# The Pharmacology of Two-Pore Domain Potassium Channels

Jordie M. Kamuene, Yu Xu, and Leigh D. Plant

## Contents



**Abstract**

Two-pore domain potassium channels are formed by subunits that each contain two pore-loops moieties. Whether the channels are expressed in yeast or the human central nervous system, two subunits come together to form a single potassium selective pore. TOK1, the first two-domain channel was cloned from *Saccharomyces cerevisiae* in 1995 and soon thereafter, 15 distinct $K_{2P}$ subunits were identified in the human genome. The human $K_{2P}$ channels are stratified into six $K_{2P}$ subfamilies based on sequence as well as physiological or pharmacological similarities. Functional $K_{2P}$ channels pass background (or "leak") $K^+$ currents that shape the membrane potential and excitability of cells in a broad range of tissues. In the years since they were first described, classical functional assays, latterly coupled with state-of-the-art structural and computational studies have

J. M. Kamuene · Y. Xu · L. D. Plant (✉)
Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA
e-mail: l.plant@northeastern.edu



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revealed the mechanistic basis of $K_{2P}$ channel gating in response to specific physicochemical or pharmacological stimuli. The growing appreciation that $K_{2P}$ channels can play a pivotal role in the pathophysiology of a growing spectrum of diseases makes a compelling case for $K_{2P}$ channels as targets for drug discovery. Here, we summarize recent advances in unraveling the structure, function, and pharmacology of the $K_2P$ channels.

**Keywords**

Background current · K2P channel · KCNK · TALK · TASK · THIK · TRAAK · TREK · TRESK · TWIK

## 1 An Introduction to Two-Pore Domain Potassium Channels

Potassium ($K^+$) channels are a superfamily of multi-subunit membrane proteins that are fundamental for physiology throughout the tree of life. $K^+$ channels are complex protein machines with a simple purpose: they open and close (gate) in a coordinated manner that allows the conduction of $K^+$ ions down their electrochemical gradient, typically from the intracellular to extracellular space in mammalian tissues. Gating occurs in response to a panoply of stimuli and shapes the resting membrane potential and the dynamics of cellular excitability by regulating the flux of $K^+$ ions. Thus, $K^+$ channels are essential for many biological processes including neuronal, muscular, and cardiac function (Enyedi and Czirjak 2010).

The superfamily of $K^+$ channels is stratified into distinct subfamilies based on structural similarities, namely the number of transmembrane domains and pore forming domains present in each subunit. The largest subfamily includes the voltage ($K_V$) channels and calcium activated ($K_{Ca}$) channels which are characterized by one reentrant pore loop (P-loop) and (typically) six transmembrane domains per subunit; holo-channels are tetramers. The inwardly rectifying $K^+$ channels ($K_{IR}$) also form as tetramers in which each subunit consists of a single P-loop and two transmembrane domains. The notion that all $K^+$ channels are tetramers changed in 1995 when TOK1 was cloned from *Saccharomyces cerevisiae* (Ketchum et al. 1995). TOK1 channels have a distinct architecture: Functional channels are dimers of subunits with eight transmembrane domains (M1-M8), intracellular amino- and carboxy-terminal tails, and two reentrant P-loops located between transmembrane domains M5-M6 and M7-M8 (Fig. 1) (Ketchum et al. 1995). Although TOK channels are not found beyond fungi, $K^+$ channel subunits with two P-loops from higher organisms were described soon after.

Unlike the $K_V$ and $K_{IR}$ channel subfamilies, discovery of the $K_{2P}$ channels was made possible using genome database mining rather than by a molecular cloning strategy (Goldstein et al. 1996; Lesage et al. 1996b; Yang and Jan 2013). In 1996, K2PØ (also called KCNKØ, or dORK) was cloned from *Drosophila melanogaster* and K2P1 (also called KCNK1 or TWIK1) was cloned from human kidney (Lesage et al. 1996b). Holo two-pore domain $K^+$ ($K_{2P}$) channels are dimers of subunits, with each subunit contributing two-P loops and four transmembrane domains to the

