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Pharmacologically targeting transient receptor potential channels for seizures and epilepsy: Emerging preclinical evidence of druggability

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Abstract

As one of the most prevalent and disabling brain disorders, epilepsy is characterized by spontaneous seizures that result from aberrant, excessive hyperactivity of a group of highly synchronized brain neurons. Remarkable progress in epilepsy research and treatment over the first two decades of this century led to a dramatical expansion in the third-generation antiseizure drugs (ASDs). However, there are still over 30% of patients suffering from seizures resistant to the current medications, and the broad unbearable adversative effects of ASDs significantly impair the quality of life in about 40% of individuals affected by the disease. Prevention of epilepsy in those who are at high risks is another major unmet medical need, given that up to 40% of epilepsy patients are believed to have acquired causes. Therefore, it is important to identify novel drug targets that can facilitate the discovery and development of new therapies engaging unprecedented mechanisms of action that might overcome these significant limitations. Also over the last two decades, calcium signaling has been increasingly recognized as a key contributory factor in epileptogenesis of many aspects. The intracellular calcium homeostasis involves a variety of calcium-permeable cation channels, the most important of which perhaps are the transient receptor potential (TRP) ion channels. This review focuses on recent exciting advances in understanding of TRP channels in preclinical models of seizure disorders. We also provide emerging insights into the molecular and cellular mechanisms of TRP channels-engaged epileptogenesis that might lead to new antiseizure therapies, epilepsy prevention and modification, and even a cure.

Keywords

Antiseizure drug (ASD); Calcium signaling; Cation channels; Epileptogenesis; Excitotoxicity; Hyperexcitability; Seizures; Transient receptor potential (TRP) channels

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

1. Introduction

Afflicting about 65 million people globally, epilepsy is a group of chronic debilitating brain conditions that have diverse etiologies and can result from genetic mutations, acute brain injuries, autoimmune conditions, brain infections, and metabolic disorders. For a large proportion of patients, however, the causes of epilepsy are unknown. Regardless of diverse etiologies, epilepsy is commonly featured by spontaneous recurrent seizures due to aberrant, excessive hyperactivity of a group of highly synchronized brain neurons (Devinsky, et al., 2018). Seizures are abrupt, uncontrollable behavioral changes with a large range of signs, such as confusion, unusual feelings and sensations, loss of consciousness, stiffness, jerking, falling, etc. The onset of seizures can be generalized or focal, depending on whether the irregular neuronal activities begin in an extensive distribution affecting both hemispheres or in a more focalized brain areas involving only one hemisphere. In addition, epilepsy is commonly associated with many neurological comorbidities, such as anxiety and depression, cognitive deficits, and psychiatric disturbances, which along with seizures themselves severely impact the quality of life for patients (Devinsky, et al., 2018; Fisher, et al., 2017a; Fisher, et al., 2017b).

Despite the remarkable advances in epilepsy research and treatment in the first two decades of this century, leading to an expanding list of the third-generation antiseizure drugs (ASDs) (Carvill, Dulla, Lowenstein, & Brooks-Kayal, 2020; Loscher & Klein, 2020; Rho & White, 2018), there are still approximately 1/3 of epilepsy patients who are inadequately treated and suffer from pharmacoresistant seizures (Janmohamed, Brodie, & Kwan, 2020). Up to date, there is no undisputed evidence showing that the use of newer ASDs leads to more seizure-free patients than the established antiseizure medications (Chen, Brodie, Liew, & Kwan, 2018; Hauser, 2018). The chronic use of current ASDs can impose a wide range of adverse effects, most commonly, ataxia, attention disturbance, blurred vision, cognitive decline, confusion, constipation, depression, diarrhea, diplopia, dizziness, drowsiness, fatigue, headache, incoordination, nausea, sedation, tremor, vertigo, vomiting, and weight gain. These systemic and neurological side effects of ASDs further complicate seizure management, particularly when polytherapy is needed (Devinsky, et al., 2018). Moreover, the current ASDs, regardless of newer or established drugs, only interrupt seizures in patients who have been diagnosed with epilepsy, and up to date, they have not been demonstrated to prevent epilepsy after precipitating events or modify the disease progression (Galanopoulou, et al., 2021; Loscher, 2020).

Most ASDs suppress seizures through at least one of the four currently known mechanisms of action: 1) modulation of voltage-gated ion channels; 2) modulation of neurotransmitter release; 3) impediment of glutamatergic excitation; 4) augmentation of GABAergic inhibition (Loscher & Klein, 2020; Sills & Rogawski, 2020). For instance, ganaxolone, a recently approved ASD, is thought to control seizures in patients with the cyclin-dependent kinase-like 5 deficiency disorder via positively modulating the $GABA_A$ receptor (Yasmen, Sluter, Yu, & Jiang, 2023). However, these four classical antiseizure mechanisms may also underlie the adverse effects of ASDs (Sills & Rogawski, 2020), and the pharmacoresistant epilepsy is likely developed due to the target-site alterations (Janmohamed, et al., 2020). As such, identification of novel drug targets may facilitate the development of safer antiseizure

therapies that engage unprecedented mechanisms of drug action to terminate acute seizures in a more efficient way and may even prevent the development of epilepsy after precipitating brain insults or modify the progression of epileptic seizures (Dey, Kang, Qiu, Du, & Jiang, 2016; Varvel, Jiang, & Dingledine, 2015). Such efforts presumably will help to overcome the seemingly insurmountable limitations in the current epilepsy medications.

