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TRPM4 Channels in Smooth Muscle Function

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Abstract

The melastatin (M) Transient Receptor Potential (TRP) channel TRPM4 is selective for monovalent cations and is activated by high levels of intracellular Ca^{2+} . TRPM4 is broadly distributed and may be involved in numerous functions, including electrical conduction in the heart, respiratory rhythm, immune response, and secretion of insulin by pancreatic β cells. The significance of TRPM4 in smooth muscle cell function is reviewed here. Several studies indicate that TRPM4 channels are critically important for pressure-induced cerebral arterial myocyte depolarization and myogenic vasoconstriction as well as autoregulation of cerebral blood flow. Regulation of TRPM4 activity in arterial smooth muscle cells is complex, and involves release of Ca^{2+} from the sarcoplasmic reticulum through inositol 1,4,5 trisphosphate receptors and translocation of TRPM4 channels to the plasma membrane in response to Protein Kinase C δ . TRPM4 is also present in colonic, urinary bladder, aortic, interlobar pulmonary and renal artery, airway, and corpus cavernosum smooth muscle cells, but its significance and regulation in these tissues is less well-characterized.

Keywords

TRP; cation channels; melastatin; smooth muscle cells; vasoconstriction; myogenic responses

Introduction

The transient receptor potential (TRP) superfamily of cation channels was unexpectedly discovered during studies of *Drosophila* phototransduction mutants that behaved as if blind in bright light and displayed abnormalities in electroretinograms [1]. Subsequent cloning of the responsible gene, *trp* [2], and sequence analysis revealed the presence of homologous ion channels in many other organisms [3]. It is now known that the human genome encodes 27 distinct TRP genes and that an additional one is present in rats and mice [4]. Mammalian TRP genes are assigned to six subfamilies designated canonical (C), vanilloid (V), melastatin (M), ankyrin (A), polycystin (P), and mucolipin (ML). This classification is based on sequence homology rather than functional similarity [5]. All TRP channels are permeable to cations with varying selectivity. Many are nonselective, two (TRPV5 and TRPV6) are highly selective for Ca^{2+} ions, and two (TRPM4 and TRPM5) are selective for monovalent cations. TRP channels are expressed as six-transmembrane domain (S1–S6) subunits with a pore-forming structure between the S5 and S6 domains. Four of these subunits assemble to form functional ion channels. Heteromultimeric channels composed of two or three different subunits can form with properties distinct from homomeric channels [6]. This situation is well characterized for TRPC channels [7–9], TRPM6/M7 [10], and TRPV5/V6 [11], but other combinations are also possible. Multiple TRP channels are

present in most, if not all, cells where they are involved in astonishingly diverse physiological functions. In general, TRP channels serve as fundamental sensors of environmental conditions at both macroscopic and cellular levels. To perform these functions, TRP channels are activated by stimuli such as chemical agonists, temperature, pH, osmolarity, light, and pressure. The reader is directed to several recent review articles for more comprehensive information about TRP channel structure [12], function [4, 13], and pathophysiology [14]. The current review is narrowly focused on the involvement of TRPM4 in the regulation of smooth muscle cell function.

Biophysical Properties of TRPM4

Two splice variants of TRPM4 have been described. The first, a short form designated as TRPM4a, was initially cloned by Xu et al. [15]. TRPM4a displays little activity and its significance is unknown. A second, longer variant was reported by Launay et al. [16] and was initially designated as TRPM4b. TRPM4b is now generally accepted to be the commonly expressed and functional isoform of the channel and will be referred to hereafter simply as TRPM4. The unitary conductance of TRPM4 is ~24 pS [16]. TRPM4 and the closely-related channel TRPM5 [17] display two defining biophysical properties: Ca^{2+} -dependent activation and selectivity for monovalent cations [16]. The relative ionic selectivity of TRPM4, as determined by substitution experiments employing HEK 293 cells expressing the recombinant human gene, is $\text{Na}^+ \approx \text{K}^+ > \text{Cs}^+ > \text{Li}^+$. Ionic selectivity is conveyed by negatively charged amino acid residues between E981 and V985 of the human TRPM4 subunit [18].

