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TITLE

ORAI channels as potential therapeutic targets in pulmonary hypertension

RUNNING TITLE

ORAI channels in pulmonary hypertension

AUTHORS

Dr. Baptiste Rode[1,2,3], Dr. Marc A. Bailey[3], Prof. Roger Marthan[1,2,4], Prof. David J. Beech[3], Dr. Christelle Guibert[1,2]

[1]INSERM, Centre de recherche Cardio-Thoracique de Bordeaux U1045, F-33000, Bordeaux, France
[2]Univ Bordeaux, Centre de recherche Cardio-Thoracique de Bordeaux, Inserm U1045, F-33000, Bordeaux, France
[3]Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Leeds, UK
[4]CHU de Bordeaux, Pôle Cardio-Thoracique, F-33000 Bordeaux, France

CORRESPONDING AUTHOR

Dr. Baptiste Rode
Phone number: (33)5 57 57 56 58
Fax number: (33)5 57 57 16 95
email address: baptiste.rode@gmail.com

SECONDARY CONTACT

Dr. Christelle Guibert
Phone number: (33)5 57 57 56 58
Fax number: (33)5 57 57 16 95
email address: christelle.guibert@u-bordeaux.fr

SUMMARY

ORAI channels regulate Ca^{2+} signalling in multiple cell types and could be targeted in the treatment of PH.

ABSTRACT

Pulmonary hypertension is a complex and fatal disease which lacks treatments. Its pathophysiology involves pulmonary artery hyper-reactivity, endothelial dysfunction, wall remodelling, inflammation and thrombosis which could all depend on ORAI Ca^{2+} channels. We review the knowledge about ORAI channels in pulmonary artery and discuss the interest to target them in the treatment of pulmonary hypertension.

KEY WORDS

ORAI/STIM, pulmonary hypertension, pulmonary artery, calcium signalling, vascular remodelling

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ABBREVIATIONS

ARC/LRC: Arachidonic acid/Leukotriene Regulated Channel
CPA: Cyclopiazonic Acid
CRP: Collagen Related Peptide
CRAC: Ca^{2+} Release Activated Ca^{2+} Channel
DAG: Diacylglycerol
EC: Endothelial Cell
ER/SR: Endo-/Sarcoplasmic Reticulum
HUVEC: Human Umbilical Vein Endothelial Cell
IP_3: Inositol triphosphate
IP_3R: Inositol triphosphate Receptor
iPAH: idiopathic Pulmonary Arterial Hypertension
HIF1α: Hypoxia Induced Factor 1 alpha
LTC4: Leukotriene C4
PA: Pulmonary Artery
PAEC: Pulmonary Artery Endothelial Cells
PAH: Pulmonary Arterial Hypertension
PASMC: Pulmonary Artery Smooth Muscle Cells
PDGF: Platelet Derived Growth Factor
PH: Pulmonary Hypertension
PLA2: Phospholipase A2
PLC: Phospholipase C
PMVEC: Pulmonary Micro-Vascular Endothelial Cells
SERCA: Sarcoendoplasmic Reticulum Ca^{2+} transport ATPase
STIM: STromal Interaction Molecule
SVEC: Saphenous Vein Endothelial Cell
TG: Thapsigargin
Treg: regulatory T cells
TRPC: Transient Receptor Potential-Canonical
VEGF: Vascular Endothelial Growth Factor

1. Pulmonary hypertension (PH) and ORAI channels: state of the art

1.1 PH pathophysiology

PH is defined by a sustained mean pulmonary artery (PA) pressure ≥25 mm Hg at rest. It is a rare, progressive chronic disease. Over the time, elevated pulmonary artery pressure induces right ventricular hypertrophy followed by dilation leading to cardiac insufficiency and eventually, death. PH can originate from a heterogeneous spectrum of conditions that have been classified into five groups (21). PH from Group 1, also referred as "Pulmonary arterial hypertension (PAH)", develops in the absence of another cardiovascular condition. This includes idiopathic PAH (iPAH), heritable forms and PAH developed secondary to drug toxicity or infection. Group 1 contains the most severe forms of PH with development of plexiform lesions and angioproliferative occlusions. PH from Groups 2-5 are secondary to other cardio-respiratory diseases: Group 2: Pulmonary hypertension due to left heart disease, Group 3: Pulmonary hypertension due to lung diseases or hypoxia, Group 4: Chronic thromboembolic pulmonary hypertension, Group 5: PH due to unclear mechanisms or mixed aetiology. Although they have different origins and development, all forms of PH share common features that lead to elevation in PA pressure including PA wall remodelling, increased PA constriction and in situ thrombosis (13) resulting from complex cellular dysfunctions and altered intercellular crosstalk (48).

