

TRPC channels in vascular cell function

Alexander Dietrich¹; Hermann Kalwa^{1*}; Thomas Gudermann²

¹Institute for Pharmacology and Toxicology, Philipps-University Marburg, Germany; ²Walther-Straub-Institute for Pharmacology and Toxicology, Ludwig-Maximilians University Munich, Germany; *present address: Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Summary

The mammalian transient receptor potential (TRP) superfamily of non-selective cation channels can be divided into six major families. Among them, the "classical" or "canonical" TRPC family is most closely related to *Drosophila* TRP, the founding member of the superfamily. All seven channels of this family designated TRPC1–7 share the common property of receptor-operated activation through phospholipase C (PLC)-coupled receptors, but their regulation by store-dependent mechanisms involving the proteins STIM and ORAi is still discussed contro-

versially. This review will focus on the proposed functions of TRPC proteins in cells of the vascular system (e.g. platelets, smooth muscle cells and endothelial cells) and will present data concerning their physiological functions analysed in isolated tissues with down-regulated channel activity and in gene-deficient mouse models.

Keywords

TRPC channels, platelets, endothelial cells, smooth muscle cells, physiological function, vascular system

Correspondence to:

Alexander Dietrich, PhD
Institute for Pharmacology and Toxicology
PhilippsUniversity Marburg
Karl-von-Frisch-Str.1, 35043 Marburg, Germany
Tel.: +49 6421 2865105, Fax: +49 6421 28 65600
E-mail: dietrica@staff.uni-marburg.de

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Introduction

The family of classical or canonical TRP cation channels (TRPC) is composed of proteins that are highly related to *Drosophila* TRP, the founding member of the TRP superfamily which is involved in the photoreceptor signal transduction pathway (for a recent review see [1]). Its seven family members can be subdivided into subfamilies on the basis of their amino acid sequence similarity. While TRPC1 and TRPC2 are almost unique, TRPC4 and TRPC5 share ~65% homology. TRPC3, –6 and –7 form a structural and functional subfamily sharing 70% to 80% homology at the amino acid level and their stimulation by diacylglycerol (DAG) (2–4) a product of phosphatidylinositol 4,5-bisphosphate cleavage by activated phospholipases C.

The common structural features of the TRPC family are: four N-terminal ankyrin repeats, six transmembrane-spanning domains and a putative pore region located between transmembrane domains 5 and 6 (see ► Fig. 1A). TRPC channels can form functional homo- and heterotetramers within two defined subgroups (TRPC1/4/5 and TRPC3/6/7) excluding TRPC2 which is a non functional pseudogene in humans (5, 6). Novel combinations of TRPC1 with TRPC4 or –5 together with TRPC3, –6 or –7 in tetrameric complexes with three different TRPC family members have also been identified in HEK293 cells, as well as in embryonic brain, but not in adult rat tissues (7). Evidence for the importance of the highly conserved pore region in the TRPC tetramer is derived from site-directed mutagenesis which results not only in the complete loss of channel activity upon heterologous expression, but also in a

dominant-negative effect of a mutated channel monomer on functional homo- or heteromeric channel tetramers (5).

In all eukaryotic cells, activation of phospholipase C(PLC)-coupled membrane receptors by hormones or neurotransmitters leads to an increase in the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. Both activation of PLC β -isozymes by G protein-coupled receptors and of PLC γ isoforms by receptor tyrosine kinases results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate and the generation of inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). While it seems clear that TRPC3/6/7 channels are directly activated by DAG and are responsible for receptor-operated Ca^{2+} entry [(ROCE) (2, 3)], the regulation of other members and their involvement in store operated Ca^{2+} entry (SOCE) is discussed controversially. SOCE occurs when inositol 1,4,5-trisphosphate (IP₃) or some other signals discharges Ca^{2+} from intracellular stores in the endoplasmic reticulum (ER). The subsequent fall in the ER Ca^{2+} concentration then signals to the plasma membrane and activates store-operated channels. It was believed that a similar cellular effect is induced by thapsigargin which blocks sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA)-pumps and results in a leakage of Ca^{2+} ions from internal stores. However, very recently it was demonstrated that the activation of TRPC7 by thapsigargin somehow results from inhibition of SERCA *per se*, rather than from depletion of Ca^{2+} stores (8). At least three general theories for a store-operated activation of TRPCs were originally proposed: conformational coupling of the IP₃-receptor and TRPCs (depicted as 1 in ► Fig. 2) coupling of store depletion to channel activation by a cytosolic influx factor (CIF) and coupling by store depletion in-