TRPM4 channel activity is dependent upon high levels of intracellular Ca^{2+} [16]. Human TRPM4 channels expressed in HEK 293 cells have an EC_{50} for Ca^{2+} -dependent activation of approximately 400 nM [16] or 15 μM [19] under whole-cell patch clamp conditions. It is not clear why these two laboratories find differences in the channel's sensitivity to intracellular Ca^{2+} , but higher levels of Ca^{2+} ($\text{EC}_{50} = 10 \mu\text{M}$) are required to activate TRPM4 channels in native vascular smooth muscle cells under whole-cell patch clamp conditions [20]. TRPM4 currents recorded from inside-out membrane patches are much less sensitive to Ca^{2+} , with an EC_{50} for activation of 370 μM [18]. Diminished Ca^{2+} sensitivity under inside-out vs. whole-cell patch clamp conditions suggests that cytosolic factors lost when membrane patches are excised influence Ca^{2+} -dependent activation of TRPM4. Consistent with this possibility, deletions of calmodulin binding sites on the C-terminus diminished Ca^{2+} -dependent TRPM4 current activation, indicating a role for calmodulin in this response [21]. Ca^{2+} -dependent activation of TRPM4 is influenced by the activity of protein kinase C (PKC). Stimulation of PKC with the phorbol compound phorbol-12-myristate-13-acetate (PMA) increases TRPM4 activity by enhancing the sensitivity of the channel to intracellular Ca^{2+} . This response is blocked by point mutations at S1145 and S1152, suggesting that PKC-dependent phosphorylation of these residues is responsible for increased Ca^{2+} -dependent channel activation [21].

Ca^{2+} -dependent activation of TRPM4 is transient under the conditions typically used for conventional whole-cell or inside-out patch clamp experiments. For example, when intracellular Ca^{2+} is maintained at 10 μM , TRPM4 currents exhibit time-dependent decay, with currents completely inactivated in most cells within 120 s in HEK and A7r5 cell expression systems [21, 22] and naïve cerebral artery smooth muscle cells [20]. Inhibition of phospholipase C (PLC) or inclusion of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate ($\text{PI}(4,5)\text{P}(2)$) in the intracellular solution prevents time-dependent decrease of TRPM4 currents [23, 24]. Sustained TRPM4 currents can be recorded from native smooth muscle cells patch-clamped using the amphotericin B perforated-patch configuration [25], a technique does not disrupt physiological intracellular Ca^{2+} signaling mechanisms. These

data suggest that time-dependent inactivation may be a consequence of the high levels of intracellular Ca^{2+} used during conventional whole-cell and inside-out patch-clamp experiments rather than an inherent property of the channel.

TRPM4 Pharmacology

Investigations into the functional significance of TRPM4 have been impeded by a lack of selective pharmacological activators and inhibitors. Decavanadate [26] and the pyrazole derivative N-[4-3,5-bis(trifluoromethyl)pyrazol-1-yl]-4-methyl-1,2,3-thiadiazole-5-carboxamide (BTP2) [27] potentiate Ca^{2+} -dependent TRPM4 activity, but these agents lack specificity. Decavanadate has effects on inositol 1,4,5 trisphosphate (IP_3) receptors and purinoreceptors (P2X) [28] whereas BTP2 inhibits TRPC3 and TRPC5 channels [29]. Flufenamic acid and clotrimazole act as gating inhibitors, and spermine and Gd^{3+} as pore blockers [19, 30–32] but these agents are not selective for TRPM4 and influence many other channels. The hydroxytricyclic derivative 9-phenanthrol may be a more useful TRPM4 inhibitory compound. In heterologous expression systems, 9-phenanthrol blocks TRPM4 currents with little effect on the closely related channel TRPM5 or the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) chloride channel [33]. Furthermore, at a concentration (30 μM , $\sim 3 \times \text{IC}_{50}$) that almost completely blocks TRPM4 activity, 9-phenanthrol does not alter TRPC3, TRPC6, voltage-gated K^+ channels (K_v), large-conductance Ca^{2+} -activated K^+ channels (BK_{Ca}), inwardly rectifying K^+ channels (K_{IR}), or L-type Ca^{2+} channel currents [34]. 9-phenanthrol also has little effect on TRPM7 currents in mouse interstitial cells of Cajal or human gastric cancer cells [35, 36]. At higher concentrations ($\sim 100 \mu\text{M}$, $10 \times \text{IC}_{50}$) 9-phenanthrol can block K^+ and voltage-dependent Ca^{2+} channels [37]. Although additional studies are necessary to fully evaluate specificity, these data suggest that 9-phenanthrol is a useful compound for studying the functional significance of TRPM4.