2. TRP cation channels

The transient receptor potential (TRP) ion channels are a group of tetrameric nonselective cation channels that are essential to the intracellular calcium homeostasis and contributes to many different physiological processes (Prakriya & Lewis, 2015). In mammals, this superfamily has 28 currently known members that can be classified into six subfamilies based on amino acid homologies: TRPA (ankyrin, A1), TRPC (canonical, C1-C7), TRPM (melastatin, M1-M8), TRPML (mucolipin, ML1-ML3), TRPP (polycystin, P1-P3), and TRPV (vanilloid, V1-V6) (Nilius & Szallasi, 2014; Samanta, Hughes, & Moiseenkova-Bell, 2018). However, in humans, TRPC2 is a pseudogene that is not expressed. Most members of TRPC, TRPM, and TRPV subfamilies are highly expressed within the brain (Wu, Sweet, & Clapham, 2010), where they can regulate a variety of cellular functions of both neurons and glial cells (Harteneck & Leuner, 2014). Particularly, the activation of TRP channels expressed by brain neurons can cause the depolarization of membrane potential to trigger the activation or inactivation of many different voltage-gated ion channels and modulate calcium signaling to regulate diverse cellular functions. In addition to these immediate actions, elevated calcium in neurons may cause some long-term effect through calcium-dependent gene regulation, which contributes to several forms of synaptic plasticity, leading to long-term alterations in the biological and pathophysiological behavior of neural circuits (Meza, Ancaten-Gonzalez, Chiu, & Chavez, 2022; Nilius & Szallasi, 2014). As such, mounting evidence from recent studies suggests that dysregulation of the TRP channel functions is involved in pathological events of various neurological and psychiatric diseases, including disorders caused by defects in genes encoding these cation channels, which are thus called hereditary TRP channelopathies (Koivisto, Belvisi, Gaudet, & Szallasi, 2022).

Deregulated calcium signaling is now well recognized as a major contributor to epileptic seizures largely because of its involvement in the aberrant synchronization of brain neurons that is thought to underlie the epileptogenic mechanisms (Penn, Segal, & Moses, 2016). Moreover, astrocytic calcium signals have been implicated in the facilitation of spreading the epileptic activities (Heuser, et al., 2018; Sasaki, et al., 2014). Particularly, calcium signaling can lead to profound effects on membrane excitability directly by calcium influx or through indirect mechanisms involving G protein-dependent pathways (Brini, Cali, Ottolini, & Carafoli, 2014; Kawamoto, Vivar, & Camandola, 2012; Yu, Nguyen, & Jiang, 2019). Among a variety of calcium-permeable cation channels that are involved in the intracellular calcium homeostasis and have been implicated in acute seizures and chronic epilepsy are TRP ion channels (Garcia-Rodriguez, Bravo-Tobar, Duarte, Barrio, & Saez, 2022). The rest of this review focuses on several TRP superfamily members (TRPV, TRPC, and TRPM) that have recently been extensively studied in various animal models for their pathogenic roles in epileptic seizures. The emerging pharmacological evidence is also highlighted to

demonstrate their druggability for the next-generation therapies to stop acute seizures and potentially epileptogenesis.

3. TRPV channels

3.1. TRPV1

Involved in many important pathological and physiological processes, TRPV1 has long been implicated in a variety of conditions, such as ischemia, epilepsy, schizophrenia, psoriasis, pneumonia, and diabetes (Gladkikh, Sintsova, Leychenko, & Kozlov, 2021). For instance, TRPV1 in cortical and hippocampal tissues from patients with mesial temporal lobe epilepsy (MTLE) is upregulated at both mRNA and protein levels when compared to the normal control subjects and is mainly found in the somas and dendrites of neurons ($NeuN^+$) and in astrocytes ($GFAP^+$) but not in microglia ($HLA\text{-}DR^+$) (Sun, et al., 2013). These findings together with its expression in both glutamatergic and GABAergic neurons suggest that TRPV1 might contribute to the neuropathogenesis of human MTLE, inspiring studies on TRPV1 as a potential therapeutic target in various preclinical models (Table 1). Interestingly, treatment with selective TRPV1 agonist OLDA dose-dependently facilitated the overall incidence of seizures induced by chemoconvulsant pentylenetetrazole (PTZ) in Wistar rats. Conversely, high-stage seizures and afterdischarge duration in rat amygdala kindling model were significantly reduced after the administration of TRPV1 antagonist AMG-9810 (Shirazi, et al., 2014). Likewise, systemic administration of TRPV1 agonist capsaicin in mice directly triggered tonic-clonic seizures. In contrast, intraperitoneal administration of TRPV1 antagonist capsazepine or genetic ablation of TRPV1 led to delayed latency to tonic-clonic seizures and reduced overall mortality in mice treated with PTZ for seizure induction (Jia, et al., 2015). These pharmacological studies suggest that the selective inhibition of TRPV1 channels might represent a novel therapeutic strategy for epilepsy (Table 1). In line with these findings, TRPV1 activation by capsaicin increased, whereas blocking TRPV1 with capsazepine reduced, the paroxysmal discharge following the maximal dentate activation (MDA) in Wistar rats. Moreover, capsaicin, when co-administered with WIN 55,212–2, an exogenous cannabinoid agonist for the CB1 receptor, was able to reduce its anticonvulsant effects. In contrast, co-administration of capsazepine potentiated the suppression by WIN 55,212–2 on the MDA response, supporting a possible crosstalk between TRPV1 and cannabinoid signaling in hyperexcitability-associated brain diseases (Carletti, Gambino, Rizzo, Ferraro, & Sardo, 2016).

