TRPV4: A trigger of pathological RhoA activation in neurological disease

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Abstract

Transient receptor potential vanilloid 4 (TRPV4), a member of the TRP superfamily, is a broadly expressed, cell surface-localized cation channel that is activated by a variety of environmental stimuli. Importantly, TRPV4 has been increasingly implicated in the regulation of cellular morphology. Here we propose that TRPV4 and the cytoskeletal remodeling small GTPase RhoA together constitute an environmentally sensitive signaling complex that contributes to pathological cell cytoskeletal alterations during neurological injury and disease. Supporting this hypothesis is our recent work demonstrating direct physical and bidirectional functional interactions of TRPV4 with RhoA, which can lead to activation of RhoA and reorganization of the actin cytoskeleton. Furthermore, a confluence of evidence implicates TRPV4 and/or RhoA in pathological responses triggered by a range of acute neurological insults ranging from stroke to traumatic injury. While initiated by a variety of insults, TRPV4–RhoA signaling may represent a common pathway that disrupts axonal regeneration and blood–brain barrier integrity. These insights also suggest that TRPV4 inhibition may represent a safe, feasible, and precise therapeutic strategy for limiting pathological TRPV4–RhoA activation in a range of neurological diseases.

Keywords

cytoskeleton; peripheral neuropathy; RhoA; spinal cord injury; stroke; TRPV4

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CONFLICT OF INTEREST
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INTRODUCTION: TRPV4 MODULATES CELL PHYSIOLOGY IN RESPONSE TO ENVIRONMENTAL STIMULI

In complex biological systems and living tissues, the ability of cells to detect external stimuli is essential for maintaining homeostasis in the face of dynamic environmental conditions. Signal transduction from the outside environment into the cell interior often involves plasma-membrane-embedded cellular "sensors" and their associated intracellular interacting protein complexes. These complexes transduce extracellular stimuli into intracellular signaling cascades, which in turn regulate downstream pathways that affect a wide range of cellular processes, including cell growth and migration, cell–cell junctional integrity, gene transcription and translation, and programmed cell death.[1]

Transient receptor potential vanilloid 4 (TRPV4) is an example of a cellular sensor with critical functions in many cell and tissue types. A member of the TRP family of ion channels, TRPV4 is a nonselective, Ca^{2+}-permeable cation channel that was first described in 2000 by the research groups of Schultz[2] and Liedtke.[3] TRPV4 is expressed at the cell surface of a variety of cell types and has been linked to a broad range of physiological processes: central osmoregulation, osmosensation in the bladder and kidney, pain signaling in keratinocytes and sensory neurons, vascular tone, epithelial and endothelial barrier integrity, and bone remodeling, among many others.[4,5] Within these tissues, TRPV4 functions to transduce a range of environmental signals into intracellular signaling cascades. TRPV4 is a mechanosensitive channel that responds to shear stress, mechanical cell stretch, and cell swelling, and it is also sensitive to moderate heat and endogenous chemical stimuli such as epoxyeicosatrienoic acid (EET) metabolites, glycerophospholipids, and anandamides.[6,7] Although the intracellular effects of TRPV4 are diverse and incompletely understood, converging evidence has highlighted a critical function of TRPV4 in influencing dynamics of the cytoskeleton and cellular morphology. Furthermore, the importance of TRPV4 for cell physiology has been highlighted by the discovery of a range of dominantly inherited diseases caused by mutations in TRPV4.[8–13] These so-called TRPV4 channelopathies result from gain-of-function missense mutations within various functional domains of the TRPV4 protein, which give rise to a spectrum of neuromuscular diseases and distinct diseases of connective tissue and bone.[14]

TRPV4 is primarily expressed at the plasma membrane where it forms a homotetrameric ion channel. Each TRPV4 subunit within the tetramer consists of six transmembrane domains, a C-terminal intracellular domain, and a large cytosolic N-terminal domain that contains the ankyrin repeat domain (ARD)[15] (Figure 1). The ARD is thought to serve a scaffolding function central to the observed effects of TRPV4 on the cytoskeleton.[16] The ARD is also adjacent to a proline-rich region (PRR), which is involved in protein–protein interactions,[17,18] and a phosphoinositide binding domain (PBD), which can interact with the plasma membrane. The PBD's interaction with the lipid membrane also exerts potent regulatory control of TRPV4 responses to thermal and hypotonic stimuli.[19] Notably, TRPV4 mutations that cause neurological disease primarily cluster in the N-terminal ARD that is believed to support a protein scaffolding function of TRPV4, highlighting the importance of this domain for TRPV4 function.[20]

