smooth muscle cells more than ten TRP isoforms have been detected (Table 1). Their expression patterns in vascular myocytes (both smooth muscle and endothelial) are discussed further below.

TRPC:

TRPC1 is broadly expressed throughout the vasculature [13, 14] and is linked to Ca^{2+} entry associated with intracellular (i.e., SR) store depletion by agonists, inhibitors of SR Ca^{2+} -ATPase

(SERCA) pumps, or strong Ca^{2+} buffering. SOC-mediated Ca^{2+} influx triggered by SR Ca^{2+} depletion is termed capacitative Ca^{2+} entry (CCE). Evidence currently favours the proposal that TRPC1 is one of the pore-forming subunits of SOC in vascular myocytes [13, 15, 16] and that TRPC1 is a key isoform responsible for CCE-induced pulmonary vasoconstriction and proliferation [14, 17, 18]. The use of siRNA and antisense technology has reinforced the proposal that TRPC1 is the molecular correlate for SOC in vascular tissues [13, 19]. TRPC1 is likely to form heteromultimeric channels (Fig. 1B), possibly with TRPC4, TRPC5, TRPC3 and TRPP2 subunits in vascular tissues [20]. Such interactions with other TRPC isoforms could be crucial in the trafficking or translocation of TRPC1 to the plasma membrane, as demonstrated in TRPC1-TRPC4 co-expression studies [9].

TRPC6 is also ubiquitously expressed amongst the vascular tissues and belongs to the diacylglycerol-activated ROC family (along with TRPC 3 and TRPC7) [21]. ROC differs from SOC in that its activation ~~occurs~~ requires receptor binding to trigger the PLC-coupled cascade. TRPC6 mRNA and protein have been widely detected in isolated systemic [19, 22-27] and pulmonary arterial SMC [28-31]. Vascular contractility was increased in TRPC6 knock-out mice [32], suggesting that TRPC6 is involved in regulating vascular tone. In a recent study, Weissman et al. demonstrated a role for TRPC6 in the contractile response of pulmonary arteries in response to acute hypoxia [33]. Selective knock-out of the TRPC6 gene ($\text{TRPC6}^{-/-}$) abolished hypoxic pulmonary vasoconstriction, and hypoxia-induced cation influx in isolated PASMC. Furthermore, there was a significant accumulation of diacylglycerol in $\text{TRPC6}^{-/-}$ mouse PASMC, further reinforcing the suggestion that TRPC6 belongs to the ROC family of Ca^{2+} channels.

In the study by Dietrich et al. [32], TRPC3 expression was enhanced in $\text{TRPC6}^{-/-}$ mice; however, TRPC3 upregulation was not functionally interchangeable with TRPC6 [32]. Whether

TRPC3 functions as a SOC [34, 35] or ROC [36, 37] is debatable, as independent studies have verified that both activation pathways can activate TRPC3 channels. A requirement for *src*-mediated tyrosine phosphorylation of the Y226 channel protein residue has recently been identified for opening TRPC3 channels [38, 39].

Other less characterized TRPC isoforms include TRPC4, TRPC5 and TRPC7. While TRPC4 is expressed in smooth muscle [19, 27, 40-42], it is predominantly expressed in the endothelium [43-48] where it plays an important role in regulating lung microvascular permeability [44], agonist-dependent vasorelaxation [43], and gene transcription [45]. In SMC, TRPC4 expression and function may regulate CCE-mediated and agonist-induced cell proliferation [41] and contraction [42]. Data describing TRPC5 expression in the pulmonary vasculature are conflicting, with some studies showing ubiquitous TRPC5 expression in PASMC and pulmonary artery endothelial cells (PAEC) [49, 50] and others showing mRNA but no protein expression [42], or vice versa [51]. TRPC7 is not widely studied in vascular tissues, although immunohistochemical analysis has described its expression in coronary and cerebral artery endothelial cells [47], as well as in aortic and renal artery SMC [27, 40, 52]. TRPC7 is thought to contribute to both ROC and SOC channel formation. It is possible that differential subunit expression of TRPC7 alongside TRPC1, TRPC3 or TRPC6 may confer the characteristic activity of either ROC or SOC [40].

Several components or mechanisms are proposed to be involved in SOC activation [20]: *i*) linkage between TRPC1 and IP₃ receptors by the scaffolding protein Homer which is regulated by the Ca²⁺ filling status of the SR [53], *ii*) involvement of stromal interaction molecule-1, which translocates (after store depletion) from the SR to the plasma membrane where it may be essential in TRP channel activation [54, 55], and *iii*) release of a diffusible Ca²⁺ influx factor

from the SR which activates SOC via a Ca^{2+} -independent phospholipase A_2 mechanism [56]. Roles for agonist-bound IP_3 receptors close to the plasma membrane interacting with TRPC channels via protein-protein reactions [57] and fusion of a Ca^{2+} entry channel with the plasma membrane [58] are also proposed.

TRPM and TRPV:

Published data on TRPM and TRPV subtypes in vascular smooth muscle and endothelium are less abundant than that for TRPC subunits. mRNA for TRPM2-4, TRPM7-8, and TRPV1-4 have been detected in both endothelium-denuded rat aorta and pulmonary artery [12]. TRPM4, TRPM7, TRPV2, and TRPV4 are currently the best characterized in the vasculature. Gated by increased $[\text{Ca}^{2+}]_i$, TRPM4 is only permeable to monovalent cations such as Na^+ and K^+ and may contribute to myogenic vasoconstriction of cerebral arteries [26]. The activity of TRPM7 is more closely related to the regulation of Mg^{2+} ; Resting or agonist-induced $[\text{Ca}^{2+}]_i$ were unaffected by siRNA in aortic SMC, whereas the permeability to Mg^{2+} was altered [59]. The impact of TRPM7 and intracellular Mg^{2+} regulation in the pulmonary vasculature is still uncertain. Of the vanilloid receptors TRPV2 appears to be involved in hypertonicity-activated cell swelling in aortic SMC [60]. Yang et al. identified mRNA for TRPV1, TRPV2, TRPV3 and TRPV4 in both PASMC and aortic SMC [59]. Finally, the polycystic disease 1-like and 2-like proteins (TRPP1 and 2) are expressed in vascular smooth muscle correlating to a SOC-like channel [61].

