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The Polysite Pharmacology of TREK K_{2P} Channels

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Abstract

K_{2P} (KCNK) potassium channels form “back-ground” or “leak” currents that have critical roles in cell excitability control in the brain, cardiovascular system, and somatosensory neurons. Similar to many ion channel families, studies of K_{2P} s have been limited by poor pharmacology. Of six K_{2P} subfamilies, the thermo- and mechanosensitive TREK subfamily comprising $K_{2P}2.1$ (TREK-1), $K_{2P}4.1$ (TRAAK), and $K_{2P}10.1$ (TREK-2) are the first to have structures determined for each subfamily member. These structural studies have revealed key architectural features that underlie K_{2P} function and have uncovered sites residing at every level of the channel structure with respect to the membrane where small molecules or lipids can control channel function. This polysite pharmacology within a relatively small (~70 kDa) ion channel comprises four structurally defined modulator binding sites that occur above (Keystone inhibitor site), at the level of (K_{2P} modulator pocket), and below (Fenestration and Modulatory lipid sites) the C-type selectivity filter gate that is at the heart of K_{2P} function. Uncovering this rich structural landscape provides the framework for understanding and developing subtype-selective modulators to probe K_{2P} function that may provide leads for drugs for anesthesia, pain, arrhythmia, ischemia, and migraine.

Keywords

K_{2P} channel; TREK subfamily; Ruthenium red; ML335/ML402; Fluoxetine; PIP_2

4.1 Introduction

Ion channel proteins facilitate the flow of bioelectricity that underlies the physiology of thought, movement, mood, and sensation [1]. The K_{2P} (KCNK) potassium channel family comprises a set of 15 members (Fig. 4.1a) of the voltage-gated ion channel (VGIC) superfamily [2] that have central roles in controlling cell excitability by producing “leak” potassium currents that are largely time and voltage-independent [3–5]. The 15 K_{2P} subtypes

comprise six subfamilies (TREK, TWIK, TASK, TALK, THIK, and TRESK) that within each subfamily are related by sequence similarities and regulation by shared types of signals [6] (Fig. 4.1a). A diverse range of stimuli affect K_{2P} s depending on the subfamily type and include physical gating commands such as pressure and temperature (TREK) [4, 7], external and internal pH (TREK, TASK, TALK, and TWIK) [4, 8], chemicals such as volatile anesthetics (TREK, TASK, THIK, TRESK) and antidepressants (TREK) [4, 9], lipids and polyunsaturated fatty acids (PUFAs) (TREK, THIK) [4, 9], and protein–protein interactions with partners such as 14–3–3, G-proteins, Protein Kinase A, and Protein Kinase C (TREK, TASK, TALK, TWIK, TRESK) [4, 9].

K_{2P} s are named for their unique architecture. Each subunit bears two pore-forming domains, PD1 and PD2, each comprising two transmembrane helices (M1–M2 and M3–M4) bridged by a pore helix (P1 and P2) and selectivity filter (SF1 and SF2) (Fig. 4.1b). K_{2P} subunits dimerize to create a channel in which the pore is intrinsically heterotetrameric due to sequence differences between PD1 and PD2. Structures have been determined for exemplars of five of the fifteen K_{2P} s subtypes (Fig. 4.1a), $K_{2P1.1}$ (TWIK-1), [10] $K_{2P2.1}$ (TREK-1), [11] $K_{2P3.1}$ (TASK-1), [12] $K_{2P4.1}$ (TRAAK), [13] and $K_{2P10.1}$ (TREK-2) [14] (Fig. 4.1c, d). These structures reveal a common protein scaffold that defines the K_{2P} family.

Similar to other VGICs [15, 16], the two transmembrane segments of the pore domains form outer (M1 and M3) and inner (M2 and M4) helices that define the pore and support the pore helices and selectivity filter [10–14]. The pore helices and selectivity filter coordinate a set of four potassium ions on the channel central axis. K_{2P} s have a unique structural feature, the CAP domain (Fig. 4.1b, c). This extracellular structural element forms an arch directly over the channel pore and creates the bifurcated extracellular ion pathway (EIP) from which the ions exit the channel after passing through the selectivity filter [10, 13]. The M1 helix is domain-swapped between the two subunits, but how this structural intertwining of the subunits impacts function, assembly, or biogenesis is not clear. Each subunit also bears sequences at both the N- and C-termini that are likely to be unstructured on their own but that provide sites for protein–protein interactions that impact function [4, 9].

Unlike other potassium channels, the K_{2P} principal gate is the selectivity filter “C-type” gate [11, 17–22]. In line with this mechanism of control, the structures of $K_{2P1.1}$ (TWIK-1), [10] $K_{2P2.1}$ (TREK-1) [11], $K_{2P4.1}$ (TRAAK), [13] and $K_{2P10.1}$ (TREK-2) [14] show that these channels lack the inner gate that is present in most other VGIC superfamily members (Fig. 4.1d). The K_{2P} structures have shown that the M4 helix is mobile and can adopt conformations that range between an “up” state and a “down” state [13, 14, 23–25]. The “down” state creates a fenestration just below the P2 pore helix that is open to the center of the membrane bilayer [10, 13, 24, 25]. These M4 conformational changes are linked to C-type gate control [17, 18, 25–27] but do not impede access from the intracellular side [26]. Intriguingly, the recent $K_{2P3.1}$ (TASK-1) [12] structure shows a “down” state in which M4 creates an intracellular barrier, termed the “X-gate” that appears to be a special feature of the TASK subfamily [12] and that highlights structural diversification of the M4 segment within the K_{2P} family.

