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REVIEW ARTICLE

Development of Non-opioid Analgesics Targeting Two-pore Domain Potassium Channels

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Abstract: Two-pore domain potassium (K2P) channels are a diverse family of potassium channels. K2P channels generate background leak potassium currents to regulate cellular excitability and are thereby involved in a wide range of neurological disorders. K2P channels are modulated by a variety of physicochemical factors, such as mechanical stretch, temperature, and pH. In the peripheral nervous system, K2P channels are widely expressed in nociceptive neurons and play a critical role in pain perception. In this review, we summarize the recent advances in the pharmacological properties of K2P channels, with a focus on the exogenous small-molecule activators targeting K2P channels. We emphasize the subtype-selectivity, cellular and *in vivo* pharmacological properties of all the reported small-molecule activators. The key underlying analgesic mechanisms mediated by K2P are also summarized based on the data in the literature from studies using small-molecule activators and genetic knock-out animals. We discuss the advantages and limitations of the translational perspectives of K2P in pain medicine and provide outstanding questions for future studies in the end.

Keywords: Pain, analgesics, ion channels, potassium channels, peripheral nervous system, central nervous system.

1. INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. Chronic pain is defined as pain that persists or recurs for longer than 3 months and is associated with significant emotional distress and/or significant functional disability [2]. In the U.S.A, chronic pain affects tens of millions of people [3]. In China, neck pain and low back pain are the top 10 causes of years lived with disability [4, 5]. Adding to this burden, currently available pain medications, like non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are only partially effective and coupled with deleterious side effects, including gastrointestinal complications, dependence and tolerance [6-8]. The development of novel classes of analgesics with high efficacy and minimum of adverse side effects represents an urgent need for pain medicine.

In the development of pain, ion channels are directly involved in the transformation of noxious stimuli, including heat, cold, mechanical forces, and chemical agents into action potentials (AP) in the primary sensory neurons. They are potential targets to disrupt pain signaling by regulating neuronal excitability of the primary nociceptors as well as the second- or higher-order neurons in the spinal cord and the brain [9, 10]. Ion channels, including voltage-gated sodium channels [11], calcium channels [12], potassium channels [13], acid-sensing ion channels [14], and transient receptor potential (TRP) channels [15], have been implicated in the pathology of pain. Several specific compounds targeting Nav1.7, TRPV1, Cav3.2, Kv7 channels failed in clinical trials due to off-target, deleterious side effects or due to improper selection of people tested in the efficacy trials [10, 16, 17]. Two-pore domain potassium (K2P) channels are a diverse family of K^+ selective ion channels. Similar to other voltage-gated ion channels, K2P channels transduce nociceptive signals provided by noxious stimuli and inflammatory mediators into changes in neuronal excitability, as their gating can be regulated by G protein-coupled receptors [9, 18]. Thus, K2P channels play a crucial role in pain signal transduction in peripheral nervous system, and are potential targets for developing new analgesics [19].

K2P channels generate background potassium currents and underlie the main resting potassium conductance that is responsible for the resting membrane potential (RMP) of cells, exerting tight control over cellular excitability. K2P channels, widely expressed in both neurons and non- neuronal cells, are involved in a wide range of physiological functions, including neuroprotection [20], anesthesia [20-

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25], sleep [22, 26, 27], nociception [19, 24, 28], chemosensing [29-31], and insulin secretion [32-34]. In the peripheral nervous system, several K2P channels are highly expressed in dorsal root ganglion (DRG) and trigeminal ganglion (TG) [35-38] and play a crucial role in acute or chronic nociceptive processing by hyperpolarizing the RMP or counteracting the AP of nociceptive neurons [39]. Their mRNA and protein levels are also altered during inflammatory and neuropathic pain conditions [35, 36, 40-47]. After K2P gene knockout or knockdown in rodents, animals showed increased sensitivity to thermal, and mechanical stimuli compared to wild type animals [40, 41, 47-52]. Thus, selective agonists targeting these K2P channels may be an attractive strategy for the development of novel analgesics.

In this review, we examine the emerging mechanisms for K2P channels involved in pain, summarize the currently available selective activators, and assess the potential of these activators as promising lead compounds for the development of innovative analgesics.