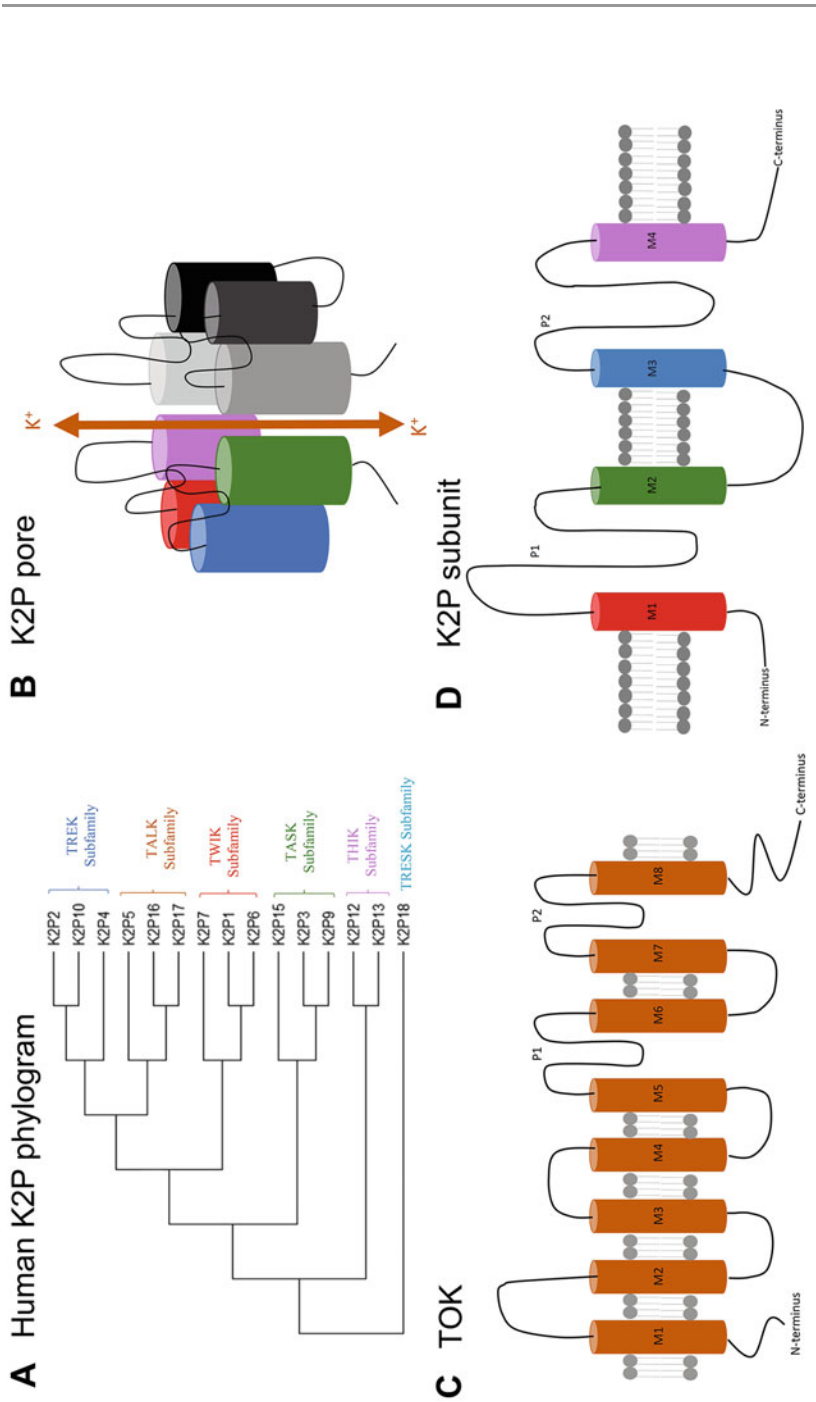


**Fig. 1** An overview of the two-pore domain potassium channels. (**a**) A phylogenic tree created using MEGA software to show the relatedness of the $K_{2P}$ subunits expressed in humans. The IUPHAR-standardized names of each subunit are in black. The phylogenic divisions of $K_{2P}$ subunits fall into the different

structure (Feliciangeli et al. 2015; Goldstein et al. 2001; Guyenet et al. 2019; Kollewe et al. 2009; Medhurst et al. 2001; Yang and Jan 2013). In general, heterologous expression of $K_{2P}$ channels produces outward $K^+$ currents under physiological conditions. The phenomenon of "background'" or "leak" $K^+$ currents has been appreciated since the 1940s and was ratified in the membrane equations of Hodgkin and Huxley (Enyedi and Czirjak 2010; Goldman 1943; Goldstein et al. 1996, 2001; Hodgkin and Huxley 1952; Lesage et al. 1996b). In the last ~25 years, numerous studies have confirmed the central role that $K_{2P}$ channels play in determining the membrane potential in a broad range of excitable and non-excitable cell types (Goldstein et al. 2001) (Fig. 1).

$K^+$ channel subunits are identified by a common sequence of amino acid residues that comprise the selectivity filter for $K^+$ ions within the conduction pore of the channel (see also chapter "Comparison of $K^+$ Channel Families"). This evolutionarily conserved structural domain is constructed from four P-loops that are held in position in the membrane between two transmembrane helixes that form the channel corpus. The surrounding architecture of the channel is comprised of transmembrane domains that correlate with their unique physiological functions. This architectural arrangement controls when the channels open and for how long (open probability), allowing the conductance of $K^+$ ions down their electrochemical gradient through the selectivity filter of the pore with high fidelity (Doyle et al. 1998). Gating of $K_{2P}$ channels is regulated by a plethora of physicochemical and mechanical stimuli including stretch, temperature, pH, and various cell signaling, and second-messenger pathways (Chemin et al. 2007; Honore 2007; Lotshaw 2007). Despite significant progress, the mechanistic basis by which each of these stimuli influences the gating machinery, and in turn the activity of $K_{2P}$ channels, remains a matter of ongoing research.

A growing body of work, first using a classical structure-function approach, and more recently via snapshots of channel structures paired with molecular dynamics simulations has revealed that extrinsic regulators typically influence the open probability of $K_{2P}$ channels via allosteric pathways and via c-type gating in particular (Bagriantsev et al. 2011, 2012; Cohen et al. 2008; Lolicato et al. 2014, 2020; Piechotta et al. 2011; Schewe et al. 2016; Zilberberg et al. 2001). Compelling evidence supports that this mode of gating results from constriction of the extracellular region of the channel, occluding the conduction pathway for $K^+$ ions (Hoshi et al. 1991; Yellen 1998). In common with data from other types of $K^+$ channel, the c-type gating of K2P channels occurs at the selectivity (SF) (Bagriantsev et al. 2011; Cohen et al. 2008; Piechotta et al. 2011). For example, binding of high affinity quaternary ammonium (QA) deep within the K2P2 channel selectivity filter revealed

**Fig. 1** (continued) subfamilies indicated, these are named for their physiological or pharmacological properties. (**b**) A cartoon depicting how the transmembrane domains and P-loops of human $K_{2P}$ channels are organized to create a single channel pore from a dimer of subunits. (**c**) A topological cartoon to show the organization of the 8-transmembrane domains (M1-M8) that comprise a TOK subunit. Note the two pore-loops (P1 and P2) between M5-M6 and M7-M8. (**d**) A topological cartoon to show the organization of the 4-transmembrane domains (M1–M4) that comprise a $K_{2P}$ subunit. Note the two pore-loops (P1 and P2) between M1-M2 and M3-M4

that the gating process was occurring at the SF (Piechotta et al. 2011; Schewe et al. 2016). Furthermore, c-type gating works in concert with the carboxy-terminal tail of the channel to mediate the response to physicochemical stimuli such as temperature and mechanical force (Bagriantsev et al. 2011, 2012), indicating that allosteric interactions can transcend the channel corpus (Bagriantsev et al. 2011, 2012; Zilberberg et al. 2001).