TRPM4 Tissue Distribution and Function

TRPM4 is broadly distributed and mRNA is expressed at high levels in the heart, pancreas, placenta, and prostate and at lower levels in kidney, skeletal muscle, liver, intestines, thymus, testis, and spleen [16]. TRPM4 is present in smooth muscle cells found in rat cerebral arteries [38], the urinary bladder of rats and guinea pigs [39], monkey and human colon [40], rat aorta [41], rat pulmonary [41] and renal (Earley, et al, unpublished observations) intralobar arteries, rat airway (Earley, et al, unpublished observations) and mouse corpus cavernosum (Werner and Earley, unpublished observations) (Table 1). TRPM4 is detected by immunolabeling in the central nervous system, including the substantia nigra pars compacta [42], the cerebral cortex, hippocampal neurons, and spinal cord motor neurons [43]. TRPM4 channels are involved in numerous physiological processes [44]. TRPM4 regulates cytokine secretion in lymphocytes and insulin secretion in pancreatic β -cells [45]. TRPM4 is involved in controlling respiratory rhythmogenesis in neurons of the pre-Bötzinger complex [46]. TRPM4 channels were recently implicated in axonal and neuronal degeneration in experimental autoimmune encephalomyelitis, a model of multiple sclerosis [43]. In the heart [47, 48], TRPM4 channels are involved in early afterdepolarizations associated with re-oxygenation following hypoxia [37]. In addition, gain of function point mutations within the TRPM4 gene are associated with an inherited form of conduction block in the heart, indicating that the channel is involved in the function of cardiac Purkinje fibers [49, 50].

Function of TRPM4 in Arterial Smooth Muscle

The resting membrane potential of vascular smooth muscle cells in the walls of arteries under physiological intraluminal pressure (60–80 mmHg) is significantly depolarized (~–45 mV) compared with the K^+ equilibrium potential (–80 mV) [51]. This is functionally significant because vascular smooth muscle cell contractility is largely regulated by Ca^{2+} influx via L-type voltage dependent Ca^{2+} channels that are sharply activated at membrane potentials between –30 and –50 mV. Thus, resting membrane potential is a major determinate of myocyte contractility, arterial diameter, and blood flow and pressure regulation.

Soon after discovery [16], TRPM4 was shown to be a critical regulator of cerebral artery smooth muscle cell membrane potential and contractility [38]. Earley et al. reported that TRPM4 mRNA was present in native cerebral artery smooth muscle cells [38]. Patch clamp experiments using the inside-out configuration identified a novel non-rectifying current (in symmetrical cationic solutions) in these cells with a unitary conductance of approximately 24 pS [38]. Like TRPM4, this 24 pS channel exhibited Ca^{2+} -dependent activation and rapid time-dependent inactivation. Ion substitution experiments demonstrated that the channel was selective for monovalent cations vs. Ca^{2+} ions [38]. The 24 pS channel was observed much more frequently in membrane patches obtained from cells acutely treated with PMA compared with controls, suggesting sensitivity to PKC activity [38]. The properties of this channel, including Ca^{2+} dependence, monovalent cation selectivity, time-dependent inactivation and sensitivity to PKC activity, are reminiscent of those reported for recombinant TRPM4 channels in HEK expression systems. To firmly establish the channel's molecular identity, antisense oligonucleotides were used to decrease expression of TRPM4 currents in native cerebral artery smooth muscle cells. The frequency of observation of the 24 pS channel in cells treated with TRPM4 antisense was much lower compared with controls, clearly establishing its identity [38].