Endothelial dysfunction is found in most types of PH (78). Pulmonary Artery Endothelial Cells (PAEC) and Pulmonary Micro-Vascular Endothelial Cells (PMVEC) produce less vasodilators (e.g. nitric oxide), more vasoconstrictors and factors that promote PASMC remodelling (e.g. endothelin-1) which overall increase the vascular tone (22). The endothelium becomes more permeable and pro-thrombotic, facilitating inflammation, cell migration and thrombus formation. In severe forms of PH, concentric proliferation of Endothelial Cells (EC) and endothelial-mesenchymal transition lead to vascular occlusion, probably due to complex mechanisms involving EC apoptosis followed by emergence of apoptotic resistant and hyperproliferative EC (48, 55, 59, 78).
Media remodelling is also a common feature in PH. Proliferation, apoptosis resistance and migration of PASMC lead to an increase in media thickness and muscularization of distal arterioles which contribute to the elevation of PA pressure (13).
Proliferation and differentiation of fibroblasts into myofibroblasts, together with extracellular matrix secretion contribute to the adventitial remodelling observed in animal models and human forms of PH (66).
Inflammation in PH is characterised by immune cells infiltrate, comprising T- and B-lymphocytes, macrophages, dendritic cells and mast cells which are found around vascular lesions in PAH patients and PH animal models. Circulating concentration of inflammatory cytokines is also elevated in patients with PAH and the presence of auto-antibodies has been detected in some cases of PAH (54).
Abnormal platelet activation has been found in patients with Group 1 and Group 4 PH (34, 80). Although it is not always clear whether platelet activation is a cause or a consequence of PH, it is likely to contribute to in situ thrombosis (34, 56, 79, 80).

All these cellular alterations are governed by multiple factors, notably hypoxia, inflammatory cytokines and growth factors such as Platelet Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) (48, 60).
Non-specific therapeutic strategies include administration of oxygen, diuretics, anti-coagulants (16). Specific pharmacotherapies available for Group 1 PH target prostacyclin, endothelin-1 and nitric oxide pathways which primarily reduce PASMC contraction and vascular cell proliferation, with possible anti-inflammatory effect (12, 35). Although they have proved efficacy, many patients do not respond (35). Voltage-gated Ca^{2+} channel blockers also show limited benefits (42). Groups 2-5 PH lack specific therapies and molecules used with Group 1 patients show unclear to no effect (7, 79). The development of new drugs targeting alternative pathways is therefore necessary.

1.2 Molecular identity and regulation of ORAI channels

Calcium (Ca^{2+}) is a key second messenger in a large variety of cellular processes. Variations in intracytoplasmic Ca^{2+}, originating either from intracellular stores or extracellular space, control different cellular processes such as secretion, migration, contraction, gene expression including in PH (18, 32, 41). Among the different Ca^{2+} channels, the non-voltage-gated ORAI channels regulate extracellular Ca^{2+} entry in almost every cell type. The three ORAI proteins (ORAI1/2/3) are expressed at the plasma membrane and form the pore of Ca^{2+}-selective channels. ORAI channels also depend on the two STromal Interaction Molecules (STIM1/2) expressed at the endo-/sarcoplasmic reticulum (ER/SR) membrane and, in the case of STIM1, at the plasma membrane. ORAI and STIM proteins form two types of Ca^{2+} channels, the store-operated Ca^{2+} Release Activated Ca^{2+} Channel (CRAC) and the receptor operated Arachidonic acid/Leukotriene Regulated Channel (ARC/LRC) (Figure 1).
The CRAC channel mediates a sub-type of the cellular function named “store-operated Ca^{2+} entry” (SOCE). SOCE happens following activation of Gq/11-coupled receptors or tyrosine kinase receptors that activate Phospholipase C (PLC) which converts plasma membrane phospholipids into Inositol triphosphate (IP_3) and Diacylglycerol (DAG). IP_3 binding to its receptor (IP_3R) allows passive depletion in ER/ES intraluminal Ca^{2+} which is sensed by STIM proteins and triggers their aggregation into puncta close to the plasma membrane. The subsequent interaction between STIM and ORAI proteins enables extracellular Ca^{2+} entry through ORAI proteins organized in hexamers at the plasma membrane. Experimental activation of SOCE is usually obtained by treating cells with the Sarcoendoplasmic Reticulum Ca^{2+} transport ATPase (SERCA) inhibitors Cyclopiazonic Acid (CPA) or Thapsigargin (TG), resulting in passive depletion in intraluminal Ca^{2+} (Figure 1). The vast majority of studies describe a CRAC channel formed of STIM1 and ORAI1 proteins. STIM2 contributes to SOCE with different sensitivity to store-depletion than STIM1 and probably regulates basal cytoplasmic Ca^{2+} concentration or SOCE under low agonist stimulation (71). ORAI2 can mediate SOCE but conflicting results exist regarding its role in enhancing or reducing SOCE (14, 72) (Figure 1). ORAI3 is also able to mediate SOCE but an ORAI3 dependent CRAC channel has only been described in a subset of breast cancer cells (45). Although the CRAC channel is considered as the main SOCE driver, SOCE can also be driven by Transient