Activation of TRPV4 by various stimuli leads to opening of the channel pore and influx of extracellular cations, particularly calcium, which initiate diverse downstream intracellular signaling cascades.[21] For example, TRPV4-mediated calcium influx been shown to affect signaling via ATP/pannexin,[22,23] nitric oxide,[24,25] phospholipase A2,[26] ERK1/2,[27] CaMKII,[28,29] and IP3,[30,31] among many others.[26] Notably, many of these signaling cascades converge on pathways that regulate cytoskeletal remodeling machinery. For example, in trabecular meshwork cells of the eye, stretch-induced TRPV4 activation leads to phosphorylation of focal adhesion kinases and cytoskeletal stress fiber formation.[32,33] In vascular endothelial cells, TRPV4 -mediated calcium influx activates phosphoinositide 3-kinase, which in turn promotes cytoskeletal remodeling during angiogenesis and tumor-driven migration.[34–36]

Classically, the function of TRPV4 in regulating the cytoskeleton has been attributed to its activity as a calcium conduit and activation of calcium-dependent signaling cascades. However, accumulating evidence suggests that TRPV4 can also regulate the cytoskeleton independent of channel activity via protein–protein interactions. For example, prior work in *C. elegans* has demonstrated ion channel-independent functions of the invertebrate TRPV4 ortholog OSM-9.[37] In addition, immunoprecipitation studies have suggested that TRPV4 interacts with tubulin and actin as well as cytoskeletal signaling molecules PKCε and CaMKII, and these interactions are independent of Ca^{2+} influx.[28] TRPV4 can also exert effects on the cytoskeleton through its interactions with PACSINs (protein kinase C and casein kinase substrate in neurons), also known as syndapins.[17,18] These proteins have diverse functions in binding to and deforming curved membranes, regulating endocytosis, and recruiting cytoskeletal remodeling machinery.[17,18,38] Thus, TRPV4 exerts control of the cytoskeleton through both modulation of intracellular calcium levels and through scaffolding of cytoskeletal modulating proteins. Recent work has further highlighted the ion channel-independent scaffolding function of TRPV4 and its powerful role in driving changes in cell shape and morphology.

TRPV4–RhoA COMPLEX REGULATES THE CYTOSKELETON

We recently identified RhoA as a TRPV4 binding partner that is regulated by both calcium influx and ion channel-independent interactions.[39] These results suggest a dual regulatory mechanism yet to be described for other TRPV4 protein–protein interactions. RhoA is an abundant and ubiquitously expressed small GTPase that functions as a master regulator of the actin cytoskeleton. It acts as a molecular switch by cycling between an inactive GDP-bound state and an active GTP-bound state in which it binds and activates downstream cytoskeletal modulating proteins, including Rho-associated coiled-coil containing kinase (ROCK), mDia, ADF/cofilin, and nonmuscle myosin. Classically, active RhoA binds the negative regulatory region of ROCK, thereby promoting phosphorylation of the ROCK target proteins myosin-light chain (MLC), MLC kinase, and LIM kinases. Phosphorylation of these proteins results in increased myosin II ATPase activity, formation of actin stress fibers, actin bundling, and changes in cell morphology[40] (Figure 2(A)). Like other small GTPases, the activation of RhoA is dynamically regulated by guanine-nucleotide exchange factors (GEFs), which facilitate GTP binding leading to activation of RhoA, and GTPase activating protein (GAPs), which hydrolyze GTP to GDP and inactivate RhoA.[41] RhoA

activity is further modulated by interactions with guanine-nucleotide dissociation inhibitors (GDIs), which bind RhoA and lock it in the GDP-bound, inactive, state.[41]

Our work demonstrated direct TRPV4–RhoA binding interactions that lead to bidirectional regulation that dynamically controls actin cytoskeletal changes. These results are consistent with a prior unbiased proteomics study that identified RhoA as a potential TRPV4 interactor. [42] Although the TRPV4 ARD has long been presumed to function as a platform for protein–protein interactions, previous studies have only demonstrated ARD interactions with calmodulin and phosphatidylinositol-4,5-bisphosphate.[43,44] We found that the switch and interswitch regions of inactive, GDP-bound RhoA directly bind to the TRPV4 ARD. In addition, we demonstrated that RhoA binding to TRPV4 suppressed ion channel activity, and conversely, TRPV4 binding to RhoA inhibited RhoA activation, likely by sequestering inactive RhoA in a manner similar to RhoGDI[45] (Figure 2(B)). Expression of TRPV4 in the presence of an antagonist led to dramatic stimulation of neurite-like processes from cultured cells, and formation of these cellular processes correlated with reduced activation of RhoA. Together, these findings demonstrate the ability of TRPV4 to promote actin cytoskeletal outgrowth and link this function with TRPV4-mediated inhibition of RhoA signaling.