In contrast to its well-studied proconvulsive roles discussed above, TRPV1 activation has also been reported to be associated with anticonvulsant effects in several preclinical models. For example, global congenital deletion of TRPV1 decreased the vulnerability to PTZ-induced seizures after repetitive hyperthermia challenges in neonatal mice (Kong, et al., 2014), suggestive of a role of TRPV1 in the pathogenesis of febrile seizures observed in newborns. However, loss of TRPV1 function prevented the excessive microglial activation and migration triggered by hyperthermic stimulation. It was further found that stimulation of microglial TRPV1 enhanced seizure susceptibility by decreasing the anti-inflammatory and neuroprotective effects of transforming growth factor β1 in microglia via interacting with toll-like receptor 4 (Kong, et al., 2019). Interestingly, systemic treatment with TRPV1

antagonists capsazepine or AMG-9810 alone reduced PTZ-provoked seizures in mice in a dose-dependent manner but decreased the anticonvulsant effects of acetaminophen in the same animal model (Suemaru, Yoshikawa, Aso, & Watanabe, 2018), suggesting that TRPV1 might be responsible for the anticonvulsant action of acetaminophen. In a lithium/pilocarpine model of status epilepticus (SE), treatment with capsaicin at a relatively low dose exacerbated neuronal death in the hippocampus and reversed neuroprotective effects of dexmedetomidine in immature rats (Tan, et al., 2020), indicating that TRPV1 activation might contribute to SE-induced neuronal death in juvenile animals. Taken together, whether TRPV1 activation leads to beneficial or detrimental effects appears to be determined by the seizure models, animal ages, and particularly, its cell type-specific distributions.

TRPV1 in the brain was induced in mice that experienced recurrent febrile seizures, particularly in activated microglia ($Iba1^+$) (Kong, et al., 2019). This result seemingly contradicts early finding that TRPV1 was not expressed in HLA-DR^+ microglia in temporal cortical tissues from MTLE patients (Sun, et al., 2013), but might be explained by the differences in seizure etiology (MTLE vs. febrile seizures), species (human vs. mouse), and microglial biomarkers (HLA-DR vs. Iba1) used in these two studies. Particularly, HLA-DR highlights the immune activation and response to brain tissue damage, whereas Iba1 emphasizes more on the cellular structure (Hendrickx, van Eden, Schuurman, Hamann, & Huitinga, 2017). Considering its discrete expression in neurons, astrocytes, and microglia in the epileptic brain (Kong, et al., 2019; Sun, et al., 2013), future studies engaging cell-type specific gene deletion (neuronal vs. microglial) are required to fully understand the complex roles of TRPV1 (anticonvulsive vs. proconvulsive) in seizure generation and subsequent neuropathogenesis.

3.2. TRPV4

Initially identified as a nonselective cationic channel permeable to calcium, TRPV4 is now known as a polymodal ionotropic receptor with a nonselective cation channel that is also permeable to potassium, magnesium, and sodium, and can be activated by a broad variety of stimuli (Zeng, Kong, Chen, & Peng, 2023). Its wide-ranging biological roles are associated with many physiological and pathological processes in the central nervous system (CNS). TRPV4 has long been implicated in the development of epilepsy of various origins, where it shows abnormal expression and activities. Both TRPV1 and TRPV4 were found upregulated in larval zebrafish after hyperthermia-induced seizures, but only the TRPV4 inhibition by RN1734, not the TRPV1 blockade by capsazepine, produced a significant reduction in hyperthermic seizure activities, an outcome that was recapitulated by NMDA receptor antagonists MK-801 and ifenprodil (Hunt, Hortopan, Gillespie, & Baraban, 2012). TRPV4 was also found elevated in the hippocampus after pilocarpine-induced SE in mice (Men, et al., 2019). Intracerebroventricular injection of TRPV4 agonist GSK1016790A in mice increased NLRP3 inflammasome activation, gliosis featured by upregulated Iba1 and GFPA, and pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, and neuronal death in the hippocampus. On the contrary, intracerebroventricular injection of TRPV4 antagonist HC-067047 markedly improved neuronal survival after pilocarpine-induced SE and abolished SE-provoked gliosis, NLRP3 inflammasome activation, and cytokine induction (Wang, et al., 2019). Given that the pharmacological inhibition of TRPV4 by HC-067047

also delayed the latency to SE, suggestive of an anticonvulsant effect (Men, et al., 2019), the broad anti-inflammatory and neuroprotective effects from HC-067047 treatment in mouse SE model are likely secondary to its antiseizure action (Table 1). Interestingly, TRPV4 activation by GSK1016790A has been shown to enhance the expression of voltage-gated potassium channel Kv4.2 and potassium channel interacting protein (KCHIP) in the hippocampus following pilocarpine-induced SE in mice, which was attenuated by TRPV4 inhibitor HC-067047 (Table 1). The TRPV4 activity-mediated elevation of Kv4.2 and KCHIP is thought to lead to an increase in the rapidly inactivating potassium current in hippocampal pyramidal neurons, which likely contributes to hyperexcitability during the early stage of epileptogenesis (Xu, et al., 2022).

Neuronal excitation can evoke astrocytic calcium transients, which in turn can modulate neuronal excitability and regulate synaptic transmission. As such, TRPV4 expression was found in approximately 30% of astrocytes within the brain, and the activation of TRPV4-expressing astrocytes may lead to the release of glutamate, which can increase excitatory gliotransmission to enhance synaptic transmission and neuronal excitability (Shibasaki, Ikenaka, Tamalu, Tominaga, & Ishizaki, 2014). Both TRPV4 and GFAP were upregulated in the hippocampus following 4-AP-induced seizures in mice, and the substantial colocalization between these two proteins suggests that astrocytic activation could be TRPV4 activation-dependent. TRPV4 inhibition by HC-067047 reduced susceptibility of mice to 4-AP-induced seizures and several key pro-inflammatory mediators, whereas treatment with GSK1016790A aggravated animal mortality (Table 1) (Zeng, et al., 2022). These findings suggest that targeting astrocytic TRPV4 activation might provide a therapeutic strategy to treat acute seizures and development of epilepsy. In addition to the well-recognized neuroinflammatory mechanisms involving astrocytes, TRPV4 channels might regulate neuronal excitability via its activation by brain temperature. The brain temperature in epileptogenic foci was found to be dramatically higher than that in other normal regions, and this increase in temperature was critical for the progression of disease where TRPV4 played a fundamental role (Shibasaki, et al., 2020). Thus, it is highly likely that cooling treatment at the epileptogenic zones might be able to suppress epileptic discharges via inhibiting TRPV4 channels.