Receptor Potential-Canonical (TRPC) channels, either in parallel or secondary to the CRAC channel activation (71). The TRPC channels are not discussed in this review.
ARC/LRC channel activation is independent of Ca^{2+} store depletion but depends on Arachidonic Acid (AA) or its metabolite Leukotriene C4 (LTC4) (84). AA can be synthesised downstream of the PLC/DAG pathway or following Phospholipase A2 activation (PLA2) (71) (Figure 1). ARC/LRC channels have been described as a heteropentameric assembly of three ORAI1 and two ORAI3 proteins with STIM1 at the plasma membrane and at the ER membrane (24, 43, 84) (Figure 1). The role of STIM2 and ORAI2 in ARC/LRC channels has not been reported (71).

Here we review the current knowledge about ORAI channels in cell types contributing to the development of PH and discuss the interest of targeting these channels as a new therapeutic strategy.

2. ORAI channels in PA and PH

2.1 EC proliferation, apoptosis and permeability

ORAI1 and STIM1 are expressed in rat, mouse and human PMVEC (69, 76) and human PAEC (1). Both STIM1- and ORAI1-knockdown inhibit thrombin-induced SOCE in human PAEC (1). However, only STIM1-inhibition (by knockdown or expression of a dominant negative) but not ORAI1-inhibition reduces TG-induced SOCE in mouse and human PMVEC (69), suggesting that ORAI1 mediates SOCE in EC only in macro-vasculature. Strict comparison between lung vascular beds will be necessary to understand the role of ORAI1 and STIM1 in PAEC and PMVEC.
In mouse and human PMVEC, ORAI2 is not detectable and the expression of ORAI3 and STIM2 is very low; their function has not been investigated (69).
There are indications that ORAI channels in PAEC and PMVEC play a role in stress and disease conditions but their exact function is unclear. Notably, basal intracellular Ca^{2+} and CPA-induced SOCE are increased in human PAEC cultured in chronic hypoxia (17) but they are decreased in PAEC freshly isolated from chronic hypoxic rats (51) possibly through a reduction in ORAI1/STIM1 interaction (82). Therefore, whether chronic hypoxia enhances or reduces Ca^{2+} signalling in PAEC and the role of ORAI channels will require further investigation.
VEGF activates an ORAI1-dependent CRAC channel in Human Umbilical Vein EC (HUVEC), Saphenous Vein EC (SVEC) and Endothelial Progenitor Cells as well as an ORAI3-dependent ARC/LRC channel in HUVEC, SVEC, Cardiac Microvascular EC and Liver EC (36, 37). HUVEC migration, proliferation and tube formation are inhibited by ORAI1- and ORAI3-knockdown (1, 36, 37). VEGF signalling is important in PH: VEGF receptor inhibition associated with hypoxia triggers severe PH in animal models and high levels of VEGF and VEGF receptor are detected in lesions form patients with PAH, suggesting their contribution to pathogenic angioproliferation (75). The role of ORAI channels in VEGF dependent PH pathogenesis remains to be determined.

In rat PMVEC, ORAI1- and STIM1-knockdown reduce Bax and Caspase-3 expression and cell death in a model of endothelial dysfunction induced by lipopolysaccharide suggesting that ORAI1 and STIM1 promote apoptosis (76).
Zhou et al reported that TG-induced endothelial permeability is more important in rat models of severe PAH than in models of mild PH and in controls. The mechanism was partially attributed to TRPC4 but the role of ORAI channels has not been investigated (85). Conflicting results have been obtained regarding the role of ORAI channels in vascular permeability. High-mobility group box 1 protein (HMGB1)-induced permeability is reduced by STIM1- and ORAI1- knockdown and non-specific CRAC blockers SKF96365 and 2-APB in HUVEC cell line EA.hy926 (86). However Stolwijk et al showed that thrombin- and histamine-induced permeability in human dermal microvascular endothelial cells depends on STIM1 but not on Ca^{2+} store release, ORAI1 expression or extracellular Ca^{2+} entry (67). Therefore, the role of ORAI channels in vascular permeability might vary depending on the vascular bed and the stimulus.
Altogether, these data suggest that ORAI channels play a role in PAEC and PMVEC proliferation, apoptosis and permeability but a better comprehension of their function in the pulmonary vasculature is necessary to evaluate their interest as a drug target in PH.