Due to selectivity for monovalent cations, TRPM4 channels are predicted to act as Na^+ influx channels in vascular smooth muscle cells under physiological conditions, as the resting membrane potential (~–45 mV) is hyperpolarized compared to the Na^+ equilibrium potential (+60 mV). Consistent with this expectation, antisense-mediated down-regulation of TRPM4 expression impaired pressure-induced smooth muscle cell depolarization and vasoconstriction in isolated rat cerebral arteries [38]. Membrane depolarization in response to the purinergic receptor agonist uridine triphosphate was not altered by downregulation of TRPM4, suggesting that the channel is specifically involved in pressure-dependent depolarization [38]. Small interfering RNA (siRNA)-mediated downregulation of TRPM4 expression has similar effects (Earley et al., unpublished observations). In addition, administration of 9-phenanthrol to isolated rat cerebral arteries with pre-developed myogenic tone resulted in hyperpolarization of the plasma membrane and relaxation to nearly passive diameter [34]. More interestingly, the membrane potential hyperpolarized to –70 mV in response to 9-phenanthrol, approaching the K^+ equilibrium potential, suggesting that TRPM4 channels are a dominant depolarizing influence in cerebral arterial myocytes [34]. Preliminary data from our laboratory indicates that 9-phenanthrol prevents the development of myogenic tone in renal intralobar arteries (unpublished observations), suggesting that TRPM4 channels are also important in vasomotor responsiveness in this vascular bed.

The physiological significance of TRPM4-dependent regulation of arterial myocyte membrane potential was elegantly revealed by Reading and Brayden, who found that administration of TRPM4 antisense oligonucleotides into the cerebral spinal fluid of the third cerebral ventricle of rats caused TRPM4 downregulation in pial artery smooth muscle

cells *in vivo* [52]. Cerebral blood flow was measured over a range of mean arterial pressures (MAP) using fluorescent microsphere methods and was found to be significantly greater at elevated MAP in animals treated with TRPM4 antisense vs. controls, indicating that cerebral arteries in these rats failed to appropriately respond to changes in perfusion pressure. These data provide compelling evidence that TRPM4 is crucially important for the autoregulation of cerebral blood flow in response to pressure changes *in vivo*.

TRPM4 knockout (TRPM4^{-/-}) mice have an unexpected cardiovascular phenotype. TRPM4^{-/-} mice are hypertensive compared to controls, likely due to elevated circulating catecholamine levels [53]. Phenylephrine-induced contraction of aortic ring segments and pressure-induced increases in vascular resistance in isolated hind limbs did not differ between TRPM4^{-/-} and wildtype mice, suggesting that agonist-induced and myogenic vasomotor responses were not affected by TRPM4 knockout [53]. The reasons for the difference between the effects of TRPM4 knockout and more acute suppression of TRPM4 expression or activity are not apparent. Possible explanations include differences in species (rats vs. mice), differences in experimental preparation (perfused hind limb preparation vs. pressure myography), and differences in arterial bed (skeletal muscle vs. cerebral). Alternatively, unintended phenotypic changes associated with TRPM4 gene knockout could be compensating for the loss of TRPM4 activity during development. Further experiments, including the use of inducible and/or tissue specific knockout animals, are needed to conclusively resolve these issues.

Ca²⁺-Dependent Regulation of TRPM4 in Smooth Muscle

Regulation of TRPM4 activity in native, contractile cerebral artery myocytes has been extensively characterized. In both heterologous expression systems and freshly-isolated cerebral artery smooth muscle cells, TRPM4 channels require high levels of intracellular Ca²⁺ for activation. Ca²⁺ levels of 10–100 μ M are typically used to record channel activity from cells patch clamped in the conventional whole-cell and inside-out configurations. This level of Ca²⁺ is much greater than global Ca²⁺ levels in vascular smooth muscle cells under normal conditions and results in rapid, Ca²⁺-dependent inactivation of TRPM4. However, native arterial myocytes transiently generate subcellular regions where localized Ca²⁺ levels are much higher than global levels. Ca²⁺ micro (or nano) domains created by Ca²⁺ influx or by Ca²⁺ released from the sarcoplasmic reticulum (SR) and are critical for numerous cellular functions. The best characterized Ca²⁺ microdomains in arterial smooth muscle cells are Ca²⁺ sparks, regions of intracellular Ca²⁺ in the 1–100 micromolar range generated by release of SR Ca²⁺ via ryanodine receptors [54]. Ca²⁺ sparks are functionally coupled to BK_{Ca} channels and are important regulators of cerebral artery tone [54]. To record currents generated by Ca²⁺ microdomains, cells are patch clamped in the amphotericin B perforated patch configuration to avoid disruption of intracellular Ca²⁺ signaling pathways. Using this method, sustained inward cation currents can be recorded from native cerebral artery smooth muscle cells voltage clamped at negative holding potentials for as long as seal viability is maintained (>30 min). These currents are referred to as “Transient Inward Cation Currents” (TICCs) [25]. TICCs have an apparent single channel conductance of ~24 pS, reverse near 0 mV in symmetrical cation solution, and are abolished when extracellular Na⁺ is substituted with the impermeant cation NMDG [25]. These properties are similar to those of recombinant TRPM4 channels. In addition, TICC activity is inhibited by the TRPM4 blocking compounds flufenamic acid and 9-phenanthrol and attenuated by siRNA-mediated downregulation of TRPM4 expression in cerebral artery myocytes [25]. These findings suggest that Na⁺ influx via TRPM4 is responsible for TICC activity in arterial smooth muscle cells.

