SYMPOSIUM REPORT

Functional role of TRPC proteins in native systems: implications from knockout and knock-down studies

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Available data on transient receptor potential channel (TRPC) protein functions indicate that these proteins represent essential constituents of agonist-activated and phospholipase C-dependent cation entry pathways in primary cells which contribute to the elevation of cytosolic Ca^{2+}. In addition, a striking number of biological functions have already been assigned to the various TRPC proteins, including mechanosensing activity (TRPC1), chemotropic axon guidance (TRPC1 and TRPC3), pheromone sensing and the regulation of sexual and social behaviour (TRPC2), endothelial-dependent regulation of vascular tone, endothelial permeability and neurotransmitter release (TRPC4), axonal growth (TRPC5), modulation of smooth muscle tone in blood vessels and lung and regulation of podocyte structure and function in the kidney (TRPC6). The lack of compounds which specifically block or activate TRPC proteins impairs the analysis of TRPC function in primary cells. We therefore concentrate in this contribution on (i) studies of TRPC-deficient mouse lines, (ii) data obtained by gene-silencing approaches using antisense oligonucleotides or RNA interference, (iii) expression experiments employing dominant negative TRPC constructs, and (iv) recent data correlating mutations of TRPC genes associated with human disease.

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TRP channels may couple plasma membrane receptors and the activation of voltage-dependent channels

Stimulation of cells with extracellular agonists leads to an increase of $[Ca^{2+}]_i$ both from Ca^{2+} release from intracellular organelles and Ca^{2+} entry across the plasma membrane (Fig. 1). This process is initiated upon the binding of a hormone or a growth factor to its receptor and activation of phospholipase C (PLC). PLC in turn converts phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Plasma membrane channels, including TRPC channels, are activated and extracellular cations, including Ca^{2+}, flow into the cell. The activation of plasma membrane channels might involve a reduction of the PIP_2 concentration or an increase of DAG or its metabolites such as polyunsaturated fatty acids (Hardie, 2003). By reduction of PIP_2 tonic PIP_2-dependent inhibition might be abolished whereas DAG or polyunsaturated fatty acids might activate TRPC channels directly. In addition IP_3 will bind to its receptor in the endoplasmic reticulum membrane which results in the release of intracellular Ca^{2+}. This process might be accompanied by the opening of Ca^{2+} release-activated or store-operated channels in the plasma membrane which allow additional cations, including Ca^{2+}, to enter the cell. When $[Ca^{2+}]_i$ reaches a threshold level Ca^{2+}-activated non-selective (CAN) channels will be activated. The TRPC-related TRPM4 and TRPM5 proteins might underlie CAN channel activity and allow Na^+ to enter the cell. Thereby, the cell is depolarized and voltage-dependent Ca^{2+} channels do open. Ca^{2+} influx mediated by voltage-gated Ca^{2+} channels then further contributes to the increase of $[Ca^{2+}]_i$ and the spatio-temporally controlled elevations of $[Ca^{2+}]_i$ in defined cell compartments as key signals for numerous cellular functions. Available data support the engagement of this signalling cascade in T-lymphocytes (Badou *et al.* 2005) and, probably, in certain smooth muscle cells. Concerning TRPC channels there is evidence

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that they can be activated by DAG, by direct interaction with the Ca^{2+} release channels of the endoplasmatic reticulum, the IP_3 receptor and the ryanodine receptors, by translocation to the plasma membrane via an exocytotic process and by store-operated mechanisms (Parekh & Putney, 2005). Whereas the molecular composition of the TRPC channels in native tissues is still elusive the subunit structure of voltage-gated Ca^{2+} channels (Ca_v) has been known for years. They represent heteromultimeric protein complexes consisting of the pore-forming $\alpha 1$ subunit and the ancillary subunits β, γ, $\alpha 2$ and δ, which modulate the expression, targeting, gating and activity of the $\alpha 1$ subunits. Recent studies indicate that individual $Ca_v\beta$ subunits not only constitute a portion of the Ca^{2+} channel but also regulate intracellular IP_3 levels (Berggren *et al.* 2004) and gene transcription (Hibino *et al.* 2003).