4. TRPC channels

4.1. TRPC3

TRPC3 is the second identified TRPC subfamily member and shares about 74% protein sequence identity with TRPC6 and TRPC7 (Yu, Li, & Jiang, 2022). TRPC3-containing cation channels are abundantly found in the brain, where they are mostly detected in the hippocampus, cortex, cerebellum, and substantia nigra and play important physiological functions (Hartmann, et al., 2008; Li, Calfa, Inoue, Amaral, & Pozzo-Miller, 2010; Mitsumura, Hosoi, Furuya, & Hirai, 2011; Roedding, et al., 2009; Zhou & Lee, 2011). However, recent evidence suggests that TRPC3 activity also contributes to many neuropathogenic processes, particularly in the epileptic brain. Congenital deletion of TRPC3 led to a reduction in the severity and duration of convulsions in mice treated by pilocarpine and markedly decreased the overall electroencephalogram (EEG) power and theta wave

activities of chemoconvulsant SE (Phelan, et al., 2017). These findings demonstrated that TRPC3 activation might be essential to the seizure generation and motivated follow-on pharmacological studies to determine the feasibility of targeting TRPC3 to interrupt acute seizures and epileptogenesis.

Pyr3, a pyrazole compound that was identified as a highly potent and selective TRPC3 inhibitor (Kiyonaka, et al., 2009), when systemically administered in mice, led to a marked reduction in the overall root-mean-square power of SE induced by pilocarpine (Table 2). EEG recording revealed that TRPC3 inhibition by Pyr3 also specifically attenuated the theta activities during the entire course of SE (Phelan, et al., 2017). Interestingly, among all TRPC subtypes, only TRPC3 was significantly elevated in the hippocampus by febrile seizures induced in rats. Moreover, direct microinjection of Pyr3 into the hippocampus reduced the seizure severity and duration and largely prevented the febrile seizure-associated brain cell death and neuroinflammation (Sun, et al., 2018). Compound JW-65 was developed as a chemical analog of Pyr3 with higher metabolic stability and less toxicity (Zhang, et al., 2021), allowing high systemic doses in animal models, which would not be possible for Pyr3. Indeed, intraperitoneal administration of JW-65 strikingly diminished the behavioral seizures in pilocarpine-treated mice, and the results were validated by EEG recording and dose-dependently reproduced in a mouse PTZ model (Table 2). Remarkably, the antiseizure effects by JW-65 appeared comparable to that caused by the genetic deletion of TRPC3 in the same animal model (Nagib, et al., 2022; Phelan, et al., 2017), validating the TRPC3 as a pharmacological target for new treatment of seizures.

4.2. TRPC5

Among the TRPC subfamily members, TRPC4 and TRPC5 proteins show the highest homology to each other, as approximately 78% of their sequences are identical (Yu, et al., 2022), underlying their similarity in gating properties. TRPC1 and TRPC4 showed the highest expression in mouse hippocampus, whereas the level of TRPC5 expression was relatively low in the same brain region (Phelan, et al., 2012). However, in mouse pilocarpine model of SE, genetic deletion of TRPC5 alone diminished the seizure severity, reduced hippocampal cell death, and largely prevented SE-associated mortality (Phelan, et al., 2013). Conversely, mice lacking both TRPC1 and TRPC4 failed to alter the seizure severity but did reduce neuronal death in hippocampal areas and lowered the overall mortality in the same SE model (Phelan, et al., 2012). These findings suggest that TRPC5 subtype likely plays more important roles in generating chemoconvulsant seizures when compared to TRPC1 and TRPC4 (Phelan, et al., 2012). Interestingly, the expression of TRPC4 and TRPC5 but not TRPC1 was elevated at both protein and mRNA levels in mouse hippocampus and cortex after traumatic brain injury (TBI) (Carver, DeWitt, Stoja, & Shapiro, 2021). Subcutaneous treatment with compound M084, an inhibitor that can block both TRPC4 and TRPC5, substantially lowered the post-TBI neuronal hyperexcitability. In addition, mice after TBI showed higher susceptibility to PTZ-induced seizures, which was also lowered by M084 (Table 2) (Carver, et al., 2021). However, whether inhibiting TRPC4 or TRPC5 alone can lead to similar outcomes remains to be determined using more selective inhibitors. As such, systemic administration of compound NU6027 in rats showed marked neuroprotection after kainate-induced SE via specifically inhibiting the gating of TRPC5 channels (Table

2) (Park, et al., 2019), although the compound was originally identified as an inhibitor of threonine-protein kinase ATR and cyclin-dependent kinase.