duced translocation of channels to the plasma membrane (reviewed in [9]). A major breakthrough in understanding SOCE was, however, the identification of the Ca^{2+} sensor STIM (10–12) and the Orai channels (13–15) in 2005 and 2006, respectively. STIM1 which is predominantly located in the ER is a single transmembrane domain protein containing two N-terminal Ca^{2+} -binding EF hands in the ER lumen and is, thus, ideally situated for detecting Ca^{2+} levels in the ER. Upon Ca^{2+} release reduced Ca^{2+} levels induce STIM redistribution to punctae and the opening of store-operated channels in the plasma membrane. The so-called Orai proteins with only four predicted transmembrane domains and intracellular C- and N-termini, were identified as the pore forming unit of store-operated channels by the analysis of T cells from patients with severe combined immunodeficiency (SCID) (reviewed in [15]). Although Orai 1 channels can multimerise with themselves to form pore forming units, it was also suggested that Orai molecules might interact with TRPC channels heterologously expressed in HEK293 cells to form store-operated channels ([16] depicted as 3 in ► Fig. 3). Moreover, evidence for the interaction of the Ca^{2+} sensor STIM1 with TRPC proteins was presented for the same heterologous expression system ([17, 18] depicted as 2 in Fig. 2).

The role of TRPC channels in the vascular system is difficult to analyse in native tissues, because there are no specific channel blockers. For this reason, gene-deficient mouse models for TRPC1 and TRPC6 were established and analysed by us (19–21). Other TRPC-deficient mouse lines and approaches to down-regulate TRPC proteins by specific small interference RNAs (siRNAs) were reported by other laboratories. In this review, we will summarise what we know about TRPC function *in vivo* by focussing on three different cell types in the vascular system (platelets, endothelial cells and smooth muscle cells; see Fig. 1B). We will also discuss possible interactions of TRPCs with STIM1 and/or Orai1 in these physiological settings. TRPC function in the heart will not be discussed further in this overview because the topic has been covered by two insightful review articles (22, 23).

TRPC function in platelets

Blood platelets are cells which are differentiated from bone marrow megakaryocytes. Upon vessel wall injury they rapidly adhere to the exposed extracellular matrix (ECM), become activated and form a platelet plug, preventing blood loss. Moreover, the contact of coagulation factors like FVII and FX circulating in the blood as well as tissue factor (TF) expressed in vascular cells (smooth muscle cells and endothelial cells) results in the activation of the coagulation cascade and subsequently leads to platelet activation and thrombus formation (24, 25). However, in arteriosclerotic arteries after plaque rupture the same processes can induce acute vessel occlusion, leading to life-threatening myocardial infarction or ischaemic stroke (reviewed in [26]). For this reason, the activation of platelets has to be tightly controlled to ensure that these processes only initiate under appropriate pathophysiological conditions. Platelets possess a variety of receptors to detect these con-

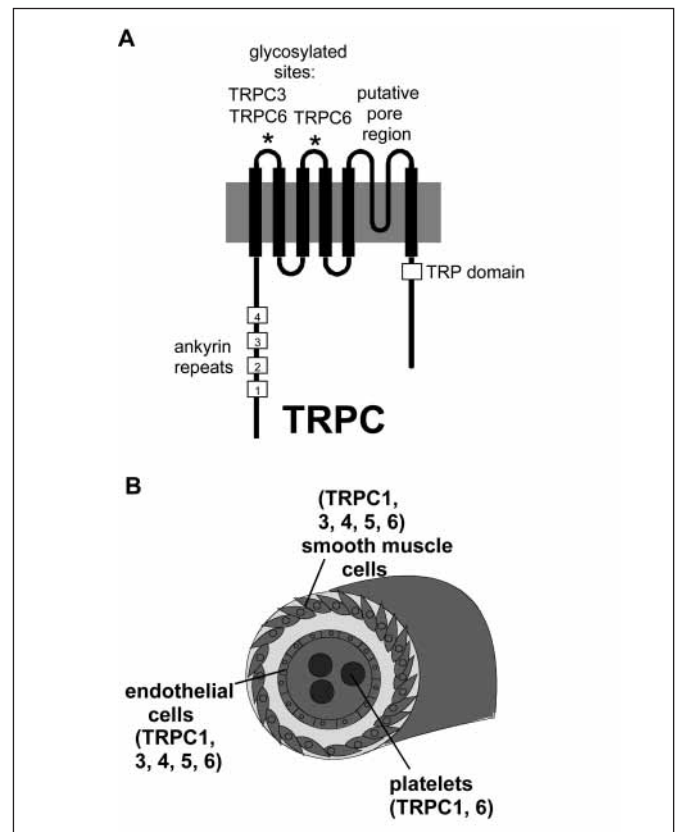


Figure 1: Plasma membrane topology (A) and expression of TRPC channels in the vasculature (B). A) TRPC members share a conserved TRP box (TRP), 4 ankyrin repeats and a putative pore region. TRPC3 channels are mono- and TRPC6 channels are doubly-glycosylated (79). See text for details. B) Topics of this review are three different cell types of the vasculature: smooth muscle cells, endothelial cells and platelets. TRPC channels which are most prominently expressed in these tissues and whose functions are discussed in the text are indicated.