2. PROPERTIES OF K2P CHANNELS

K2P channels are encoded by 15 KCNK genes and are divided into six subgroups [53] {tandem of pore domains in a weak inward rectifying K^+ (TWIK) [54]; TWIK-related alkali-activated sensitive K^+ (TALK) [55]; TWIK-related K^+ (TREK) [56]; TWIK-related acid-sensitive K^+ (TASK) [57]; tandem pore domain halothane inhibited K^+ (THIK) [58]; and TWIK-related spinal cord K^+ (TRESK) [59]} based on genetic sequence, structure, and function similarity [53]. K2P channels share some sequence homology varying from 28% to over 78% [56, 60, 61]. Each K2P protein contains four transmembrane segments (M1-M4) and two-pore domains (P1 and P2), with an extended M1-P1 extracellular loop and cytosolic N- and C-termini. For all K2P channels, selectivity filter has the particularly conserved sequence T-X-G-X-G.

Although K2P channels lack a traditional voltage-sensing domain, most K2P channels exhibit strong voltage-dependent gating due to an ionic check valve located at the selectivity filter [62]. In most K2P channels, the steady-state current-voltage relationship under physiological conditions (with 4–5 mM extracellular K^+) shows marked outward rectification [56, 60, 63]. Their regulation is mediated by diverse physical and metabolic inputs, such as mechanical stimulation, temperature, intra- or extra-cellular pH, interaction with lipids or other endogenous regulators, phosphorylation, and some volatile anesthetics [64].

Since the crystal structure of K2P channels was revealed [65-67], a substantial number of mechanisms for selective activators targeting K2P channels was demonstrated. Several activators (Table **1**) can enhance the opening of K2P channels via binding to the transmembrane helix, cytosolic N-, C-termini, or the selectivity filter [68-71]. There are also some common mechanisms in tuning K2P activity by these activators. For example, negatively charged activators like 5,6,7,8-tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine (BL-1249), 2,7-dichloro-9,9-dimethyl-10-[2-(1H-tetrazol-5-yl)-ethyl]-9,10-dihydro-acridine (ML67-33) and 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid (DCPIB) bind below the selectivity filter, where their negative charge promotes K^+ binding to the pore cavity, and thereby alter ion occupancy in the selectivity filter [72]. However, the biological consequences of these compounds activating K2P channels remain unknown, which is likely due to poor *in vivo* pharmacological profile or low selectivity [73, 74].

3. TREK

TREK channel subfamily comprises TREK-1, TREK-2 and TRAAK. They share some part of amino acid sequence varying from 45-78% [60]. TREK-1, TREK-2, and TRAAK can assemble as homodimers, and can form heterodimers among themselves as well as with other members of K2P subfamily (Table **2**). Several small molecules have been found to activate TREK channels (Table **1**). Some molecules interact with the transmembrane helix, with two possible activation mechanisms based on the dependency of C-termini. For example, fenamates, a class of NSAIDs, are involved in interaction with the N-termini of TREK channels [69]; ML67 and ML67-33 interact in a direct way with TREK-1, without coupling the pore from the polymodal C-termini sensor [68], while the action of BL-1249 requires the C-terminus tail of TREK-1 by binding below the selectivity filter [72]. Another cryptic modulator pocket located in the P1/M4 interface has been found to accommodate N-[(2,4-dichlorophenyl)methyl]-4-(methanesulfonamido) benzamide (ML335), N-[2-(4-chloro-2-methylphenoxy)ethyl]thiophene-2-carboxamide (ML402) [75], and (1S,3R)-3-((4-(6-methylbenzo[d]thiazol-2-yl)phenyl)carbamoyl)cyclopentane-1-carboxylic acid (C3001a) [76]. The mechanisms of caffeic acid derivatives [77] and 4,4′ -(hexafluoroisopropylidene) bis (p-phenyleneoxy) dianiline (GI-530159) [78] activating TREK channels remain unclear.