4.3. TRPC6

TRPC6 subtype, like TRPC3, is also extensively distributed in many regions of the brain, particularly in the hippocampus, cortex, amygdala, cerebellum, and substantia nigra (Yu, et al., 2022). However, unlike TRPC3, TRPC6 was downregulated in the hippocampus following pilocarpine-induced SE in rats (Kim, Ryu, Kim, & Kang, 2013), suggesting divergent roles of these two TRPC subfamily members in the seizing brain. Indeed, intracerebroventricular infusion of hyperforin, a natural compound that selectively activates TRPC6, protected hippocampal pyramidal neurons from SE-provoked brain cell death (Table 2), an outcome mimicking that from TRPC3 inhibition by Pyr3 (Kim, et al., 2013). In line with these findings, intracerebroventricular infusion of siRNA in rats targeting TRPC6 raised susceptibility to seizures, increased neuronal excitability, exacerbated neuronal death in dentate granule cells, but protected hippocampal cells in CA1 and CA3 regions after pilocarpine SE (Kim & Kang, 2015). However, in another study, both TRPC3 and TRPC6 were found upregulated in cortical tissues from patients with intractable TLE and in the hippocampus of mice after pilocarpine SE (Zeng, et al., 2015). Intriguingly, TRPC6 depletion by intracerebroventricular injection of an anti-TRPC6 antibody reduced dendritic arborization and spine density of hippocampal CA3 pyramidal neurons, whereas microinjection of an anti-TRPC3 antibody decreased aberrant-sprouted mossy fiber collaterals in the CA3 region of SE mice (Zeng, et al., 2015). The reasons for the contradicting expression of TRPC3 and TRPC6 in the brain after SE are unknown but might be related to different species and seizure induction protocols used in these two independent studies. Nevertheless, these two TRPC subfamily members could be differentially involved in the synaptic reorganization of mossy fiber pathway following prolonged seizures.

4.4. TRPC7

As the latest TRPC subfamily member discovered, TRPC7 is widely expressed in peripheral tissues including eye, heart, kidney, lung, intestine, and pituitary gland (Zhang & Trebak, 2014). TRPC7 is also expressed in the CNS but with functions largely unknown, and to date, there is only one study on TRPC7 channels in chemoconvulsant seizures. Congenital ablation of TRPC7 was reported to diminish the seizure induction in pilocarpine-treated mice, accompanied by reduced SE-associated mortality rate. EEG recording and analysis also revealed a decrease in gamma wave activities in TRPC7 knockout mice after pilocarpine treatment when compared to wildtype animals (Phelan, Shwe, Abramowitz, Birnbaumer, & Zheng, 2014). Further, electrophysiological study showed a reduction in epileptiform burst firing in hippocampal CA3 pyramidal neurons and the lack of high-frequency stimulation-induced long-term potentiation at CA3 and CA1 synapses in TRPC7-deficient mice. Taken together, TRPC7 might play an essential role in the initiation of acute seizures given that the epileptiform burst firing in the CA3 area is dependent on neuronal activity and considered as an essential early step of seizure generation. However, there is no pharmacological study to validate these results and determine the feasibility of TRPC7 as a therapeutic target for seizures and epilepsy.

5. TRPM channels

5.1. TRPM2

TRPM2 is widely expressed in virtually all tissues and organs, but its expression in the CNS is the highest among all TRP channels (Fonfria, et al., 2006). TRPM2 is mostly known for its important contributions to the pathogenesis of CNS conditions, such as aging, ischemic stroke, neurodegeneration, neuropathic pain, and bipolar disorder (Belrose & Jackson, 2018). However, emerging evidence suggests that TRPM2 is also involved in epileptic seizures. In an early study, TRPM2 in hippocampal neurons was found to interact with EF-hand motif-containing protein 1 (EFHC1) and contribute to the phenotypes of juvenile myoclonic epilepsy via mediating the disruptive functions of EFHC1 mutations on biological processes including neuronal apoptosis (Katano, et al., 2012). A more recent study revealed that the congenital ablation of TRPM2 in mice led to considerable antiseizure effects in PTZ, maximal electroshock, and chronic kindling models, decreased acute seizure-related neuronal death, improved cognitive functions, and alleviated brain inflammation. Importantly, the neuroprotection observed in these TRPM2 knockout mice might be related to the downregulation of PARP1/BNIP3/AIF/Endo G apoptotic pathway in cortical neurons (Zheng, et al., 2020). However, another study by the same research group showed that genetic ablation of TRPM2 in mice increased susceptibility to seizures induced by PTZ and enhanced neuronal excitability in the hippocampal CA1 region likely via inhibiting the Kv7 potassium channels (Ying, et al., 2022). The reasons for these inconsistent findings remain unclear, and future studies engaging post-SE ablation or pharmacological inhibition of TRPM2 might help to solve this contradiction.

5.2. TRPM3

TRPM3 is a heat-activated cation channel and its functions in noxious heat sensation has been well established. Like other TRP channels, the activation of TRPM3 requires phosphatidylinositol 4,5-bisphosphate. In addition to the central pore commonly found in other TRP channels, TRPM3 has an alternative pore, which shows inwardly rectifying characteristics (Zhao & Rohacs, 2021). TRPM3 is also highly expressed in the human brain (Fonfria, et al., 2006), where its roles remain largely elusive. However, recent studies reveal that mutations in TRPM3 are frequently associated with neurodevelopmental disorders, pointing to some important roles of TRPM3 in the immature brain. Particularly, a close association has been widely reported between mutations in human TRPM3 gene and the developmental and epileptic encephalopathy (DEE) (Dyment, et al., 2019; Gauthier, et al., 2021; Kang, et al., 2021), a heterogeneous group of brain disorders characterized by epilepsy and the associated intellectual disability. These DEE-associated mutations are mostly gain-of-function mutations but may have distinct effects on the TRPM3 gating via different mechanisms, including enhanced basal activity, elevated sensitivity to stimulation by high temperatures and the endogenous neurosteroid pregnenolone sulfate, as well as changed response to ligand modulation, leading to channel overactive (Van Hoeymissen, et al., 2020; Zhao, Yudin, & Rohacs, 2020). Interestingly, these mutants can be inhibited by selective TRPM3 antagonist isosakuranetin and ASD primidone, which can directly inhibit the TRPM3 ion channels (Krugel, Straub, Beckmann, & Schaefer, 2017). However, it remains to be determined whether the antiseizure effects of primidone should be attributed

to its direct inhibition of TRPM3. On the other hand, it would also be interesting to know whether currently known TRPM3 inhibitors, such as isosakuranetin and liquiritigenin, have potential to be developed to treat other forms of epilepsy, besides DEE.