K₂Ps have roles in a multitude of physiological responses and pathological conditions such as action potential propagation [28, 29], anesthetic responses [30, 31], microglial surveillance [32], sleep duration [33], pain, [34–36] arrhythmia [37], ischemia [30, 38, 39], cardiac fibrosis [40], depression [41], migraine [42], intraocular pressure regulation [43], pulmonary hypertension [44], acute respiratory distress syndrome [45], and cancer [46]. Despite these clear physiological roles, the pharmacology of K₂Ps is generally poor [9, 47] and has been a barrier to understanding K₂P function. The paucity of reagents to probe K₂P activity has motivated multiple efforts that have begun to define new K₂P modulators [11, 36, 48–52] and key structural aspects of K₂P channel pharmacology [11, 14, 53, 54].

K₂Ps are thought to be potential therapeutic targets for pain [47, 55, 56], anesthesia [9, 47], arrhythmia [57, 58], ischemia [59], depression [60], and migraine [9, 61]. Although there are no approved drugs that target K₂Ps specifically, recent advances in the discovery of new classes of a variety of K₂P modulators should enable elaboration of a suite of new pharmacological tools directed at this channel family [11, 12, 36, 48, 49, 51, 62–64].

4.2 The TREK Subfamily: Model Polymodal Ion Channels

The TREK subfamily comprising K_{2p}2.1 (TREK-1), K_{2p}10.1 (TREK-2), and K_{2p}4.1 (TRAAK) is both the most extensively studied K_{2p} family and the only subfamily for which structures of each subtype are known [11, 13, 14] (Fig. 4.1a). TREK channels are polymodal ion channels that respond to diverse physical and chemical gating cues including temperature, pressure, pH, and modulatory lipids [7, 65]. The sensors for these signals reside in different parts of the channel. The intracellular C-terminal tail is key to modulation by temperature [17, 18, 66–68], pressure [18, 69, 70], intracellular pH [70, 71], responses to lipids such as phosphoinositol [4, 5] bis-phosphate (PIP₂) [68, 72, 73], and control by phosphorylation [17, 74, 75]. The sensor for extracellular pH is a histidine [19, 76] located in the loop that connects the P1 helix to the CAP domain [11]. Gating cues from the extracellular pH sensor [19, 76] and the C-terminal tail [17, 18] converge on the selectivity filter C-type gate and make this channel element the nexus of signal integration and functional control [11, 17, 18, 21, 22].

TREK subfamily channels are found throughout the central and peripheral nervous system [4, 28, 29, 34, 47, 77], the eye [43], and the heart [58]. Since their discovery, TREK subfamily channels have been implicated as therapeutic targets for pain, ischemia, and depression [47, 60, 65, 78]. K_{2p}2.1 (TREK-1) and K_{2p}10.1 (TREK-2) share ~65% sequence similarity, whereas K_{2p}2.1 (TREK-1) and K_{2p}4.1 (TRAAK) share only ~40% similarity [79–81]. Most of this sequence divergence is embedded in the N- and C-terminal cytoplasmic regions. In line with the high degree of conservation in the core elements of the channel, the structures of homodimers of each of the TREK subfamily members are similar to each other [11]. The three subtypes also have been shown to heterodimerize and provide further functional diversity from this subfamily [42, 82–84]. An important consequence of heterodimerization is that it creates a channel in which the PD1 and PD2 domains are all different from each other. How these differences manifest in functional diversification remains to be defined. Understanding how the similarities and differences within the TREK

subfamily contribute to function is of critical importance for developing pharmacological tools and potential therapeutics targeted toward this complex subfamily.

Both activation and inhibition of TREK channels have proposed therapeutic benefits. The ability of TREK channels to stabilize the membrane potential and reduce excitability together with their high expression in sensory neurons gives activators of this K_{2P} subfamily the potential to function as analgesics or anesthetics [35, 47, 85] and as agents against migraine. [42] Interestingly, the original studies of $K_{2P2.1}$ (TREK-1) knockout mice indicated that inhibition of this channel might have a role in mitigating depression [41]. Pharmacologically relevant antipsychotics have also been reported to inhibit $K_{2P2.1}$ (TREK-1) [86]. Consequently, there has been an effort to explore $K_{2P2.1}$ (TREK-1) inhibitors, such as the peptide spadin [87, 88] and small-molecule norfluoxetine [60] as new directions for treating depression. Inhibiting $K_{2P2.1}$ (TREK-1) with such agents would facilitate membrane depolarization, but how such effects could result in modulation of the mental disease remains unclear and harder to understand than the links between TREK channel activation and pain suppression.

Because TREK subfamily channels are readily studied in a variety of experimental systems from potassium transport deficient yeast [48] to *Xenopus* oocytes [11, 22, 52, 54, 75, 80], to transfected mammalian cells [11, 22, 75, 80, 81, 89], to reconstitution assays using purified channels [13, 27, 51, 90], this K_{2P} subfamily has been a key model for understanding K_{2P} channel biophysics in general and is leading the way in demonstrating the potential of K_{2Ps} as pharmacological targets [47].