Apart from modulating RhoA through sequestration and inhibition, we also found that TRPV4 could activate RhoA through ion channel activity and calcium influx. Stimulation of TRPV4 and increased intracellular calcium triggered rapid, time-locked activation of RhoA, followed by actin stress fiber formation and cytoskeletal contraction. Interestingly, TRPV4–RhoA complexes became dissociated in response to hypotonic stress, indicating that certain environmental stimuli are able to trigger release of RhoA from the inhibitory effect of TRPV4. Our results are in alignment with prior work showing stimulation of RhoA activity by calcium[46,47] and TRPV4-mediated activation of RhoA-dependent pathways.[36] Indeed, other recent studies have shown that TRPV4-mediated calcium influx increases RhoA activation and downstream ROCK activity as well as F-actin levels in endometrial cancer cells, endothelial cells, and trabecular meshwork cells of the eye.[32,36,48] Consistent with a central role for ROCK, our unpublished results demonstrate that inhibition of ROCK with the specific inhibitor Y-27632 blocks TRPV4-calcium-mediated cytoskeletal changes in HEK293T cells despite calcium-dependent RhoA activation (Figure 3). Together, these results point to a critical role for TRPV4 in regulating RhoA activation, downstream ROCK activation, and resulting cytoskeletal changes in response to extracellular stimuli. Interestingly, there is also precedent for regulation of RhoA and related Rho GTPases by other TRP channels, suggesting that TRPV4–RhoA regulation may be one aspect of a more general role for TRP channel-mediated regulation of the cytoskeleton.[49] Importantly, TRPV4 channel-mediated regulation of RhoA may have implications for pathological pathways activated in a range of human diseases.

PATHOLOGICAL RhoA ACTIVATION IN NEUROLOGICAL DISEASES

RhoA has been implicated in the pathogenesis of multiple neurological diseases, suggesting that maintenance of the unique properties of the nervous system requires precise RhoA regulation. RhoA plays particularly important roles in neurons and in vascular endothelial

cells of the nervous system.[50–52] In general, RhoA activation within axonal growth cones of developing neurites drives actin cytoskeletal changes that restrain neurite outgrowth and/or promote neurite retraction.[53] In the context of neuronal injury, RhoA and ROCK activation inhibit axonal regeneration and subsequent reinnervation of target tissues. In mouse models of spinal cord injury (SCI), for example, levels of RhoA increased at the site of injury, and RhoA inhibition promoted motor recovery.[54–56] In animal models of traumatic brain injury (TBI), activation of RhoA and ROCK signaling promoted dendritic spine retraction, whereas inhibition of RhoA signaling pathways led to improved motor and cognitive outcomes.[57]

RhoA also contributes to neurological disease by modulating the barrier function of neural vascular endothelial cells. The blood–brain barrier is formed by endothelial cells that line the blood vessels of the brain and restrict the movement of ions, molecules, and cells from the blood into the surrounding neural tissue. Critical components of this barrier are tight junctions and adherens junctions between endothelial cells, which tether neighboring cells together and provide a physical barrier that limits the diffusion of blood constituents across the endothelial layer.[58] RhoA and ROCK regulate and maintain these junctions via modulation of the actin cytoskeleton that anchors these protein complexes.[59] Disruption of blood–brain barrier permeability has been identified as a pathological hallmark of a wide range of neurological disease states including those associated with acute neurological injury, such as stroke, TBI, and SCI; demyelinating diseases such as multiple sclerosis; neurodegenerative diseases such as Alzheimer's disease; and chronic neurological sequelae of COVID-19 infection.[58,60,61] In neurological conditions associated with pathological blood–brain barrier disruption, such as ischemic stroke, RhoA signaling pathways are upregulated within brain vascular endothelial cells at sites of neurovascular insult.[62,63] In a mouse model of TBI, inhibition of RhoA via TIMP1-induced FAK phosphorylation and attenuated blood–brain barrier permeability and loss of junctional proteins.[64] A similar rescue of blood–brain barrier permeability and junctional protein expression by RhoA/ROCK inhibition was demonstrated in a mouse model and an in vitro brain endothelial cell model of inflammatory blood–brain barrier disruption using lipopolysaccharide.[65] Furthermore, in a rat model of SCI, inhibition of ROCK ameliorated blood–spinal cord barrier disruption and improved motor outcomes.[66] These results were attributed to inhibition of ROCK-mediated activation of the actin cytoskeletal modulating activities of ADF/cofilin and myosin phosphatase. Recent work has also suggested that RhoA activation downstream of the SARS CoV-2 spike protein contributes to blood–brain barrier disruption.[67]