TREK channels are highly expressed in the nervous system including brain, spinal cord, TG and DRG. In the peripheral nervous system, they are expressed in both small- and medium-sized neurons. TREK-1 channels are co-localized with TRPV-1, substance P and isolectin-B4 (IB4) positive fibers [40]; and TREK-2 channels are mainly associated with $IB4^+$ fibers [37]. It has been found that the protein or mRNA levels of TREK channels were altered during the generation of pathological pain. In the model of chemotherapy-induced neuropathic pain, it was shown that the mRNA expression of TREK channels was downregulated in mice [41]. In acute pancreatitis, the mRNA levels of TREK-1 and TREK-2 in DRG neurons were also downregulated [76]. In other pain models, including chronic constriction injury [46, 47], spinal nerve axotomy [37] or complete Freund's adjuvant induced inflammatory pain [35, 40], changes in the mRNA levels of TREK channels were contradictory. The underlying mechanisms for the altered protein or mRNA expression remain to be investigated in pathological pain.

Behavioral experiments suggest that TREK knockout mice were more sensitive to mechanical stimuli [40, 44, 48]. TREK-2 controlled thermosensation of moderate temperatures between 40℃ and 46℃ for heat and between 20℃ and 25℃ for cold [44]; meanwhile, TREK-1 and TRAAK channels, individually or in combination, controlled the perception of aversive temperatures, above 46℃ and below 17℃

Table 1. Summary of the small-molecule activators of K2P channels.

Name	Target	Selectivity	*In vivo*	References
ML335, ML402	TREK	Activate TREK-1 TREK-2, not TRAAK	ND	[75]
ML67, ML67-33	TREK	Activate TREK-1, TREK-2, TRAAK	ND	[68]
BL-1249	TREK	Activates TREK-1 and TREK-2, higher potency than TRAAK	ND	[72, 73, 110, 111]
GI-530159	TREK	Activates TREK-1/2, not TRAAK	ND	[78]
Caffeic acid derivatives	TREK-1	ND	Analgesic effect *in vivo*	[77, 112, 113]
2-APB	TREK-2	Activates TREK-2 with higher potency	ND	[114-117]
11-deoxy prostaglandin F2α	TREK-2	Activates TREK-2, inhibits TREK-1	ND	[118]
DCPIB	TREK	Activates TREK-1, TREK-2	ND	[119]
C3001a	TREK	Activates TREK-1, TREK-2, higher potency than TRAAK	Analgesic effect *in vivo*	[76]
RNE28	TREK-1	Activates TREK-1, higher potency than TREK-2, TRAAK	Analgesic effect *in vivo*	[71]
NPBA	TASK-3	Activates TASK-3, not TASK-1	ND	[120]
CHET3 and derivates	TASK	Activate TASK-3, TASK-1	Analgesic effect *in vivo*	[70]
(+)-cis-Dioxolane iodide	TRESK	Activates TRESK	ND	[90]
OXA-22 Iodide	TRESK	Activates TRESK	ND	[90]
Oxotremorine methiodide	TRESK	Activates TRESK	ND	[90]
Acetyl-beta-methylcholine chloride	TRESK	Activates TRESK	ND	[90]
Carbachol	TRESK	Activates TRESK	ND	[90]
Arecoline hydrobromide	TRESK	Activates TRESK	ND	[90]
Spiperone hydrochloride	TRESK	Activates TRESK	ND	[90]
Phorbol 12-myristate 13-acetate	TRESK	Activates TRESK	ND	[90]

Abbreviations: ML335, N-[(2,4-dichlorophenyl) methyl]-4-(methanesulfonamido) benzamide; ML402, N-[2-(4-chloro-2-methylphenoxy) ethyl]thiophene-2-carboxamide; ML67-33, 2,7-dichloro-9,9-dimethyl-10-[2-(1H-tetrazol-5-yl)-ethyl]-9,10-dihydro-acridine; BL-1249, 5,6,7,8-tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine; GI-530159, 4,4 ' -(hexafluoroisopropylidene) bis (p-phenyleneoxy) dianiline; 2-APB, 2-aminoethoxydiphenyl borate; DCPIB, 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid; C3001a, (1S,3R)-3-((4-(6-methylbenzo[d]thiazol-2-yl)phenyl)carbamoyl)cyclopentane-1-carboxylic acid; RNE28, (E)-2-cyano-3-(furan-3-yl) acrylic acid; NPBA, N-(2-((4-nitro-2-(trifluoromethyl)phenyl)amino)ethyl)benzamide; CHET3, (E)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-(4-hydroxy-6-((p-tolylthio)methyl) pyrimidin-2-yl) guanidine; ND, not determined.

