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INVITED REVIEW

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The diacylglycerol-sensitive TRPC3/6/7 subfamily of cation channels: functional characterization and physiological relevance

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Abstract Among the "classical" or "canonical" transient receptor potential (TRPC) family, the TRPC3, -6, and -7 channels share 75% amino acid identity and are gated by exposure to diacylglycerol. TRPC3, TRPC6, and TRPC7 interact physically and coassemble to form functional tetrameric channels. This review focuses on the TRPC3/6/7 subfamily and describes their functional properties and regulation as homomers obtained from overexpression studies in cell lines. It also summarizes their heteromultimerization potential in vitro and in vivo and presents initial data concerning their physiological functions analyzed in isolated tissues with downregulated channel activity and gene-deficient mouse models.

Keywords DAG-sensitive TRPC3, TRPC6, TRPC7 · Non-selective cation channels · Functional characterization · Physiological function · Smooth muscle cells · Pulmonary arterial hypertension · Bayliss effect

Introduction

Receptor-operated cation channels are gated in response to agonist binding to a membrane receptor distinct from the channel protein itself. Over the last decade, a large family of mammalian homologues of the *Drosophila* transient receptor potential (TRP) visual transduction channel has been identified (for review see [12]). The family of classical or canonical TRP cation channels (TRPCs) is composed of proteins that have the highest homology to *Drosophila* TRP. Its seven family members can be subdivided into subfamilies on the basis of their amino acid homology. Whereas TRPC1 and TRPC2 are almost unique, TRPC4 and TRPC5 share a roughly 65% identity. TRPC3, TRPC6, and TRPC7 form a structural and functional subfamily, sharing 70–80% identity at the amino acid level and their common sensitivity towards diacylglycerol (DAG) [23, 48].

Members of the TRPC3/6/7 subfamily display common structural features of the TRPC family: they contain four N-terminal ankyrin repeats, six transmembrane-spanning domains, a putative pore region located between transmembrane domains 5 and 6, and a highly conserved TRP box containing the EWKFAR motif and a proline-rich region (Fig. 1). Support for the relevance of the pore region for TRPC function is derived from site-directed mutagenesis studies resulting not only in the complete loss of channel activity on heterologous expression, but also in a dominant-negative effect of a mutated channel monomer on functional homo- or heteromeric channel tetramers [25]. On the contrary, the function of the EWKFAR motif and the neighboring proline-rich region forming the TRP box remains unclear.

Due to the lack of discriminating channel blockers, it is not possible to define the physiological relevance of TRPC homo- or heteromers for complex organ functions in the whole organism. To overcome these limitations, targeted gene inactivation in embryonic stem cells and subsequent production of gene-deficient mouse models for each of the channels and channel subfamilies is necessary to analyze TRPC channel function in vivo.

This review will focus on the TRPC3/6/7 family and describe their functional properties and regulation as homomers obtained from overexpression studies in cell lines. It will also summarize their heteromultimerization potential in vitro and in vivo and will present preliminary data concerning their physiological functions analyzed in isolated tissues with down-regulated channel activity and in gene-deficient mouse models (Table 1).

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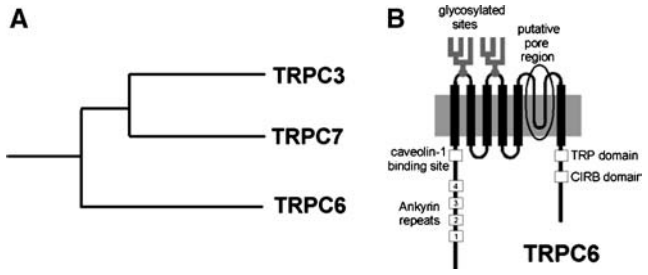

Fig. 1 **a** Phylogenetic tree of the "canonical" transient receptor potential (TRPC)3/6/7 subfamily. **b** Structural elements of the TRPC6 cation channel. The TRP domain contains an EWKFAR motif conserved in all TRPC, TRP vanilloid, and TRP melastatin family members. The role of the calmodulin-inositol 1,4,5-trisphosphate receptor-binding domain (*CIRB domain*) is discussed in the text

Biophysical properties

TRPC3, TRPC6, and TRPC7 are non-selective cation channels displaying double rectification with single-channel conductancies of 66, 35, and 25 pS, respectively [23, 26, 59, 88]. The relative ion permeability, P_{Ca}/P_{Na}, ranges from 5 for TRPC6 to 1.6 for TRPC3. However, the functional characterization of members of the TRPC3/6/7 subfamily following heterologous overexpression of their cDNA in various different cultured cell lines gave rise to contradictory results regarding intrinsic channel properties, such as activation mechanisms as well as ion selectivity.