Given the central role of RhoA signaling in neuropathological states, pharmacological approaches to limit RhoA activity have been examined in disease models and in humans, albeit with mixed success. In humans, the ROCK inhibitor netarsudil is approved for the treatment of refractory glaucoma.[68] In animal models, studies of RhoA pathway inhibition have most often utilized the bacterial endotoxin C3 transferase (a Rho GTPase inhibitor that targets RhoA as well as RhoB and RhoC) and ROCK inhibitors Y-27632 and fasudil (HA-1077). In addition to animal studies, fasudil has also been used in several clinical trials for a range of neurological diseases.[69–72] Targeting RhoA or ROCK signaling can improve some aspects of pathogenicity in disease models, but it can also exacerbate others.

[73] These results are not surprising given the myriad essential roles of RhoA in multiple cell types and tissues. For example, a recent study of a mouse model of SCI found that while inhibition of RhoA activity in neurons promoted regeneration, specific deletion of RhoA within astrocytes was detrimental.[52] As opposed to therapies directly targeting RhoA, strategies to specifically inhibit pathological RhoA activation in relevant tissues and/or cell types could theoretically avoid the pitfalls of more generalized inhibition. We propose that TRPV4 may be such a therapeutic target.

TRPV4 AS A REGULATOR OF PATHOLOGICAL RhoA ACTIVATION IN THE NERVOUS SYSTEM

Strikingly, many of the same neurological diseases associated with excessive RhoA and ROCK signaling are also associated with increased TRPV4 activation. Particular examples of coincident RhoA and TRPV4 activation have come from animal models of stroke, TBI, and SCI. These studies have highlighted the importance of endothelial cell TRPV4 function in blood–brain and blood–spinal cord barrier integrity in these conditions.[74–77] These findings are consistent with single-cell and bulk RNA sequencing studies of nervous tissue from humans and mice showing that TRPV4 is primarily expressed in endothelial cells of the neurovasculature.[78–82] In contrast, there is surprisingly little evidence for widespread expression of TRPV4 in human and mouse neurons.[78,83] In the mouse spinal cord, unbiased expression profiling has demonstrated TRPV4 expression in a small subpopulation of sensory neurons, but very little expression in alpha motor neurons.[79,84] However, given that small numbers of clustered ion channels can elicit robust signaling events,[85–87] the absence of high-level expression does not necessarily rule out an important functional role of TRPV4 in neurons. Studies to evaluate the role of TRPV4 in neurons, in some cases using exogenous expression, have shown that TRPV4 ion channel function can influence neurite outgrowth, similar to RhoA.[28] TRPV4 colocalized with actin-rich structures within neuronal growth cones, and ion channel activation led to rapid changes in morphology of actin-rich lamellipodia and filopodia.[28] Activation of TRPV4 in cultured neurons caused disruption of axonal integrity with varicosity formation and neurite retraction.[29,88] In a *Drosophila* model of TRPV4 neuropathy, expression of gain-of-function TRPV4 mutations led to axonal and dendritic degeneration, consistent with TRPV4-mediated inhibition of neurite outgrowth.[29] Importantly, degeneration of dendrites and axons in this model could be rescued by inhibition of Rho1, the fly homolog of RhoA, further linking TRPV4-mediated cytoskeletal changes with RhoA signaling.[39]

While these studies suggest a potential role for TRPV4 and RhoA in neurons, the extent to which TRPV4 is expressed within neurons of the brain, spinal cord, and peripheral nerves remains debatable. In contrast, a functional role for TRPV4 in neural vascular endothelial cells is more robustly established. Like RhoA, TRPV4 has been implicated in maintaining endothelial barrier function and affecting blood–brain and blood–spinal cord barrier permeability. In rodent models of SCI, endothelial cell TRPV4 was found to be upregulated in response to injury in conjunction with disruption of the blood–spinal cord barrier.[74] Furthermore, in mice subjected to SCI, knockout of TRPV4 prevented blood–spinal cord barrier breakdown and degradation of tight junction proteins with associated

improvement in behavioral outcomes. Similarly, a mouse model of hemorrhagic stroke demonstrated upregulation of TRPV4 expression and concurrent RhoA pathway activation with the corresponding disruption of blood–brain barrier integrity.[76] In this model, inhibition of TRPV4 reduced RhoA and ROCK-dependent stress fiber formation resulting in preserved blood–brain barrier integrity. Moreover, in a mouse model of post-ischemic brain injury, TRPV4 knockout decreased loss of tight junction proteins and blood–brain barrier disruption in ischemic tissue, further highlighting the importance of TRPV4 activity in the response to neurovascular injury.[75] Importantly, whether TRPV4 and RhoA play a similar role in regulating blood–nerve barriers of the peripheral nervous system has yet to be established. Furthermore, in addition to a direct role in regulating cytoskeletal changes in endothelial cells, TRPV4 channel activation can also trigger inflammatory signaling cascades that may further exacerbate tissue injury and contribute to pain sensation, as is reviewed elsewhere.[89–91]