TRP Channels in Agonist-mediated Vascular Contraction and Vascular SMC Proliferation

TRP channels play an important functional role in the regulation of vascular tone, being involved in agonist mediated vascular contractility and mitogen-mediated smooth muscle cell

proliferation. Phenylephrine-induced contractions in the canine pulmonary artery can be attributed to SOC being inhibited by SK&F 96365 [62]. Interestingly, serotonin-induced pulmonary vasoconstriction is attributed to ROC as it is insensitive to SOC inhibitors and to store depletion by cyclopiazonic acid [63]. TRPC1 and TRPC6 are currently thought underlie ROC responsible for agonist- and hypoxia-induced vasoconstriction of pulmonary arteries [31, 33] as well as in SMC from aorta [64, 65], portal vein [22, 25, 66], and mesenteric arteries [67]. One recent study, however, disputes the involvement of TRPC6-encoded ROC channels in chronic hypoxia-induced pulmonary hypertension [33]; TRPC6^{-/-} mice exhibited no significant changes in heart mass and pulmonary artery muscularization in response to chronic hypoxia (compared to their wild-type littermates). Conversely, expression of TRPC channels can also promote vasoconstriction via SOC-mediated mechanism in these vascular tissues [16, 18, 42, 49, 68-73]. TRPM4 may function as a mechanosensor to promote pressure-induced vasoconstriction in cerebral arteries [26] and TRPV4 may cause hypotonicity-induced airway contraction [74].

An intimate relationship between $[Ca^{2+}]_i$ and cell proliferation exists and TRP channels may indeed be responsible for increased Ca^{2+} influx stimulating proliferation. Indeed, enhanced proliferation is associated with augmented CCE via TRP-mediated SOC channels in PASMC [14, 17, 28, 29, 41, 71].

Functional Interaction of TRPs with the Cytoskeleton and within Caveolae

The cytoskeleton and plasma membrane structures may play an important role not only in TRP localization to the plasma membrane, but also to TRP function itself. Caveolae are cholesterol-rich compartments in the plasma membrane. Caveolae function to centralize cooperative receptors, ion channels, transporters, signaling moieties, and effectors within plasma

membrane microdomains [75] (Fig. 3). These regions are important sites for ligand-mediated activation of receptors and Ca^{2+} entry in both smooth muscle and, more prominently, in endothelial cells [76, 77] and are closely associated with the endoplasmic or sarcoplasmic reticulum [78], as shown in Figure 3.

Caveolin-1 (cav-1), the main structural protein in caveolae may play a crucial role in the plasma membrane localization of both TRPC1 and TRPC3. Indeed, ET-1-induced vascular contraction and TRPC1 co-localization with cav-1 are decreased by caveolae disruption [16, 79-81]. TRPC1 and cav-1 have been proposed to physically interact. An N-terminal cav-1 binding motif on the TRPC1 gene is involved in the binding of cav-1 and the regulation of TRPC1 plasma membrane localization and the integrity of lipid rafts [82, 83]. Indeed, functioning of TRPC1 or capacitative Ca^{2+} entry is decreased by cholesterol depletion [16, 80], suggesting that TRPC function is dependent upon the formation of caveolae. In addition, cav-1 may regulate both TRPC1 localization and function via interaction with the caveolin-scaffolding domain, thereby enhancing ER/SR-SOC channel interactions [82, 84].

Other TRPC channels have also been implicated in having functional co-localization with Ca^{2+} signaling pathways. For example, TRPC4 has been shown to interact with a PDZ domain on NHERF (Na^+ - H^+ exchanger regulatory factor) which makes interaction with PLC possible [85]. Remodeling of the actin cytoskeleton enables coupling between IP_3 type II receptors and TRPC1 channels in platelets [86] and actin stabilization prevents the internalization of TRPC3 channels and loss of CCE [87, 88]. Recently, Cioffi and co-workers demonstrated that the activation of SOC channels in PAEC necessitates the interaction of TRPC4 with cytoskeletal protein 4.1 [48].

Lately, Ca^{2+} entry via the reverse mode of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, which is functionally expressed in cultured human PASMC, is suggested to contribute to store depletion-mediated increases in $[\text{Ca}^{2+}]_i$ [89] (Figs. 2 and 3). Potentially, blockade of the reverse mode of the NCX could be a novel therapeutic approach for treatment of pulmonary hypertension [89]. Rosker and colleagues had previously shown that TRPC3 interacts with the NCX providing a novel idea in TRP-NCX-mediated Ca^{2+} signalling [90]. Supporting data included *a*) the extracellular Na^+ dependence of Ca^{2+} signals generated by TRPC3 over-expression in HEK293 cells and *b*) potent suppression of TRPC3-mediated Ca^{2+} entry by inhibition of the NCX with KB-R9743 [90]. Cell fractionation and co-immunoprecipitation experiments demonstrated co-localization of TRPC3 and NCX1 in low density membrane fractions [90]. It is thus possible that, as both TRPC3 and NCX expression are detected, this signalling pathway is localized in caveolae (Fig. 3).