4.3 The Polysite Pharmacology of TREK Channels

Structural studies of TREK K_{2Ps} have revealed a strikingly rich structural landscape for functional control, especially given their modest size (~70 kDa). Binding sites for small molecules are found at every layer of the protein starting from its extracellular side through the portion that interacts with the membrane bilayer inner leaflet (Fig. 4.2). This polysite pharmacology comprises four defined binding sites for small molecules or lipids: the Keystone inhibitor site [54], the K_{2P} modulator pocket, [11] the Fenestration site [14, 53], and the Modulatory lipid site [11]. Each offers a distinct structural environment and mechanism for controlling K_{2P} function.

4.3.1 The Keystone Inhibitor Site: Block by Polynuclear Ruthenium Amines

The trinuclear oxo-bridged ruthenium amine ruthenium red (RuR) [91] is a polycation with many biological applications [92], including a ~50 year legacy of use as an inhibitor of diverse ion channels. RuR has been shown to inhibit three K_{2P} channels, two from the TREK subfamily, $K_{2P4.1}$ (TRAAK) [93, 94] and $K_{2P10.1}$ (TREK-2) [93], as well as $K_{2P9.1}$ (TASK-3) [95–97]. Functional studies showed that a negatively charged residue at the base of the K_{2P} CAP domain comprises a key RuR sensitivity determinant in the natively RuR sensitive channels $K_{2P9.1}$ (TASK-3) [95–97] and $K_{2P10.1}$ (TREK-2). Further, placing a negatively charged residue at the CAP base is sufficient for rendering a non-RuR sensitive K_{2P} responsive to RuR inhibition [54, 93]. Hence, this negative residue is both necessary and sufficient for RuR sensitivity in the context of a K_{2P} channel.

Structural studies of a RuR-sensitive $K_{2P}2.1$ (TREK-1) mutant, I110D [54], revealed that RuR inhibits K_{2P} s in a 1:1 stoichiometry matching functional studies [93, 94] and places one ruthenium amine moiety directly over the channel pore while the remainder of the RuR molecule occupies one of the two branches of the extracellular ion pathway (EIP). This “finger in the dam” mechanism provides both, and even an electrostatic and physical barrier that prevents the flow of potassium ions through the selectivity filter.

RuR interacts directly with the negatively charged residues that form the RuR-sensitivity determinant and that constitute the “Keystone inhibitor site” at the base of the CAP domain. The principal mode of binding is through a multipronged interaction made by the two acidic residues at the Keystone inhibitor site with multiple RuR elements. This sort of direct engagement of RuR by multiple acidic sidechains is likely to contribute to RuR block of other classes of RuR-sensitive channels where the binding site is thought to be rich in acidic residues such as TRP channels [98–105], the mitochondrial calcium uniporter (MCU) [106–109], CALHM calcium channels [110–112], ryanodine receptors [113, 114], and Piezo channels [115, 116]. The dinuclear ruthenium amine, Ru360 [117], an inhibitor of the mitochondrial calcium uniporter [106, 118, 119] not previously known to affect potassium channels also binds to the Keystone inhibitor site in a similar way, although due to its reduced electrostatic interactions relative to RuR, Ru360 is a weaker blocker ($IC_{50} = 0.287$ vs. $11.3 \mu\text{M}$, for RuR and Ru360, respectively) [54] (Fig. 4.2).

Once sites of modulator action are known, it is possible to use the structural information to alter the protein or the ligand to create molecules having new properties. Using a structure-based protein engineering approach, our lab-created RuR super-responder $K_{2P}2.1$ (TREK-1) mutants having IC_{50} s in the low nanomolar range by placing acidic residues at Asn147 site at the external mouth of the selectivity filter in conjunction with the I110D mutation ($IC_{50} = 12.7 \text{ nM}$) [54]. Because of the shared pore architecture among K_{2P} s, this strategy is generalizable to other K_{2P} members to create subtypes endowed with a high-affinity RuR sensitivity and could provide a means for exploring their functions. The demonstration that compounds such as RuR and Ru360 can block K_{2P} function by reaching through the EIP raises the possibility of identifying other classes of molecules that could work similarly. Two interesting directions for making subtype-selective modulators directed at the Keystone inhibitor site would be to capitalize on the renewed interest in synthesizing novel polyruthenium amine derivatives [120] or to design compounds having moieties that interact with the Keystone inhibitor site but that also make specific contacts to non-conserved features of CAP exterior. Biologics, such as nanobodies, may be particularly suited to this type of molecular recognition mode as one can envision that a long variable loop from the nanobody could reach through the EIP to block the pore while other parts of the protein recognize subtype-specific features of the CAP exterior and EIP entryway.

4.3.2 The K_{2P} Modulator Pocket: A Cryptic Small Molecule Binding Site for K_{2P} Control

The K_{2P} modulator pocket (Fig. 4.2) is unrelated to any previously known small molecule binding pocket in the VGIC superfamily and was discovered in structural studies of $K_{2P}2.1$ (TREK-1) with two novel activators, ML335 (N-[(2,4-dichlorophenyl)methyl]-4-(methanesulfonamido) benzamide) and ML402 (N-[2-(4-

chloro-2-methylphenoxy)ethyl]thiophene-2-carboxamide (Fig. 4.2) [11]. This L-shaped pocket is found in the P1–M4 interface, an intersubunit interface involved in C-type gating [17, 18]. Both compounds bind in similar ways and act as molecular wedges that stabilize the P1–M4 interface and directly activate the channel selectivity filter C-type gate [11, 22]. In the unliganded structure, the K_{2P} modulator pocket is occluded by P1–M4 interface interactions that require small movements of few residues to open, making this pocket a cryptic site that relies on conformational change similar to cryptic sites described for soluble proteins [121]. Rigidification of the P1–M4 interface is central to channel activation [11, 22]. The observation that these two compounds stabilize this intersubunit interface highlights the general importance of intersubunit interfaces as sites of channel control.