TRPCs are members of the TRP family of cation channels and, to date, at least 28 TRP homologues have been identified in mammalian genomes. According to their primary structure, mammalian TRP proteins are classified into six subgroups, in addition to the TRPC subgroup, TRPV, TRPM, TRPP, TRPML and TRPA (Montell *et al.* 2002; Montell, 2005). The seven members of the TRPC subfamily (classical or canonical TRPs) are structurally related to *Drosophila* TRP and to each other (> 30% amino acid sequence identity within the amino-terminal 750–900 amino acid residues) and differ mainly within their carboxy-terminal regions. There is evidence that TRPC1, TRPC4 and TRPC5 or TRPC3, TRPC6 and TRPC7, respectively, do specifically interact and form heteromultimeric protein complexes (Strubing *et al.* 2001, 2003; Hofmann *et al.* 2002; Goel *et al.* 2002). Accordingly coexpression of TRPC1, TRPC4 and TRPC5 can be readily detected in hippocampal neurones (Fig. 1) although in most other tissues and cells expression patterns of the three genes differ substantially (Freichel *et al.* 2004).

Concerning the possible functions of TRP proteins there is ample evidence that phospholipase C-dependent processes are involved in TRPC channel gating (see above). However, the data available have been mainly obtained after overexpression of the TRPC cDNAs in common cell culture systems such as HEK 293 cells (human embryonic kidney cell line). These cells were introduced originally because they readily overexpress foreign proteins (Pritchett *et al.* 1988; Flockerzi *et al.* 2005). Moreover mRNA isolated from HEK 293 cells have been used as a source to clone the full length cDNAs of the human TRPC1 (Zhu *et al.* 1995) and TRPC3 (Zhu *et al.* 1996). Accordingly it is not unlikely to assume that endogenous TRPC1 and TRPC3 proteins are already expressed in these cells at decent concentrations. These proteins can build endogenous homo- and heteromultimeric TRPC1, TRPC3 and TRPC1/TRPC3 channels which may contribute to currents recorded from these cells. It is not difficult to foresee that the expression of additional foreign TRPC proteins in these HEK cells will further aggravate the interpretation of whole-cell current recordings and to define the contribution of the same TRPC protein to these currents. Unfortunately, reliable anti-TRPC antibodies which might allow the monitoring of endogenous TRPC protein expression are scarce (Ong *et al.* 2002; Flockerzi *et al.* 2005).

TRPC-deficient mouse models and gene silencing of TRPC genes in primary cells

Few methods overcome the restraints of overexpression systems and the lack of compounds known to block or to activate TRPC-mediated cation entry into cells with sufficient potency and specificity. To characterize the functional relevance of individual TRPC proteins in their native environment for cation entry into cells, for isolated

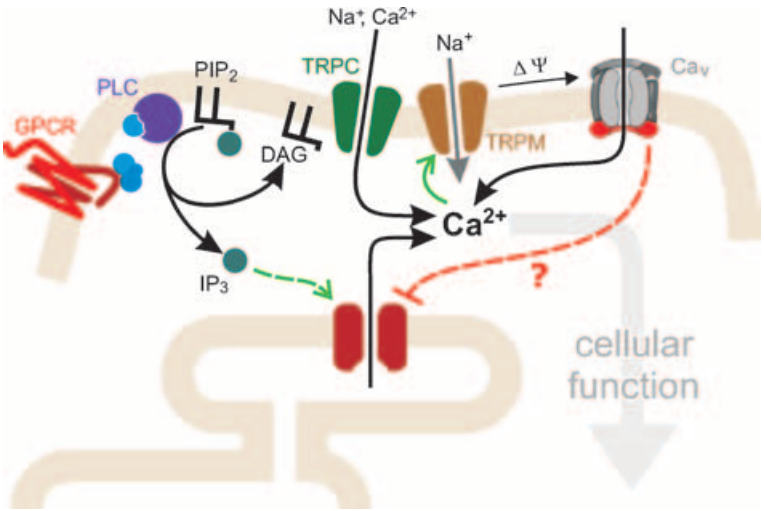

Figure 1. Assumed roles of TRPC proteins for regulation of the membrane potential and voltage-dependent ion channels
GPCR, G protein-coupled receptor; PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; Ca_v, voltage-gated Ca^{2+} channel; $\Delta\Psi$, change in membrane potential.

organ physiology and for complex systemic functions in a whole organism we and others therefore used (i) targeted gene inactivation in embryonic stem cells to generate mice carrying null alleles of TRPC genes, (ii) silencing of TRPC transcripts in primary isolated cells using antisense oligonucleotides or RNA interference, or (iii) expression of dominant negative TRPC variants. In addition, for the first time spontaneous mutations within one TRPC gene were linked to a hereditary human disease. Based on segregation studies in several families and direct identification of missense mutations in affected individuals two groups described independently an essential role for the TRPC6 gene in glomerular slit diaphragm-associated channel activity in the kidney.