Differential Physiological Functions of TRP Channels in PASMC and PAEC

Interestingly, the expression of TRPs in smooth muscle and endothelial cells may lead to different physiological effects. As described above, TRP channel mediated Ca^{2+} influx in vascular SMC causes vasoconstriction and also stimulates the proliferation of smooth muscle cells and medial hypertrophy. Conversely, Ca^{2+} influx into EC stimulates the production of both endothelium-derived relaxing (~~EDRF~~, e.g., nitric oxide, prostacyclin) and hyperpolarizing factors (~~EDHF~~). By opening Ca^{2+} -activated K^+ channels, increased $[\text{Ca}^{2+}]_i$ in EC also promotes K^+ efflux to the intercellular space between EC and SMC, which induces membrane hyperpolarization in SMC by activating inward rectifier K^+ channels [91]. All the processes ultimately lead to EC-dependent vasodilation.

Several studies have demonstrated an important role for Ca^{2+} influx via SOC in vascular endothelial cells. In *TRPC4*^{-/-} mice, a strong correlation has been established between TRPC4, ATP- and acetylcholine-induced Ca^{2+} influx, and relaxation in aortic EC [44]; thrombin-induced Ca^{2+} influx was abolished in PAEC isolated from *TRPC4*^{-/-} mice [43, 44]. Overexpression of TRPC3 has been directly associated to increased Ca^{2+} influx in response to ATP and bradykinin in PAEC [92]. Bradykinin activates Ca^{2+} influx in mesenteric arterial EC which express TRPC1 and TRPC3 channel mRNA [93]. TRPC1, especially, is implicated in SOC function in PAEC [16, 48].

Endocannabinoid-activated TRPV1 and TRPV4 channels have been recently implicated in the control of vascular tone. In mesenteric artery, endocannabinoid-mediated vasodilation may also be regulated by these channels as the TRPV1-specific inhibitor, capsazepine, reduced relaxation in response to anandamide [94]. Furthermore, 2-arachidonoyl-glycerol activated TRPV1-mediated Ca^{2+} influx by phosphorylating vasodilator-stimulated phosphoprotein (VASP) which may, in turn, contribute to vascular relaxation by stimulating protein kinases G and/or A [95].

Important functional roles for TRP isoforms in the regulation of vascular permeability have also been proposed. Thrombin-induced elevation of $[\text{Ca}^{2+}]_i$ in EC activates pathways resulting in endothelial cell contraction and disassembly of vascular endothelial barriers, which subsequently increases vascular permeability [96, 97]. Permeability of the microvasculature of the lung in response to hypoxia correlates with an increased expression of TRPC4 and enhanced CCCE in PAEC [44, 45]. Additional supporting evidence for the role of TRPC in regulating pulmonary vascular permeability includes: *a*) TRPC4-dependent Ca^{2+} entry in mouse lung vascular EC increases microvascular permeability [44, 98]; *b*) over-expression of TRPC1 augments, and anti

TRPC1 antibody decreases, thrombin- and VEGF-induced increases in vascular permeability [99], and inhibition of TRPC1 by antisense oligonucleotides reduces store-operated Ca^{2+} entry [100]; c) VEGF-mediated increase vascular permeability is mimicked by TRPC6-activating agents OAG and flufenamic acid [101]; and d) activation of TRPV1 by VASP is associated with increased vascular permeability [95].

Upregulated TRP Expression and/or Function in Pulmonary and Essential Hypertension

Arterial pressure is a function of cardiac output and vascular resistance. Hypertension occurs due to increased cardiac output and/or elevated vascular resistance. In both the systemic and pulmonary vasculature, sustained vasoconstriction, obliteration of small arteries due to physical occlusion of the vascular lumen by thromboemboli, and severe vascular wall thickening are the major underlying mechanisms for the increased resistance to blood flow, and subsequently for the increased arterial pressure. What follows is a discussion of how TRP channels contribute to the onset and maintenance of pulmonary and essential (systemic) hypertension.

Pulmonary Hypertension:

One of the hallmarks of severe PAH is pulmonary arterial medial hypertrophy due to increased PASMC proliferation. TRP channels, mainly those encoded by TRPC isoforms, have been implicated in the development of pulmonary vascular medial hypertrophy in PAH. TRPC3 and TRPC6 mRNA and protein are upregulated in PASMC from idiopathic PAH patients [30], and subsequently enhanced Ca^{2+} influx through SOC/ROC may partly account for the raised $[\text{Ca}^{2+}]_i$ levels in PASMC from these patients [17]. In addition to upregulation of TRPC3 and TRPC6, the mRNA and protein expression of NCX1 and caveolin-1/2, and the number of

caveolae on the surface membrane are also significantly increased in PASMC from idiopathic PAH patients compared with cells from normal subjects and normotensive patients with cardiopulmonary diseases [89, 102].