ML335 and ML402 are remarkably selective, activating $K_{2P2.1}$ (TREK-1) and $K_{2P10.1}$ (TREK-2) but not $K_{2P4.1}$ (TRAAK) [11] (Fig. 4.3). This strong subtype selectivity originates from a single lysine residue on the N-terminal end of M4 that engages in a cation– π interaction with the upper ring of each of the activators (Fig. 4.2). The equivalent residue in $K_{2P4.1}$ (TRAAK) is glutamine and exchanging $K \rightarrow Q$ in $K_{2P2.1}$ (TREK-1) and $Q \rightarrow K$ in $K_{2P4.1}$ (TRAAK) at this position is sufficient for rendering the former insensitive to ML335 and ML402 activation and the latter sensitive to activation by both compounds [11]. The importance of a single amino acid difference in an otherwise conserved small molecule binding pocket underscores the potential for exploiting local differences and structural knowledge to develop subtype-selective K_{2P} modulators.

The K_{2P} modulator pocket is unique to K_{2Ps} [11]. Currently, there are no known natural ligands for this site, but it seems unlikely that such a well-defined binding site is only recognized by two unnatural small molecules. Stabilization of the P1–M4 interface is central for integrating gating cues that arise in other parts of the protein, particularly the C-terminal tail [11, 17, 18, 22]. Because the K_{2P} modulator pocket is in the center of this interface, it seems very likely that there are natural compounds such as lipids, metabolites, signaling molecules, or regulatory proteins that target this site. Hence, understanding the normal function of this part of the channel and whether Nature has exploited natural compounds to affect K_{2P} activity through the K_{2P} modulator pocket remains an important direction for future study.

4.3.3 The Fenestration Site: A Binding Site for Activators and Inhibitors

The K_{2P} M4 transmembrane helix is a key moving element and serves as a means to convey gating cues from temperature [17, 18, 25, 26], pressure [18, 26, 27], and phosphorylation [17] to the C-type gate. Structural studies of TREK subfamily channels have defined two extreme positions of the M4 helix termed “up” and “down” [13, 14, 23–25]. The “down” state creates a fenestration just below the P2 pore helix that is open to the center of the membrane bilayer [10, 13, 24, 25], the “Fenestration site” (Fig. 4.2). Structural studies of $K_{2P10.1}$ (TREK-2) have shown that this site binds to the K_{2P} inhibitors fluoxetine and norfluoxetine [14]. These compounds bind to a site defined by the lower part of the P2 pore helix and M4 (Fig. 4.2) and require the M4 helix to adopt the “down” position.

Remarkably, crystal structures of a $K_{2P10.1}$ (TREK-2) complex with a brominated version of an activator, the fenamate BL-1249, although not defining the entire compound, strongly

indicate that this molecule and perhaps other activators bind to the Fenestration site created by the “down” M4 position [53]. How can the binding of a small molecule to the same site yield opposite functional outcomes of inhibition and activation? Clearly, the answer cannot be in the stabilization of the M4 “down” state over the “up” state as the binding of both inhibitors and activators to the Fenestration site requires an M4 “down” conformation [14, 53]. Interestingly, it is suggested that activators such as BL-1249 use their tetrazole moiety to create a binding site for potassium ions in the central cavity and thereby stabilize the selectivity filter C-type gate [53]. Given this type of mechanism, it is notable that the norfluoxetine structure places the norfluoxetine amine just below the selectivity filter where its expected positive charge could provide an unfavorable modification to the potassium ion conduction pathway that would lead to channel inhibition (Fig. 4.2). The fenestration site is commonly found in the VGIC superfamily of which K_{2P} s are members and serves as the site of action for multiple types of activators of different classes of potassium channels [53]. Understanding the relationship between the occupation of this site, effects on the selectivity filter C-type gate, and the relationship between the properties of activators and inhibitors that can inhabit this site is an important challenge for further development of K_{2P} modulators.

4.3.4 The Modulatory Lipid Site: PIP₂ and the C-Terminal Tail

PIP₂ is an important modulatory lipid for TREK subfamily channels [68, 72, 73]. The likely site of PIP₂ action has been located in a series of $K_{2P}2.1$ (TREK-1) structures [11, 22]. These show the presence of a phospholipid that co-purified with the channel and that was bound to a groove at the M1/M2/M4 interface (Fig. 4.2). The phosphoinositol headgroup contacts an electro-positive patch on the C-tail comprising five residues implicated in PIP₂ modulation (Arg297, Lys301, Lys302, Lys304, and R311) [68, 72] (Fig. 4.2). This same stretch of the C-terminal tail also contains the intracellular proton sensor site, Glu306 [71], and inhibitory phosphorylation site, Ser300 [74]. The key PIP₂-interacting residues are in a portion of the channel that is most affected by movements of M4 between the “up” and “down” positions. Hence, it seems likely that regulatory impacts of the modulatory lipid, intracellular pH sensor, and phosphorylation site on the C-terminal tail are all tightly intertwined with M4 motions [122]. Further study is needed to unravel these interactions, to understand whether other lipids reported to impact TREK channel function, such as phosphatidyl serine and phosphatidic acid [68, 123], compete with PIP₂ at this site, whether this site can be targeted by small molecules, and to define how changes in this lower part of the channel impact the dynamics and function of the C-type gate.