TRPC1. TRPC1 is expressed in brain, heart, kidney, endothelium, ovaries, testis and in smooth muscle cells (Freichel *et al.* 2004; Inoue *et al.* 2004; Montell, 2005). The mode of activation of TRPC1-mediated cation entry is still under discussion but may accompany PLC-dependent processes and depletion of intracellular Ca^{2+} stores (Zitt *et al.* 1996; Montell, 2005). So far no results have been published on TRPC1-deficient mice. However, the TRPC1 gene was inactivated by somatic cell gene targeting in the DT-40 B lymphocyte cell line (Mori *et al.* 2002). These DT-40 cells lack expression of TRPC1 transcripts and protein as shown by Northern blot and immunocytochemical studies. Stimulation of the B cell receptor or application of thapsigargin leads to an elevation of $[Ca^{2+}]_i$ and this elevation was significantly impaired in the TRPC1-deficient cells. In addition, targeting of the TRPC1 gene resulted in a reduction of the occurrence of Ca^{2+} currents activated by dialysis of these cells with IP_3 by 80%, of the number of Ca^{2+} oscillations evoked upon B cell receptor stimulation, and of the nuclear factor of activated T cell (NF-AT)-dependent gene expression (Mori *et al.* 2002). In another study, antisense oligonucleotides directed against TRPC1 mRNA were injected into endothelial cells isolated from human pulmonary artery. Upon stimulation with thapsigargin the injected cells responded with a smaller elevation of the intracellular Ca^{2+} concentration compared to non-injected cells. The extent of reduction of TRPC1 protein in the antisense injected endothelial cells has not been shown in this study. (Brough *et al.* 2001). To study TRPC1 function in neurones, Kim *et al.* (2003) generated a mutated TRPC1 protein by replacing the phenylalanine residue at position 561 of the primary structure by an alanine residue (TRPC1/F561A). In contrast to wild-type TRPC1, this mutated TRPC1 did not induce significant inward currents when overexpressed in the Chinese hamster ovary cell line (CHO cells) constitutively expressing the metabotropic glutamate receptor type 1 (mGluR1). Transfection of the TRPC1/F561A mutant into cerebellar Purkinje cells resulted in a 49% reduction of mGluR-evoked slow excitatory postsynaptic currents (EPSCs) whereas fast transmission mediated by AMPA-type glutamate receptors remained unaffected. The mGluR1 receptor is coupled to PLC via G_q and the results obtained are in line with PLC-mediated gating of TRPC1. Recently, TRPC1 has been implicated as a component of a mechanosensitive cation channel in *Xenopus* oocytes, which transduces membrane stretch into cation flux across the cell membrane (Maroto *et al.* 2005). However, in primary cells from mammals there is so far no evidence for any TRPC being activated by mechanical stimuli. Singh *et al.* (2001) introduced TRPC1 into the salivary gland of rats by adenovirus-mediated gene transfer. They demonstrated that after TRPC1 overexpression saliva secretion increased upon stimulation with a muscarinic receptor agonist. In parallel the muscarinic agonist-induced Ca^{2+} entry was increased in cells isolated from infected salivary glands (Singh *et al.* 2001).