As shown in Figure 4, membrane receptors (G protein-coupled receptors and receptor tyrosine kinases), TRP-encoded SOC/ROC, and NCX are randomly distributed on the plasma membrane in normal PASMC. However, in PASMC from idiopathic PAH patients, the increased caveolin/caveolae may render the upregulated TRP channels and NCX1 in close proximity to receptors (or ligand-bound receptors), thereby enhancing agonist- or mitogen-mediated Ca^{2+} influx through TRP-formed ROC/SOC as well as inward Ca^{2+} transport via reverse mode NCX1 (see Figs. 2 and 3). Furthermore, increased caveolae and upregulated TRPC/NCX in caveolae may lead to endocytosis or internalization by interacting with endocytic proteins (e.g., clathrin, dynamin), TTP (SH3BP4, a SH3-containing protein) [103], and receptor proteins. The endocytosis of the ligand- and TRP/NCX-enriched caveolae would make the internalized vesicles (which contain high $[\text{Ca}^{2+}]$) more efficiently “reach” downstream Ca^{2+} -sensitive targets such as the SR/ER in the perinuclear area, compartmentalized signalling protein complexes, nuclear envelope, mitochondria, and contractile apparatus. The entrapped ligands and high concentration of Ca^{2+} in the internalized caveolae/vesicles may provide a sustained stimulation on receptors and cause a massive rise in $[\text{Ca}^{2+}]$ in the “designated” area inside of cell where Ca^{2+} is required for activating signal transduction proteins and transcription factors. The internalized vesicles may also play a role in, efficiently and selectively raising $[\text{Ca}^{2+}]$ in the nucleus, activating Ca^{2+} -sensitive nuclear proteins and transcription factors, and regulating gene transcription (Fig. 4). With continuous activation of receptors on the plasma membrane, cytoplasmic PLC- γ can bind to TRPC in the internalized vesicles and increase channel insertion

into the plasma membrane [104, 105]. Therefore, the upregulated TRP/NCX, increased number of caveolae, and enhanced Ca^{2+} entry, as well as the cycling between the internalization of ligand- and TRPC/NCX-enriched caveolae and the re-insertion of vesicular TRPC/NCX onto the plasma membrane would all contribute to enhancing contraction, proliferation and migration in PASMC from idiopathic PAH patients.

Similarly, TRPC1 and TRPC6 expression are both enhanced in normal PASMC treated with serum and growth factors [17] or maintained in sustained hypoxia [31, 72]. Inhibition of TRPC1 and TRPC6 using antisense oligonucleotides and TRPC6-specific siRNA decrease SOC currents, inhibits Ca^{2+} influx, reduces $[\text{Ca}^{2+}]_i$ and inhibits PASMC proliferation [14, 30]. Wang et al. demonstrated increased mRNA and protein expression of TRPC1 and TRPC6 channels and enhanced capacitative Ca^{2+} entry via SOC in PASMC from rats and mice exposed to chronic hypoxia, as well as in hypoxia-inducible factor-1 (HIF-1)-transfected hypoxic PASMC [71]; HIF-1 is a transcription factor known to contribute greatly to the progression of vascular remodelling and hypoxic pulmonary hypertension [106].

Most of the discussion insofar has centered on the physiological and pathogenic role of TRP in vascular smooth muscle cells. However, as indicated in Table 1, TRP subunits are also expressed in endothelial cells. As in PASMC, TRPC channels' activation also raises $[\text{Ca}^{2+}]_i$ through capacitative Ca^{2+} entry via SOC and receptor-mediated Ca^{2+} entry through ROC in PAEC. The end-effect may vary depending on the origin of the PAEC. We previously reported that sustained hypoxia increased activating protein-1 (AP-1) transcription factor binding activity by enhancing Ca^{2+} influx via La^{3+} -sensitive TRPC4-encoded SOC channels in human PAEC [45]. In the same study, enhanced TRPC4 expression correlated with increased SOC-dependent Ca^{2+} influx [45]. We thus speculated that upregulated AP-1-responsive gene expression in PAEC

would result in increased generation of pro-proliferative or vasoconstrictive products in PAEC (e.g., ET-1, PDGF, and VEGF). These factors would then act on PASMC via paracrine mechanisms to stimulate PASMC proliferation and, ultimately, pulmonary vascular remodelling in patients with hypoxia-mediated pulmonary hypertension.

An alternative theorem regarding the role of TRPC in PAEC has also been proposed, centering on the role of PAEC as the physical barrier between the lumen and PASMC. Disruption of this endothelial barrier by inflammatory mediators results in the formation of interendothelial gaps which allow circulating vasoconstrictors and mitogens (e.g., serotonin, PDGF, thrombin) to penetrate into the vascular medial layer and act on newly available PASMC. An increase in $[Ca^{2+}]_i$ in PAEC underlies their contraction; a number of studies have identified enhanced SOC function as the cause for the increased $[Ca^{2+}]_i$ in PAEC. Some groups proposed that the increased expression of TRPC1 might underlie PAEC contraction and vascular endothelial barrier dysfunction [107, 108]. However, there is also evidence to suggest that TRPC4 may also play a significant role in PAEC contraction and regulating endothelial permeability. Tiruppathi et al. demonstrated that TRPC4 knockout mice exhibit decreased endothelial leakage and decreased SOC-mediated CCE when stimulated with thrombin [44, 98]. Alvarez et al. [109] ‘proved’ both claims regarding TRPC1 and TRPC4 when they showed that their downregulation may be involved in an adaptive mechanism that limits endothelial permeability during chronic heart failure.

Systemic Hypertension:

Although the alteration of cation influx channels has been described in systemic hypertension, the precise underlying mechanisms are still to be elucidated. A putative role for

TRP channel isoforms in the pathogenesis of pulmonary hypertension has been described above, however Liu et al. were among the first to compare TRPC expression and function in normotensive controls and spontaneously hypertensive rats [110] and essential hypertension patients [111]. In monocytes from hypertensive patients and rats, increased protein expression of TRPC3 and TRPC5 isoforms was associated with a consequent increase of Ca^{2+} influx. This response was blocked by SK&F 96365 and by knockdown of TRPC3 [110] and TRPC5 [111] using targeted siRNA.