ditions and adhere in response to well defined stimuli (reviewed in [27]). Agonist binding to receptors like those for ADP, thromboxane A₂ and thrombin activates PLC β isozymes via Gq-proteins, while collagen-, integrin- and C-type lectin-like receptors directly stimulate PLC γ . In both cases, however, production of DAG and IP₃ will produce an elevation of the cytosolic Ca^{2+} concentration from intracellular sources as well as Ca^{2+} influx from the extracellular medium. The elevated $[\text{Ca}^{2+}]_i$ contributes to various steps of platelet activation such as reorganisation of the actin cytoskeleton necessary for shape change (28), degranulation, or inside-out activation of integrins (29). In megakaryocytic cells, TRPC1, -2, -3, -4 and -6 were detected by RT-PCR experiments (30). Because platelets do not possess a nucleus with genomic DNA but use pre-mRNA for processing and functional expression of proteins (31), reverse transcription PCR (RT-PCR) and siRNA technology can not be used as efficiently as in other cell types to detect TRPC expression and function. For this reason, TRPC proteins are identified with antibodies whose specificity is the subject of considerable debate and controversy (32). While TRPC1 has been reported

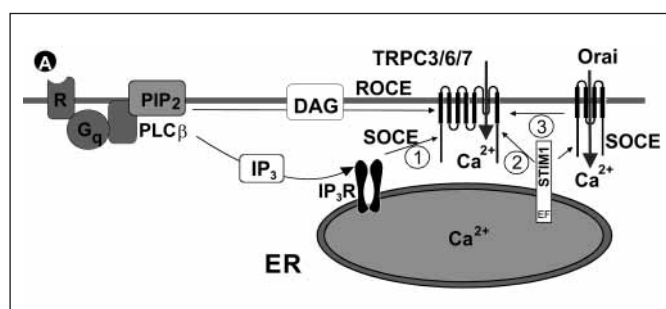


Figure 2: Proposed regulatory mechanisms of TRPC3/6/7 channel activity. After binding of an agonist (A) to its receptor (R), G protein (G_q)-activated phospholipase C- β (PLC β) cleaves phosphatidylinositol 4,5-bisphosphate (PIP $_2$) into inositol-1,4,5 trisphosphate (IP $_3$) and diacylglycerol (DAG). TRPC3/6/7 channels can be activated by DAG resulting in receptor-operated Ca $^{2+}$ entry (ROCE). Involvement of TRPCs in store-operated Ca $^{2+}$ entry (SOCE) is also discussed. Three possibilities for a store-operated regulation of TRPC channels are indicated in this figure (1) conformational coupling of activated IP $_3$ receptors to the channel protein (2) interaction of STIM1 the Ca $^{2+}$ sensor in the endoplasmic reticulum (ER) with TRPC channels or (3) interaction of store-operated Orai channels with TRPC channels in heteromeric complexes. See text for a more detailed description of the mechanisms.

to be expressed in the plasma membrane of the platelets associated with TRPC4 and TRPC5 (33), other reports found it only to be expressed in very low amounts in internal membranes (32). Moreover, conformational coupling between the IP $_3$ -receptor2 (IP $_3$ R2) and TRPC1 was proposed to be the major SOCE pathway in platelets (34) based mainly on *in vitro* studies using the same TRPC1 antibody of questionable quality to reduce Ca $^{2+}$ influx in response to thrombin and thapsigargin. The model suggested that Ca $^{2+}$ store release triggers coupling between the IP $_3$ R2 and TRPC1, thereby activating TRPC1 as the store-operated channel in the plasma membrane resulting in Ca $^{2+}$ entry (34). But, as outlined above, induction of store-depletion by thapsigargin is not sufficient evidence for store-operated regulation of a TRPC channel (8). Therefore, the function of TRPC1 in platelet was tested in a collaborative approach with the help of our TRPC1-deficient mouse model. Most interestingly, no significant differences in platelet function (e.g. activation, aggregation, haemostasis and thrombosis) were observed in TRPC1 $^{-/-}$ platelets (35). Moreover, SOCE is completely unaffected in TRPC1 $^{-/-}$ platelets, while STIM1- and Orai1-deficient platelets showed only a marginal SOCE compared to wild-type cells (35, 36). The same research group, however, was able to show the importance of STIM1 in mice bearing a single amino acid change (D84G) in the EF hand of this protein (called Sax mutation after the Bulgarian king Saxcoburgotski who suffered from a heavy bleeding disorder) (37). While animals homozygous for the mutation (*Sax/Sax*) have a high mortality in the embryonic stage due to severe haemorrhages in different regions of the body, heterozygous (*Sax/+*) mice were born, but still displayed multiple defects (37). Platelets of these mice were found to be pre-activated and were rapidly cleared from the blood inducing a macrothrombocytopenia and a profound bleeding phenotype (37). If now TRPC1 $^{-/-}$ mice were crossed with the EF-hand