TRPC2. TRPC2 is a pseudogene in humans containing six mutations that generate premature stop codons (Liman & Innan, 2003). In rodents, a functional TRPC2 gene is expressed in the vomeronasal organ (VNO) and possibly in the testis (Vannier *et al.* 1999; Jungnickel *et al.* 2001). The VNO neurones and their associated projections represent a set of olfactory neurones which is anatomically distinct to the main olfactory epithelium. The VNO is found in most terrestrial vertebrates but is likely to be vestigial in humans. In rodents, the VNO gains access to water-soluble chemical cues carried by the nasal mucus and sends fibres to the accessory olfactory bulb, which in turn projects to discrete loci of the ventromedial hypothalamus via the mediocortical amygdala. The VNO pathway was previously suggested to play a primary role in the detection of non-volatile pheromones and in eliciting behavioural and physiological responses to conspecifics and thereby in mating and intraspecies aggressive behaviour. Results from two independently generated mouse lines (Stowers *et al.* 2002; Leypold, 2002) show a loss of both sex discrimination and male–male aggression in TRPC2-deficient males. Additionally, lactating females are docile and fail to initiate aggressive attacks on intruder males, and TRPC2−/− males vigorously mount other males. TRPC2−/− males mate normally with females indicating that male–female sexual behaviour appears normal. TRPC2-deficient males are fertile since matings with TRPC2−/− males produced normal litter sizes (Stowers *et al.* 2002; Leypold *et al.* 2002) although the acrosome reaction of murine sperms was significantly inhibited in the presence of an anti-TRPC2 antibody *in vitro* (Jungnickel *et al.* 2001). VNO neurones isolated from TRPC2−/− mice do not show the specific increase in spiking rate upon application of urine pheromones, although they are electrically active under basal conditions. The transduction mechanisms of mammalian pheromone signalling by VNO neurones

involves phospholipase C (PLC) activation, and recently, the search for ion conductances activated by pheromone signals that are regulated by PLC activity lead to the identification of a DAG-activated cation channel in the dendritic tips of these neurones. This DAG-activated cation channel is defective in TRPC2−/− VNO neurones indicating that in mice TRPC2 proteins are principal subunits of this DAG-gated channel (Lucas *et al.* 2003).

TRPC3. TRPC3 RNA has been shown to be enriched in the adult human brain (Zhu *et al.* 1996) but another study showed that in rats the TRPC3 protein is predominantly expressed in the brain during a relatively narrow developmental period before and after birth (Montell, 2005). Several modes of activation of TRPC3-mediated Ca^{2+} entry were reported including direct activation by DAG analogues (Hofmann *et al.* 1999; Montell, 2005). Very recently, TRPC3 has been shown to be involved in the development of the highly specialized neuronal network and movements of axonal growth cones. The chemotropic axon guidance is determined by the sensing of extracellular cues that are translated into intracellular signals including activation of PLC and elevation of the intracellular Ca^{2+} concentration mediated at least in part through the activation of agonist-activated cation channels but also of voltage-gated Ca^{2+} channels (Henley & Poo, 2004). Li *et al.* showed that the rise of $[Ca^{2+}]_i$ and growth cone turning induced by brain-derived neurotrophic factor (BDNF) was abolished by transfection of rat cerebellar granule cells with short interfering RNAs (siRNAs) directed against TRPC3 mRNA as well as dominant negative TRPC3 and TRPC6 constructs (Li *et al.* 2005). In contrast, Ca^{2+} elevations or chemoattraction of axonal growth cones were not altered by siRNA-based strategies aimed at impairing TRPC1 expression. However, RNAi-mediated gene silencing of the TRPC1 homologue in *Xenopus* TRP-1 (also referred to as xTRPC1) leads to an almost 70% reduction of xTRPC1 protein expression in spinal neurones from *Xenopus* embryos, and both growth cone turning and elevation of $[Ca^{2+}]_i$ following stimulation with the agonist netrin-1 were abolished (Wang & Poo, 2005).

Based on results from RT-PCR studies TRPC3 might also be expressed in smooth muscle cells and endothelial cells from blood vessels (Freichel *et al.* 1999; Inoue *et al.* 2001, 2004; Beech *et al.* 2004). In line with TRPC3 expression in vascular smooth muscle cells treatment of rat cerebral arteries with oligonucleotides directed against TRPC3 mRNA reduces the depolarization of smooth muscle cells and the degree of constriction of isolated vessel rings by 61% and 37% upon application of 10^{-6} M and 10^{-5} M UTP, respectively, whereas myogenic responses were not affected (Reading *et al.* 2005).