Together, animal models show a benefit of TRPV4 channel inhibition in the context of neurological injuries in which RhoA is known to be activated and detrimental. Furthermore, the association of TRPV4 and RhoA in the same pathological conditions and recent data demonstrating linked TRPV4–RhoA signaling cascades suggests that TRPV4 may be an important regulator of pathological RhoA activation. Thus, by acting as a sensor of mechanical disruption and/or injury, TRPV4 may be a critical and pharmacologically tractable node in pathological RhoA activation across a wide range of neurological insults (Figure 4).

TRPV4 INHIBITION IS A PROMISING THERAPEUTIC ROUTE TO TREAT NEUROLOGICAL DISEASE

Since the discovery of TRP channels as mechanically gated ion channels important for sensory function,[92] there has been substantial interest in potential pharmacologic applications of small molecules and tissue-specific delivery strategies for targeting these channels.[93,94] Importantly, while gain of function mutations in TRPV4 cause human disease, TRPV4 knockout does not display any overt deleterious phenotypes in mice. [95–97] Several naturally occurring pharmacological compounds targeting TRPV4 have been discovered, and several other synthetic compounds have been developed. Small-molecule antagonists include nonselective inhibitors such as ruthenium red, and more selective inhibitors such as HC-067047, GSK2193874, GSK205, and the orally bioavailable GSK2798745.[98–100] These inhibitors have been successfully used in vivo without obvious side effects, and GSK2798745 has been tested in Phase II clinical trials for pulmonary edema and chronic cough (NCT03372603).[101] While these trials did not meet efficacy endpoints for these disease indications, the antagonist drug was well tolerated without any significant adverse effects (NCT02119260).[101] This drug is currently being tested in a Phase I clinical trial for diabetic macular edema (NCT04292912), motivated by work implicating TRPV4 in the regulation of blood–retinal barrier integrity.[102]

Pharmacological TRPV4 antagonists have been successful at inhibiting several neuropathological processes in animal models of neurological disease.[76,103–106] However,

to more firmly establish the connections between TRPV4 activation and RhoA signaling as well as the therapeutic potential of pharmacological targeting of TRPV4, further studies are needed. In animal models of neurological injury, such as stroke, TBI, and SCI, activation or inhibition of TRPV4 needs to be combined with more detailed assessment of RhoA pathway activation. In vitro, Rho activation assays, RhoA biosensors, and immunohistochemistry for active RhoA mediators can be readily utilized to correlate RhoA activation with TRPV4 channel activity. In vivo approaches to monitor RhoA pathway activity are more challenging, but could utilize immunohistochemistry for activated RhoA or phosphorylation of RhoA targets such as MLC, myosin phosphatase (MYPT1), and ADF/cofilin, as well as a RhoA–FRET-based biosensor mouse.[107–109] These approaches could be coupled with in vivo experiments utilizing genetically encoded calcium indicators, such as GCaMP, to monitor TRPV4 ion channel activity in real time.[109]

Understanding TRPV4–RhoA contributions to disease will also require more detailed assessment of cell-type-specific activation of downstream pathways. While global RhoA knockout is lethal, cell-specific knockout in endothelial cells has been tested using Cre-recombinase approaches.[110] Similarly, the generation of tissue-specific TRPV4 knockout mice would allow for direct interrogation of TRPV4 function in potentially relevant cell types, including endothelial cells, glia, and neurons. Such approaches will be critical to elucidating cell-specific contributions of TRPV4 in various nerve injury paradigms, as well as providing clarity regarding whether TRPV4 and RhoA regulate blood–nerve barriers in the peripheral nervous system in a similar fashion to what has been demonstrated in the central nervous system. This issue is particularly relevant given that studies of TRPV4 in different peripheral nerve injury models have produced somewhat conflicting results. For example, pharmacologic TRPV4 inhibition was found to be beneficial in mouse models of chemotherapy-induced neuropathy[111,112] and temporomandibular pain,[113] whereas global knockout of TRPV4 worsened outcomes in a mouse model of peripheral nerve transection. [114] The latter result may relate to disruption of Schwann cell-specific TRPV4 functions as there is evidence of a beneficial role for TRPV4 in regulating cytoskeletal changes that support glial migration to sites of inflammation and injury.[115] Together, these results and unanswered questions underscore the need to better define cell type-specific functions of TRPV4 and RhoA as well as biodistribution and nervous system penetration of TRPV4-acting drugs.