mutant *Stim1*^{Sax/+} mice in which platelet Orai channels are constitutively opened (37), the basal Ca $^{2+}$ levels in their platelets remained unchanged (35). However, if a STIM1–TRPC1 interaction as reported in heterologous expression systems (18) was important in platelets adjacent to or in complexes with Orai channels (16), Ca $^{2+}$ levels should have been decreased. Therefore, *in vivo* evidence for STIM-TRPC and TRPC-Orai interaction is completely missing in platelets, and TRPC1 function in the plasma membrane of platelets is questionable. The role of TRPC1 in internal membranes, especially the dense tubular system, is still an interesting target for further investigations. This structure is, like the ER in other mammalian cells, the major intracellular Ca $^{2+}$ pool in platelets.

In contrast to TRPC1, there is a consensus about the prominent expression of TRPC6 in the plasma membrane of both human and murine platelets. Receptor-operated TRPC6 activation in platelets, independent of Ca $^{2+}$ release from the intracellular stores was demonstrated. TRPC6 phosphorylation by PKG and PKA was also shown in platelets. However, this phosphorylation does not affect Ca $^{2+}$ entry through the channels (32). A store-operated activation of TRPC6 by thapsigargin was reported, but, as outlined above, thapsigargin-induced store depletion is not sufficient evidence for SOCE (8). The role of TRPC6 in the plasma membrane is clearly unknown, and further investigations in TRPC6-deficient platelets from the TRPC6 $^{-/-}$ mouse model (19) are required.

TRPC function in endothelial cells

The endothelium is a thin layer of cells which line the interior surface of all blood vessels and form an interface between circulating blood in the lumen and the vessel wall. Endothelial cells are a specialised type of epithelial cells which reduce blood flow turbulence. Other important functions of the endothelium are to regulate the transport of liquids across the semi-permeable vascular endothelial barrier and to serve as a borderline between the coagulation factors circulating in the blood and TF, the primary initiator of coagulation within the vascular wall (25, 38). Vascular inflammation induces changes in endothelial cell shape and consequently increases in endothelial permeability which is induced by gaps between endothelial cells after Ca $^{2+}$ influx. Several reports demonstrated the involvement of Ca $^{2+}$ entry through TRPC1, -4 and -6 channels in the disruption of the barrier function in pulmonary arteries (39–41). The TRPC1-mediated signal may be associated with Rho activation or PKC α phosphorylation of TRPC1 as well as mechanisms involving nuclear factor κ B (NF- κ B) signalling (42, 43). Deletion of TRPC4 in TRPC4 $^{-/-}$ mice inhibits increases in lung vascular permeability to about 50% (40). Cultures of lung endothelial cells from TRPC4 $^{-/-}$ mice fail to respond to either thrombin or a PAR-1 agonist peptide with enhanced Ca $^{2+}$ influx (40). Moreover, isolated perfused TRPC4-deficient lungs showed a reduced PAR-1 receptor-mediated increase in vascular permeability (40). The abnormal Ca $^{2+}$ influx in TRPC4 $^{-/-}$ endothelial cells was associated with a lack of thrombin-mediated actin-stress fiber formation as well as a reduced cellular retraction response (40).

The inability of thrombin to induce actin-stress fiber formation may be a direct result of TRPC4 interaction with protein 4.1, an endothelial cytoskeletal protein (44). In addition, the intracellular C-terminal region of TRPC4 has been shown to interact with cytoskeletal α II- and β V-spectrin and to regulate plasma membrane expression of TRPC4 (45).

TRPC6- (41) and TRPC1/4 (46)-mediated Ca^{2+} entry were proposed to induce the resultant changes in endothelial cell shape in response to inflammatory agonists (e.g. thrombin and bradykinin). Vascular endothelial growth factor (VEGF) also increases vascular permeability by stimulating endothelial Ca^{2+} entry. In human microvascular endothelial cells, the VEGF-induced cation current has characteristics similar to those of VEGF-mediated TRPC currents in cells heterologously expressing VEGFR2 and TRPC3 or -6 (47), while another group also implicated TRPC1 in VEGF-induced vascular hyper-permeability (48). In conclusion, only a careful comparison of endothelial cell responses from different TRPC-deficient mice with those from wild-type mice could help to identify which channel or channel combination is essential for changes in vascular permeability.