$K_{2P}$ channels assemble as dimers with each subunit composed of four transmembrane domains and two P-loops, one between the M1 and M2 helices and one between the M3 and M4 helices (Fig. 1b, c) (Brohawn et al. 2012; Goldstein et al. 2001; Kollewe et al. 2009; Lolicato et al. 2017; Miller and Long 2012). In addition, the first extracellular loop of each $K_{2P}$ subunit (linking the M1 to M2 helices) contributes to a "cap-domain" located above the axis of the $K^+$ selectivity filter. This structure bifurcates the pathway for $K^+$ ions and is proposed to render $K_{2P}$ channels insensitive to many classical $K^+$ channel blockers (such as protein toxins) by shielding the extracellular mouth of the pore via steric hindrance (Fig. 2) (Lolicato et al. 2017; Miller and Long 2012; Piechotta et al. 2011; Zuniga and Zuniga 2016). The extracellular cap-domain is formed when the two extracellular helices (E1 and E2) assemble (Şterbuleac 2019). The cap-domain has not been observed in other $K^+$ channels and was first revealed upon elucidation of the structure of K2P1 (TWIK1) and K2P4 (TRAAK) by X-ray crystallography. The placement and movement of the transmembrane helices allow the channel to adopt the two unique states, "up" and "down" (Brohawn et al. 2012; Miller and Long 2012; Şterbuleac 2019). Transitioning from the "up" to the "down" states reveals fenestrations which allow molecules to interact with the channel's inner pore (Feliciangeli et al. 2015; Şterbuleac 2019). The cap-domain has been observed on all K2P channel structures solved to date, including K2P1, K2P2, K2P3, K2P4, and K2P10 (Brohawn et al. 2012; Dong et al. 2015; Lolicato et al. 2017; Miller and Long 2012; Pope et al. 2020; Rödström et al. 2020).

The unique topology of $K_{2P}$ channels is shared among 15 human genes designated "*KCNK"* by the Human Gene Organization nomenclature (Lesage and Barhanin 2011; Yang and Jan 2013) (Table 1). These genes encode 15 $K_{2P}$ channel subunits that are classified into six subfamilies based on similarities in structural and functional properties: **t**andem of pore domains in a **w**eak **i**nward rectifying $\mathbf{K^+}$ channel (TWIK); **T**WIK-**re**lated $\mathbf{K^+}$ channel (TREK); **T**WIK-related **a**cid **s**ensitive $\mathbf{K^+}$ channel (TASK); **T**WIK-related **al**kaline pH-activated $\mathbf{K^+}$ channel (TALK); **T**WIK-**re**lated **s**pinal cord $\mathbf{K^+}$ channel (TRESK); and **t**andem pore domain **h**alothane-**i**nhibited $\mathbf{K^+}$ channel (THIK) (Table 1). To mitigate the variance in the pharmacological and physiological attributes that were subsequently associated with different members of each subfamily the nomenclature of the $K_{2P}$ channels "$K_{2PX}$" was designated by the International Union of Basic and Clinical Pharmacology (IUPHAR) (Table 1). However, the descriptive names of these channels have utility and remain in common use.

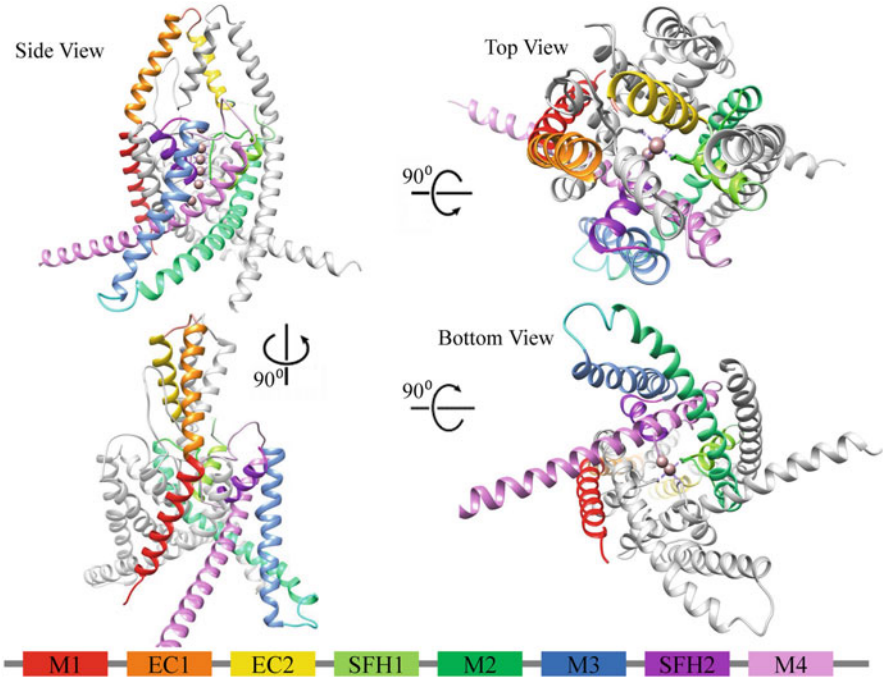


**Fig. 2** The architecture of a $K_{2P}$ channel. An overview of the three-dimensional architecture of K2P2 (Crystal structure, PDB ID: 6CQ6) showing views from the side, the top (extracellular), and the bottom (intracellular) of the channel. The helices of one subunit are colored to reflect the segments of a single subunit: four transmembrane domains (M1–M4); two portions of extracellular loop1 that contribute to the cap-domain (EC1 and EC2); two selectivity filter helices (SFH1 and SFH2), one contributes to each P-loops. Images were rendered from the PDB files indicated using UCSF Chimera software (https://www.rbvi.ucsf.edu/chimera)

## 2 The Role of $K_{2P}$ Channels in Pathology and Pain Signaling

Numerous studies have linked $K_{2P}$ channels to cardiac and neuronal diseases. In this section we highlight examples. $K_{2P}$ channels have also been linked to neurodevelopmental disorders including Birk-Barel syndrome. $K_{2P}$ channels have also been linked to neurodevelopmental disorders including Birk-Barel syndrome. This rare genetic disease is associated with mutation of the glycine residue at position 236 (Gly236) to arginine (a positively charged residue) in the KCNK9 gene (encodes K2P9, also called TASK3) and is characterized by intellectual disability, hypotonia and hyperactivity. Two-electrode voltage-clamp (TEVC) studies of WT and mutant channels expressed in *Xenopus* oocytes revealed that while wild type (WT) channels passed measurable currents, mutant channels had no measurable current. In addition, co-expression of mutant channel with either WT