In addition to TRPC, other TRP isoforms may also be implicated in systemic hypertension. Mg^{2+} is the second-most prominent divalent cation in vascular smooth muscle cells, and TRPM channels can regulate Mg^{2+} transport. Increased $[\text{Mg}^{2+}]_i$ can attenuate agonist-induced vasoconstriction [112]; vascular SMC proliferation observed during hypertension may additionally be regulated by extracellular Mg^{2+} [113]. In aortic and mesenteric artery SMC, TRPM7 is upregulated by chronic treatment with angiotensin II and aldosterone [114, 115]. More importantly, TRPM7-deficient cells do not proliferate in response to angiotensin II. In a related study, TRPM7 was found to be downregulated in mesenteric artery SMC from spontaneously hypertensive rats compared to their normotensive controls. The blunted expression of TRPM7 correlated with a significantly decreased $[\text{Mg}^{2+}]_i$ [115].

TRPV1 is found mainly in a family of primary afferent capsaicin-sensitive sensory neurons which project to cardiovascular and renal tissues [116]. It is also proposed to be involved in the pathophysiology of salt-induced hypertension. TRPV1 expression and function appear to be linked to the ability to compensate for salt-induced increases in blood pressure. More specifically, TRPV1 is activated and its expression is upregulated during high salt intake in Dahl salt-resistant rats, thereby acting to prevent salt-induced increases in blood pressure. In contrast,

TRPV1 expression and function are impaired in Dahl salt-sensitive rats, rendering these animals sensitive to salt load in terms of blood pressure regulation [117]. As added support for this finding, Deng et al. demonstrated that activation of TRPV1 in hypertensive rats by rutaecarpine led to an increase in calcitonin-gene related peptide (CGRP) release and a subsequent decrease in blood pressure [118].

Genetic Variations in TRP Genes and Their Correlation with Pulmonary and Essential Hypertension

The discovery of specific genetic mutations predisposing to both familial and idiopathic PAH may accelerate the understanding of the mechanisms in the pathogenesis of PAH. In patients with idiopathic PAH, a C-to-G single-nucleotide polymorphism (SNP) has been identified in the promoter region (*nt.* -254) of TRPC6 gene; the allele frequency of the -254(C→G) SNP is significantly higher in idiopathic PAH patients than in normal subjects and patients with secondary pulmonary hypertension [119]. Genotype analysis demonstrated that 6.3% of idiopathic PAH patients carried homozygous -254G/G, whereas none of the normal subjects did. Moreover, the -254(C→G) SNP creates a binding sequence for the transcription factor nuclear-factor-κB (NF-κB). Functional analyses showed that the -254(C→G) SNP enhanced NF-κB-mediated promoter activity and stimulated TRPC6 expression in PASMC. Inhibition of NF-κB attenuated TRPC6 expression in PASMC of idiopathic PAH patients harbouring the -254G allele [119]. These results suggest that the -254(C→G) SNP may predispose individuals to high-risk of idiopathic PAH by upregulating TRPC6 expression in PASMC and, additionally, by linking abnormal TRPC6 transcription in PASMC to NF-κB, an inflammatory transcription factor [119].

Recently, monogenic human hypertension has been linked to mutations in the gene coding for WNK4, a kinase of the WNK family which regulates the expression of TRPV4. Fu and colleagues have shown that co-expression of WNK4 down-regulates TRPV4 function by decreasing its cell surface expression in HEK-293 cells [120]. Collectively this study demonstrates functional regulation of TRPV4 by WNK4 and speculates that this pathway may impact systemic Ca^{2+} balance [120].

TRP Channels as Potential Therapeutic Targets.

Development of novel and effective therapeutic approaches for patients with pulmonary hypertension and essential hypertension is important. The functional correlation of TRP channel expression with changes in blood pressure in both essential and pulmonary hypertension highlights these channels as potential therapeutic targets for the abrogation of vasoconstriction and SMC proliferation characteristic of hypertensive disease states. Indeed, bosentan, an endothelin receptor antagonist which is currently approved by FDA for the treatment of PAH, has been shown to inhibit ET-1- and PDGF-mediated PASMC growth in association with the downregulation of TRPC6 channel protein expression [29]. New evidence also suggests that targeting of other TRP isoforms may also prove beneficial in attenuating essential hypertension in some models [118].

Therapeutic strategies should focus not only on inhibiting TRP channel expression and function, but also on disrupting the functional “partners” and microenvironment for TRP channels. Therefore, agents should be developed which *a)* inhibit TRP channel trafficking and re-insertion; *b)* disrupt functional and physical coupling of TRP channels with membrane receptors and transporters, intracellular organelles, and IP_3 receptors; *c)* downregulate

STIM/Orai-1 or other messengers and intermediates that promote store depletion-mediated Ca^{2+} ; and *d*) disrupt caveolae by inhibiting cholesterol and caveolin production.

Conclusion

This review serves to highlight the importance of TRP channels as integral pathogenic components and as potential new drug targets to combat the detrimental effects of vasoconstriction and vascular remodelling (due to SMC proliferation) in hypertensive disease states (Fig. 5). In the vasculature, several cation-permeable TRP channels have been identified as being involved in both the physiological and pathophysiological regulation of vascular tone and smooth muscle cell proliferation. In the pulmonary vasculature, increased Ca^{2+} influx via store- and receptor-operated Ca^{2+} channels encoded by TRP genes may underlie both the enhanced vascular medial hypertrophy as well as the endothelial permeability which contributes to both pulmonary vascular remodeling and pulmonary edema. In essential hypertension, findings are not as clear, especially as it relates to non-TRPC channel isoforms. What is apparent is that TRPC channel upregulation is a strong promoter of vascular SMC proliferation in both types of hypertension. The potential protective role of TRPV and TRPM channels *vis à vis* blood pressure regulation in essential hypertension provides us with a novel therapeutic target in the treatment of hypertension.