A major drawback in the analysis of TRPC channels is the lack of specific pharmacological tools. TRPC channels are blocked by lanthanum (La^{3+}) and gadolinium (Gd^{3+}) ions [64], and most intriguingly, 50% inhibitory concentration (IC_{50}) values for Gd^{3+} ions differ between heterologously expressed channel homomers and endogenously occurring TRPC heteromers [64, 72]. Specifically, the IC_{50} values for La^{3+} ions for homomeric channel complexes vary from 4 μM to 6 μM for TRPC3 and TRPC6, respectively [20, 27, 30]. A complete suppression of TRPC7 was achieved by 100 μM La^{3+} [48]. The non-specific cation channel blocker flufenamate, however, may represent a pharmacological tool to differentiate between TRPC3, TRPC6, and TRPC7. In human embryonic kidney (HEK)293 cells expressing the recombinant mouse TRPC6 as well as in rabbit portal vein myocytes [27] and in A7r5 cells endogenously expressing TRPC6 [29], flufenamate has been reported to reversibly enhance currents mediated by TRPC6, whereas TRPC3 and TRPC7 were inhibited by the drug. Unfortunately, the potentiating effect of flufenamate could neither be reproduced in a HEK293 cell line permanently expressing TRPC6 [3].

Whereas the members of the TRPC3/6/7 subfamily share many common biophysical properties, they differ remarkably in their constitutive channel activity. When expressed in HEK293 cells, TRPC3 and TRPC7 display elevated basal channel activity that can be still substantially increased by agonist challenge, but not by emptying of intracellular Ca^{2+} stores (summarized in [66]). On the contrary, TRPC6 is a tightly receptor-regulated channel with minimal basal activity [23]. A structural analysis of heterologously expressed TRPC3

Table 1 Tissue distribution of members of the "canonical" transient receptor potential (*TRPC*)3/6/7 subfamily

Subtype	Species	Method of detection[a]	Tissue distribution[b]	Reference
TRPC3	Human	MTN	Brain	[88]
	Human	ISH	Arterial endothelial cells, arterial smc	[80]
	Human	WB	Pulmonary artery smc	[81]
	Mouse	WB	Pontine neurons	[35, 37]
	Rat	ISH	Cerebellar Purkinje cells	[49]
	Rat	WB	Cerebral arteries	[55]
	Pig	RT-PCR	Aortic endothelial cells	[1]
	Mammals	WB, RT-PCR	Smc	[4]
TRPC6	Human	MTN	Lung, placenta, ovary, spleen, ubiquitous	[24]
	Human	WB	Platelets	[21]
	Human	WB	Pulmonary artery smc	[82]
	Human	ISH	Arterial endothelial cells, arterial smooth muscle cells	[80]
	Mouse	MTN	Lung, brain	[7]
	Rat	ISH	Dentate gyrus granule cells; cerebral cortical neurons	[49]
	Rat	WB	Pulmonary artery smc	[38]
	Rat	WB	Cerebral arteries smc	[77]
	Rabbit	WB	Portal vein smc	[27]
	Mammals	WB, RT-PCR	Smc	[4]
TRPC7	Human	ISH	Arterial endothelial cells,	[80]
	Mouse	MTN	Heart, lung, eye, hindbrain, spleen, testis	[48]
	Mouse	ISH	Cerebellar Purkinje cells, olfactorial bulb, hippocampus	[48]
	Mammals	WB, RT-PCR	Smc	[4]

[a] *MTN* Multiple-tissue Northern blotting, *ISH* in situ hybridization, *WB* Western blotting, *RT-PCR* reverse transcriptase-polymerase chain reaction
[b] *smc* Smooth muscle cells

by glycosylation scanning mutagenesis revealed a naturally occurring glycosylation site in the second extracellular loop of the cation channel [69]. Although its functional significance remains poorly understood, the introduction of a second glycosylation site in the second extracellular loop in order to imitate the situation in TRPC6 reduced the high TRPC3 basal activity [14].