**Table 1** The 15 mammalian K2P channels

| K2P subfamily | Channel name | Gene name | Common name |
|---|---|---|---|
| Tandem pore domain halothane-inhibited channel (THIK) | K2P12 | KCNK12 | THIK2 |
| | K2P13 | KCNK13 | THIK1 |
| The TWIK-related spinal cord $K^+$ channel (TRESK) | K2P18 | KCNK18 | TRESK |
| TWIK-related alkaline pH-activated $K^+$ channel (TALK) | K2P5 | KCNK5 | TASK2 |
| | K2P16 | KCNK16 | TALK1 |
| | K2P17 | KCNK17 | TALK2 |
| Tandem of pore domains in a weak inward rectifying $K^+$ channel (TWIK) | K2P1 | KCNK1 | TWIK1 |
| | K2P6 | KCNK6 | TWIK2 |
| | K2P7 | KCNK7 | kcnk8 |
| TWIK-related $K^+$ channel (TREK) | K2P2 | KCNK2 | TREK1 |
| | K2P10 | KCNK10 | TREK2 |
| | K2P4 | KCNK4 | TRAAK |
| TWIK-related acid sensitive $K^+$ channel (TASK) | K2P3 | KCNK3 | TASK1 |
| | K2P9 | KCNK9 | TASK3 |
| | K2P15 | KCNK15 | TASK5 |

The 15 unique K2P channels expressed by mammals. Abbreviations: *TWIK* **t**andem of pore domains in a **w**eak **i**nward rectifying **$K^+$** channel, *TREK* **T**WIK-**re**lated **$K^+$** channel, *TASK* **T**WIK-related **a**cid **s**ensitive **$K^+$** channel, *TALK* **T**WIK-related **al**kaline pH-activated **$K^+$** channel, *TRESK* **T**WIK-**re**lated **s**pinal cord **$K^+$** channel, *THIK* **t**andem pore domain **h**alothane-**i**nhibited **$K^+$** channel, *TRAAK* **T**WIK-**r**elated **a**rachidonic-**a**cid-stimulated **$K^+$** channel. See Two P domain potassium channels in the IUPHAR/BPS Guide to Pharmacology Database https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79 for more information about each individual channel

or K2P3 channels (which form functional heterodimers with K2P9) resulted in decreased current (Barel et al. 2008). Using the bacterial $K^+$ channel KcsA to generate a homology model structure, Barel and colleagues determined that the expected location of the Gly236 residue was in the ion conduction pathway. It was therefore postulated that a mutation to arginine may result in the disruption of physical and electrostatic interactions in the pore that would diminish current by impeding the conduction of $K^+$ ions.

KCNK18 gene encodes for K2P18 or the TRESK channel and is primarily expressed in trigeminal root ganglion (TRG) and dorsal root ganglion (DRG). Truncations and other mutations in KCNK18 have been associated with familial migraine (Lafrenière et al. 2010). Expression of mutant K2P18 channels resulted in decreased current density when expressed in oocytes. This observation led Lafrenière and colleagues to propose that an increase in the functional expression of WT K2P18 could protect against migraines and that as yet unidentified mutations in KCNK18 could lead to an increase in migraine risk (Lafrenière et al. 2010).

Following whole exome sequencing (WES) studies conducted on patients with arrhythmic disorders, Decher and colleagues identified a heterozygous K2P2 mutation (Ile267Thr) in a patient with right ventricular outflow tract ventricular tachycardia (RVOT-VT). When expressed in *Xenopus* oocytes, K2P2 Ile267Thr channels have decreased current compared to WT channels. Further, co-expression of WT and

mutant channel resulted in reduced current density in what is known as a "dominant-negative" behavior (Decher et al. 2017). It was found that the mutant channel was more permeable to sodium ($Na^+$) ions, unlike WT channels. This change in ion selectivity of the channel was attributed to the mutation of the isoleucine residue in the second pore loop to threonine. A change in the selectivity of K2P channels that permits an increase in the conductance of sodium has previously been observed for development-related alternative-translation initiated truncation variants of K2P2 and for mutation in K2P1 (Thomas et al. 2008). Following their observations, Decher and colleagues sought to reverse this defect in ion selectivity by finding drugs that would "rescue" the channel. Incubation of the channel with the following K2P2 blockers verapamil (62 μM) and fluoxetine (80 μM) and activators 2-APB (50 μM) and riluzole (500 μM) did not alter the selectivity of the channel (Decher et al. 2017). In contrast 5 μM of BL-1249 rescued channel function. Authors hypothesized that BL-1249 may be binding at a unique site that differs from the other compounds.

$K_{2P}$ channels are expressed ubiquitously across excitable and non-excitable tissues (Lesage 2003; Lesage and Lazdunski 2000). Several $K_{2P}$ channels are expressed in the TRG and DRG (Mathie and Veale 2015). The DRG and TRG somatosensory neurons give rise to the peripheral axonal fibers that innervate various tissues including the skin, muscle, and viscera and ascend to the spinal cord (DRG) or brainstem (TRG) (Mathie and Veale 2015; Plant 2012). Damage-sensing (nociceptive) somatosensory neurons detect and respond to noxious stimuli through activation of Aδ fibers which are lightly myelinated neurons that respond to localized pain, and via C-fibers which are unmyelinated neurons that are activated by a range of noxious stimuli (Plant 2012). Aα and Aβ fibers are myelinated fibers that respond to innocuous, mainly mechanical stimuli (Plant 2012). $K_{2P}$ channels expressed in the DRG and TRG modulate neuronal excitability and response to noxious and innocuous mechanical stimuli.

Using a rat neuropathic pain model, Pollema and colleagues demonstrated that following spared nerve injury (SNI) levels of mRNA for KCNK3 and KCNK9 (that encode for K2P3 and K2P9 channels, respectively), were downregulated compared to sham controls. Downregulation of these K2P channels following SNI implicates these channels in neuropathic pain phenotypes. Interestingly, four weeks post SNI, only mRNA for KCNK1 (which encodes for K2P1) remained downregulated hinting at the importance of this channel in maintaining the neuropathic pain phenotype (Pollema-Mays et al. 2013). Contrary to this study, another group found that while still using the SNI model, intrathecal delivery of K2P18 in an adenovirus vector reduced the response of rats to neuropathic pain (Zhou et al. 2013).