The ability to record sustained TRPM4 current activity as TICC allowed detailed analysis of Ca^{2+} -dependent activation mechanisms in arterial myocytes. Removal of extracellular Ca^{2+} did not acutely affect TRPM4 activity recorded from native cerebral artery smooth muscle cells, suggesting that Ca^{2+} influx is not directly involved [25, 55]. However, blocking the sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) rapidly inhibited activity, indicating that Ca^{2+} released from the SR is necessary for TRPM4 activity in these cells [25, 55]. Inhibition of IP_3 receptors with Xestospongin C attenuated TICC activity, whereas block of ryanodine receptors had no effect on these currents [25]. In addition, TICC activity was stimulated by the membrane permeable IP_3 analog Bt- IP_3 [55]. These data suggest that TRPM4 channels are activated by Ca^{2+} released from SR via IP_3 receptors (Figure 3) [25, 55]. Further evidence of functional coupling between TRPM4 channels and IP_3 receptors was provided by conventional whole-cell patch clamp experiments utilizing the Ca^{2+} buffers ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) or bis-ethane-N,N,N',N'-tetraacetic acid (BAPTA) in the pipette solution. BAPTA and EGTA have identical steady-state kinetics and Ca^{2+} binding affinities but significantly differ in their binding rate constants [56]. BAPTA binds free Ca^{2+} at a rate that is two orders of magnitude faster than EGTA [56]. As such, when present at equimolar concentrations, BAPTA will bind Ca^{2+} released from a point source much more rapidly than EGTA, limiting diffusion. Accordingly, Ca^{2+} microdomains, with a Ca^{2+} source-to-sensor distance of greater than 50 nm, are defined by Ca^{2+} -dependent responses that are equally disrupted by identical concentrations of BAPTA and EGTA [57] whereas, Ca^{2+} nanodomains, with a Ca^{2+} source-to-sensor distance less than 50 nm, are defined by Ca^{2+} -dependent responses that are significantly interfered with by BAPTA, but not by equimolar concentrations of EGTA [57]. In whole-cell patch clamp experiments, activation of TRPM4 was observed when 10 mM EGTA but not 10 mM BAPTA was included in the recording pipette [55]. This differential effect on channel activity by equal concentrations of BAPTA or EGTA indicates that IP_3 receptor-mediated activation of TRPM4 occurs within spatially restricted domains, suggesting that TRPM4 channels and IP_3 receptors are located no more than 50 nm apart (Figure 1). Furthermore, loss of TRPM4 channel activity in the presence of BAPTA provides electrophysiological evidence that the IP_3 receptor-mediated activation of TRPM4 channels depends on Ca^{2+} release rather than physical coupling between TRPM4 and IP_3 receptors. Regulation of TRPM4 by this mechanism may not be unique to arterial smooth muscle cells, as functional coupling between TRPM4 and IP_3 receptor has been reported for the neonatal mouse preBötzinger complex, where the channels contribute to neuronal control of respiratory rhythms [58].