[48]. These results show that TREK-1, TREK-2, and TRAAK channels appear to have complementary roles in thermosensation. *In vitro*, C-fibers from TREK knockout mice showed increased spike firing [40, 44, 48]. Also, TREK-2 knockdown could depolarize the RMP of neurons by 10 mV [37]. These electrophysiological results were consistent with the behavioral phenotypes, indicating that TREK channels contributed to modulate the excitability of nociceptive neurons. In chronic inflammatory pain model, knockout animals displayed an increased heat hypersensitivity compared with wild type [40, 48]. In neuropathic pain, knockout mice showed increased cold sensitivity in the model of

Table 2. Summary of the heterodimers of K2P channels.

Heterodimers	Validation	Physiological Function	References
TREK-1/TREK-2	Co-IP	Involved in the mechanism of pain	[40, 48, 76, 121] [61, 122]
TREK-1/TRAAK	SiMPull; TIRF imaging; FRET; PLA		
TREK-2/TRAAK			
TREK-1/TRESK TREK-2/TRESK	SiMPull	Involved in the mechanism of MA	[80]
TREK-1/TWIK-1	Co-IP; BiFC; MY2H; PLA	Mediate passive conductance and fast glutamate release in cortical astrocytes	[123, 124]
TREK-2/TWIK-1	Co-IP	ND	
TRAAK/TWIK-1	Co-IP	ND	
TASK-1/TASK-3	Co-IP	Mediate the pH and isoflurane-sensitive K^+ currents in hypoglossal motoneurons	[125, 126]
TASK-3/TWIK-1	FRET; Co-IP	Comprise the acid-sensitive K^+ currents and respond to halothane in cerebellar granule cells.	[127]
TASK-1/TWIK-1			
TASK-1/TALK-2	BiFC; FRET; Co-IP	ND	[128]
THIK-1/THIK-2	FRET; PLA	ND	[129]

Abbreviations: Co-IP, co-immunoprecipitation; SiMPull, single-molecule pull-down; TIRF, total internal reflection fluorescence; FRET, fluorescence resonance energy transfer; PLA, proximity ligation assay; BiFC, bimolecular fluorescence complementation; MY2H, membrane yeast two-hybrid; ND, not determined.

chronic constriction injury and oxaliplatin-induced neuropathy [44, 48]. In the nodes of Ranvier of trigeminal myelinated afferent nerves, TREK-1 and TRAAK were involved in rapid AP conduction, and knockdown of TREK-1 and TRAAK channels resulted in a significant reduction of tactile responses in rats [79]. Royal *et al.* found that TREK-1 and TREK-2 were relevant to migraine associated with TRESK; and double knockout mice for TREK-1 and TREK-2 presented a migraine-like allodynia phenotype [80]. Particularly, TREK-1 channel, acting downstream from the μ opioid receptor, was a crucial contributor of morphine-induced analgesia in mice, while it was not involved in morphine-induced constipation, respiratory inhibition, and dependence [18, 71]. The evidence suggests that TREK channels play a crucial role in nociception and pathological pain.

Consistent with studies using knockout animals, agonists targeting TREK channels exhibit analgesic effects. Qiu *et al.* [76] identified a small molecule, (1S,3R)-3-((4-(6-methylbenzo[d]thiazol-2-yl) phenyl) carbamoyl) cyclopentane-1-carboxylic acid (C3001a), as a selective TREK-1/2 activator against other K2P channels. C3001a, binding to the P1-M4 cryptic pocket with the residues Tyr285, Phe149, Thr156, Phe160, Trp290, and Ile293, was effective at largely increasing K^+ currents about 4.5-fold at 10 μM carried by TREK-1, TREK-2, with a relatively lower efficacy (~ 2.4-fold at 10 μM) on TRAAK. *In vitro*, C3001a was found to increase K^+ conductance, hyperpolarize the RMP, and reduce the excitability in a subset of nociceptive DRG neurons. *In vivo*, the pharmacokinetic profile showed that blood brain barrier was weakly permeable to C3001a. In acute and chronic pain models, C3001a was effective in attenuating thermo hyperalgesia and mechanical allodynia. Notably, C3001a had positive effects on relieving pancreatic inflammation in the model of acute pancreatitis. Recently, Busserolles *et al.* [71] found that (E)-2-cyano-3-(furan-3-yl) acrylic acid (RNE28), binding to the N-terminus of TREK-1, could activate TREK-1 with an E_{max} of 4.46-fold at 100 μM. *In vivo*, RNE28 was found to be effective in reducing inflammatory pain and neuropathic pain. Importantly, administration of RNE28 did not induce constipation, respiratory depression, sedation, or rewarding effects.