4.4 Subtype Specific Modulators in the TREK Subfamily

The growing progress in developing modulators for the TREK subfamily has been well-reviewed recently elsewhere [9, 47] and is, therefore, not reiterated here. As new modulators are uncovered, one key question is whether it will be possible to create subtype-specific modulators that can not only distinguish among the various K_{2P} subfamilies but also among the different subtypes within a subfamily. Such a high level of target selectivity would provide powerful tools for delineating the biological functions of these channels and could provide starting points for the development of subtype-selective pharmacology for these therapeutically relevant targets.

With respect to subtype selectivity, currently characterized TREK subfamily activators mostly fall into two categories. There are many examples of molecules that affect all three members of the TREK subfamily such as Riluzole [89, 124], BL-1249 [52], fenamates [36], ML67–33 [48], 2-Aminoethoxydiphenyl Borate (2-APB) [125, 126], and C3001a [85]. Some of these, such as BL-1249 [52] show limited selectivity for $K_{2p}2.1$ (TREK-1) and $K_{2p}10.1$ (TREK-2) over $K_{2p}4.1$ (TRAAK). The second category of compounds activate $K_{2p}2.1$ (TREK-1) and $K_{2p}10.1$ (TREK-2) but not $K_{2p}4.1$ (TRAAK) and include ML335 [11], ML402 [11], GI-530159 [127], and aristolochic acid (AristA) [128]. Given that $K_{2p}2.1$ (TREK-1) and $K_{2p}10.1$ (TREK-2) have sequences that are more similar to each other than they are to $K_{2p}4.1$ (TRAAK) (Fig. 4.1a), it is not surprising that $K_{2p}4.1$ (TRAAK) exhibits different responses to some modulators. There is a report of a $K_{2p}2.1$ (TREK-1) selective activator, Compound 36 [36], based on studies of less selective caffeic acid ester activators [36, 56, 63], but a detailed understanding of this high degree of selectivity remains to be defined. The compounds T2A8 and T2A9 are also reported to activate $K_{2p}10.1$ (TREK-1) with some selectivity [62]. The fact that there are already compounds showing some degree of selectivity provides an encouraging sign that developing activators having better subtype selectivity is a goal that can be reached.

TREK subfamily inhibitors show slightly more selectivity than activators and follow the same pattern having broadly acting inhibitors and inhibitors showing some selectivity. ML45 [48] and TKDC [129] inhibit all three TREK subfamily channels. There are a set of molecules that inhibit $K_{2p}2.1$ (TREK-1) and $K_{2p}10.1$ (TREK-2) but not $K_{2p}4.1$ (TRAAK), namely, antipsychotics [86], fluoxetine [84], and norfluoxetine [130]. A series of inhibitors (T2I1–10) unable to discriminate between $K_{2p}2.1$ (TREK-1) and $K_{2p}10.1$ (TREK-2) have also been reported [62], but whether these compounds affect $K_{2p}4.1$ (TRAAK) remains to be established. The polyruthenium blockers RuR and Ru360 show an unusual inhibition profile. RuR and Ru360 inhibit $K_{2p}10.1$ (TREK-2) but not $K_{2p}2.1$ (TREK-1) [54, 93, 94]. $K_{2p}4.1$ (TRAAK) is sensitive to RuR but the mechanism of inhibition must be different from the “finger in the dam” mechanism as $K_{2p}4.1$ (TRAAK) lacks the defining acidic residue in the Keystone inhibitor site [54]. Ru-TRAAK-1 and Ru-TRAAK-2 inhibit $K_{2p}4.1$ (TRAAK) as well as K_{2ps} from other subfamilies, such as $K_{2p}1.1$ (TWIK-1), $K_{2p}3.1$ (TASK-1), and $K_{2p}18.1$ (TRESK) [51]. The action of these compounds on other TREK subfamily members has not been reported, but given their ability to inhibit K_{2ps} outside of the TREK subfamily, it would be surprising if they did not also show some activity against the more closely related $K_{2p}2.1$ (TREK-1) or $K_{2p}10.1$ (TREK-2). The peptide spadin has been reported to act as a $K_{2p}2.1$ (TREK-1)-selective inhibitor [87, 88], but its mechanism of action remains unclear. T2A3, T2A8, T2A9, and the bioactive lipid 11-deoxyprostaglandin-F2 α form an unusual class $K_{2p}2.1$ (TREK-1) inhibitors that are reported to also act as $K_{2p}10.1$ (TREK-2) activators [62]. How such dual action occurs is not known, but has been proposed to involve a short part of the P2–M4 loop [62]. As with the activators, the growing examples of subtype-selective inhibitors indicate that developing better and more selective inhibitors of the TREK subfamily should also be feasible, especially as more structural data about how such molecules interact with K_{2ps} becomes available.