Other agonists regulate production of vasoactive compounds e.g. nitric oxide (NO) via TRPC channel-mediated Ca^{2+} entry. For instance, in primary aortic endothelial cells of TRPC4-/- mice, acetylcholine-induced Ca^{2+} entry is reduced markedly, resulting in a significant decrease in endothelium-dependent NO-mediated vasorelaxation of blood vessels (49). These findings indicate that TRPC4 is part of the Ca^{2+} influx signal transduction pathway regulating vascular tone. However, TRPC5 might also be involved in this process, because treatment of bovine aortic endothelial cells with siRNA against TRPC5 prevented NO-induced Ca^{2+} entry (50). This study also demonstrated that NO caused cysteine S-nitrosylation of TRPC5 on two cysteine residues in the S5-S6 loop region which are conserved in TRPC1 and TRPC4. The authors proposed a positive feed back loop in which Ca^{2+} influx-mediated by NO-induced nitrosylation of TRPC channels leads to increased endothelial nitric oxide synthase (eNOS) activity, which increases NO production resulting in enhanced smooth muscle relaxation (50). The described data are, however, in clear contrast to other publications which describe NO as an inhibitor of Ca^{2+} entry in endothelial cells ([51, 52] reviewed in [53]). Along these lines, inhibition of TRPC3 activity by protein kinase G-dependent phosphorylation was demonstrated to protect endothelial cells from the detrimental effect of excessive NO and Ca^{2+} (54, 55). Because TRPC3-/- (56) and TRPC5-/- mice (57) are now available, it is essential to verify the molecular mechanism in TRPC-deficient endothelial cells in comparison to wild-type cells.

Hypoxia sensed by endothelial cells activates transcription factors leading to the production of growth factors, which stimulate smooth muscle cell proliferation, resulting in vascular remodelling and hypertension (58). One of the transcription factors involved in this process is AP-1, which regulates Ca^{2+} -sensitive genes. Culture of human pulmonary arterial endothelial cells under hypoxic conditions results in increased TRPC4 mRNA and protein expression (59). This is accompanied by enhanced binding of AP-1 to a number of AP-1-responsive genes involved in proliferation. Ex-

pression of siRNA against TRPC4 in endothelial cells prevented hypoxia-induced increases in TRPC4 expression and AP-1 binding. Thus, TRPC4 appears to be involved in mediating some aspects of hypoxia-induced gene expression and cell proliferation.

TRPC3 channels have been suggested to serve as redox sensors which monitor oxidative stress in endothelial cells. Porcine aortic endothelial cells express TRPC3 and display oxidant-induced cation currents with properties similar to those of TRPC3 (60, 61). Expression of the N-terminus of TRPC3, which acts as a dominant negative TRPC3 fragment, abolished the oxidant-induced cation current and reduced membrane depolarisation in endothelial cells (60). However, TRPC3 may not be acting alone in this process, because it was demonstrated recently that heteromeric channels composed of TRPC3 and -4 form redox-sensitive channels both in native endothelial cells and when heterologously expressed in HEK293 cells (62). Expression of dominant negative constructs for either TRPC3 or -4 are able to suppress oxidant-induced channel activity in both systems (62). It may be interesting to test these hypotheses by comparing primary endothelial cells from TRPC3-/- and TRPC3/4-/- double deficient mice with those of wild-type mice.

Endothelial cell migration plays an important role in vascular remodelling and regeneration. Recently, a TRPC5/6 activation cascade has been shown to take part in the regulation of endothelial cell migration (63). Treatment of bovine aortic endothelial cells with lysophosphatidylcholine (lysoPC), which inhibits cell migration by blocking non-voltage-gated Ca^{2+} channels (64), induced translocation of TRPC5 and -6 to the plasma membrane. Treatment of endothelial cells with siRNA against either TRPC5 or -6 reduced lysoPC-induced increases in $[\text{Ca}^{2+}]_i$ and cell migration. Similar results were obtained in TRPC6-deficient endothelial cells, which also showed a lack of lysoPC-induced translocation of TRPC5 to the plasma membrane. However, this report is the only one demonstrating the existence of TRPC5/6 heteromeric channels while other reports excluded this hetero-multimerisation (5-7).

In human umbilical vein endothelial cells, 11,12-epoxygenase-derived epoxyeicosanotrienoic acids (EETs) some of the predominant mechanically produced metabolites from arachidonic acid are found to facilitate the translocation of the TRPC6 protein to caveolin-1-rich cell membrane areas. This event leads to enhanced Ca^{2+} influx into endothelial cells and prolongation of the membrane hyperpolarisation in response to bradykin (65). 11,12-EETs from endothelial cells can also directly activate large conductance K_{Ca} (BK_{Ca}) channels in vascular smooth muscle cells to induce hyperpolarisation and vasorelaxation (reviewed in [66]). In summary, TRPC channels support endothelial functions like vascular regeneration, increased permeability and endothelium-derived NO-mediated vasorelaxation of smooth muscle cells. Nevertheless, the exact role of these channels still needs to be elucidated.