Loucif *et al.* [78] reported that 4,4′ -(hexafluoroisopropylidene) bis (p-phenyleneoxy) dianiline (GI-530159), a selective activator of TREK-1 and TREK-2 with EC_{50} of 0.9 μM in HEK293 cells, could reduce the firing frequency of DRG neurons by activating TREK channels, and lead to a small hyperpolarization of the RMP at 1 μM. *In vivo*, Vivier *et al.* [77] found that an analogue of caffeic acid derivatives was effective at inhibiting acetic acid-induced writhing. However, the potential of these activators in relieving pathological pain *in vivo* remains to be further evaluated.

In conclusion, TREK-1, TREK-2, and TRAAK channels, as important modalities for sensory neurons to detect and regulate nociceptive signals and pathological pain are important analgesic targets. For these activators of TREK family, more efforts should be focused on *in vivo* evaluations. Meanwhile, TREK channels are also expressed in other tissues, such as heart, lungs, and gut [56, 81, 82]. Particularly, TREK-1 channel plays a potential role in repolarization, conduction of cardiomyocytes [83, 84], and modulating cardiac morphology and function [85]. The possible effects on cardiac function should be assessed when TREK-1 activator is used as analgesic. On the other hand, whether TREK channels in the central nervous system (CNS) play a role in

pain remains underexplored. Chronic pain is usually associated with mood disorders, such as anxiety and depression [86]. In the brain, inhibition of TREK-1 channel was found to be anti-depressive [87, 88], while in the peripheral nervous system, activation of TREK channels was proved to be analgesic. It would be very interesting to assess if and how TREK channels affect the comorbidity of chronic pain and mood disorders in CNS. These unsolved questions may provide guiding insights for precisely modulating the function of TREK channels in relieving pain.

4. TRESK

TRESK gene shows less than 34% amino acid sequence similarity to other K2P channels [59]. TRESK channel is inhibited by arachidonic acid [59] but directly activated by calcineurin after $G_{\alpha}q$ receptor stimulation and a subsequent rise in intracellular calcium [89]. There are few pharmacological tools modulating TRESK channel. With high-throughput screens, Bruner *et al* [90] yielded 8 activators of TRESK with low efficacy (Table **1**). Wright *et al.* found that the anti-amoebic drug, cloxyquin, was effective in activating TRESK with EC_{50} of 3.2 μM in U2OS cell line [91]. However, the activation mechanism and the selectivity against other K2P channels for these activators remain unknown.

TRESK channel was first found to be expressed in the spinal cord [59]. Further studies found that TRESK was expressed abundantly in sensory neurons of DRG and TG [92, 93], especially in small and medium diameter sensory neurons [94]. TRESK mediates major background potassium currents in primary afferent neurons and regulates neuronal excitability in both normal and pathological states [51, 52, 94]. In various models of chronic pain, the expression of TRESK was downregulated, which might be associated with decreased calcineurin. In the pain model associated with bone metastasis, tumor-associated production of vascular endothelial growth factor (VEGF) activated the receptor VEGFR2 on DRG neurons, which increased the calcineurin inhibitor Down syndrome candidate region 1 (DSCR1) in abundance. Moreover, DSCR1 decreased calcineurin-mediated activation of the transcription factor, namely nuclear factor of activated T cells, thereby reducing the transcription of the gene encoding TRESK [95].