PKC-Dependent Regulation of TRPM4 in Smooth Muscle

TRPM4 activity is stimulated by PKC [20, 21, 38, 59]. PKC is also involved in the development of myogenic tone in cerebral arteries [20, 60, 61], suggesting that PKC may influence myocyte contractility via TRPM4. Consistent with this hypothesis, the PKC activator PMA elicits TRPM4-dependent smooth muscle cell membrane depolarization and cerebral artery constriction [20]. The effects of PKC stimulation on the subcellular distribution of TRPM4 protein were investigated. Using cell surface biotinylation and total internal reflection fluorescence microscopy (TIRFM), Crnich and colleagues showed that stimulation of PKC activity with PMA caused TRPM4 channel protein to rapidly traffic to the plasma membrane in A7r5 cells, primary cerebral artery myocytes, and intact cerebral arteries [62]. PMA-induced translocation of TRPM4 to the plasma membrane was blocked by selective inhibition of $\text{PKC}\delta$, but was insensitive to inhibition of $\text{PKC}\alpha$ and $\text{PKC}\beta$ [62]. In addition, siRNA-mediated downregulation and pharmacological inhibition of $\text{PKC}\delta$ caused TRPM4 channels to rapidly translocate from the plasma membrane into the cytosol [63], suggesting that basal $\text{PKC}\delta$ activity is required to maintain TRPM4 channels at the plasma membrane. Predictably, TRPM4 activity was attenuated by block of $\text{PKC}\delta$ activity

[63], and suppression of PKC δ expression hyperpolarized smooth muscle cells and impaired vessel constriction in response to both PMA and increases in intraluminal pressure in intact cerebral arteries [62]. These findings suggest that PKC-induced increases in Ca²⁺-dependent activation of TRPM4 activity may result from elevated levels of channel protein at the cell surface. Furthermore, these data suggest that smooth muscle cell excitability can be regulated by dynamic trafficking of TRPM4 channels to and from the plasma membrane.

Hypothetical Unified Scheme for Regulation of TRPM4 Activity in Cerebral Artery Smooth Muscle Cells

Evidence discussed above suggests that TRPM4 channel activity is an important determinant of cerebral artery smooth muscle cell resting membrane potential and contributes to pressure-induced vasoconstriction and autoregulation of blood flow. Data also indicate that in cerebral arterial myocytes, TRPM4 channels are activated by a combination of Ca²⁺ nanodomains created by Ca²⁺ released from the SR through IP₃ receptors and by PKC δ -mediated translocation of TRPM4 channels to the plasma membrane. The activity of Phospholipase C (PLC) could potentially unify these two pathways. PLC cleaves PtdIns(4,5)P₂ into the second messengers diacylglycerol (DAG) and IP₃. IP₃ elevates Ca²⁺ release from IP₃ receptors and DAG stimulates PKC activity. Thus, it is possible that PLC activity accelerates the generation of TRPM4-activating Ca²⁺ nanodomains and promotes translocation of TRPM4 channels to the plasma membrane (Figure 2). A preliminary report from my laboratory is consistent with this possibility [64], but further verification is necessary. If this scheme is correct, the upstream activators of PLC in smooth muscle cells are of considerable interest. G-protein coupled receptors that are stimulated by vasoconstrictor agonists such as endothelin-1, purinergic nucleotides, and angiotensin II are well-characterized activators of PLC. In addition, the concept that G-protein coupled receptors are involved in pressure-induced vasoconstriction has been put forth [65], but this idea remains controversial [66]. Nonetheless, it remains possible that G-protein-mediated activation of PLC could influence smooth muscle cell membrane potential and contractility in response to vasoconstrictor agonists as well as intraluminal pressure by activating TRPM4, but significant additional work is required to test this hypothesis.

Function of TRPM4 in Other Types of Smooth Muscle

TRPM4 channels are present (Table 1) and functional in several types of smooth muscle outside of the vasculature. Dwyer et al. recorded basally active non-selective cation currents in freshly-isolated colonic smooth muscle cells from humans and monkeys that are reminiscent of TICC_s recorded from native cerebral artery myocytes [40]. These currents were partially inhibited when Na⁺ was replaced with the impermeant ion N-methyl-D-glucamine (NMDG) and attenuated with the TRPM4 blocker 9-phenanthrol [40]. In addition, NMDG substitution significantly hyperpolarized the resting membrane potential of these cells [40]. These findings suggest that Na⁺ influx via TRPM4 channels contributes to the maintenance of resting membrane potential in colonic smooth muscle cells. Two reports by Smith et al. indicate that TRPM4 channels are present in rat and guinea pig detrusor smooth muscle cells that make up the wall of the urinary bladder [67, 68]. TICC-like currents that were sensitive to 9-phenanthrol were recorded from these cells. In addition, 9-phenanthrol reduced the amplitude and frequency of spontaneous and pharmacologically-induced contractions and the amplitude of electrical field stimulation-induced contractions of isolated bladder strips, suggesting an important role for TRPM4 in urinary bladder function. TRPM4 mRNA was also detected by RT-PCR in rat pulmonary intralobar artery and aortic smooth muscle, but the channel's functional significance in these tissues has not been reported [41]. Preliminary findings also suggest that TRPM4 is present in the corpus cavernosum of mice. Precontracted corpus cavernosum strips are relaxed by 9-phenanthrol,