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Table 1: TRP expression in vascular tissues

		<i>TRPC</i>							<i>TRPV</i>				<i>TRPP</i>	<i>TRPM</i>								Refs
	Species	1	2	3	4	5	6	7	1	2	3	4	2	1	2	3	4	5	6	7	8	
Aorta (fresh and A7R5)	R, M	c	a	c	c	c	c	a	c	c	a	c	c		c	a	a			a	A	[13, 19, 27, 32, 40, 59, 60, 64, 114, 121, 122]
Caudal artery	R	a		a			a															[16]
Cerebral arteriole	Rab	a		a	a	a	a															[13, 123]
Cerebral artery	M, H, R	c	a	c	c	c	c	c		c		c	a				a					[13, 23, 26, 32, 47, 60, 70, 124-126]
Coronary artery	H	b		b	b	b	b															[47, 94, 101]
Mesenteric artery	M, R, F, Rab	c		a	c	a	c		a	c									c	c		[19, 60, 67, 101, 114, 115]
Pulmonary artery	R, H, M	c	a	c	c	a	c	a	c	a	a	c			c	a	a			a	c	[14, 17, 18, 28, 33, 41, 42, 49, 51, 52, 59]
Renal artery	R, C	c		c	c	c	c	a														[27, 52, 127]
VEC *	M, H, R	c		c	c	c	c	c				c										[43, 47, 93, 125, 128, 129]
PAEC	H, R, B	a	a	a	c	a	a	a	a	a		a		a		a	a		a	a	a	[28, 45, 48, 49, 92, 107-109]

H, human; M, mouse; R, rat; Rab, rabbit; C, canine; B, bovine; F, frog; VEC, vascular endothelial cell; PAEC, pulmonary artery endothelial cell

‘a’, mRNA identified only; ‘b’, protein identified only; ‘c’, both mRNA and protein identified

* VECs correspond to endothelial cells from the middle cerebral arteriole, coronary and mesenteric artery, and aorta

Figure Legends:

Figure 1. Structure of TRP Channels. A: Representative topological structure of TRPC1, TRPV1, TRPM7, and TRPP (PKD2). Six transmembrane domains (TM1-TM6), ankyrin repeats or ankyrin-like domains (A), pore region (P), TRP box, caveolin-scaffolding (CSD) and coiled-coil (CC) domains are shown. B: Subunit arrangement of TRP monomers into functional homo- or hetero-tetrameric channels with a central ion-conducting pore.

Figure 2. Receptor-mediated increase in $[Ca^{2+}]_{cyt}$ in pulmonary artery smooth muscle cells (PASMC). A: Upon activation of membrane receptors including G protein-coupled receptors (GPCR) and receptor tyrosine kinases (RTK) by ligands, store-operated cation channels (SOC) are activated by store depletion as a result of IP_3 -mediated Ca^{2+} mobilization from the sarcoplasmic reticulum (SR). B: Receptor-operated cation channels (ROC) are activated by diacylglycerol and PKC activation. Opening of SOC and ROC not only causes Ca^{2+} influx, but also Na^+ influx. The increased cytoplasmic $[Na^+]$ in close proximity to the Na^+/Ca^{2+} exchanger (NCX) activates the reverse mode of NCX and promotes inward Ca^{2+} transport. Increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is a major trigger for PASMC contraction, proliferation and migration.

Figure 3. Potential mechanisms involved in ligand-mediated regulation of intracellular $[Ca^{2+}]_{cyt}$ through receptors, TRP channels, NCX1, and caveolin colocalized within caveolae. Caveolin is a structural protein that not only promotes the formation of caveolae, but also helps localize TRPC and NCX to the plasma membrane of caveolae. The structure of caveolae also helps entrap extracellular or intercellular ligands that activate the receptors and channels. Because of the close

proximity to each other and to ER/SR, the GPCR/RTK, TRP channels, and NCX in the caveolae would significantly enhance the agonist-mediated increase in $[Ca^{2+}]_{cyt}$.

Figure 4. Proposed mechanisms involved in the pathological role of upregulated TRPC and NCX as well as increased caveolin and caveolae in PASMC proliferation. In comparison to normal PASMC, TRPC and NCX1 expression are upregulated and caveolin and caveolae are increased in PASMC from idiopathic PAH patients. The increased number of the ligand- and TRP/NCX-enriched caveolae would not only enhance agonist-mediated increase in $[Ca^{2+}]_{cyt}$ by enhancing receptor-mediated Ca^{2+} influx through ROC and inward Ca^{2+} transportation through the reverse mode of NCX1, but also make the receptor/channel closer to intracellular organelles (e.g., the ER/SR). The close proximity to the ER/SR would not only enhance receptor-mediated Ca^{2+} mobilization but also augment store depletion-mediated Ca^{2+} influx. Furthermore, internalization or endocytosis of the ligand- and TRP/NCX-enriched caveolae (which contain 1.8 mM $[Ca^{2+}]$) would make the high $[Ca^{2+}]$ -contained vesicles be able to reach the ER/SR and mitochondria in the perinuclear area and the nuclear envelope and nucleus to provide Ca^{2+} for functional and transcriptional needs. The dynamic cycling between the internalization and the re-insertion of TRPC/NCX in the caveolae or the cytoplasmic vesicles would serve as a potential mechanism for initiating and maintaining sustained PASMC contraction, proliferation and migration.