So far, the origins of subtype selectivity for TREK modulators are understood for only two sites at the level of the atomic interactions, the Keystone inhibitor site, and the K_{2p}

modulator pocket. For the Keystone inhibitor site, the negative charge at the Keystone inhibitor site is the principal determinant controlling RuR and Ru360 inhibition [54] (see Sect. 1.3.1). How RuR affects K_{2P}4.1 (TRAAK) remains unknown, as this channel lacks the negative residue at the Keystone inhibitor site and is inhibited with a stoichiometry higher than the 1:1 interaction of the Keystone inhibitor site [93, 94]. The basis of the subtype selectivity of the ML335 and ML402 activators [11] arises from a single lysine in the K_{2P} modulator pocket that controls subtype selectivity (see Sect. 1.3.2). Although not yet mapped in atomic detail, BL-1249 shows a ~10-fold selectivity for K_{2P}2.1 (TREK-1) and K_{2P}10.1 (TREK-2) over K_{2P}4.1 (TRAAK) that originates from differences in the M2/M3 helix interface [52]. Understanding the molecular origins of the subtype specificity for this compound as well as GI-530159 [127], aristolochic acid (AristA) [128], and C3001a [85] will require further studies that combines both structural and functional approaches.

4.5 Perspectives on K_{2P} Channel Polysite Pharmacology

Structural data for complexes of K_{2P}2.1 (TREK-1) and K_{2P}10.1 (TREK-2) with various modulators have uncovered a surprisingly large number of unique sites for small molecule control present at every level of the channel structure with respect to the membrane. These sites are arranged above (Keystone inhibitor site [54]), at the level of (K_{2P} modulator pocket [11]), and below (fenestration [14] and modulatory lipid [11] sites) the structure that is at the heart of channel function, the C-type gate [17, 18, 20–22]. It seems likely that there is a fifth site within the channel cavity, as there is good functional evidence that this architectural feature is targeted in TREK channels by alkylammonium pore blockers [9, 53] and this shared K_{2P} architectural element is the site of crystallographically defined small molecule block of K_{2P}3.1 (TASK-1) [12]. All of these sites have functions that are crucial for the normal functioning and modulation of K_{2P}s. The intersection of small molecule modulators and key sites of channel modulation emphasizes the importance of building an integrated understanding of modulator action and the basic mechanisms of channel function.

The structural pharmacology of the Keystone inhibitor site, K_{2P} modulator pocket, and Fenestration site has been defined by crystal structures of K_{2P}s complexed with nonnatural compounds that exert powerful effects on channel function. These findings highlight two key outstanding questions: To what extent has Nature capitalized on these control points to influence TREK channel activity? and What are the naturally occurring modulators that target these sites? Answering such questions will be important for developing a better understanding of the roles of K_{2P}s in physiological responses. One known natural modulator of great physiological interest whose site remains to be defined on the TREK subfamily is the site of action of the activator arachidonic acid [75, 131]. From a structural perspective, although K_{2P}4.1 (TRAAK) was the first member of the TREK subfamily structurally characterized [13] and received its name due to its sensitivity to arachidonic acid [131], it remains the only channel in the TREK subfamily lacking any modulator-bound structures. Given the fact that this channel stands apart with respect to the selectivity of many modulators (Fig. 4.3), defining the site of arachidonic acid action in the TREK subfamily along with obtaining structural data for small molecule:K_{2P}4.1 (TRAAK) complexes will provide important guides for unraveling natural mechanisms of channel modulation and better templates for subtype-selective modulator discovery.

The clear crosstalk between various K_{2P} moving parts and the C-type gate [17, 18, 52] raises the question of whether the action of modulators at the various sites influence each other. The strength of polyruthenium amine block at the Keystone inhibitor site is not influenced by C-type gate stabilization by compounds occupying the K_{2P} modulator pocket [54], but the extent to which there may be crosstalk among the other sites remains to be evaluated. Understanding such interactions could provide insight into how to boost the efficacy of current modulators and will refine our understanding of how signals from various parts of the channel impinge on the C-type gate.

The current structural knowledge of modulator sites provides a framework to discover a new chemical matter that can affect K_{2P} function in novel ways. Such molecules may be engineered to block or enhance the consequences of various physical and chemical stimuli or to modify the channel chemically so that its biogenesis, distribution, and interaction with other cellular components can be followed in complex cell types and tissues. Besides further crystallographic studies of new K_{2P}: modulator complexes, an obvious key advance will be to understand structural consequences of heterodimer formation [42, 82–84] and to image K_{2P}s in lipid membrane environments using cryo-electron microscopy (cryo-EM) so that interactions between the channel and bilayer can be better understood. The initial burst of activity surrounding K_{2P} structural pharmacology is the first of many waves that will fill out our understanding of this important ion channel family and should lead to new and novel therapeutic strategies for a host of brain, cardiac, and nervous system diseases.