indicating that TRPM4 contributes the maintenance of tonic contraction of this tissue (Werner and Earley, unpublished observations). Collectively, these studies suggest that TRPM4 may be important for establishing the resting membrane potential of many types of smooth muscle cells.

Conclusions

Pharmacological and molecular evidence demonstrate an important role for TRPM4 channels in the regulation of rat cerebral artery smooth muscle cell resting membrane potential and contractility, which critically influences autoregulation of cerebral blood flow in response to elevations in intraluminal pressure. Regulation of TRPM4 activity in arterial myocytes is complex, and involves release of Ca^{2+} from the SR through IP_3 receptors and translocation of TRPM4 channels to the plasma membrane mediated by the activity of PKC δ . Although the channel appears to be important for myogenic regulation, it remains unresolved how increases in intraluminal pressure are translated into elevated TRPM4-dependent cation current activity. Conceptually, increases in pressure and stretch of arterial myocytes could directly increase TRPM4 activity if the channel is inherently mechanosensitive. In support of this possibility, one report demonstrates increased activity of TRPM4 channels expressed in HEK cells in response to application of negative pressure to the recording electrode, implying direct mechanosensitivity [69]. However, a preliminary study from my laboratory was unable to replicate these findings, but provides evidence that TRPM4 channels are indirectly activated by stretch through a phospholipase C-dependent pathway [70]. Resolution of this issue and achieving a better understanding of mechanosensing in the vascular in general are important areas for future studies. It is also important to consider TRPM4 channels in the context of diseases affecting smooth muscle cells and determine if the channel or its regulatory pathways are viable targets for pharmaceutical development.

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Figure 1. Stimulation of TRPM4 Activity by Ca^{2+} Nanodomains Created by Ca^{2+} released from the Sarcoplasmic Reticulum via IP_3 Receptors

In freshly isolated smooth muscles, TRPM4 activation requires Ca^{2+} released from the SR through proximal IP_3 receptors. The Ca^{2+} concentration profile comparing the buffering capacity of the slow and fast Ca^{2+} chelators EGTA and BAPTA on $[\text{Ca}^{2+}]_i$ as a function of distance from a single IP_3R Ca^{2+} release site. Simulation data using CalC software v. 6.0 [71] was re-plotted from Gonzales and Earley [55]. From: Gonzales and Earley, Microcirculation, *in press*. [22].

Figure 2. Hypothetical Unified Scheme for Regulation of TRPM4 Activity in Cerebral Artery Smooth Muscle Cells

PM, plasma membrane; GPC, G-protein Coupled Receptor; PLC, Phospholipase C; PtdIns(4,5)₂, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP₃, inositol 1,4,5-trisphosphate; IP₃R, inositol 1,4,5-trisphosphate receptors; TRPM4, transient receptor potential melastatin 4; ΔV_m, change in membrane potential; VDCC, voltage-dependent Ca²⁺ channel.

Table 1

Expression of TRPM4 Channels in Smooth Muscle

Tissue	Evidence	References
Rat cerebral and cerebellar resistance artery smooth muscle	RT-PCR Immunocytochemistry Western blot	[25, 38, 52, 55, 62]
Rat and guinea pig urinary bladder (detrusor) smooth muscle	RT-PCR Immunohistochemistry Immunocytochemistry Western blot	[67, 68]
Monkey and human colic smooth muscle	RT-PCR	[40]
Rat pulmonary intralobar artery smooth muscle	RT-PCR	[41]
Rat aortic smooth muscle	RT-PCR	[41]
Mouse corpus cavernosum smooth muscle	RT-PCR Immunohistochemistry	Werner and Earley (unpublished)
Rat renal intralobar artery smooth muscle	RT-PCR Immunocytochemistry	Earley et. al (unpublished)
Rat airway smooth muscle	Immunocytochemistry	Earley et.al (unpublished)