An additional consideration is the tissue-specific expression pattern of TRPV4, both in health and in response to neurological injury. While the lack of specificity of available TRPV4 antibodies has limited immunohistochemical approaches to define cellular expression, assessments of TRPV4 transcript and protein levels have demonstrated upregulation in response to brain, nerve, and SCI.[74,77,113] Further work using complementary methods, such as TRPV4 reporter mice,[116] will be vital to deriving a more complete picture of TRPV4 expression within relevant cell types under normal and disease conditions. Such knowledge could guide the implementation of tissue-specific TRPV4 inhibition strategies to target the most relevant cell types in a given pathological state.[94]

In addition to defining cell type-specific contributions of TRPV4 and RhoA function, it will be critical to define temporal aspects of pathological TRPV4–RhoA pathway activation in

various neurological disease states. As neurological injury triggers a complex cascade of pathological events evolving over disparate timescales, from hours to days to weeks,[117] there may be discrete windows during which the negative contribution of TRPV4–RhoA activation is maximal and in which therapeutic intervention would be most beneficial. Elucidation of these temporal aspects of pathological TRPV4 function will likely require careful longitudinal assessment of the impacts of pharmacological TRPV4 inhibition in animal models of disease.

CONCLUSIONS

In this review, we highlight accumulating evidence that activation of TRPV4 and RhoA is linked to pathological events that drive actin cytoskeletal change in response to neurological injury. This collective work supports a model in which TRPV4 acts as a critical gatekeeper of RhoA-mediated cytoskeletal changes in multiple pathological states. As a cell surface-expressed ion channel that can be modulated by available drugs, TRPV4 is an attractive and tractable pharmacological target that may allow precise inhibition of pathological RhoA activation associated with nerve injury and blood–brain barrier disruption. Driven by this important insight, experimental approaches to precisely delineate TRPV4 expression patterns, kinetics of activation, and downstream pathological RhoA activation may illuminate a range of therapeutic opportunities for currently untreatable neurological insults.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

ARD	ankyrin repeat domain
EET	epoxyeicosatrienoic acid
GAP	GTPase activating protein
GDI	guanine-nucleotide dissociation inhibitor
GEF	guanine-nucleotide exchange factor

MLC	myosin-light chain
MYPT	myosin phosphatase
PBD	phosphoinositide binding domain
PRR	proline-rich region
ROCK	Rho-associated coiled-coil containing kinase
SCI	spinal cord injury
TBI	traumatic brain injury
TRPV4	transient receptor potential vanilloid

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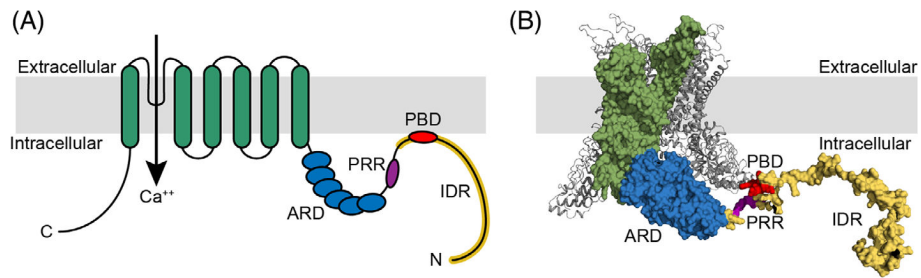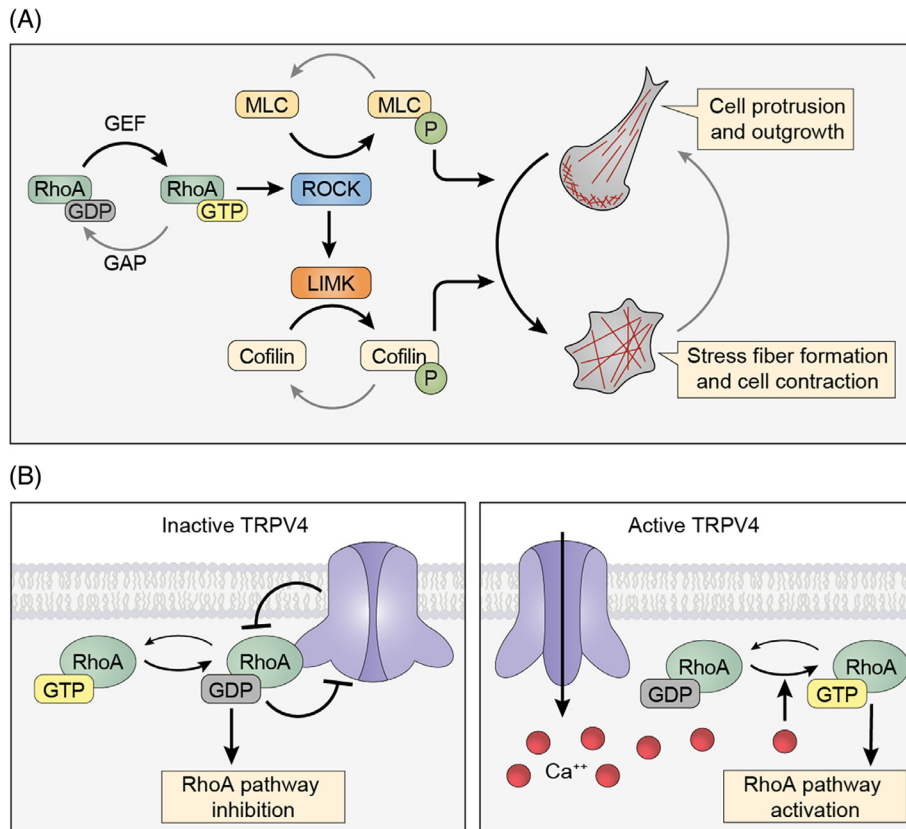
FIGURE 1.
Structure of transient receptor potential vanilloid 4 (TRPV4). (A) Schematic of TRPV4 structural domains including transmembrane domains (green) with the ion channel pore between S5 and S6, the ankyrin repeat domain (ARD, blue), the proline-rich region (PRR, purple), PIP$_2$ binding domain (PBD, red), and the intrinsically disorder region (IDR, yellow). (B) Crystal structure of xenopus tropicalis TRPV4 demonstrating structural domains highlighted in (A).