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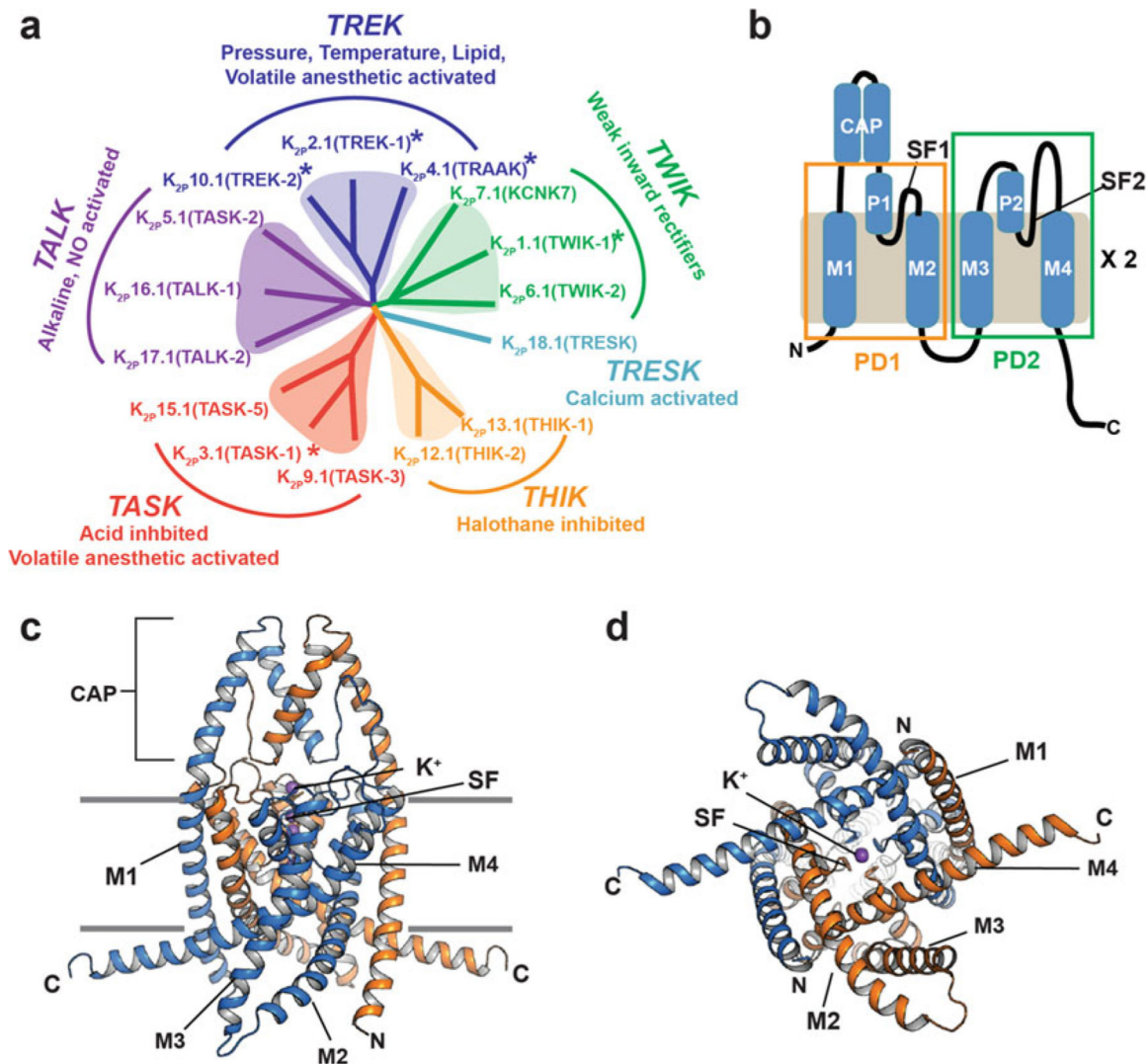


Fig. 4.1. K_{2P} channel relationships and architecture (a) K_{2P} channel dendrogram. Subfamilies are indicated. Asterisks indicate structurally characterized K_{2Ps} . (b) K_{2P} subunit diagram. Pore domains 1 and 2 (PD1 and PD2), transmembrane helices (M1–M4), pore helices (P1 and P2), selectivity filters (SF1 and SF2), and CAP domain are indicated. (c and d) Cartoon diagram of the $K_{2P2.1}$ (TREK-1) structure (PDB:6CQ6) [11]. Chains are colored marine and orange. Potassium ions are purple. (c), side view, (d), cytoplasmic view. Channel elements are labeled as in “b”