Regulation of channel activity

In all eukaryotic cells, activation of phospholipase C (PLC)-coupled membrane receptors by hormones leads to an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Both activation of PLCβ isozymes by G-protein-coupled receptors and of PLCγ isoforms by receptor tyrosine kinases results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol 1,4,5-trisphosphate (IP_3) and DAG. The increase of the $[Ca^{2+}]_i$ is initially caused by the release of Ca^{2+} from IP_3-sensitive intracellular stores, and is followed by an influx of cations from the extracellular space through activated TRP and other yet unknown channels. Two mechanisms by which PLC-linked receptors activate Ca^{2+} entry across the plasma membrane have been extensively discussed lately: store-operated Ca^{2+} entry (SOC) and receptor-operated Ca^{2+} entry. SOC occurs when IP_3 or some other signal discharges Ca^{2+} from intracellular stores in the endoplasmic reticulum (ER), and the subsequent fall in the ER Ca^{2+} concentration then signals to the plasma membrane activating store-operated channels. Experimentally, a similar cellular effect is evoked by thapsigargin or cyclopiazonic acid, which blocks sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA) pumps, resulting in leakage of Ca^{2+} ions from internal stores. Neither the exact mechanisms for this signaling pathway nor the molecular identity of the SOC channels are known with certainty so far. However, there are at least three general theories for the mechanisms of signaling. One theory suggests the release from the ER of a diffusible messenger of still unknown identity, called Ca^{2+} influx factor (CIF), which would activate SOC channels. It has recently been postulated that CIF does not directly activate SOC channels but releases intracellular phospholipase A_2 ($iPLA_2$) from constitutive inhibition by calmodulin. Lysophospholipids as reaction products of $iPLA_2$ activity are subsequently thought to be able to induce SOC currents in isolated inside-out patches [61]. The second theory, known as conformational coupling [5, 28], involves agonist-bound IP_3 receptors in close vicinity to the plasma membrane, which are able to activate membranous Ca^{2+}-permeable ion channels by direct protein–protein interaction. In a third mechanism, a Ca^{2+} entry channel transported in a cytoplasmic vesicle is proposed to fuse with the plasma membrane to mediate SOC [51, 78]. In accord with this notion, recent data suggest that TRPC channels are held in reserve and are rapidly translocated to the cell membrane in response to a stimulus [6, 10].

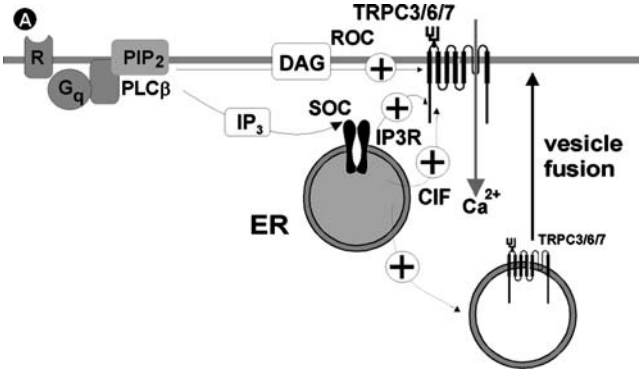

Fig. 2 Proposed regulatory mechanisms of TRPC3/6/7 channel activity. After binding of an agonist (*A*) to its receptor (*R*), G-protein (G_q)-activated phospholipase C-β (*PLC*β) cleaves phosphatidylinositol 4,5-bisphosphate (*PIP_2*) into inositol-1,4,5 trisphosphate (*IP_3*) and diacylglycerol (*DAG*). TRPC3/6/7 channels can be activated by DAG, resulting in receptor-operated Ca^{2+} influx (*ROC*). Involvement of TRPC3 and TRPC7 in store-operated Ca^{2+}-influx (*SOC*) is also discussed. For the regulation of SOC by the filling status of internal Ca^{2+} reservoirs in the endoplasmatic reticulum (*ER*), three hypothesis are currently known: (1) direct or indirect activation by a soluble Ca^{2+} influx factor (*CIF*), (2) conformational coupling of activated IP_3 receptors to the channel protein, or (3) fusion of a cytoplasmic vesicle transporting a Ca^{2+} entry channel with the plasma membrane. (See text for a more detailed description of the mechanisms.) A possible activation of TRPC3 and TRPC6 by tyrosine kinases and via phospholipase Cγ is not shown, but discussed in the text