5.3. TRPM8

Known as the cold and menthol receptor 1, TRPM8 shows the most discrete expression patterns in human bodies among all TRP channels. Particularly, it has the highest expression in prostate, followed by liver but virtually is undetectable in other tissues and organs including the CNS (Fonfria, et al., 2006). However, TRPM8 agonist menthol has been reported to enhance tonic, but not phasic, GABAergic inhibition, suggesting a role of TRPM8 in regulating neuronal activity (Zhang, et al., 2008). Indeed, systemic treatment with M8-B, a TRPM8 antagonist that can lower the body temperature, increased the latency to febrile seizures in rat pups and led to a substantial anticonvulsant effect on PTZ-induced seizures but had no effect on electroshock-provoked convulsions (Table 3) (Zandi, Zaniani, Moghimi, & Roohbakhsh, 2019). Given that reducing body temperature can prevent febrile seizures, the antiseizure effects of M8-B is likely related to its thermoregulation, which thus may affect other temperature-sensitive TRP channels in epilepsy including TRPV4 (Shibasaki, et al., 2020). In addition, menthol was found to exert TRPM8-independent antiseizure effects in prefrontal cortex pyramidal neurons via blocking sodium channels and resembling the *in vitro* action of carbamazepine, a conventional ASD targeting voltage-gated sodium channels (Szulczyk & Spyrka, 2022), highlighting the limitation of using menthol to study TRPM8. As such, genetic global deletion of TRPM8 in mice exacerbated the PTZ-induced seizures and penicillin G potassium-provoked epileptiform discharges, both of which were largely suppressed by intracortical microinjection of TRPM8 agonist WS-3 in wildtype animals (Moriyama, et al., 2021). These findings together suggest that TRPM8 activation might provide a promising pharmacological strategy to manage seizures (Table 3).

6. Conclusions and future perspectives

6.1. What are promising?

Mounting evidence from recent studies employing both genetic and pharmacological strategies support that several members of the TRP superfamily have consistent implications in seizures of various etiologies. Among these, TRPV1, TRPV4, TRPC3, and TRPM8 likely represent the most promising TRP channels as therapeutic targets for acute seizures and potentially epilepsy. These calcium-permeable channels are often expressed at basal levels under normal conditions but can be substantially upregulated in seizing brain, particularly in the epileptic foci, demonstrating their druggability. Antiseizure effects, neuroprotection, and many other therapeutic benefits by several pharmacological compounds that target these excitatory cation channels are widely reported in various animal models, ruling out any model- or species-specific findings from these studies (Tables 1–3). In addition, no adverse effects of these potential therapeutic agents have been reported in these studies, indicative of their safe uses *in vivo*.

6.2. What are missing?

It is noted that the pharmacodynamic and pharmacokinetic profiles (e.g., potency, selectivity, *in vivo* half-life, and brain penetration) of some of these agents are either marginal or have not been reported (Tables 1–3). For instance, TRPV4-selective antagonist RN-1734 has a moderate potency (IC_{50}: 2.3 μM) and is only about 13-fold selective against TRPV3 and TRPM8 channels (Vincent, et al., 2009). Likewise, JW-65, a recently developed TRPC3-selective inhibitor, has favorable plasma half-life (3.1 h) and brain penetration (brain-to-plasma ratio: 0.3) in mice (Nagib, et al., 2022), but it also non-negligibly blocks TRPC6 and TRPC7 channels (Zhang, et al., 2021). Future efforts in medicinal chemistry should be directed to continually improve their drug-like properties, which are essential to their development into new pharmacotherapies for seizure disorders.

In parallel to the emerging evidence from pharmacological studies, findings using various constitutive knockout mice also reveal that TRP channels have wide-ranging functions in health and disease (Wu, et al., 2010). However, global congenital deletion of TRP channels in mice could lead to some developmental adjustments and induce compensatory responses, which are quite common among some TRP subfamilies (Yu, et al., 2022). These confounding issues may underlie the controversial findings derived from studies on mice lacking TRPV1 or TRPM2. As such, in the future, adult-stage ablation or conditional, cell type-specific deletion should be used to better understand the roles of TRP channels in epilepsy, particularly, the adult-onset forms.

It is well known that seizures in human patients and experimental animals can be affected by many biological variables, such as age, weight, sex, etc., and, particularly, there is a growing recognition on sex differences in epilepsy and the associated comorbidities (Christian, Reddy, Maguire, & Forcelli, 2020). It should be pointed out that male animals (mice and rats) were exclusively used in most of these preclinical studies except one in which larval zebrafish were utilized as the model (Tables 1–3). This deficit should be rectified in the future studies via using both male and female animals to comply with the ARRIVE guidelines for animal research (Percie du Sert, et al., 2020) and to better understand the candidacy of TRP channels as drug targets for seizures and epilepsy by avoiding sex-biased findings.

6.3. Selectivity may not matter

It should also be noted that some of the TRP superfamily members can form heteromeric complex channels with others, and this is especially true in certain brain areas. For example, TRPC3 isomers alone can polymerize to form operational homomeric channels, but they can also multimerize with other subtypes of TRPC, particularly TRPC6 and TRPC7 due to their similarities in amino acid sequence, three-dimensional structure, signal transduction, and expression pattern (Yu, et al., 2022). The heteromerization among different TRP channel members can be cell type-specific, function-dependent, and disease-related (Zhang & Trebak, 2014). It’s unclear whether TRP homomultimers or heteromultimers are chiefly responsible for the pathogenic processes in the epileptic brain. If culprit is the latter, targeting a specific TRP member by highly selective compounds may not provide satisfactory therapeutic benefits. This possibility and the potential compensation among the

TRP superfamily members, which is actually quite common (Wang, et al., 2020), would explain, at least partially, why the genetic deletion of a single TRP channel (e.g., TRPC3) was not able to fully terminate seizures in most of these studies. As such, less selective compounds that can act on multiple TRP superfamily members might have advantages and should not be abandoned for evaluations in animal seizure models.