In vivo, TRESK knockout mice exhibit increased thermal sensitivity [25, 51] and mechanical thresholds [52]. Isobutylalkylamide, an inhibitor of TRESK, could evoke flinching and licking behaviors in rats [42]. In DRG, knocking down TRESK increased neuronal excitability (indicated by depolarized RMP, reduced rheobase current, and increased AP numbers), mechanical hypersensitivity, thermal hyperalgesia, and spontaneous pain in normal rats [95]. In pathological states, overexpressing TRESK in DRG neurons inhibited neuronal hyperexcitability and relieved nociceptive behaviors [95].

In TG, TRESK, has been directly linked to migraine with aura *via* a causally linked 2-bp frameshift mutation (F139WfsX24), which caused premature truncation of TRESK ("TRESK-MT"). This mutation was shown to produce a non-functional protein, and TRESK-MT had been shown to induce hyperexcitability of TG neurons [93]. A recent research found that TRESK was able to form heterodimers with TREK-1 and TREK-2. TRESK-MT can specifically co-assemble with TREK-1 and TREK-2 to downregulate their function, strongly inhibiting TRESK, TREK-1, and TREK-2 currents, leading to the enhancement of TG neurons excitability [80].

Thus, mechanistic studies above strongly suggest TRESK as a promising analgesic target. However, specific activators for TRESK have been lacking.

5. TASK

TASK subfamily consists of TASK-1, TASK-3, and TASK-5. TASK-5 was non-functional in recombinant system [96]. TASK-1 and TASK-3 are closely related to each other with 62.3% overall identity [63]. They are sensitive to extracellular pH [57, 63] and are also modulated by G protein-coupled receptors [97]. TASK-1 and TASK-3 can function as homo or heteromeric channels [98].

In DRG small-sized neurons, TASK-3 was expressed in TRPM-8, TRPV-1, or tyrosine hydroxylase positive nociceptive neurons, and about 95% of TASK-3^{+} neurons express TASK-1 subunit, suggesting the possible formation of TASK-3/TASK-1 heterodimers in neurons [70, 99]. In pathological states, the expression of TASK-1 and TASK-3 was altered but not consistently among different pain models. In neuropathic pain induced by spared nerve injury, spinal nerve ligation and inflammatory pain induced by complete Freund's adjuvant, TASK-3 was downregulated [35, 36, 70, 100], while injection of 1% formalin increased the protein levels of TASK-3 [100].

TASK-3 or TASK-1 knockout mice showed hypersensitivity to cold and heat [70, 101]. In chronic pain models, TASK-3 knockout mice displayed aggravated mechanical allodynia, spontaneous pain, and thermal hyperalgesia in neuropathic and inflammatory pain [70]. Recently, Liao *et al.* [70] discovered (E)-1-(2,3-dihydrobenzo[b] [1,4] dioxin-6-yl)-2-(4-hydroxy-6-((p-tolylthio) methyl) pyrimidin-2-yl) guanidine (CHET3) as a highly selective activator for TASK-3 with EC_{50} of 1.4 μM. *In vitro*, CHET3 was effective at modulating the excitability of TASK-3 containing DRG neurons demonstrated by using electrophysiology and calcium imaging. *In vivo*, CHET3 prolonged the response time of tail flicking in noxious cold (5℃) and heat (46℃ and 52℃) stimuli. In inflammatory and neuropathic pain, CHET3 could alleviate mechanical allodynia and thermo hyperalgesia. Especially, CHET3 was more effective on cold hyperalgesia than pregabalin, which is a first-line agent for the treatment of neuropathic pain. Notably, Liao *et al.* systematically evaluated possible side effects after activation of TASK-3 and found that CHET3 had no effects on locomotion activities, body temperature, respiratory and cardiovascular functions. This work firmly established TASK-3 as a promising analgesic target. Terbinafine, an anti-fungal drug, was identified as a TASK-3 activator [102]. García *et al.* [100] found that terbinafine, by intrathecal injection, reduced allodynia in formalin-induced inflammatory pain and spinal nerve ligation induced neuropathic pain in rats.

Liao *et al.* [70] found a higher expression of TASK-3 in TG (~14%). It may be worth evaluating TASK-3 channels in TG-associated pathological pain in trigeminal neuropathy

and migraine. In addition, in the CNS, TASK-3 had been reported to be associated with depression [103] and sleep disorders [26, 27]. The biological mechanisms of TASK-3 in these CNS diseases need to be further explored using the newly discovered selective small molecules.