## 2.1 K2P Channel Pharmacology

Although multiple lines of evidence support a role for $K_{2P}$ channels in pain physiology, pharmacological options that target these proteins remain elusive. Given that present pharmacophores lack the ability to selectively inhibit $K_{2P}$ channels, development of selective pharmacological agents is therefore imperative in order to study

distinct characteristics of each channel. Intensive efforts to identify selective, potent, and efficacious pharmacophores are in progress. For example, Bagriantsev and colleagues utilized a 384 well plate yeast-based screening assay to identify $K_{2P}$ blockers and activators in a high-throughput fashion. They began by screening a library containing 106,281 small molecules for their ability to inhibit the growth of yeast expressing K2P2. From this screen the library of small molecules was narrowed to 320 compounds that were selected for their ability to inhibit 44–99% of growth (Bagriantsev et al. 2013). A dose-response screen revealed 61 compounds that successfully prevented the growth of yeast expressing K2P2. TEVC experiments conducted in *Xenopus* oocytes revealed that 2 inhibitors ML45, ML58 and 3 activators ML12, ML42, and ML67 altered K2P2 channel activity (Bagriantsev et al. 2013). Bagriantsev et al. selected the activator ML67 which caused an ~11 fold ($EC_{50}$ 213 ± 1.2 μM) increase in K2P2 channel current for further characterization. Through TEVC experiments it was found that the compound activated closely related channels (K2P10, $EC_{50}$ ~ 250 μM) but not the more distantly related K2P3 channel. Substitution of a tricyclic ring to the ML67 compound yielded the compound ML67–33 which was 5 times more potent than the other ML-67 derivatives (Bagriantsev et al. 2013). Mutations at the P1 pore helix (Gly1371) and M4 (Trp275) of K2P2 resulted in decreased channel activity. Conversely, triple glycine mutations at the C-terminal lead to channels that could be activated by the compound. As a result, the authors postulated that ML-67-33 mediates its effects on K2P2 activity by modulating the C-type gate. Compounds such as ML-67-33, a selective and potent activator of K2P2 channels, provide an approach by which similar compounds could be developed and assayed. In this chapter we provide a concise summary of the pharmacology and regulation of $K_{2P}$ channels in that they may be explored further toward the development of novel pharmacophores.

## 3 The THIK Channels: K2P12 and K2P13

The THIK subfamily is composed of THIK2 (K2P12, *KCNK12*) and THIK1 (K2P13, *KCNK13*) channels (Girard et al. 2001; Rajan et al. 2001). The mammalian K2P12 and K2P13 channels share 64% homology as well as a similar pore region structure (Renigunta et al. 2014). While K2P13 channels are expressed ubiquitously, K2P12 channels are expressed in the lungs, spleen, and brain (Rajan et al. 2001). When expressed heterologously in *Xenopus* oocytes, only K2P13 channel activity can be measured while K2P12 channel activity is largely undetectable. K2P13 currents are activated by arachidonic acid and inhibited by halothane, quinidine, and weakly by hypoxia (a ~ 13% reduction compared to control when $Po_2$ is decreased to 20 mmHg) (Table 2) (Campanucci et al. 2005; Enyedi and Czirjak 2010; Feliciangeli et al. 2015; Renigunta et al. 2014).

K2P12 channels are one of five channels: K2P1 (TWIK1), K2P6 (TWIK2), K2P7 (kcnk8), and K2P15 (TASK5) that are classified as electrically silent channels because they do not pass measurable $K^+$ current in either native cells or in

**Table 2** Modulators of THIK subfamily of K2P channels[a]

| Channel | Activators | Inhibitors |
|---|---|---|
| K2P13 (THIK1, KCNK13) | Arachidonic acid (Rajan et al. 2001) | Halothane (Rajan et al. 2001); Quinidine (Chatelain et al. 2013; Rajan et al. 2001); Hypoxia (Campanucci et al. 2005) |
| K2P12 (THIK2, KCNK12) | No known modulators | Halothane (Rajan et al. 2001) |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

heterologous expression systems (Renigunta et al. 2014). Two groups reported that lack of detectable K2P12 channel activity was a result of the channel possibly being sequestered in the endoplasmic reticulum (ER) and thus resulting in low expression of the channel at the cellular membrane (Blin et al. 2014; Chatelain et al. 2013). However, detection of K2P12 channel activity is possible under specific circumstances. Thus, it was found that substitution of a proline residue within M2 helix or deletion of 18 to 19 AA found in the N-terminus (corresponding to an ER retention/retrieval signaling motif) results in the appearance of macroscopic K2P12 activity that is comparable to K2P13 (* Chatelain et al. 2013; Renigunta et al. 2014). Removal of the AAs from the N-terminus however prevented the channel from being activated by arachidonic acid even though it could still be inhibited by both halothane and quinidine (Renigunta et al. 2014). Of great physiological relevance, heterodimerization of K2Ps 12 and 13 results in functional channels presumably because K2P13 masks the ER retention motif on the K2P12 subunit (Bayliss et al. 2019).

## 4 The TRESK Subfamily: K2P18

The TRESK subfamily contains only the K2P18 channel, encoded by *KCNK18* (Sano et al. 2003). Discovery of K2P18 in 2003 was made possible following the completion of the human genome project (Sano et al. 2003). Sano and colleagues utilized the human draft sequencing data to clone the K2P18 subunit from the complementary DNA of the spinal cord. Subsequence expression analysis found mRNA transcript for KCNK18 throughout the central and peripheral nervous systems (Bayliss et al. 2019; Enyedi et al. 2012; Enyedi and Czirjak 2015; Gada and Plant 2019; Tulleuda et al. 2011; Weir et al. 2019). In rodents, expression of K2P18 has also been detected in the spleen, thymus, and testis (Enyedi and Czirjak 2010). K2P18 channels contribute to the leak or background $K^+$ current which plays an important role in the regulation of neuronal excitability (Hwang et al. 2015). When studied using symmetrical $K^+$ solutions, K2P18 channels displayed outward rectification (Lengyel et al. 2018; Sano et al. 2003). Tulleuda and colleagues reported a decrease in channel activity following neuronal injury, which alters neuronal excitability and thus changes "pain pathways."