6.4. Multiple roads to treatment

Most pharmacological studies to date have been specifically focusing on the effects of these TRP-targeting compounds on acute seizures. Future research should also be directed to evaluate their effects on the development of spontaneous recurrent seizures after precipitating brain insults, such as TBI, stroke, brain infection, *de novo* SE, brain tumor, etc. Outcomes will help to determine whether targeting TRP channels can lead to the prevention of acquired epilepsy (i.e., epileptogenesis), modification of the disease progression, or even a cure, the ultimate goal in epilepsy management that no current FDA-approved drugs have been demonstrated to be able to achieve (Chen, et al., 2023; Jiang, Santhakumar, & Zhu, 2022; Loscher, 2020). Another crucial but unsolved issue that substantially impedes the current antiseizure medication is pharmacoresistance, which is commonly observed in more than 30% patients with epilepsy (Janmohamed, et al., 2020). Therefore, it is also important to test these TRP-targeting compounds in preclinical models of refractory epilepsy and to determine whether they can enhance the sensitivity to current ASDs when seizures become pharmacoresistant (Löscher, 2017).

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Data Availability

No data was used for the research described in the article.

Abbreviations

AIF	apoptosis-inducing factor
4-AP	4-aminopyridine
ARRIVE	Animal Research: Reporting of In Vivo Experiments
ASD	antiseizure drug
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
CNS	central nervous system
DEE	developmental and epileptic encephalopathy
EEG	electroencephalogram

EFHC1	EF-hand motif-containing protein 1
Endo G	endonuclease G
GABA	γ-aminobutyric acid
GFAP	glial fibrillary acidic protein
HLA-DR	human leukocyte antigen - DR isotype
Iba1	ionized calcium-binding adapter molecule 1
IL-1β	interleukin 1β
IL-6	interleukin 6
KCHIP	potassium channel interacting protein
MDA	maximal dentate activation
NeuN	neuronal nuclear antigen
NMDA	*N*-methyl-D-aspartate
OLDA	*N*-oleoyldopamine
PARP1	poly (ADP-ribose) polymerase 1
PBCH	*N*-phenyl-*N'*-(4-benzyloxyphenoxycarbonyl)-4-chlorophenylsulfonyl hydrazide
PTZ	pentylenetetrazole
SE	status epilepticus
TBI	traumatic brain injury
TLE	temporal lobe epilepsy
TNF-α	tumor necrosis factor α
TRP	transient receptor potential channels

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Table 1.

Pharmacological studies on TRPV channels in animal models of seizures and epilepsy.

Pharmacological Targets	Animal Models	Therapeutic Agents	Major Outcomes	References
TRPV1	PTZ-induced seizures in adult male Wistar rats	OLDA (0.01, 0.1 and 1 μg/animal, i.c.v.)	Facilitated the overall incidence of seizures	Shirazi et al., 2014
TRPV1	Amygdala kindling in adult male Wistar rats	AMG-9810 (0.3, 3 and 30 μg/animal, i.c.v.)	Reduced high-stage seizures and afterdischarge duration	Shirazi et al., 2014
TRPV1	Adult male Kunming mice	Capsaicin (40 mg/kg, i p.)	Promoted tonic-clonic seizures	Jia et al., 2015
TRPV1	PTZ-induced seizures in adult male Kunming mice	Capsazepine (0.05 and 0.5 mg/kg, i.p.)	Delayed the latency to tonic-clonic seizures and reduced mortality	Jia et al., 2015
TRPV1	MDA in adult male Wistar rats	Capsaicin (1, 5, and 10 mg/kg, i.p.)	Increased paroxysmal discharge and MDA response	Carletti et al., 2016
TRPV1	MDA in adult male Wistar rats	Capsazepine (0.5, 1, and 2 mg/kg, i.p.)	Reduced paroxysmal discharge and MDA response	Carletti et al., 2016
TRPV1	PTZ-induced seizures in adult male ICR mice	Capsazepine (1, and 3 mg/kg, i.p.)	Showed antiseizure activity alone but reduced anticonvulsant effects of acetaminophen	Suemaru et al., 2018
TRPV1	PTZ-induced seizures in adult male ICR mice	AMG-9810 (1, 3, and 10 mg/kg, i.p.)	Showed antiseizure activity alone but reduced anticonvulsant effects of acetaminophen	Suemaru et al., 2018
TRPV1	Lithium/pilocarpin e-induced seizures in immature male SD rats	Capsaicin (0.1 mg/kg, i.p.)	Increased neuronal death in hippocampus and reversed the neuroprotection of dexmedetomidi ne after SE	Tan et al., 2020
TRPV1	HT-induced seizures in larval zebrafish	Capsazepine (100 μM)	Had no effect on HT seizure activity	Hunt et al., 2012
TRPV4	HT-induced seizures in larval zebrafish	RN-1734 (0.5–1 mM)	Produced a significant reduction of HT seizure activity	Hunt et al., 2012
TRPV4	Pilocarpine-induced seizures in young adult male ICR mice	HC-067047 (10 μM/ mouse, i.c.v.)	Improved neuronal survival and diminished gliosis, inflammasome activation, and cytokine induction	Wang et al., 2019
TRPV4	Pilocarpine-induced seizures in young adult male ICR mice	HC-067047 (10 μM/ mouse, i.c.v.)	Delayed SE onset and reduced connexin expression	Men et al., 2019
TRPV4	Pilocarpine-induced seizures in young male ICR mice	HC-067047 (10 μM/ mouse, i.c.v.)	Attenuated the increased protein levels of Kv4.2 and KCHIP2	Xu et al., 2022
TRPV4	Pilocarpine-induced seizures in young male ICR mice	GSK1016790 A (1 μM/ mouse, i.c.v.)	Increased hippocampal protein levels of Kv4.2 and KCHIP2	Xu et al., 2022
TRPV4	4-AP-induced seizures in young male adult C57BL/6 mice	HC-067047 (10 μM/ mouse, i.c.v.)	Reduced seizure susceptibility, neuronal death, inflammatory cytokines, and improved neurobehavioral functions	Zeng et al., 2022
TRPV4	4-AP-induced seizures in young male adult C57BL/6 mice	GSK1016790 A (1 μM/ mouse, i.c.v.)	Increased animal mortality	Zeng et al., 2022