6. THIK

THIK-1 was first described in conjunction with its closely related partner, THIK-2 [58]. THIK-1 was activated by high concentration of arachidonic acid and inhibited by halothane [58].

In the nervous system, THIK-1 was found to expressed in microglia and neurons [38, 58, 104, 105]. In DRG, all small neurons and large groups of medium and large DRG neurons express THIK-1; and THIK-1 is expressed in $IB4^+$ and tyrosine kinase receptor A positive nociceptive neurons [38]. In inflammatory pain induced by complete Freund's adjuvant, Haskins *et al.* [38] found that the expression of THIK-1 was decreased, and siRNA-mediated knockdown of THIK-1 in DRG increased the duration and number of spontaneous feet lifting in rats. Recently, Madry *et al.* [105] reported that THIK-1 was functionally expressed in microglia. THIK-1 was involved in maintenance of the RMP; and pharmacologically blocking THIK-1 by using non-selective blockers tetrapentylammonium and quinine or genetic deletion of THIK-1 reduced microglial ramification, surveillance, and IL-1β release in hippocampus and cortex. Microglia-mediated inflammatory response is critically involved in chronic pain [106]. The involvement of microglia THIK-1 in pain remains to be investigated.

7. OTHER K2P CHANNELS: TWIK-1

TWIK-1 was the first identified member of K2P channel family [54]. Due to non-measurable (or very small) TWIK-1 current both in recombinant cell lines and *in situ*, there are only a few studies evaluating the physiological function of TWIK-1.

For the role of TWIK-1 in the pain process, it was only reported that TWIK-1 was expressed in large- and medium-sized neurons of DRG, without overlap with TRPV-1 or $IB4^+$ staining. The expression of TWIK-1 was strongly decreased 1, 2 and 4 weeks after neuropathic injury [36]. The physiology of TWIK-1 in the pain process remains largely unexplored.

CONCLUSION AND FUTURE DIRECTIONS

To sum up, recent studies in rodents strongly suggest K2P channels as promising targets for relieving pain. We highlight the activation of TREK-1 devoid of morphine-induced side effects, the activation of TASK-3 in neuropathic pain and the activation of TRESK in MA as promising directions to be pursued. However, there remain several open questions regarding the mechanisms of K2P channels involved in the pain: 1. What is the molecular basis for the regulation of mRNA or protein level of K2P during the pain process? 2. Are these K2P channels engaged in the central sensitization of the spinal cord or the brain in chronic pain? It also should be noted that glial cells, including microglia and astrocytes, play critical roles in pain [106] and express some K2P channels [105, 107]. It remains largely unknown whether K2P channels in glia modulate pain. 3. As reported, K2P channels are involved in depression and anxiety [70, 87, 103]. Meanwhile, chronic pain can enhance negative emotional states [108, 109]. Whether K2P channels are involved in the mechanisms of pain and comorbidities is not completely understood. 4. K2P channels are interactive in the pain process. Are non-specific activators of K2P channels more effective to alleviate pain? 5. K2P channels are also expressed in non-nervous tissue, such as the cardiovascular system, gastrointestinal system and so on. Systematic evaluation of the possible side effects remains a translational challenge. The answers to these questions would be paramount to the further development of K2P-based innovative pain pharmacotherapy.

LIST OF ABBREVIATIONS

AP	=	Action Potential
CNS	=	Central Nervous System
DRG	=	Dorsal Root Ganglion
IB4	=	Isolectin-B4
K2P	=	Two-pore Domain Potassium
NSAIDs	=	Non-steroidal Anti-inflammatory Drugs
RMP	=	Resting Membrane Potential
TALK	=	TWIK-related Alkali-activated Sensitive K^+
TASK	=	TWIK-Related Acid-Sensitive K^+
TG	=	Trigeminal Ganglion
THIK	=	Tandem Pore Domain Halothane Inhibited K^+
TREK	=	TWIK-related K^+
TRESK	=	TWIK-related Spinal Cord K^+
TRP	=	Transient Receptor Potential
TWIK	=	Tandem of Pore Domains in a Weak Inward Rectifying K^+

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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