K2P18 shares ~19% sequence homology with other members of the K2P family (Lengyel et al. 2018; Sano et al. 2003). Despite this, human K2P18 is predicted to be structurally like the rest of the K2P channels. It however differs in that its intracellular loop found between the second and third transmembrane domains is longer (>120 amino acids (AA) compared to the 20–30 AA in the other K2P channels) and its C-terminal is shorter (30 AA long compared to the ≥120 AA in the other K2P channels) (Enyedi and Czirjak 2015; Sano et al. 2003). In contrast to most other K2P channels, K2P18 has a short C-terminal tail. This structural difference may indicate differential regulation of K2P18, including how regulatory events might allosterically influence the activity of the channel. (Braun et al. 2015).

The activity of K2P18 channels is enhanced by volatile (inhaled) anesthetics (e.g., isoflurane, sevoflurane, halothane, desflurane) but is inhibited by local anesthetics, including bupivacaine, tetracaine, ropivacaine, mepivacaine, lidocaine, as well as unsaturated fatty acids (Table 3) (Czirjak et al. 2004; Liu et al. 2004). Like most K2P channels, K2P18 channels are sensitive to differences in extracellular and intracellular pH, however the degree of sensitivity differs in the human ortholog compared to rodent orthologs (Lotshaw 2007). In contrast to other K2P channels, K2P18 is modulated by the cytosolic concentration of $Ca^{2+}$ ions. Thus, K2P18 channels are regulated by activation of $G\alpha_q$-coupled receptors, which lead to downstream release of $Ca^{2+}$ from intracellular stores (Table 3). However, a series of elegant studies by Czirják et al. showed that the direct application of $Ca^{2+}$ ions to the inside of the membrane was insufficient to stimulate K2P18 in off-cell patches, suggesting that additional cytoplasmic factors are required to activate the channels (Czirjak et al. 2004). Subsequent studies found that the $Ca^{2+}$-dependent activation of K2P18 is mediated by the calmodulin-dependent protein phosphatase, calcineurin, which interacts with the C-terminal tail of the channel (Czirjak et al. 2004). This regulatory mechanism that activates K2P18 channels can be inhibited by pharmacological inhibitors of calcineurin such as cyclosporine. In addition, mutant channels that lack the calcineurin binding site are still subject to regulation by a novel-type of protein kinase C (Pergel et al. 2019).

## 5 The TALK Subfamily: K2P5, K2P16, and K2P17

The TALK family includes the K2P5, (TASK2, *KCNK5*), K2P16 (TALK1, *KCNK16*), and K2P17 (TALK2, TASK4, *KCNK17*) channels (Decher et al. 2001; Girard et al. 2001; Reyes et al. 1998). K2P16 and K2P17 channels share 37% homology (Lotshaw 2007). When K2P5 was first cloned from human kidney it was assigned to the TASK subfamily. However, it was later reassigned to the TALK subfamily because it had more sequence similarity (~30%) to K2P16 and K2P17 and, in addition, its pH sensitivity was in the alkaline range, similar to that of K2P16 and K2P17 (Enyedi and Czirjak 2010; Lotshaw 2007; Reyes et al. 1998). In humans, K2P5 expression has been detected in the kidneys, pancreas, and liver. Transcripts for KCNK5 were also detected in DRG and spinal cord (Medhurst et al. 2001) (Enyedi and Czirjak 2010). In humans, mRNA for KCNK17 has been found in the

**Table 3** Modulators of the TRESK subfamily of K2P channels[a]

| Channel | Activators | Inhibitors |
|---|---|---|
| **K2P18** (TRESK, KCNK18) | Volatile anesthetics (Liu et al. 2004)<br>Calcium (Czirjak et al. 2004) | Local anesthetics (Czirjak et al. 2004; Liu et al. 2004)<br>Unsaturated fatty acids (Sano et al. 2003) |
| | Gαq (Czirjak et al. 2004) | Cyclosporin (Czirjak et al. 2004) |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

liver, heart, pancreas, and lungs while K2P16 channels appear to be expressed exclusively in the pancreas (Duprat et al. 2005; Girard et al. 2001; Lotshaw 2007).

All TALK subfamily channels are activated by extracellular and intracellular alkalinization and inhibited by extracellular acidification (Cid et al. 2013) (Table 4). The pH-sensing of K2P5 requires Arg244; substitution of this amino acid with neutral residues abolishes the response of the channel to changes in alkalization of the extracellular pH ($pH_o$) (Niemeyer et al. 2007). Protonation of Arg244 residue lowers $K^+$ occupancy of the selectivity filter resulting in pore-blockade (Cid et al. 2013).

TALK channels are also sensitive to changes in the intracellular pH ($pH_i$) (Niemeyer et al. 2010). It is postulated that lys245, located on the C-terminus of K2P5, acts as a sensor for $pH_i$ (Cid et al. 2013). Given the findings, it may be that the regulation of K2P5 channel activity by $pH_o$ and $pH_i$ occurs via effects on independent gates (Cid et al. 2013; Niemeyer et al. 2010); however, the mechanistic details that subserve this idea are yet to be elucidated.

K2P5 activity can be inhibited by Gβγ subunits of the heterotrimeric G protein (Anazco et al. 2013) (Table 4). Añazco and colleagues suggested that Gβγ modulation plays a role in the channel's ability to react to changes in cell volume (this is a result of neutralization of a lysine residue in the C-terminus that is important for inhibition by Gβγ). Although modulation of K2P5 by Gβγ is possible, it remains an open question in the field. Evidence to support Gβγ-modulation of K2P channel activity can be found in the K2P2 channels (Woo et al. 2012). Finally, Duprat and colleagues demonstrated that both K2P16 and K2P17 channels can be activated by nitric oxide (NO) and reactive oxygen species (ROS) (Table 4) (Duprat et al. 2005).