2.2 PASMC proliferation and migration

ORAI1 and STIM1 expression has been detected in human, mouse and rat PASMC (10, 19, 27, 46, 47, 63, 77). Their expression correlates with the proliferative phenotype of PASMC: treatment of proliferative rat PASMC with factors inducing differentiation into contractile phenotype (TGF-β and heparin) leads to lower expression of ORAI1 in the case of TGF-β and ORAI1and STIM1 in the case of heparin (19). CPA-induced SOCE is reduced by ORAI1- or STIM1-knockdown and increased by STIM1-overexpression in cultured PASMC (39, 47). Similarly, in rat aortic smooth muscle cells, ORAI1- and STIM1-expression is higher in proliferative cells than in freshly isolated cells. ORAI1- and STIM1-knockdown reduce TG-induced SOCE and also proliferation and migration of proliferative aortic smooth muscle cells (53) raising the possibility of a role of ORAI and STIM1 in PASMC remodelling.
Hypoxia regulates ORAI1 and STIM1 expression and function in PASMC. The expression of ORAI1 is enhanced in distal pulmonary artery of chronic normobaric hypoxic rat and mice (21 days, 10% O_2) (77). This study reports no change in STIM1 expression, however STIM1 expression is increased in distal pulmonary artery of chronic hypobaric hypoxic rats (21 days, 380 mmHg) (28, 77). Although different cell types are present in the distal pulmonary artery, most in vitro studies show that chronic hypoxia enhances ORAI1 and STIM1 expression in rat and human PASMC (11, 27, 28, 62, 77) which leads to an increase in TG- or CPA-induced SOCE (11, 28, 77). The mechanisms by which hypoxia enhances ORAI1 and STIM1 proteins expression are not clear. The Hypoxia Induced Factor 1 alpha (HIF1α) plays a role in PASMC remodelling in response to hypoxia (61) but does not seem to regulate ORA1 expression (77). However, chronic hypoxia-induced increase in ORAI1 and STIM1 expression might depend on the production of hydrogen peroxide (H_2O_2) (11). In addition to increasing ORAI1 and STIM1 protein expression, hypoxia can also activate ORAI channels as acute hypoxia was shown to induce a STIM1- and ORAI1-dependent SOCE (39, 46), possibly by increasing

ORAI1/STIM1 interaction through a H_2O_2 dependent mechanism (11). The role of ORAI1 and STIM1 in hypoxia induced remodelling is poorly studied. Only one study shows that STIM1-knockdown inhibits hypoxia induced NFATc3 nuclear translocation and proliferation in rat PASMC (28).
In human PASMC, PDGF increases CPA-induced SOCE and ORAI1 and STIM1 expression through the AKT/mTOR pathway (49). PDGF-induced SOCE, migration and proliferation are ORAI1- and STIM1-dependent in aortic (8) and airway smooth muscle cells (65, 68). Although not demonstrated in PASMC, these studies suggest that ORAI1 and STIM1 might mediate PDGF dependent remodelling in PH.