At present, the gating mechanism(s) of TRPC channels (summarized in Fig. 2) and especially of the DAG-sensitive TRPC3/6/7 subfamily still remain(s) elusive, and it is a highly contentious issue as to whether TRPC channels are involved in SOC entry at all.

TRPC3 was initially described as a receptor-operated as well as a thapsigargin-activated cation channel by a study overexpressing the cation channel in HEK293 cells [86]. Heterologous expression in Chinese hamster ovary cells, however, resulted in Ca^{2+}-activated TRPC3 channel activity [88]. The latter results were confirmed by expressing a heart-specific splice variant of rat TRPC3 in *Xenopus* oocytes [47]. In a subsequent study by Zhu et al. [87], the activation of TRPC3 was carefully re-examined in an HEK293 cell line stably expressing high levels of TRPC3. The authors concluded that TRPC3 is an agonist-operated cation channel, but it is apparently not activated by store depletion.

Moreover, in DT40 chicken B lymphocytes, TRPC3 expressed at low levels was found to be activated by depletion of Ca^{2+} stores, whereas at higher channel densities in the cell membrane, TRPC3 activity was increased by receptor coupling to PLC isoforms [71, 72]. These results support the hypothesis that the cellular expression level of the channel protein may critically influence its functional characteristics (reviewed in [52]).

In contrast to the results mentioned above, electrophysiological analysis of an HEK293 cell line stably expressing TRPC3 provided evidence for a

store-operated activation mechanism via conformational coupling. In excised membrane patches, TRPC3 currents previously elicited by store depletion were abolished after extensive washing, and could be recovered by addition of brain microsomal membranes containing agonist-bound IP_3 receptors [31]. This first piece of direct evidence for TRPC channel activation by IP_3 receptors was subsequently complemented by the identification of contact sites in both proteins [8], and corresponding peptides mimicking these contact sites were able to suppress or activate Ca^{2+} influx when acting at heterologously expressed TRPC channels as observed in Ca^{2+} imaging experiments [8]. Moreover, by competitive binding of Ca^{2+}/calmodulin and IP_3 receptors to the same site [**c**almodulin-**IP**3 **r**eceptor-**b**inding domain (**CIRB** domain)] in the intracellular C terminus of all members of the TRPC family, a putative Ca^{2+}/calmodulin-dependent inactivation mechanism of these cation channels was proposed [63]. In agreement with this notion, application of the calmodulin inhibitor calmidazolium resulted in enhanced TRPC3 channel activity [85]. These mechanisms of activation and deactivation have been challenged, because in a DT40 cell line devoid of all three forms of IP_3 receptors, TRPC3 is activated by agonists to the same extent as in wild-type cells [74], yet it was recently discovered that these cells still express fragments of IP_3 receptors that could bind to TRPC6 [19] and probably other TRPC channels. However, in the same TRPC3-expressing HEK293 cell line previously used to develop the conformational coupling hypothesis, TRPC3 activation was demonstrated to function independently of the IP_3 receptor [65]. A recent mutagenesis study then revealed that the CIRB domain of TRPC3 is involved in channel targeting to the cell membrane without requiring functional interaction with either calmodulin or IP_3 receptors [76]. To conclude, the latter data strongly support the view of a receptor-operated TRPC3 gating mechanism that is mediated by the second messenger DAG, without involvement of protein kinase C (PKC) activity [23, 65]. Along these lines, a recent report by Singh et al. [60] demonstrated that receptor-dependent activation of TRPC3 is associated with exocytosis-mediated plasma membrane insertion, whereas thapsigargin had no effect on subcellular localization of TRPC3 [60]. Very recently, however, a longer variant of TRPC3 (TRPC3a) shows Gd^{3+}-resistant (5 μM) activation by store depletion when expressed in HEK293 cells stably expressing TRPC3a and analyzed by single-cell fluorometry [79].