Figure 5. Role of TRP channels in the development of sustained vasoconstriction and vascular remodelling in hypertension. Upregulated TRPC channels in smooth muscle (and endothelial) cells would increase the activity of both ROC and SOC cation channels and enhance Ca^{2+} influx

when membrane receptors are activated by circulating agonists and mitogens. The resultant increase in $[Ca^{2+}]_{cyt}$ triggers SMC contraction and stimulates SMC proliferation, and ultimately causes sustained vasoconstriction and vascular wall thickening. Since the vascular resistance is inversely proportional to the 4th power of the intraluminal radius of arteries, a small decrease in radius as a result of vasoconstriction and vascular wall thickening would significantly increase the vascular resistance and ultimately increase the arterial pressure. In the systemic vasculature, decreased function and expression of TRPV1 and TRPM channels may contribute to causing SMC contraction by decreasing CGRP and cAMP in sensory neurons and by decreasing cytoplasmic $[Mg^{2+}]$ in smooth muscle cells.

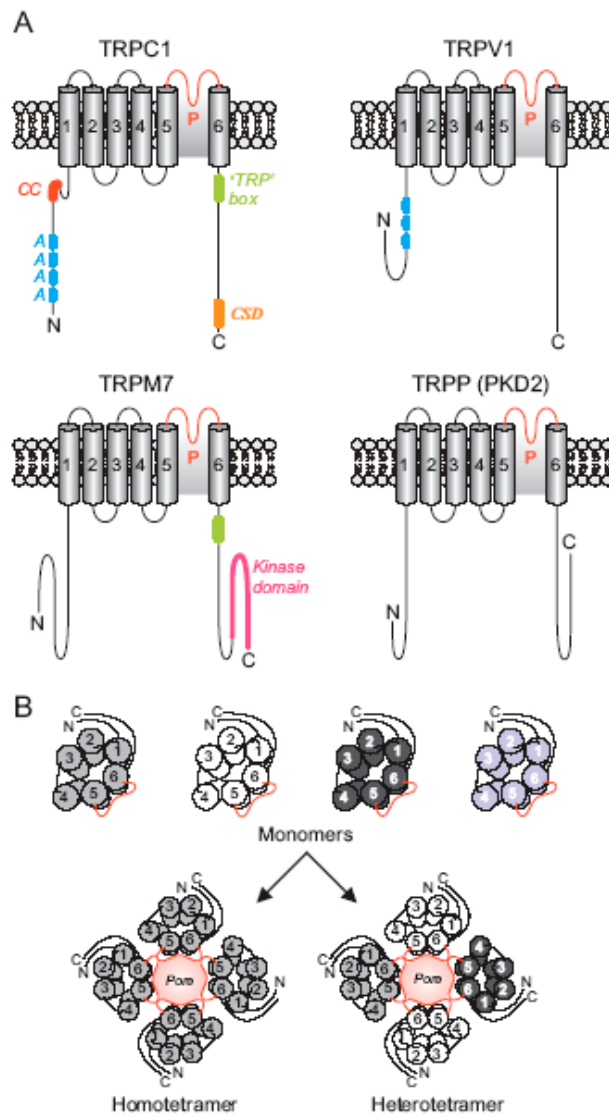


Figure 1

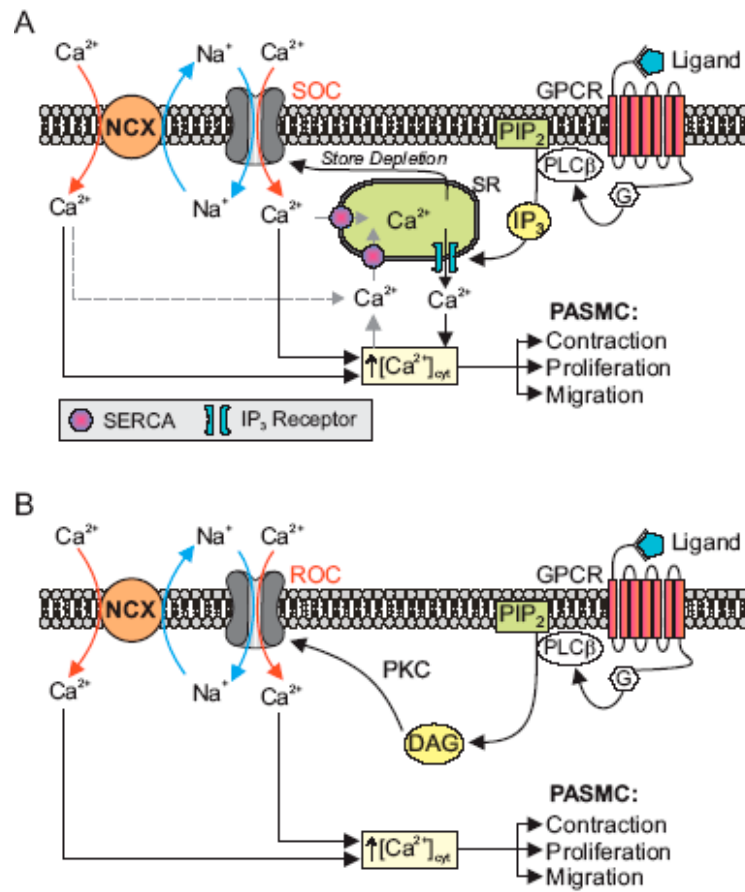


Figure 2

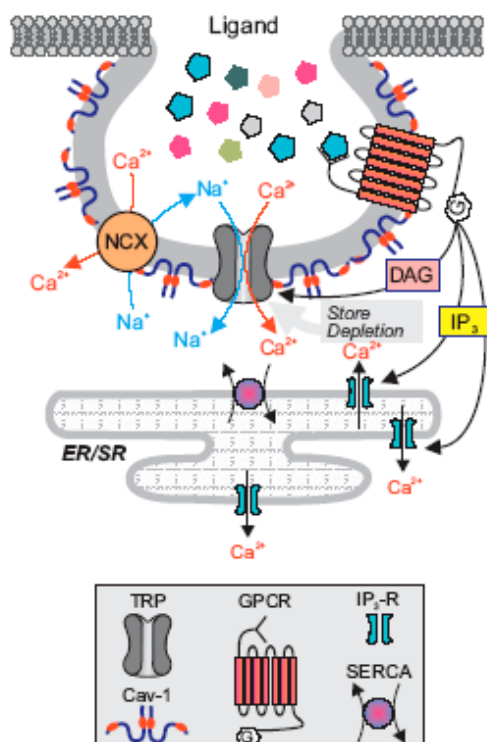


Figure 3

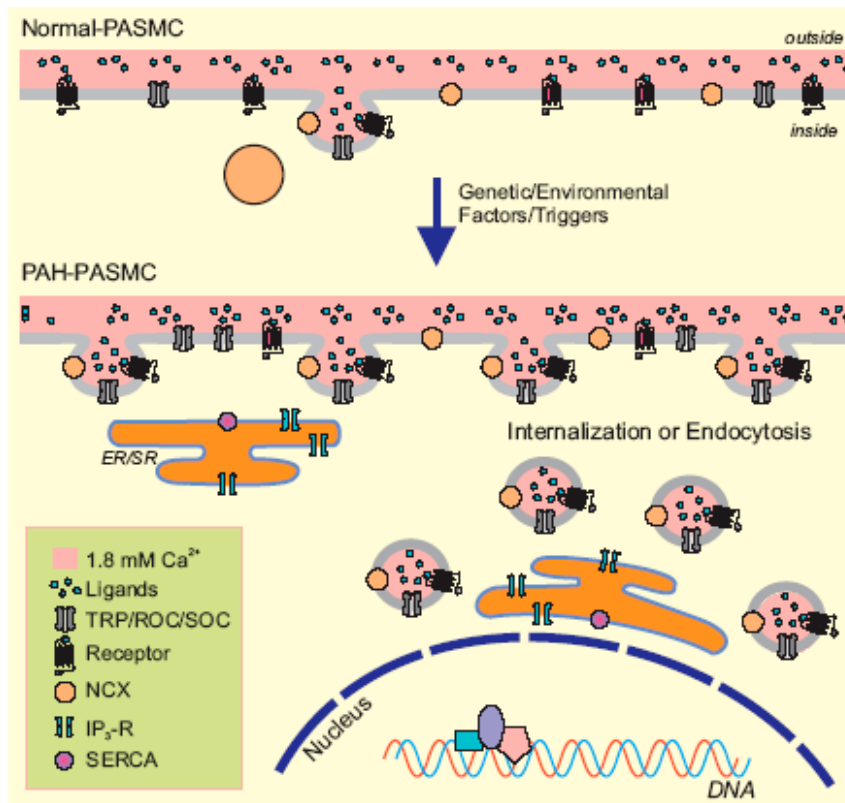


Figure 4

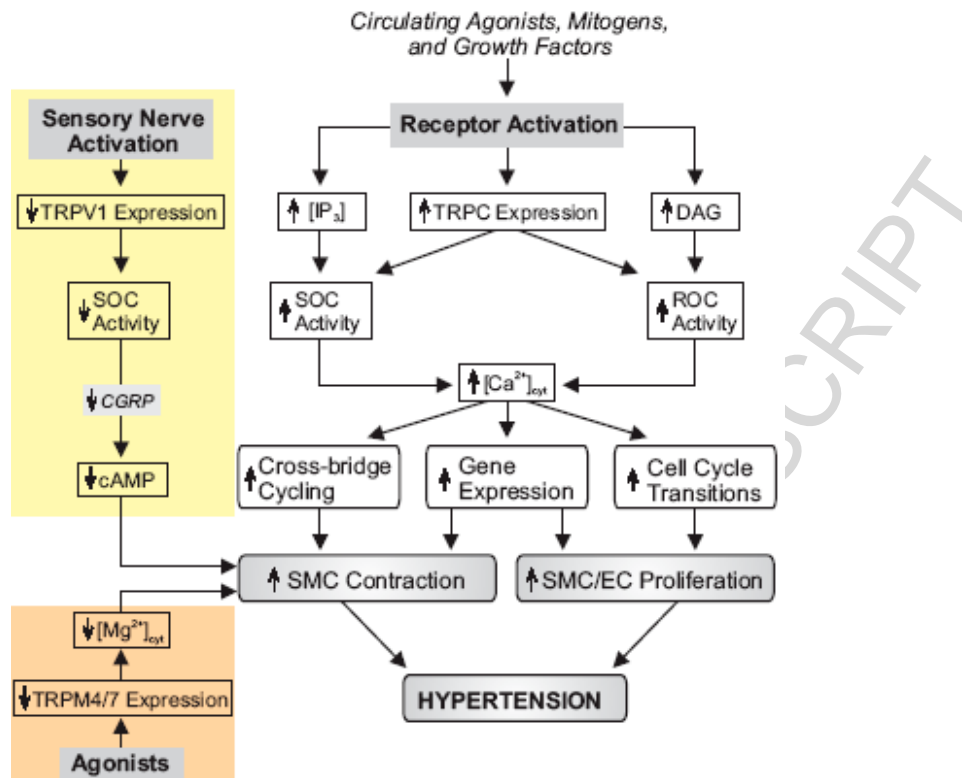


Figure 5