ORAI2 and STIM2 are also expressed PASMC (10, 19, 27, 46, 47, 63, 77). Like STIM1 and ORAI1, STIM2 and ORAI2 expression is higher in proliferative (cultured) than in contractile (freshly isolated) rat PASMC and treatment of proliferative rat PASMC with factors inducing differentiation into contractile phenotype (TGF-β and heparin) leads to lower expression of ORAI2 and STIM2 (19). However, unlike ORAI1 and STIM1, ORAI2- and STIM2-knockdown or STIM2-overexpression have little to no effect on CPA-induced SOCE in PASMC (10, 19, 27, 39, 46, 47, 63, 77). Similarly, ORAI2- and STIM2-knockdown has no effect on TG-induced SOCE, proliferation and migration in cultured aortic smooth muscle cells (53) and STIM2-knockdown has no effect on PDGF-induced SOCE and migration in airway smooth muscle cells (68), suggesting a minor role of ORAI2 and STIM2 in vascular smooth muscle cells.
Nevertheless, the role of ORAI2 and STIM2 in PASMC might be important under stress and pathologic conditions. Like ORAI1 and STIM1, ORAI2 expression is enhanced in distal pulmonary artery of chronic hypoxic rat and mice (77) and STIM2 and ORAI2 expression is increased in PASMC cultured in chronic hypoxia (27, 62, 63, 77). ORAI2-knockdown reduces chronic hypoxia-induced increase in CPA-induced SOCE although the effect seems less important than ORAI1-knockdown (77). Contrary to ORAI1, hypoxia induced expression of ORAI2 depends on HIF1α, indicating a distinct regulation mechanism (77).
Nicotinamide phosphoribosyltransferase (NAMPT), a pro-inflammatory molecule which plasma concentration is increased in patients with PAH, induces the expression of ORAI2 and STIM2, but not ORAI1 and STIM1 in human PASMC. NAMPT-induced human PASMC proliferation is reduced by ORAI2- and STIM2-knockdown, indicating they play a role in response to inflammation (10).
Similarly, the expression of ORAI2 and STIM2, but not STIM1, was found increased in PASMC from patients with iPAH as compared to PASMC from normal subjects (63, 64). Basal level of intracellular Ca^{2+}, NFATc2 nuclear translocation, CREB-, STAT3-, AKT-phosphorylation, Bcl-2/Bax expression ratio and proliferation are enhanced in PASMC from patients with iPAH. Interestingly, these parameters can be reduced by STIM2-knockdown in PASMC from patients with iPAH and enhanced by STIM2-overexpression in control PASMC (63, 64). Although the role of ORAI1, ORAI2 and STIM1 in these mechanisms is not studied, the results suggest a pivotal role for STIM2 in Ca^{2+} regulation, proliferation and anti-apoptotic phenotype of PASMC in iPAH.

Finally, ORAI3 is expressed in rat PASMC (19, 77), its expression correlates with PASMC proliferation (19) but is not modified by chronic hypoxia (11, 77). Globally the role of ORAI3 in PASMC has not been studied. Interestingly, in rat PASMC, serotonin activates a store-independent Ca^{2+} entry which depends on AA synthesis via the PLC/DAG/DAG-lipase pathway (15, 25). This signalling pathway seems of particular importance in serotonin induced Ca^{2+} signalling and contraction in chronic hypoxia (57) and could regulate the serotonin-dependent remodelling in PH (40). Part of the Ca^{2+} entry might depend on AA metabolism via the cytochrome P450 epoxygenase pathway leading to TRPV4 activation (15) but whether serotonin also activates an ORAI and STIM dependent ARC/LRC type channel is to be determined. An ARC/LRC type channel, activated by LTC4, has been described in rat aortic and carotid artery smooth muscle cells (24, 83). ORAI3 expression and LTC4-induced currents are increased in smooth muscle cells from rat carotid artery media and neointima after balloon injury (24) and ORAI3-silencing reduces neointima formation (24), showing a role of the ARC/LRC channel in smooth muscle cell remodelling.
In total, there are multiple indications that ORAI channels regulate PASMC remodelling which suggest a possible benefit of inhibiting these channels in the treatment of PH.

2.3 Fibroblasts

The role of ORAI channels in vascular fibroblasts has not been studied. However, TG-induced SOCE is massively reduced in Mouse Embryonic Fibroblasts (MEF) from STIM1- or ORAI-knockout mice and in skin fibroblast from patients carrying a mutation that abolishes STIM1 or ORAI1 expression (26, 33, 38, 50, 52). In all cases of ORAI1 or STIM1 deficiency, SOCE can be rescued by transient expression of the wild-type protein showing, the important role of ORAI1 and STIM1 for SOCE in fibroblasts. TG-induced SOCE is also reduced in MEF from STIM2-knocknout mice but the effect is less important than STIM1- or ORAI1-knockout (26, 50). As far as we know, there are no data available about ORAI2 and ORAI3 in fibroblasts. Interestingly, pharmacological inhibition of the CRAC channel reduces extracellular matrix proteins secretion in cardiac fibroblast (58, 81), suggesting a role in fibrosis.
ORAI channels in fibroblasts seem to regulate SOCE and fibrosis but their expression and function in PA fibroblasts still has to be studied.

2.4 Immune cells in inflammation and auto-immunity

There are currently no data about the role of ORAI channels in immune cells in the pathogenesis of PH. ORAI channels have a complex roles in the immune system as they both allow pro-inflammatory cytokine secretion and are necessary in regulatory T cells (Treg) to prevent auto-immunity (20). Nevertheless, moderate inhibition of the CRAC channel by drug administration could at the same time limit inflammation, preserve immune response to infection and preserve Treg function (20). Interestingly, oral administration of the CRAC channel blocker AMG1 prevents inflammation in experimental encephalomyelitis (EAE). In this mouse model of autoimmune disease of the central nervous system, inflammation is mediated by autoreactive Th1 and Th17 cells and promoted by defective Treg cells (31).