Vascular smooth muscle cells

Almost all TRPC family members are expressed in vascular smooth muscle cells and can be detected by their mRNAs via RT-

PCR experiments (reviewed in [67]). Because TRPC2 is a pseudogene in humans and TRPC7 is only expressed in coronary artery smooth muscle cells, we will focus on the function of TRPC1, -3, -4, -5, and -6 in vascular smooth muscle cells.

Smooth muscle cells provide not only structural integrity for the vessel but also precise regulation of vascular tone and blood pressure. However, a striking feature of smooth muscle cells is their cellular heterogeneity in different vessels making it difficult to talk about smooth muscle cells in general. Moreover, in response to various environmental stimuli, including growth factors, cytokines, mechanical influences, and various inflammatory mediators smooth muscle cells change their phenotype. The quiescent contractile vascular smooth muscle cells undergo transcriptional changes resulting in both the down-regulation of contractile proteins and concurrent up-regulation of proteins supporting a proliferative, so-called "synthetic" phenotype. This switch from the contractile to the proliferative and migratory phenotype is believed to have evolved as a mechanism of vascular repair during injury or vascular adaptation. Although this phenotypic switch is under tight control and is often reversible, it contributes to pathophysiological processes such as arteriosclerosis, hypertensive microvessels, vein graft failure and restenosis following percutaneous intervention. This switch can be induced *in vitro* by adding high concentrations of serum to the smooth muscle cells resulting in up- and down-regulation of different ion channels, receptors and Ca^{2+} pumps (reviewed in [68]). In pulmonary artery smooth muscle cells, TRPC1 and TRPC6 expression increases following serum stimulation (69, 70). Most interestingly, TRPC up-regulation was also observed in patients with idiopathic pulmonary arterial hypertension (IPAH) which is characterised by excessive proliferation of smooth muscle cells in the pulmonary artery. Up-regulation of TRPC3 and -6 in smooth muscle cells of these patients was detected recently (71) and siRNA directed against TRPC6 markedly attenuated proliferation of these cells *in vitro*. Smooth muscle cell proliferation results in neointima formation, which is an important component of arteriosclerosis but also occurs after vascular injury. Most interestingly it was reported that TRPC1 expression is also up-regulated in neointima from mice, rats, pigs and humans (72) and *in vitro* treatment of neointima with a TRPC1-antibody inhibits cell proliferation (72). Studies in human pulmonary arterial smooth muscle cells demonstrated that enhanced proliferation was associated with an increase in TRPC4 expression, and treatment of these cells with siRNA against TRPC4 inhibited ATP-induced proliferation (73). TRPC4 up-regulation was preceded by increases in phosphorylated CREB, and was prevented by blockage of purinergic receptors, as well as inhibition of CREB phosphorylation (73). Migration of vascular smooth muscle cells is another important component of neointima formation. Sphingosine-1-phosphate (S1P) binding to G protein-coupled receptors (GPCR) stimulates smooth muscle cell migration and induces Ca^{2+} entry in cultured human saphenous vein smooth muscle cells (74). Both primary smooth muscle cells from human saphenous veins and human saphenous veins express TRPC1 and -5, and treatment of these cells with a dominant negative construct of TRPC5 or a TRPC5-antibody inhibited

S1P-induced smooth muscle cell migration (75). To summarise, TRPC1, -4 and -5 seem to play a major role in Ca^{2+} -induced smooth muscle cell proliferation and migration.

Vascular smooth muscle cell contraction is activated by $\alpha 1$ -adrenergic agonists and can be blocked by suppressing TRPC6 expression (76), and activation of TRPC6 was also shown in a A7r5 smooth muscle cell line stimulated by vasopressin (77). Both reports agree on the important role of TRPC6-induced cation influx for smooth muscle cell contraction and a subsequent increase in blood pressure. Therefore, we expected smooth muscle contraction and blood pressure to be decreased in a TRPC6-deficient mouse line. Much to our surprise, we observed airway smooth muscle hyperreactivity after exposure to bronchoconstrictors and higher agonist-induced contractility in isolated tracheal (78) and aortic rings (19) prepared from these mice. Furthermore the systemic BP was elevated (19, 78). These effects are explained by *in vivo* replacement of TRPC6 by TRPC3-type channels which are closely related, but constitutively active (79), resulting in enhanced basal and agonist-induced cation entry into smooth muscle cells which increases smooth muscle contractility (19, 78). Moreover, smooth muscle cells from TRPC6-/- aorta or cerebral arteries are more depolarised and demonstrate enhanced spontaneous and agonist-induced Ca^{2+} entry (19). TRPC6 has also been shown to be involved in the regulation of myogenic tone in vascular smooth muscle cells of small resistance vessels. The elevation of intravascular pressure in these vessels increases myogenic tone, a phenomenon already described by Arthur Bayliss in 1902. Treatment of rat cerebral arteries with antisense oligonucleotides directed against TRPC6 inhibited vasoconstriction-induced by elevating intravascular pressure, and suppressed pressure-induced depolarisation of vascular smooth muscle cells (80). However, TRPC6-deficient mice displayed an earlier onset of the so-called Bayliss effect, excluding an exclusive role of TRPC6 for this effect. Along these lines, UTP-induced depolarisation of cerebral arteries and subsequent contraction of smooth muscle cells is inhibited by treatment with antisense oligonucleotides for TRPC3 (81), demonstrating again an important role of TRPC3 in these cells. Changes in TRPC3 expression has also been proposed to be a factor involved in essential hypertension, because spontaneously hypertensive rats express abnormally high levels of TRPC3 compared to wild-type control animals (82, 83).