As opposed to the situation with TRPC3, store-operated activity of **TRPC6** has never been much of an issue, although high-affinity IP_3 receptor peptide interaction with the TRPC6 C terminus has been proven biochemically [63]. Functional analysis of mouse TRPC6 clearly demonstrated a receptor-activated, store-independent cation channel [7]. As TRPC6 activation was inhibited by pre-treatment with a PLC inhibitor but could not be activated by application of IP_3, the role of DAG as an additional messenger as well as additional DAG derivates, e.g., arachidonic acid, was characterized further [23]. In isolated inside-out patches, membrane-permeable [1-oleoyl-2-acetyl-*sn*-glycerol (OAG)] as well as naturally occurring DAGs (1-stearoyl-2-arachidonoyl-*sn*-glycerol and 1-stearoyl-2-linoleoyl-*sn*-glycerol) were able to activate TRPC6 in a membrane-delimited fashion. Of note, DAG stimulation of TRPC6 is independent of PKC activation, as deduced from the ineffectiveness of various PKC inhibitors or downregulation of PKC by long-term phorbol ester treatment [23]. More recently, TRPC6 has also been reported to be sensitive to the arachidonic acid metabolite 20-hydroxyeicosatetraenoic acid [3].

Similarly, TRPC3 and TRPC7 are also activated by DAG. However, most interestingly, it was discovered that DAG activation is not an exclusive characteristic of the TRPC3/6/7 family, because DAG sensitivity of TRPC2 and TRPC5 was reported recently [33, 43]. The exact location of a putative binding site for diacylglycerols in TRPC proteins is still elusive, because an OAG-insensitive splice variant of TRPC6, (TRPC6B, [84]) characterized by means of fluorometry turned out to be activated by DAG when analyzed by electrophysiological methods [29].

The regulation of TRPC6 by calmodulin was carefully assessed, leading to an overall picture different from that of TRPC3. Calmodulin inhibitors like calmidazolium and trifluoperazine had an inhibitory effect on receptor-operated Ca^{2+} influx into TRPC6-expressing HEK293 cells, indicating a stimulatory impact of Ca^{2+}/calmodulin on TRPC6 channel activity [9]. The latter concept was further extended by the observation that accelerated TRPC6 activation by the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) most probably involves phosphorylation by calmodulin-dependent kinase II, an effect that was not noted for the closely related TRPC7 protein [59].

TRPC7 was characterized as a non-selective cation channel activated by DAG secondary to receptor stimulation, but clearly unaffected by store depletion. In contrast to these initial findings, cloning of human TRPC7 and stable expression of the protein in HEK293 cells resulted in store-operated channel activity [56]. To shed further light on this discrepancy, both clones were recently reanalyzed in HEK-293 cells and compared to TRPC3 and TRPC6. Whereas stable expression of both TRPC7 clones in HEK293 cells resulted in a receptor- and store-operated mode of activation, transient expression in the same cells revealed only receptor-operated activation of TRPC7 [39]. Both clones shared the high basal activity of TRPC3, and enhancement of channel activity by IP_3 was demonstrated recently [59]. Similar to TRPC6, TRPC7 is regulated by Ca^{2+} on both sides of the plasma membrane involving complex calmodulin-dependent and independent mechanisms [59].

Another receptor-dependent signaling event that modulates TRPC3 and TRPC6 activity was recently discovered [22, 73]. Inhibition of **tyrosine kinases** in HEK293 cells stably expressing TRPC3 abolished

activation of the ion channel by muscarinic receptor stimulation and by DAG. Receptor and DAG-dependent activation was lost, following expression of a dominant-negative mutant of Src or expression of TRPC3 in a Src-deficient cell line [73] placing further emphasis on the role of tyrosine phosphorylation for TRPC3 function. Fyn, another member of the Src family of protein tyrosine kinases, increases TRPC6 channel activity. Stimulation of epidermal growth factor receptor entails tyrosine phosphorylation of TRPC6, and Fyn and TRPC6 physically interact in mammalian brain as well as after heterologous expression in COS-7 cells [22].