AMG1 treatment reduces pro-inflammatory cytokine secretion by Th1 and Th17, without affecting immuno-suppressive properties of Treg (31), indicating a potential way to reduce Th1/Th17 dependant inflammation in PH (33, 54).

2.5 Platelet activation and thrombosis

Human and murine platelets express ORAI1 and STIM1 (6). Platelets from STIM1-knockout mice, ORAI1-knockout mice or mice expressing the dominant negative ORAI1-R93W have reduced TG-induced SOCE, SOCE induced by pro-coagulant factors thrombin and Collagen related Peptide (CRP), and phosphatidylserine surface exposure (a key step in the coagulation process) (2, 4, 9, 23, 74). Despite these functional alterations, haemostasis seems to be moderately altered in mice with STIM1-knockout haematopoietic lineage and unaltered in mice with ORAI1-knockout haematopoietic lineage (2, 9, 74) suggesting a limited role of ORAI channels under physiological conditions. However, mice with ORAI1- or STIM1-knockout platelets are protected against different models of thrombus formation (2, 9, 74). Notably, mice with ORAI1-knockout hematopoietic lineage are protected against a model of lethal pulmonary thromboembolism (9). van Kruchten et al showed that SOCE and in vitro thrombus formation induced by a combination of convulxin and thrombin (two platelet activators) are reduced in human platelets treated with different CRAC channel inhibitors, including the CRAC channel specific inhibitors Synta66 and GSK-7975A. The same study showed that treatment of mice with 2-APB reduced brain infarct in a model of ischemic stroke (73). Although 2-APB is not specific to the CRAC channel and investigation in PH is necessary, this study suggests that pharmacological inhibition of the CRAC channel could be used to limit thrombosis.
Human and murine platelets also express ORAI2, ORAI3 and STIM2 (6). The role of ORAI2 in platelets has not been studied. STIM2 seems of minor importance since CRP-induced Ca^{2+} signal and in vitro thrombus formation is unaltered in platelets from STIM2-knockout mice (23). AA seems to increase ORAI1/ORAI3 interaction in human platelets, suggesting the existence of an ARC/LRC type channel (5). The exact role of ORAI2, ORAI3 and STIM2 in platelets is therefore to be determined.

2.6 Cardiac remodelling

This review focuses on pulmonary artery but ORAI channels are also expressed in the heart and might contribute to right ventricular failure in PH. ORAI1 and STIM1 are expressed in cardiomyocytes where they have been shown to mediate cardiac hypertrophy and dilation (for review, see (3)). Interestingly, a recent study indicates a role of ORAI1 and STIM1 in right ventricular remodelling in a monocrotaline rat model of PH (30). ORAI1 and STIM1 are also expressed in cardiac fibroblasts and might contribute to cardiac fibrosis (58, 81).

3. Clinical significance of ORAI channels and perspectives for PH

Data presented in this review suggest that ORAI channels have a limited role under normal physiological conditions but might be important in PA remodelling, inflammation and

thrombosis in PH (Figure 2). Importantly, the potential role of ORAI channels in PDGF,VEGF and serotonin signalling, cytokine secretion and fibrosis might allow to target these therapeutically relevant but unexploited pathways (29). It is tempting to assume that inhibition of ORAI channels will have multiple beneficial effects but extensive study of their role in PA function and PH pathogenesis is necessary. In particular, STIM2, ORAI2 and ORAI3 are usually neglected but could have important role in the pathophysiology.

There is currently no molecule targeting ORAI channels available for medical use. However the CRAC channel inhibitor CM4620 developed by Calcimedica has been approved for clinical trial in the treatment of acute pancreatitis and intervention on ORAI channels has been suggested to treat inflammation, cancer or cardiovascular diseases (20, 44, 70) showing a growing interest of these channels for drug development. Therefore the study of ORAI channels in PH should be strongly encouraged.