Treatment of cultured arteries with an antibody to TRPC1 inhibited SOCE-induced contractions (84, 85), and adenovirus-mediated overexpression of human TRPC1 in rat pulmonary artery rings led to enhanced SOCE and enhanced SOCE-mediated contraction (86). In aortic smooth muscle cells from TRPC1-deficient mice, the amount of SOCE was indistinguishable from wild-type cells (20). Down-regulation of STIM1, however, completely abolished SOCE in these cells (20). The latter data clearly indicate that SOCE is completely independent of TRPC1 and no functionally relevant STIM1-TRPC1 interaction exists, at least in aortic smooth muscle cells.

In contrast to the systemic vasculature, the pulmonary circulation responds to hypoxia by constricting pulmonary arteries and diverting blood flow to the well-ventilated areas of the lung to en-

sure maximal oxygenation of the venous blood. This alveolar hypoxia-mediated vasoconstrictive phenomenon is known as acute hypoxic pulmonary vasoconstriction (HPV) and was first described by von Euler and Liljestrand in 1946. Sustained pulmonary vasoconstriction (chronic HPV) is often accompanied by vascular remodelling, i.e. the muscularisation 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, resulting from intimal fibrosis and medial hypertrophy is extensive. As outlined above, up-regulation of TRPC3 and -6 was observed in smooth muscle cells of IPAH patients (71) and in a rat model of pulmonary hypertension, TRPC1 and -6 up-regulation was shown to require the expression of hypoxia-inducible factor 1 (HIF-1) (87, 88). For these reasons, we analysed pulmonary arterial pressure in isolated lungs during acute (<20 min) and prolonged (60–160 min) hypoxia. Much to our surprise, acute HPV was completely absent in TRPC6^{-/-} mice, while the vasoconstriction after prolonged hypoxia was not significantly different in TRPC6^{-/-} mice compared to wild-type mice (21). These data show for the first time that the acute hypoxic vasoconstrictor response and the prolonged phase are regulated by different molecular mechanisms. TRPC6 is only indispensable for acute HPV and its loss cannot be compensated by up-regulation of other TRPC channels. Moreover, the lack of acute HPV in TRPC6^{-/-} mice has profound physiological relevance because partial occlusion of alveolar ventilation provoked severe hypoxaemia in TRPC6^{-/-}, but not in wild-type mice (21). However, vascular as well as cardiac remodelling after chronic hypoxia of three weeks was not significantly different in TRPC6^{-/-} mice compared to control mice (21). As for the systemic vasculature, small arterial vessels rather than large vessels are responsible for the regulation of the vascular tone. Most researchers, however, analyse the larger and easily isolated pulmonary arteries, although the TRPC expression pattern is significantly different in small and large pulmonary arteries (21, 89, 90). Most interestingly, TRPC3, which is up-regulated in TRPC6-deficient mice, is only expressed in large, but not in small precapillary pulmonary arterial smooth muscle cells (21, 89, 90). For this reason, we analysed Ca²⁺ influx in small precapillary pulmonary arterial smooth muscle cells (PASMC) from TRPC6-deficient and wild-type mice after priming the cells with endothelin-1. Hypoxic incubation of wild-type PASMC resulted in an increase in [Ca²⁺]_i, which was completely absent in PASMC from TRPC6^{-/-} mice (21). Most interestingly, Ca²⁺ influx was completely dependent on extracellular Ca²⁺ (21), excluding a proposed contribution of STIM1 and Orai, as well as store-operated TRPC channels (18). It is notable that the entry of Ca²⁺-ions in response to hypoxia is mostly carried by voltage-gated Ca²⁺ channels, because nifedipine, a potent blocker of these channels, almost completely inhibited acute HPV in isolated lungs and Ca²⁺ influx in wild-type PASMC (21). These data support a model in which Na⁺ influx through TRPC6 channels leads to membrane depolarisation and activation of voltage-gated Ca²⁺ channels (91–94). Most interestingly, TRPC6 seems to be activated by hypoxia-induced DAG accumulation, which can be mimicked by inhibition of DAG degradation to phosphatidic acid through DAG-kinases (21).