## 6 The TWIK Subfamily: K2P1, K2P6, and K2P7

Following its initial description in 1996, K2P1 (TWIK1, *KCNK1*) was observed to have low channel activity in heterologous expression systems (Goldstein et al. 1998; Lesage et al. 1996b; Pountney et al. 1999). However, since mRNA transcripts for KCNK1, the gene that encodes for the K2P1 subunit, are found in the kidney, placenta, lungs, heart, and the brain (Gaborit et al. 2007; Lesage et al. 1996b; Talley et al. 2001), several groups pursued potential cellular and biophysical mechanisms that would limit the activity of K2P1 channels. Data to support three

**Table 4** Modulators of TALK subfamily of K2P channels[a]

| Channel | Activators | Inhibitors |
|---|---|---|
| K2P5 (TASK2, KCNK5) | Alkaline pHo and pHi (Cid et al. 2013; Niemeyer et al. 2007) | Gβγ (Anazco et al. 2013) |
| K2P16 (TALK1, KCNK16) | Alkaline pHo and pHi (Cid et al. 2013; Niemeyer et al. 2007); Nitric oxide (NO) and Reactive oxygen species (ROS) (Duprat et al. 2005) | No known modulators |
| K2P17 (TALK2, KCNK17) | | No known modulators |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

hypotheses have been presented: SUMOylation of K2P1 channels at the plasma membrane; rapid endocytosis of K2P1 channels from the plasma membrane, and hydrophobic dewetting of the channel pore.

SUMOylation is an enzyme-mediated post-translational modification pathway that links a ~100 amino acid Small Ubiquitin-like MOdifier (SUMO) protein to the epsilon amine-group of lysine residues in specific motifs (Hay 2005). Although SUMOylation was not thought to occur at the plasma membrane, the process was shown to inhibit the activity of K2P1 channels because $K^+$ selective currents were observed when SUMO was removed from the channel by a SUMO-specific proteases (SENPs), or when the SUMOylation site (K2P1-Lys274) was mutated to prevent SUMO-binding (Plant et al. 2010; Rajan et al. 2005). SUMOylation is now known to regulate the activity of an array of ion channels in multiple tissues. The process is rapid, reversible, and dynamic and is often challenging to capture biochemically. In keeping with observations of numerous soluble SUMO substrates, such as transcriptional regulators, SUMOylation of K2P1 channels is labile and is often not observed when cells and tissues are studied after detergent purification (Feliciangeli et al. 2007; Hay 2005). Therefore, SUMOylation is typically studied in live cells using real-time electrophysiology, spectroscopy, and microscopy (Plant et al. 2010).

Studies in MDCK and HEK293 cells found that the low activity of K2P1 could be attributed to rapid, endocytic recycling of the channel from the plasma membrane (Feliciangeli et al. 2010, 2015). The process is dynamin-dependent based on analysis of a di-isoleucine motif: mutation of Ile293 and Ile294) resulted in measurable currents upon heterologous expression. Further, K2P1 was found to associate with ARF6, a small G protein that modulates endocytosis at the apical surface of epithelial cell (Decressac et al. 2004).

Following the resolution of the crystal structure of human K2P1, molecular dynamic simulations (MDS) of ion permeation identified a "hydrophobic cuff" in the inner vestibule of the channel, below the selectivity filter, comprised of four residues: Leu146 on M2 and Leu261 on M4, from each subunit (Aryal et al. 2014; Miller and Long 2012). MDS revealed that stochastic motion of the cuff restricted the access of water molecules to the internal entrance of the pore, creating an

energetic barrier to the permeation of $K^+$. Based on this model, substitution of Leu146 with hydrophilic residues resulted in a K2P1 channel variant that passed robust currents in *Xenopus* oocytes (Aryal et al. 2014; Chatelain et al. 2012).

Determining how SUMOylation, the hydrophobic gating barrier, and rapid endocytosis contribute individually or together to the regulation of K2P1 in native cells remains an area of active study that is spurred on by the observation that K2P1 knockout mice exhibit altered physiology in several tissues, including pancreatic β cells and the kidney (Chatelain et al. 2012; Nie et al. 2005). Similarly, K2P1 has been shown to play a key physiological and developmental role in the atria of transgenic zebrafish (Christensen et al. 2016). K2P1 has also been shown to mediate arrhythmogenic depolarization of cardiac myocytes exposed to low concentrations of $K^+$ associated with hypokalemia (Gotter et al. 2011). A part of the enigmatic character of K2P1 can be attributed to heterodimerization with K2P3 and K2P9 subunits in rat neurons and with K2P2 in rat astrocytes (Hwang et al. 2014; Plant et al. 2012). The resultant heteromeric channels have distinct properties. For example, the activity of K2P1-K2P3 and K2P1-K2P9 channels is increased by volatile, halogenated ester-based anesthetics and is subject to regulation by the SUMO pathway (Plant et al. 2012).

The TWIK subfamily is also composed of K2P6 (TWIK2, *KCNK6*) and the K2P7 (Kcnk8, *KCNK7*) channels. K2P6 was described by two independent groups (Chavez et al. 1999; Pountney et al. 1999) and shares 34% sequence identity with K2P1. In contrast, K2P7 is more closely related to K2P6 (94% homology) (Lesage and Lazdunski 2000; Lotshaw 2007). K2P6 and K2P7 are expressed in peripheral tissues and peripheral blood leukocytes, respectively (Lesage and Lazdunski 2000; Medhurst et al. 2001).

In native cells all TWIK channels have low channel activity and as a result they are sometimes considered to be electrically silent (Bockenhauer et al. 2000; Renigunta et al. 2014), limiting functional characterization of the channels as well as the development of selective pharmacological tools (Lotshaw 2007). When active, K2P1 and K2P6 currents are inhibited by barium, quinine, or quinidine (Table 5) (Lesage et al. 1996b). Separately, K2P1 channels can also be inhibited by intracellular (Lesage et al. 1996b) as well as extracellular acidification (Plant et al. 2010). K2P1 is also regulated by PKC activation by phorbol esters such as PMA, which enhances channel activity (Table 5) (Lesage et al. 1996b).