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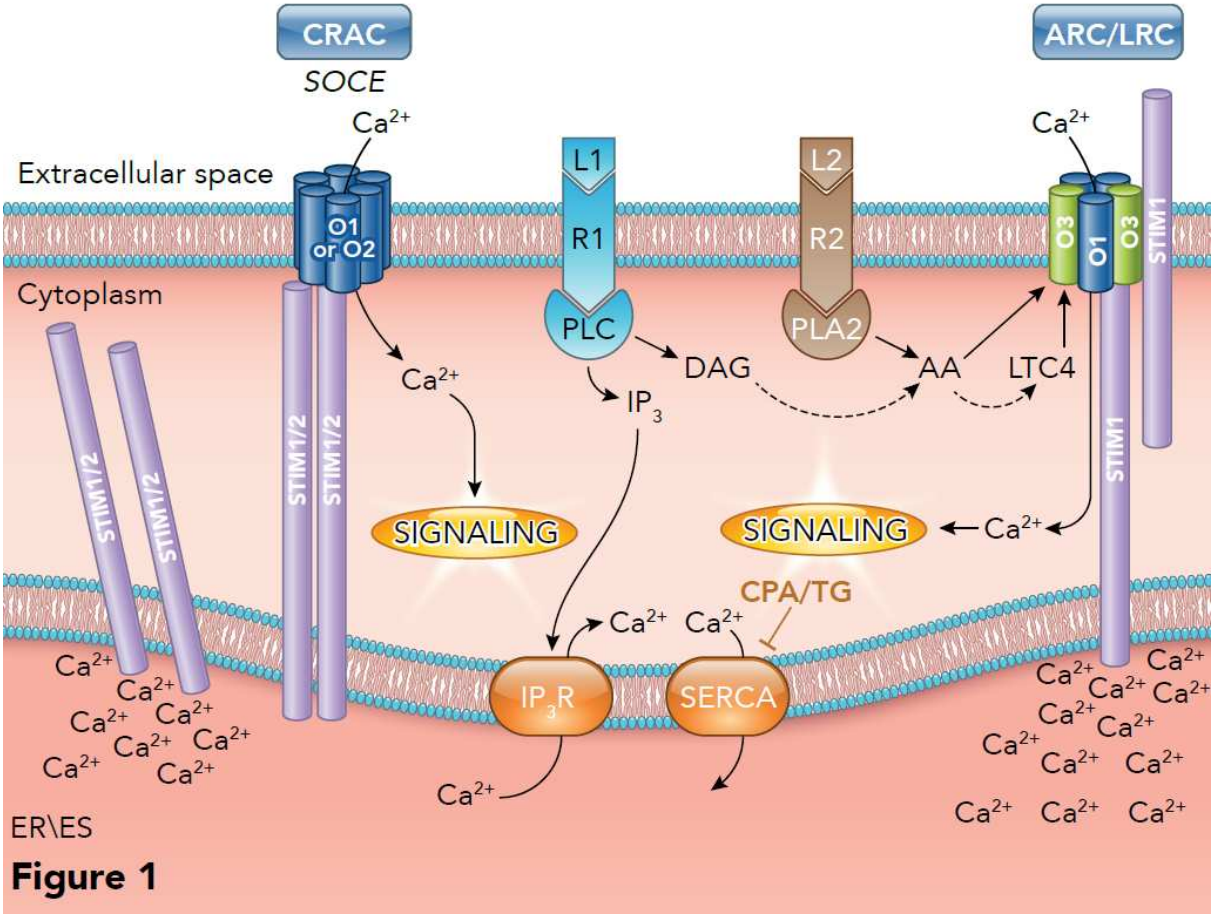
FIGURE LEGENDS

FIGURE 1: Activation mechanisms of ORAI channels
On the left hand side: the CRAC channel. Binding of a ligand (L1) to its receptor (R1) activates the PLC which synthesizes IP_3 and DAG. IP_3 binds to the IP_3R at the ER\ES membrane, allowing Ca^{2+} release. The reduction in intraluminal Ca^{2+} concentration triggers STIM1 or STIM2 aggregation and their interaction with ORAI1 or ORAI2 (O1 or O2), enabling Ca^{2+} entry. The CRAC channel can also be activated by treatment with the SERCA pump inhibitors CPA or TG. On the right hand side: the ARC/LRC channel. AA is synthesised either downstream of DAG synthesis by the PLC or after binding of a ligand (L2) to its receptor (R2) coupled to the PLA2. The ARC/LRC channel can be activated either by AA itself or by its metabolite LTC4. The ARC/LRC channel depends on ORAI1 (O1), ORAI3 (O3) and STIM1 at ER\ES membrane or at the plasma membrane.

FIGURE 2: Functions regulated by ORAI channels in the pulmonary artery in PH
Functions regulated by ORAI channels are indicated for each cell type. Functions are in bold when clearly demonstrated in PA, in italic when extrapolated from other tissues or diseases and with a question mark when unclear.