TRPC3 and TRPC6 are also targets for **PKC**-dependent inhibition, thus providing a potential feedback loop initiated by DAG-activated PKC [67, 75]. At the molecular level, a single highly conserved serine residue immediately downstream of the TRP domain in the C termini of all members of the TRPC family, appears to be essential for PKC-mediated negative regulation of TRPC3 [67].

An interesting structural aspect was recently identified in TRPC3. The channel protein contains a partial **PH-like domain** that interacts with the complementary partial PH domain of phospholipase $C\gamma_1$ to facilitate lipid binding and cell surface expression of TRPC3 [70].

TRPC3 expression and its proposed physiological role

TRPC3 was the second cloned gene of the TRPC family [86] and is the most abundant TRPC channel in brain. The expression pattern is most pronounced in cerebellar Purkinje and in olfactory mitral cells [49]. In neurons, TRPC3 appears to be an integral component of a signal transduction cascade initiated by brain-derived growth factor (BDNF), involving PLCγ generation of IP_3 and an increase in $[Ca^{2+}]_i$ [35]. In line with this assumption, TRPC3 protein was immunoprecipitated with the receptor tyrosine kinase TrkB, which is activated by BDNF. During brain development, the spatial and temporal expression patterns of both TrkB and TRPC3 were found to coincide, and TRPC3 may contribute to the BDNF-induced signaling cascade in pontine neurons [35]. However, the channel characterized in pontine neurons differs from heterologously expressed TRPC3 with regard to its single-channel conductance, mean open time, and sensitivity to IP_3. Until now, it is unclear whether other channel subunits or accessory proteins are needed to reconstitute the distinct biophysical properties observed in pontine neurons. Two recent reports stress the relevance of these additional proteins. Expression of dominant-negative forms of TRPC3 and TRPC6 in neurons inhibited BDNF-induced growth cone turning [37], and the interaction of TRPC3 with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor complex was demonstrated very recently in neuronal and epithelial cells [60]. Most interestingly, a vesicle-associated protein 2-dependent exocytosis of TRPC3 in these cells was suggested [60].

Oxidative stress may also result in activation of mammalian TRPC proteins. Endothelial cells express an oxidant-activated, non-selective cation channel that functions as a redox sensor in the vascular endothelium, and a dominant-negative form of TRPC3 abolishes the oxidant-induced current [1]. These experiments suggest that either TRPC3 or a channel capable of heteromultimerizing with TRPC3 contributes to this current. On the basis of these studies, it is imaginable that oxidative stress in the mammalian brain may result in constitutive activation of TRP proteins, which would then initiate cell death due to uncontrolled influx of cations such as Ca^{2+}. Moreover, HEK293 cells overexpressing TRPC3 show an increase of basal membrane conductance after exposure to the lipophilic peroxide *tert*-butyl hydroperoxide, whereas wild-type cells are characterized by a remarkable resistance to oxidative stress [17].

The mammalian signal transduction cascade activated by light differs fundamentally from *Drosophila*, where TRPs are the key players [46]. Recently, a photopigment called melanopsin was discovered that signals more as an invertebrate opsin than as a classical vertebrate rod and cone opsin. Melanopsin has been proposed to be the photopigment of the intrinsically photosensitive retinal ganglion cells that control circadian and pupillary adjustments by projections to the brain distinct from the visual system. Coexpression of melanopsin with TRPC3 in HEK293 cells or coexpression of TRPC3 with melanopsin and arrestin in *Xenopus* oocytes results in light-triggered membrane depolarization and increased $[Ca^{2+}]_i$ [54, 50], indicating a role of TRPC3 channels in the mammalian eye.

It is still elusive if non-selective cation channels like TRPC3/6/7 can contribute to molecular complexes that form Ca^{2+} release activated Ca^{2+} channels in T-cells, but thapsigargin-activated Ca^{2+} entry depends on the presence of TRPC3 in Jurkat T-cells [53].