FIGURE 2.

Transient receptor potential vanilloid 4 (TRPV4) and RhoA-dependent pathways regulate the actin cytoskeleton. A. Schematic depicting the activity cycle of RhoA and downstream effectors. RhoA is activated by guanine nucleotide exchange factors (GEFs), which promote GTP loading, and inactivated by GTPase activating proteins (GAPs), which promote hydrolysis of GTP to GDP. Active RhoA leads to activation of ROCK, which promotes phosphorylation of myosin light chain (MLC) and activation of nonmuscle myosin. ROCK also activates LIMK, which increases activation of ADF/Cofilin. Increased activation of RhoA-dependent signaling leads to actin stress fiber formation and cellular contraction. (B) Schematic depicting TRPV4–RhoA functional interactions. Inactive TRPV4 channels bind GDP-bound, inactive RhoA and reduce the active pool of RhoA. Activation of TRPV4 ion channel activity leads to release of RhoA, calcium-mediated RhoA activation, and resultant cytoskeletal changes
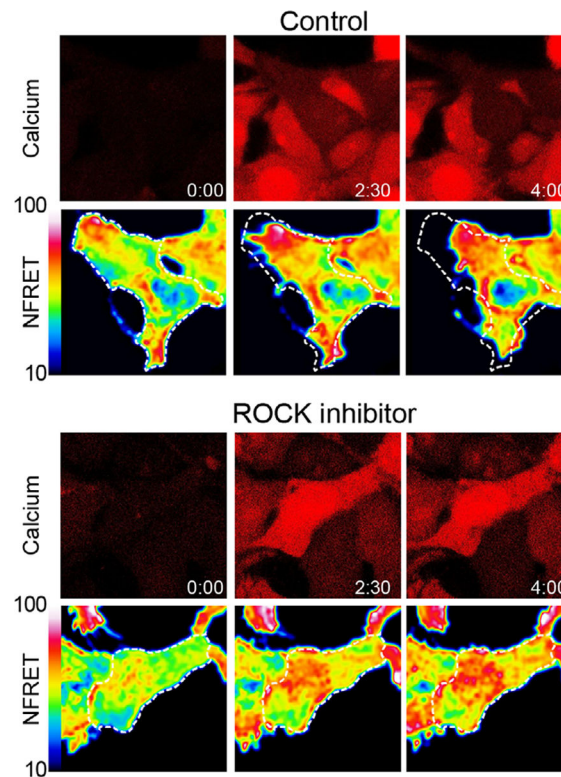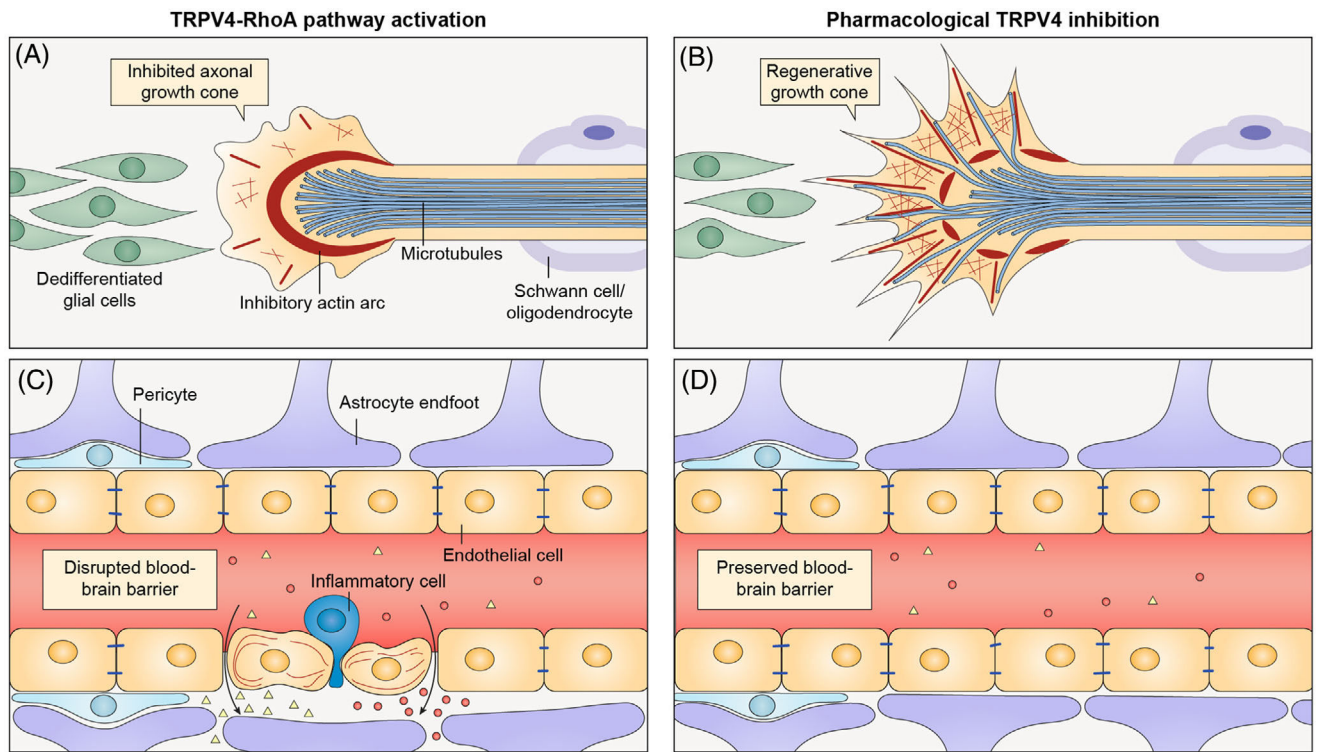
FIGURE 3.