Therefore, initial priming by endothelin-1 results in PLC activation producing a basal DAG concentration which is not sufficient for TRPC6 activation. Hypoxia-induced DAG accumulation which activates TRPC6 might be induced by further activation of PLC, phospholipase D (PLD), which produces DAG from phosphatidic acid, or inhibition of DAG-degrading DAG kinases (reviewed in [22]). Further studies involving siRNA-mediated down-regulation of these proteins in precapillary pulmonary arterial smooth muscle cells will identify the exact molecular mechanism of TRPC6 activation during acute HPV.

Stretch-activated channels (SAC) which respond to mechanical stress as well as cell swelling are thought to be responsible for the pressure-induced vasoconstriction, e.g. the Bayliss effect described above. Characteristics of these channels were already described in vascular smooth muscle cells (reviewed in [95]). Two TRPC channels TRPC1 and TRPC6 were proposed to be activated by mechanical stimulation (96, 97). Patch clamp studies suggested an intriguing association of TRPC1 with the endogenous mechanosensitive cation channel (MSCC) in *Xenopus* oocytes (96). After fractionation of the *Xenopus* membrane proteins, the most efficient fraction was found to be rich in a ~80 kD protein immunoreactive to an TRPC1 antibody. Overexpression of human TRPC1 protein in *Xenopus* oocytes or the hamster cell line CHO-K1 remarkably enhanced, whereas microinjection of antisense TRPC1 copy RNA (cRNA) into the oocytes greatly reduced the MSCC activity (96). However, in smooth muscle cells from isolated cerebral arteries, we could not find any differences in mechanically-activated cation currents and pressure-induced vasoconstriction between TRPC1-deficient and wild-type mice (20). In addition, another report found that the MSCC activity was not significantly different between non-transfected and TRPC1-transfected CHO-K cells, and concluded that the observed MSCC may reflect the endogenous MSCCs present in the expression system (98). Similar conflicting evidence has been obtained regarding the apparent mechanosensitivity of TRPC6 channels. While one report proposed a common bilayer-dependent mechanism for mechanical activation of expressed TRPC6 channels (97), Gottlieb et al. found no TRPC6-mediated MSCC activity in a heterologous expression system (98). Moreover, a careful analysis of TRPC6 mechanosensitivity suggest that mechanical activation of TRPC6 channels is mediated by accumulation of DAG through mechanosensitive GPCR-activation, rather than by direct stimulation of TRPC6 by mechanical stimuli (99). Along these lines, two other indirect mechanisms result in stimulation of TRPC6-activity after mechanical stretching of smooth muscle cells. Mechanical stress can activate phospholipase A₂ (PLA₂) to produce arachidonic acid which can be converted into 20-hydroxyeicosatetraenoic acid (20 HETE) or EETs by ω -hydroxylases or epoxygenases, respectively (reviewed in [95]). One study already reported that exogenously applied 20-HETE could directly induce small cationic currents in TRPC6-expressing cells (100). However, these currents were atypical and did not show the characteristic doubly rectifying I-V curves demonstrated in HEK293 cells heterologously expressing TRPC6. Very recently, it was demonstrated that potentiation of TRPC6 activity by mechanically-activated receptors was decreased by

siRNA-mediated down-regulation of cytosolic PLA₂ and pharmacological inhibition of ω-hydroxylases (101). Therefore, intracellular produced 20-HETE is indeed able to directly activate TRPC6 channels to initiate pressure-induced contraction (101). In addition, the mechanically produced 11,12-EETs are able to promote translocation of TRPC6 channels to the plasma membrane leading to increased acute hypoxic pulmonary vasoconstriction which is absent in TRPC6-deficient smooth muscle cells (102).

Conclusions

At present, it is still enigmatic why nature provides us with several TRPC channels transcribed from different genes or produced by alternative splicing of pre-mRNAs (reviewed in [103]) in different tissues. However, future experiments with wild-type and gene-deficient mice will work out which unique and indispensable roles exist for TRPC channels in specific physiological processes in platelets, endothelial cells and smooth muscle cells. As already alluded to in this article, TRPC proteins may play a central role in the control of vascular smooth muscle tone, endothelial permeability and platelet function, because they are likely molecular correlates of receptor-operated cation entry in these cells. TRPCs may therefore be attractive novel drug targets to tackle pathophysiological states such as hypertension, oedema formation and bleeding disorders.

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