As revealed by expression of antisense oligodeoxynucleotides, TRPC3 seems to be responsible for the uridine triphosphate-induced depolarization and constriction of intact cerebral arteries [55], whereas TRPC6 has been proposed to play a critical role in the intravascular pressure-induced depolarization and constriction of small arteries and arterioles [77] known as the Bayliss effect.

In most smooth muscle tissues, coexpression of TRPC3 and TRPC6 can be readily observed ([80]; see [4] for a recent review) probably resulting in heteromeric channels of yet unknown composition, functional properties, and biological roles. As mentioned before, only the identification of TRPC3 activity in its physiological settings or the phenotypic analysis of TRPC3-deficient mice will shed further light on these important issues.

TRPC6 expression and its proposed physiological role

The full-length cDNA of mouse TRPC6 was isolated from brain [7], whereas human TRPC6 was cloned from

placenta [23]. TRPC6 is most prominently expressed in lung tissues as deduced from Northern blot analysis [7]. Three splice variants with shorter amino termini were additionally cloned from rat lung [84].

As mentioned above, a recent study indicated an important role for TRPC6 in conjunction with TRPC3 for the growth cone turning of neurons depending on a BDNF gradient [37]. Although TRPC6 expression in brain is lower than that of TRPC3, in situ hybridization and histochemistry revealed TRPC6 expression exclusively in the dentate granule cell layer of the adult mouse brain [49].

There is increasing evidence that TRPC6 is an intrinsic constituent of receptor-operated cation entry involved in numerous physiological processes. In human platelets, thrombin-activated cation influx is independent of store depletion, consistent with the observation that TRPC6 is highly expressed in these cells [21]. In this model system, TRPC6 does not serve as a substrate of tyrosine kinases, but is phosphorylated in a cAMP-dependent manner [21]. It is well documented that phosphoinositide 3 kinase activation resulting in the production of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) triggers platelet aggregation by inducing a Ca^{2+} influx [42]. In accord with this concept, TRPC6 was recently identified as the putative molecular correlate of a PIP_3-sensitive Ca^{2+}-entry system in platelets, Jurkat T-cells, and RBL-2H3 mast cells [68].

In situ hybridization revealed expression of TRPC6 in the endothelial and smooth muscle cells of human coronary and cerebral arteries [79], and there are numerous studies providing evidence for an important role of TRPC6 in vascular and pulmonary smooth muscle cells. By comparative biophysical characterization and gene suppression using antisense oligonucleotides, TRPC6 was suggested to be the molecular correlate of the α_1-adrenoceptor-activated, non-selective cation channel in vascular smooth muscle cells [27] and the vasopressin-activated cation channel in an aortic smooth muscle cell line [29]. In addition, TRPC6 is responsible for the mechanical stimulation resulting in the Bayliss effect ([77]; see above). Myogenic constriction of resistance arteries results from Ca^{2+} influx through voltage-gated Ca^{2+} channels subsequent to membrane depolarization, but the precise location of TRPC6 in the signaling pathway elicited by elevated intravascular pressure still remains poorly understood.

Recently, expression studies revealed that PDGF-mediated proliferation of pulmonary artery smooth muscle cells (PASMC) is associated with c-Jun/STAT3-induced upregulation of TRPC6 expression [81]. In this context, it is intriguing to note that excessive PASMC proliferation, a major cause of the elevated pulmonary vascular resistance in patients with idiopathic pulmonary arterial hypertension (IPAH), also correlates with overexpression of TRPC6 and TRPC3 proteins in PASMC. In line with these data, downregulation of TRPC6 by TRPC6-specific small interfering RNA attenuated IPAH–PASMC proliferation [82]. Moreover, TRPC6 expression is upregulated in pulmonary arteries of rats kept under chronic hypoxic conditions to induce pulmonary hypertension. As expected, OAG-induced cation entry was significantly increased in hypoxia-treated PASMC as compared to control cells [38].