Stimulation of transient receptor potential vanilloid 4 (TRPV4) leads to RhoA activation and ROCK-dependent cytoskeletal changes. Time-lapse confocal microscopy images of stable, inducible TRPV4-expressing HEK293T cells (T-Rex-TRPV4) expressing a RhoA FRET biosensor (RhoA2G-mVenus-mTFP) and loaded with Cal590 calcium indicator demonstrate dynamics of calcium influx, RhoA activation, and cytoskeletal changes after stimulation of TRPV4. In the top panel, addition of the TRPV4 agonist GSK1016790A (100 nM) leads to calcium influx and increased RhoA activation (indicated by increased normalized FRET (NFRET)) followed by cellular contraction over 4 min. In the bottom panel, addition of the ROCK inhibitor Y-26732 (10 μM) blocks cytoskeletal changes downstream of TRPV4-mediated RhoA activation. Detailed methods were described previously[39]

FIGURE 4.
Schematic of the potential role of transient receptor potential vanilloid 4 (TRPV4) inhibition in limiting neurological injury. (A) In injured neurons, activation of TRPV4 and RhoA leads to ROCK-dependent bundling of actin at the base of the axonal growth cone, thereby restricting microtubule entry into the growth cone. (B) Inhibition of TRPV4 could potentially reduce RhoA activation and create a regeneration-permissive environment within the axonal growth cone. Specific TRPV4–RhoA functions in glia require further study. (C) In injured vascular endothelial cells of the brain and spinal cord, TRPV4 activation leads to RhoA-dependent disruption of the blood–brain barrier with subsequent extravasation of blood constituents and inflammatory cells, which worsen local tissue injury. (D) Inhibition of TRPV4 could potentially reduce RhoA-dependent blood–brain barrier breakdown, thereby limiting the extent of surrounding tissue injury