CRAC
SOCE
ARC/LRC
Extracellular space
Cytoplasm
ER\ES
O1 or O2
L1
R1
PLC
L2
R2
PLA2
O3
O1
O3
STIM1
STIM1/2
STIM1/2
STIM1/2
STIM1/2
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AA
LTC4
IP3
SIGNALING
SIGNALING
CPA/TG
IP3R
SERCA
Ca2+

Figure 1

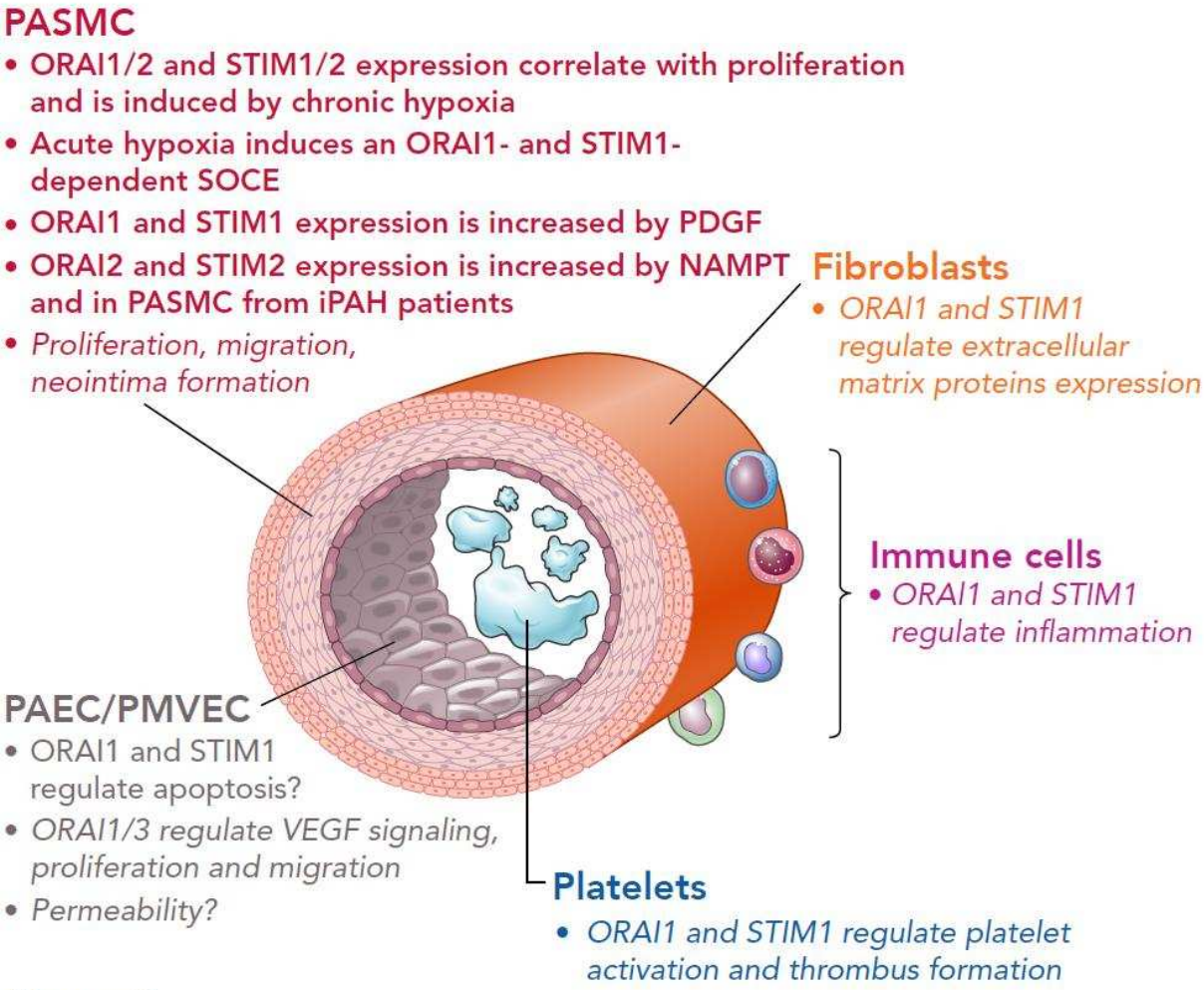
PASMC
• ORAI1/2 and STIM1/2 expression correlate with proliferation and is induced by chronic hypoxia
• Acute hypoxia induces an ORAI1- and STIM1-dependent SOCE
• ORAI1 and STIM1 expression is increased by PDGF
• ORAI2 and STIM2 expression is increased by NAMPT and in PASMC from iPAH patients
• Proliferation, migration, neointima formation
Fibroblasts
• ORAI1 and STIM1 regulate extracellular matrix proteins expression
Immune cells
• ORAI1 and STIM1 regulate inflammation
PAEC/PMVEC
• ORAI1 and STIM1 regulate apoptosis?
• ORAI1/3 regulate VEGF signaling, proliferation and migration
• Permeability?
Platelets
• ORAI1 and STIM1 regulate platelet activation and thrombus formation

Figure 2