TRPC4. TRPC4 is expressed in brain, endothelium, kidney, retina, testis and adrenal gland and the mode of activation may differ in these cell systems (Freichel *et al.* 2004; Montell, 2005). The endothelium that lines the inner side of blood vessels plays a critical role in the preservation of normal vessel functions. Agonist-activated Ca^{2+} entry is involved in many of these functions, and transcripts of several TRP genes, including TRPC1, TRPC2, TRPC3, TRPC4, TRPC6, TRPV4, TRPM4 and TRPM7, were readily detected in primary isolated endothelial cells from mouse aorta (MAECs) (Freichel *et al.* 1999, 2001, 2004; Wissenbach *et al.* 2000; Nilius *et al.* 2003). Based on experiments in isolated cells from wild-type and TRPC4−/− mice, TRPC4 proteins were identified as indispensable components of store-operated Ca^{2+} channels in MAECs acting either as channel-forming subunits or as constituents of the channel complexes that are essential for channel activation (Freichel *et al.* 2001). Additionally, agonist-activated Ca^{2+} entry into TRPC4−/− endothelial cells is drastically decreased leading to a markedly impaired regulation of vascular tone. In addition, following stimulation with thrombin, which activates G protein-coupled proteinase-activated receptor-1 (PAR-1), the Ca^{2+} influx in endothelial cells isolated from the lung of neonatal TRPC4−/− mice was also markedly reduced (Tiruppathi *et al.* 2002). Thrombin-induced Ca^{2+} entry is a critical determinant of endothelial permeability and, hence, thrombin-induced decreases of transendothelial resistance of cultured endothelial monolayers as a measure of endothelial cell retraction efficiency were significantly smaller in TRPC4−/− confluent monolayers than in wild-type controls. Similar results were observed when changes of microvascular permeability was studied by perfusing the intact circulation of isolated lungs. In these *ex vivo* preparations the agonist-activated permeability was reduced by about 50% (Tiruppathi *et al.* 2002). These results clearly support the conclusion that TRPC4 is, or is part of, a Ca^{2+} entry pathway in macrovascular endothelium such as in MAECs. The expression of various other TRP genes additional to TRPC4 in MAECs and other types of endothelial cells raises the possibility that Ca^{2+}-entry channels in these cells may be formed by TRP heteromers (Strubing *et al.* 2001, 2003; Hofmann *et al.* 2002; Goel *et al.* 2002).

Within the central nervous system, a functional role for TRPC4 proteins was recently revealed within unique types of synaptic terminals of the thalamic network, the so-called F2 terminals. F2 terminals derive from dendrites of local GABAergic interneurones and are situated in a triadic synaptic arrangement within a glomerular neuropil of the dorsal lateral geniculate nucleus (Pape *et al.* 2004). The release of γ-aminobutyric acid (GABA) from F2 terminals is modulated by extrathalamic input systems acting on metabotropic receptors and it was found that this release occurs in a Ca^{2+}-dependent manner. In TRPC4−/− mice, the 5-hydroxytryptamine (5-HT)-induced increase

of GABA release from the thalamic interneurones is largely reduced. In contrast, the increase of GABA release upon stimulation with an agonist of metabotropic glutamate receptors or inhibition of GABA release by acetyl-β-methylcholine is unchanged, indicating that F2 terminals are still functional in TRPC4−/− mice (Munsch *et al.* 2003). The regulation of the GABAergic component within these local circuit interactions by extrathalamic input systems may be critical for the gating of signals through the thalamus depending on the sleep–wake cycle. Therefore, by regulating F2-mediated signals through these systems, TRPC4 might contribute to the state-dependent processing of visual information, such as visual contrast sensitivity, or might be operative as a state-dependent gain control of retinogeniculate transmission in general (Pape *et al.* 2004).

In addition to these thalamic interneurones, TRPC4 is expressed in a variety of other cells within the central nervous system, such as in the olfactory bulb, septal nuclei, hippocampus, cortex and cerebellum. Additionally, TRPC4 transcripts were readily identified in uterus, ovary and kidney by Northern blot analysis and could be detected in pancreatic islets using RT-PCR. Results of glucose tolerance tests do not differ between wild-type and TRPC4-deficient mice and ongoing studies are aimed at revealing the possible functions of TRPC4 in pancreatic islets.