Recently, we were able to present initial results on the phenotype of mice deficient in TRPC6. Based on the above reviewed data, we predicted that loss of TRPC6 function would lead to a diminished vascular smooth muscle tone and hypotension. Unexpectedly, we observed higher agonist-induced contractility in aortic rings prepared from these mice, and elevated systemic blood pressure that was further increased by inhibition of nitric oxide synthase [13]. These effects could be explained by in vivo replacement of TRPC6 by TRPC3-type channels that are closely related, but constitutively active resulting in enhanced basal and agonist-induced cation entry into smooth muscle cells, leading to increased smooth muscle contractility [15, 45]. Because the expression pattern of TRPC3 and TRPC6 overlaps in most tissues (reviewed in [4]), a heteromeric TRPC3/6 channel complex most probably represents the molecular correlate of the non-selective cation influx into smooth muscle cells (reviewed in [18]).

TRPC7 expression and its proposed physiological role

TRPC7 was first cloned from mouse brain, and its function was analyzed after heterologous expression in HEK293 cells [48]. TRPC7 is expressed in heart, lung, and eye, with lower levels of transcripts in brain, spleen, and testis [48], but it is not expressed endogenously in HEK293 cells [56].

In contrast to TRPC3 and TRPC6, TRPC7 was not detected in smooth muscle cells of human coronary and cerebral arteries by in situ hybridization, although strong hybridization signals for all three channel mRNA were seen in endothelial cells [80]. There are no data available about its physiological functions, and a gene-deficient mouse model is not available yet.

Multimerization potential of the TRPC3/6/7 subfamily

As originally demonstrated for *Drosophila* TRP (reviewed in [46]), TRPCs form homomeric, but also heteromeric channel complexes with other TRPCs and accessory proteins. This fact may contribute to the observation that it has proven difficult to ascribe unequivocally native non-selective cation currents to molecularly defined TRPCs expressed heterologously. The basic principles of homo- and hetermultimerization of heterologously expressed TRPC channel monomers were defined by four different approaches: cellular co-trafficking of TRPC subunits, differential functional suppression by dominant-negative subunits, fluorescence resonance energy transfer, and coimmunoprecipitation [25]. All experimental approaches employed led to the

conclusion that TRPCs assemble into homo- and heterotetramers within the confines of TRPC subfamilies. Only TRPC1 has the ability to form channel subunits with the TRPC4/5 subfamily, whereas TRPC2 is not able to interact with any other TRPC channel. These results were confirmed by systematic coimmunoprecipitation of TRPCs from isolated rat brain synaptosomes [16]. Recently, novel combinations of TRPC1–TRPC4/5 together with TRPC3/6 were identified in HEK293 cells as well as in embryonic brain but not in adult rat tissues [62]. Moreover, native TRPC3/6 heteromers were identified in epithelial cells [2], and TRPC2 appears to interact with TRPC6 in murine erythroblasts [11].

These discrepant findings may be reconciled by the presence of embryonic cell-specific factors stabilizing such complexes. In general, TRPC channel complexes may also be organized in supramolecular signaling complexes with accessory proteins in native tissues. Such a complex, called signalplex, was already identified in photoreceptors of *Drosophila melanogaster* [46], and there is evidence for TRPC1 function in complexes with the adaptor protein homer [83]. Furthermore, TRPC1 and TRPC3 are assembled in caveolar lipid rafts in multimolecular signaling complexes containing PLCβ, $G\alpha_{q/11}$, IP3R, SERCA, ezrin, and caveolin 1 [40, 41]. Along these lines, colocalization and functional interaction of TRPC3 and the Na^+/Ca^{2+} exchanger 1 was discovered recently [57].

Moreover, the interaction of the second ankyrin repeat in TRPC6 with MxA, a member of the dynamin superfamily was described recently, although its functional role remains unclear [44], and transient coexpression of Alzheimer's disease-linked presenilin 2 mutants abolished agonist-induced TRPC6 activation without affecting agonist-induced endogenous Ca^{2+} entry in HEK293 cells [34].

Conclusion

At present, the exact physiological role of the TRPC3/6/7 subfamily is still largely unknown. Hopefully, the overt lack of specific pharmacological blockers will be at least partially overcome by the use of small interfering RNA. As already pointed out in this review, the TRPC3/6/7 are likely molecular correlates of receptor-operated cation entry especially in smooth muscle cells [32]. DAG-sensitive TRPCs may therefore be attractive novel drug targets to tackle pathophysiological states such as asthma, hypertension [58, 82], and pulmonary diseases [36].

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