Abbreviations: HT, hyperthermia; ICR, Institute of Cancer Research; i.c.v., intracerebroventricular; i.p., intraperitoneal; KCHIP, K^+ channel interacting protein; MDA, maximal dentate activation; PTZ, pentylenetetrazole; SD, Sprague-Dawley; SE, status epilepticus.

Table 2.

Pharmacological studies on TRPC channels in models of seizures and epilepsy.

Pharmacological Targets	Animal Models	Therapeutic Agents	Major Outcomes	References
TRPC3	Pilocarpine-induced seizures in young adult male SD rats	Pyr3 (0.1 mg/kg/day, i.c.v.)	Reduced hippocampal TRPC3 and protected hippocampal pyramidal neurons	(Kim et al, 2013)
TRPC3	Pilocarpine-induced seizures in young adult male C57BL/6 mice	Anti-TRPC3 antibody (1:6.5, 2 μL, i.c.v.)	Decreased mossy fiber sprouting after SE	(Zeng et al., 2015)
TRPC3	Pilocarpine-induced seizures in adult male C57BL/6 mice	Pyr3 (3 mg/kg, i.p.)	Reduced the overall RMS power of SE and theta activities during SE	(Phelan et al., 2017)
TRPC3	HT-induced FS in juvenile SD rats	Pyr3 (1 mg/kg, i.h.)	Reduced neuronal cell death and neuroinflammation	(Sun et al., 2018)
TRPC3	Pilocarpine-induced seizures in young adult male C57BL/6 mice	JW-65 (100 mg/kg, i.p.)	Diminished behavioral and electrographic seizures	(Nagib et al., 2022)
TRPC3	PTZ-induced seizures in young adult male C57BL/6 mice	JW-65 (20 or 100 mg/kg, i.p.)	Elongated latencies to MJ and GTCS in a dose-dependent manner	(Nagib et al., 2022)
TRPC5	Kainate-induced seizures in adult male SD rats	NU6027 (100 μg/kg, i.p.)	Had no effect on seizure severity but protected neurons in the piriform cortex and hippocampus	(Park et al., 2019)
TRPC5	Post-TBI and PTZ-induced seizures in adult male C57BL/6J mice	M084 (10 mg/kg, s.c.)	Decreased post-TBI neuronal hyperexcitability and PTZ-provoked kindling	(Carver et al., 2021)
TRPC6	Pilocarpine-induced seizures in young adult male SD rats	Hyperforin (6 μM, 0.5 μL/h, i.c.v.)	Increased hippocampal TRPC6 and protected hippocampal pyramidal neurons	(Kim et al, 2013)
TRPC6	Pilocarpine-induced seizures in young adult male C57BL/6 mice	Anti-TRPC6 antibody (2 μL, i.c.v.)	Decreased the dendritic arborization and spine density of hippocampal CA3 neurons	(Zeng et al., 2015)

Abbreviations: FS, febrile seizure; GTCS, generalized tonic-clonic seizure; HT, hyperthermia; i.c.v., intracerebroventricular; i.h., intrahippocampal; i.p., intraperitoneal; RMS, root mean square; s.c., subcutaneous; MJ, myoclonic jerk; PTZ, pentylenetetrazol; SD, Sprague-Dawley; SE, status epilepticus; TBI, traumatic brain injury.

Table 3.

Pharmacological studies on TRPM channels in models of seizures and epilepsy.

Pharmacological Targets	Animal Models	Therapeutic Agents	Major Outcomes	References
TRPM8	Febrile seizures induced in male Wistar rat pups	M8-B (3, 6, and 9 mg/kg, i.p.)	Increased the latency to the first febrile seizure	Zandi et al., 2019
TRPM8	PTZ-induced seizures in male Wistar rat pups	M8-B (3, 6, and 9 mg/kg, i.p.)	Increased the latencies to the stage-2 and stage-4 seizures and reduced the stage-5 seizure duration	Zandi et al., 2019
TRPM8	Electroshock-induced convulsions in male Wistar rat pups	M8-B (3, 6, and 9 mg/kg, i.p.)	Had no significant effect on convulsions	Zandi et al., 2019
TRPM8	PTZ-induced seizures in male adult C57BL/6 mice	WS-3 (0.1, 0.3, and 1 mM, 0.1 μL/min, i.c.)	Exacerbate d PTZ-induced seizures	Moriyama et al., 2021
TRPM8	PG-induced seizures in male adult C57BL/6 mice	WS-3 (0.1, 0.3, and 1 mM, 0.1 μL/min, i.c.)	Exacerbate d PG-induced epileptiform discharges	Moriyama et al., 2021

Abbreviations: i.c., intracortical; i.p., intraperitoneal; PG, penicillin G potassium; PTZ, pentylenetetrazole.