TRPC5. The predominant site of expression of TRPC5 is the central nervous system (Freichel *et al.* 2004; Montell, 2005). Here, TRPC5 transcripts (Fig. 2) and proteins (Greka *et al.* 2003) are abundant in hippocampal neurones. To address the function of TRPC5 in a native system, a TRPC5 mutant (DN-TRPC5) harbouring an exchange of three amino acids between the fifth and sixth predicted transmembrane domain was expressed in cultured hippocampal neurones. Co-transfection of this construct together with wild-type TRPC5 was reported to abolish carbachol-induced whole-cell currents that are obtained after TRPC5 cDNA transfection alone. Transfection of DN-TRPC5 in dissociated hippocampal neurones induced an increased length of their filopodia, the finger-like projections characteristic of growth cones. Furthermore, the neurite length and also the filopodia perimeter were increased under these conditions. Based on experiments in the same cellular system it was recently concluded that the translocation of TRPC5 proteins from intracellular vesicles to the plasma membrane, which was shown to depend on the activity of phosphatidylinositol 4-phosphate 5-kinase (PIP(5)Kα), is a key step for TRPC5-mediated regulation of neurite extension rates (Bezzerides *et al.* 2004). Based on these results TRPC5 and potentially the TRPC5-mediated Ca^{2+} entry can be regarded as an important determinant of axonal growth and growth cone morphology. Notably, no results for a TRPC5-related role in growth cone turning upon chemotropic stimulation were reported although this process was shown to be related to the expression of other TRPC proteins such as TRPC3 and TRPC6 or TRPC1 depending on the neuronal system analysed. Apparently, the available

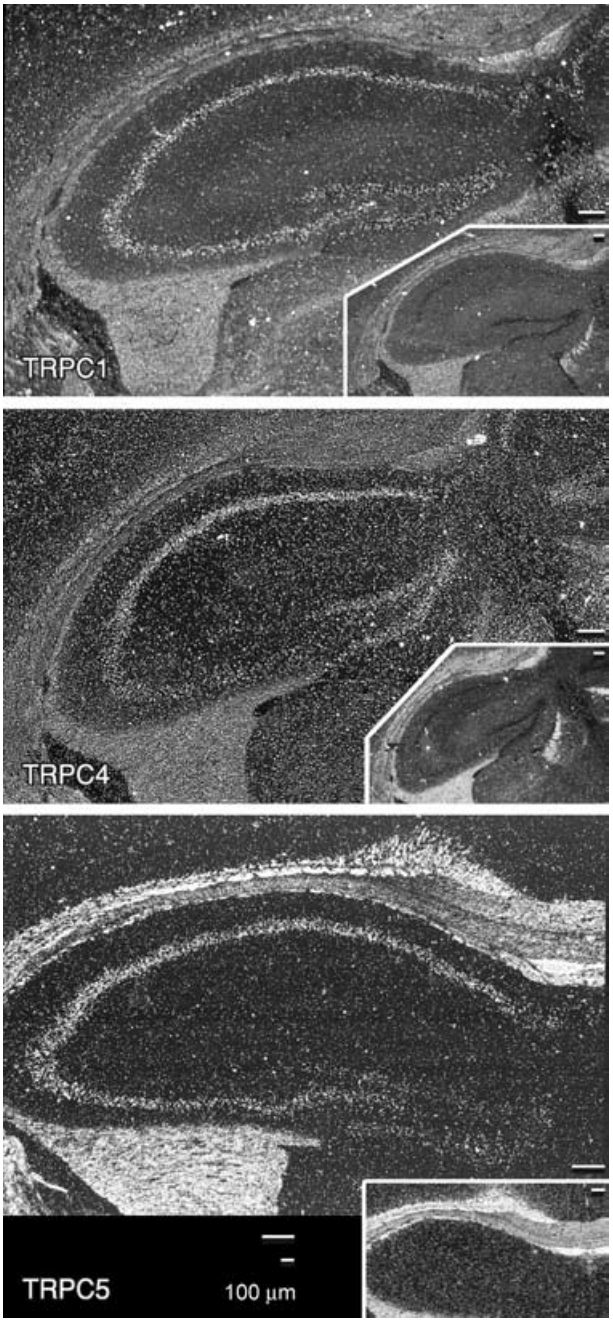

Figure 2. Expression of TRPC1, TRPC4 and TRPC5 transcripts in mouse hippocampus
In situ hybridization experiments of horizontal sections through the mouse brain hippocampus hybridized with antisense cRNA probes specific for mouse TRPC1 (corresponding nucleotides 4–358, accession number U40980) and for TRPC4 and TRPC5 as described (Philipp *et al.* 1998). No hybridization signals were detected with the corresponding sense cRNA probes (insets).

results obtained from studies in isolated neurones may indicate that TRPC proteins show a specific subcellular localization pattern and are assigned to distinct functional tasks within separate compartments of a particular cell type.