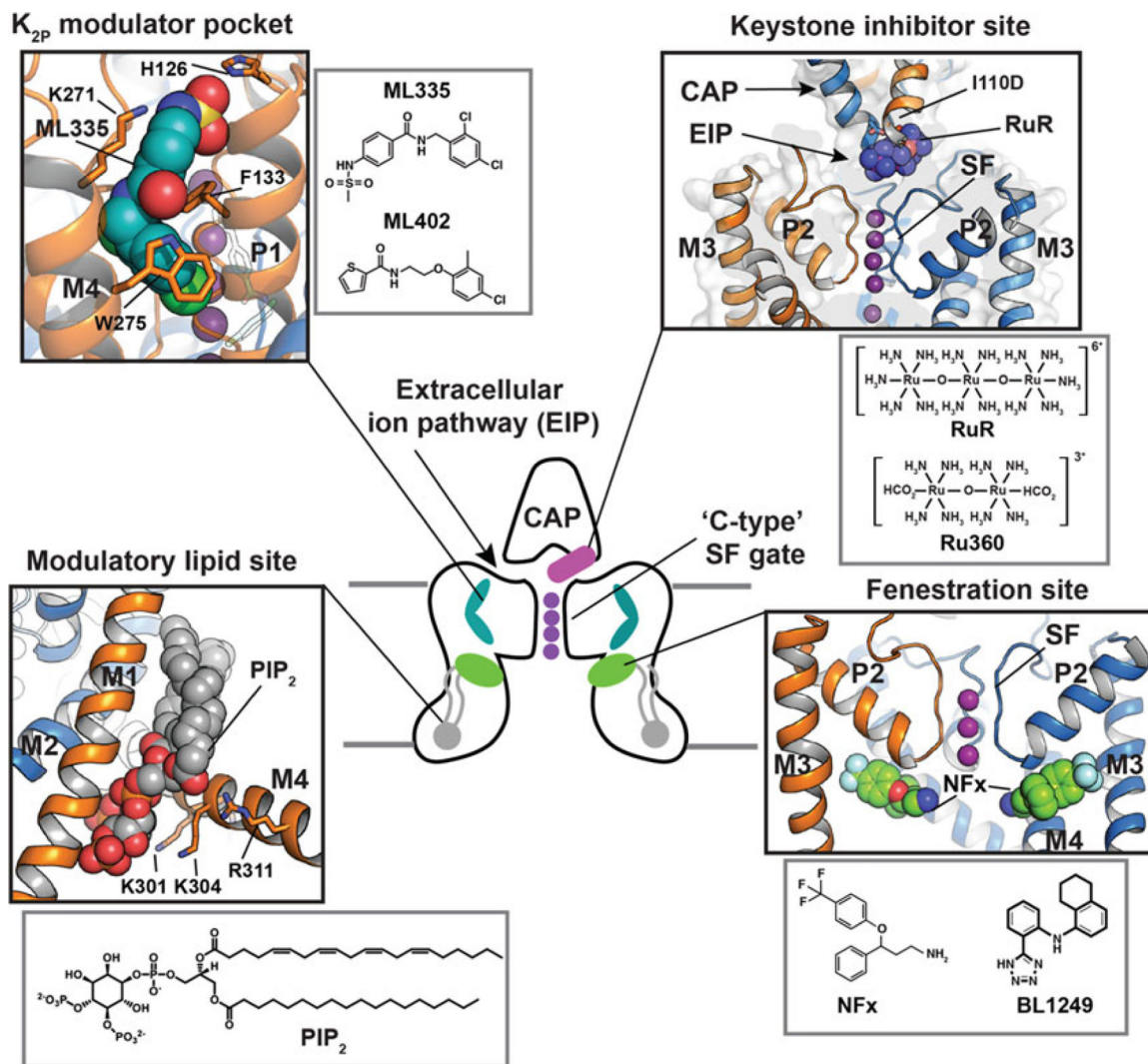


Fig. 4.2. Polysite model of TREK subfamily modulation. Central cartoon shows the locations of structurally defined K_{2P} small molecule binding sites including the Keystone inhibitor site (magenta), K_{2P} modulator pocket (cyan), fenestration site (green), and modulatory lipid site (grey). CAP and "C-type" SF gates are indicated. Potassium ions are shown (purple). Grey lines denote the lipid bilayer. Black boxes show the details of the individual sites. Grey boxes show modulator chemical structures

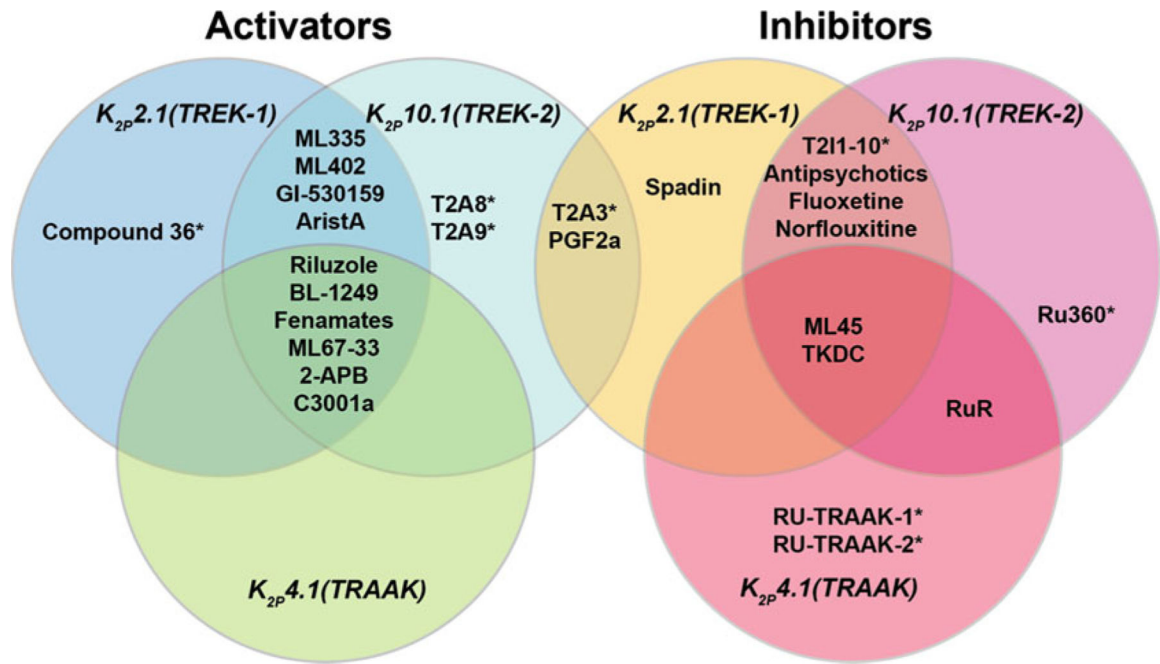


Fig. 4.3. Selectivity profiles of TREK subfamily small molecule activators and inhibitors. Asterisks indicate modulators lacking a complete profile of subtype selectivity