TRPC6. TRPC6 proteins are expressed in the lung, the brain (Montell, 2005) and in smooth muscle cells, and their expression levels were reported to correlate with cell proliferation of pulmonary artery smooth muscle cells (PASMCs) (Yu *et al.* 2004). Furthermore, expression of TRPC6 was shown to be increased in lung tissues and PASMCs from patients with idiopathic pulmonary hypertension and, hence, siRNA-mediated knockdown of TRPC6 expression markedly attenuated proliferation of PASMCs from these patients (Yu *et al.* 2004).

Recently, initial results were presented that describe the phenotype of TRPC6-deficient mice obtained by targeting the TRPC6 gene. In TRPC6−/− mice, a higher contractility in isolated tracheal and aortic rings was observed following application of the agonists methacholine and phenylephrine, respectively, as well as a reduced agonist-induced expiration rate (Dietrich *et al.* 2003). The density of inward currents that were induced by perfusion of the cells with the DAG derivate 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) and that were carried to a large extent by Na^+, were significantly higher in cerebrovascular artery smooth muscle cells isolated from TRPC6−/− mice. The increased current densities were also observed in non-stimulated cells indicating a higher basal and agonist-induced cation entry in TRPC6−/− smooth muscle cells (Mederos y Schnitzler *et al.* 2004). Accordingly, an increase in the responsiveness of peripheral resistance arteries to vasoconstrictors and in the mean arterial blood pressure was described, and the intravascular pressures necessary to induce myogenic constriction due to the Bayliss effect were shifted to the left towards lower values (Dietrich *et al.* 2004). The observed increased smooth muscle tone both under basal conditions and after agonist stimulation might be due to the formation of TRPC3 homo-oligomeric channel complexes in TRPC6-deficient smooth muscle cells, because mRNA expression of TRPC3 appears to be up-regulated two- to threefold in TRPC6−/− mice.

In an earlier study, TRPC6 expression was knocked down using an antisense oligodesoxynucleotide approach in cultured rat cerebral arteries. In this study, vasoconstriction induced by elevating intravascular pressure was inhibited by 70% to 80% in TRPC6 antisense-treated arteries compared with control arteries which were treated with sense oligonucleotides (Welsh *et al.* 2002). Differences in the results from the different approaches might be due to the varying degrees of reduction of TRPC6 protein expression, which was in the range of 60% (Welsh *et al.* 2002) in the knock-down approach. Antisense oligonucleotides were also used to down-regulate TRPC6 expression in cultured rabbit portal vein myocytes (Inoue *et al.* 2001). Here, the density of a cation current activated by the α-adrenoceptor agonist phenylephrine was markedly decreased after treatment with the TRPC6 antisense oligonucleotide compared with cells treated with the control TRPC6 sense oligonucleotides.

Expression of the TRPC6 protein in glomerular podocytes was demonstrated recently by immunostaining. These podocytes represent a central component of the renal filtration barrier (Pavenstadt *et al.* 2003). Furthermore, the TRPC6 gene was mapped as a candidate gene for focal and segmental glomerulosclerosis (FSGS), a hereditary kidney disorder leading to proteinuria and renal failure (Winn *et al.* 2005; Reiser *et al.* 2005). Sequencing of the TRPC6 gene of FSGS-affected individuals yielded six missense mutations in independent families; these mutations were located in the amino- or carboxyterminal parts of the protein. After expression in cell culture systems, some but not all of the TRPC6 mutants induced inward currents with larger densities than those induced by non-mutant TRPC6. Apparently the causative mutation of FSGS occurs in the TRPC6 gene raising the possibility that TRPC6 may be a useful therapeutic target in chronic kidney disease.

Résumé

The use of gene ablation and gene silencing approaches has already enabled tremendous progress towards the identification of functional roles for TRPC proteins in their native environment, in primary cell systems, in isolated organs and whole organisms. It is apparent that TRPCs like other members of the TRP superfamily of cation channels play decisive physiological roles and if mutated could lead to human disease.

The concepts demonstrated by the genetic approaches summarized above will certainly further encourage the efforts to screen for potent and specific agonists and/or antagonists of these TRPC cation channels which might be useful not only as experimental pharmacological tools but also as potential therapeutics.

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