## 7 The TREK Subfamily: K2P2, K2P10, and K2P4

The TREK subfamily is composed of K2P2 (TREK1, *KCNK2*), K2P10 (TREK2, *KCNK10*), and K2P4 (TWIK-related arachidonic-acid-stimulated $K^+$ channel or TRAAK, K2P4, *KCNK4*) channels (Bang et al. 2000; Fink et al. 1998). In humans K2P2 and K2P10 tissue expression overlaps in the CNS and periphery tissues while K2P4 expression is most notable in the neurons (Lesage et al. 2000; Meadows et al. 2000; Medhurst et al. 2001). K2P10 channel shares 65% sequence similarity to K2P2 and 45% similarity to K2P4 (Bang et al. 2000; Ozaita and Vega-Saenz de

**Table 5** Modulators of TWIK subfamily of K2P channels[a]

| Channel | Activators | Inhibitors |
|---|---|---|
| K2P1 (TWIK1, KCNK1) | pHo (Rajan et al. 2005) (deSUMOylated channel); PKC (Lesage et al. 1996b) | Barium (Lesage et al. 1996b); Quinine or Quinidine (Lesage et al. 1996b); Acid pHi (Lesage et al. 1996b) |
| K2P6 (TWIK2, KCNK6) | No known modulators | Barium (Lesage et al. 1996b); Quinidine (Lesage et al. 1996b) |
| K2P7 (Kcnk8/ KCNK7) | No known modulators | No known modulators |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

Miera 2002). The K2P2 and K2P10 channels exhibit similar outward rectification (Lesage et al. 2000; Maingret et al. 1999; Medhurst et al. 2001). The differences between K2P2 and K2P10 currents can be seen when comparing unitary currents of the two channels under high extracellular concentration of $K^+$. Under this condition K2P10 exhibits inward rectification (Lesage et al. 2000; Maingret et al. 1999; Medhurst et al. 2001) while both K2P2 and K2P4 exhibit Goldman-Hodgkin-Katz (GHK) rectification (Fink et al. 1998).

The TREK subfamily of K2P channels are noted for their sensitivity to mechanical stimuli. These mechanosensitive channels are modulated by numerous physicochemical stimuli including pH, temperature, mechanical stress (stretch, shear, and swelling), polyunsaturated fatty acids (PUFAs), anesthetics (volatile), and protein phosphorylation (Table 6) (Lotshaw 2007; Maingret et al. 1999). K2P2 channels are also activated by an acidic $pH_i$ (Maingret et al. 2000), likely due to protonation of a glutamic acid residue at position 306 (Glu306). Protonation of this residue is an important regulator of the response of K2P2 channels to mechanical stimulation (Honore et al. 2002).

TREK channels are also activated by heat (Kang et al. 2005; Maingret et al. 2000). Thus, at 37 °C K2P2 channels exhibit outward rectification (Kang et al. 2005; Maingret et al. 2000) that is lost upon cooling (Kang et al. 2005; Maingret et al. 2000). K2P2 and K2P10 are also activated by halogenated volatile anesthetics such as chloroform, ether, halothane, isoflurane (Table 6) (Lesage et al. 2000; Maingret et al. 2000). Halothane is a more effective activator of K2P10 while chloroform is a more efficacious activator of K2P2 (Lesage et al. 2000). All the TREK subfamily channels are activated by riluzole, a neuroprotective drug that transiently activates K2P2 and K2P10 but permanently activates K2P4 (Lesage et al. 2000). The mechanism by which riluzole exerts its effect is believed to be a result of PKA inhibition as a result of cAMP accumulation (Lesage et al. 2000). In 2001, Bockenhauer and colleagues demonstrated that PKA phosphorylation of serine-348 (Ser348) results in an altered voltage-dependence of K2P2 channels, effectively reducing the open probability and thereby the channel activity (Bockenhauer et al. 2001).

**Table 6** Modulators of TREK subfamily of K2P channels[a]

| Channel | Activators | | | Inhibitors |
|---|---|---|---|---|
| K2P2 (TREK1, KCNK2) | NO (Koh et al. 2001) | Acid pHi (Maingret et al. 1999), Volatile anesthetics (Chloroform, ether, halothane, isoflurane) (Lesage et al. 2000; Maingret et al. 2000), Mechanical stress (Lesage et al. 2000; Medhurst et al. 2001), | PUFA (Lesage et al. 2000; Meadows et al. 2000; Medhurst et al. 2001), Riluzole (Lesage and Lazdunski 2000) Heat (Kang et al. 2005; Maingret et al. 2000), | Gαs and Gq (Lesage et al. 2000), Quinidine (Lesage et al. 2000) |
| K2P10 (TREK2, KCNK10) | Gαi (Lesage et al. 2000) | | | |
| K2P4 (TRAAK, KCNK4) | Alkaline pHi (Kim and Gnatenco 2001) | No known modulators | | No known modulators |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

Inhibition of K2P2 and K2P10 but not K2P4 was demonstrated to be mediated by activators of protein kinases (Table 6). Lesage and colleagues found that co-expression of K2P10 and $G\alpha_s$-coupled receptor 5HT4 resulted in decreased channel activity when the receptors were activated by 5-hydroxytryptamine. In contrast, co-expression K2P10 and $G\alpha_i$-coupled mGluR2 receptors increased channel activity upon stimulation by glutamate (Lesage et al. 2000). Lastly, co-expression of K2P10 and the $G\alpha_q$-coupled receptor mGluR1 resulted in inhibition of channel activity upon stimulation of mGluR1 by glutamate (Lesage et al. 2000). Signaling through $G\alpha_q$ results in activation of phosholipase C (PLC) which results in the hydrolysis of $PIP_2$ into diacylglcerol (DAG) and inositol 1,4,5-triphosphate (IP3) production. Lesage et al. postulated that inhibition of the channel may be a result of activation of protein kinase C (PKC) by DAG (Fig. 3).

## 8 The TASK Subfamily: K2P3, K2P9, and K2P15

The TASK subfamily is composed of K2P3 (TASK1, *KCNK3*), K2P9, (TASK3, *KCNK9*), and K2P15 (TASK5, *KCNK9*) channels (Duprat et al. 1997; Kim and Gnatenco 2001; Kim et al. 2000). The K2P3 channel was first isolated based on its sequence homology to K2P1 and K2P2 (Duprat et al. 1997). In general, the TASK channels share low sequence similarity with other K2P channels (<30%) however, amongst each other TASK channels share relatively high sequence similarity (>50%) (Ashmole et al. 2001; Duprat et al. 1997, 2007; Kim et al. 2000). TASK channels are expressed in most tissues with notable expression in the placenta and pancreas (Ashmole et al. 2001; Duprat et al. 1997; Kim et al. 2000; Rajan et al. 2000). While K2P3 and K2P9 can form functional homodimers or heterodimers,

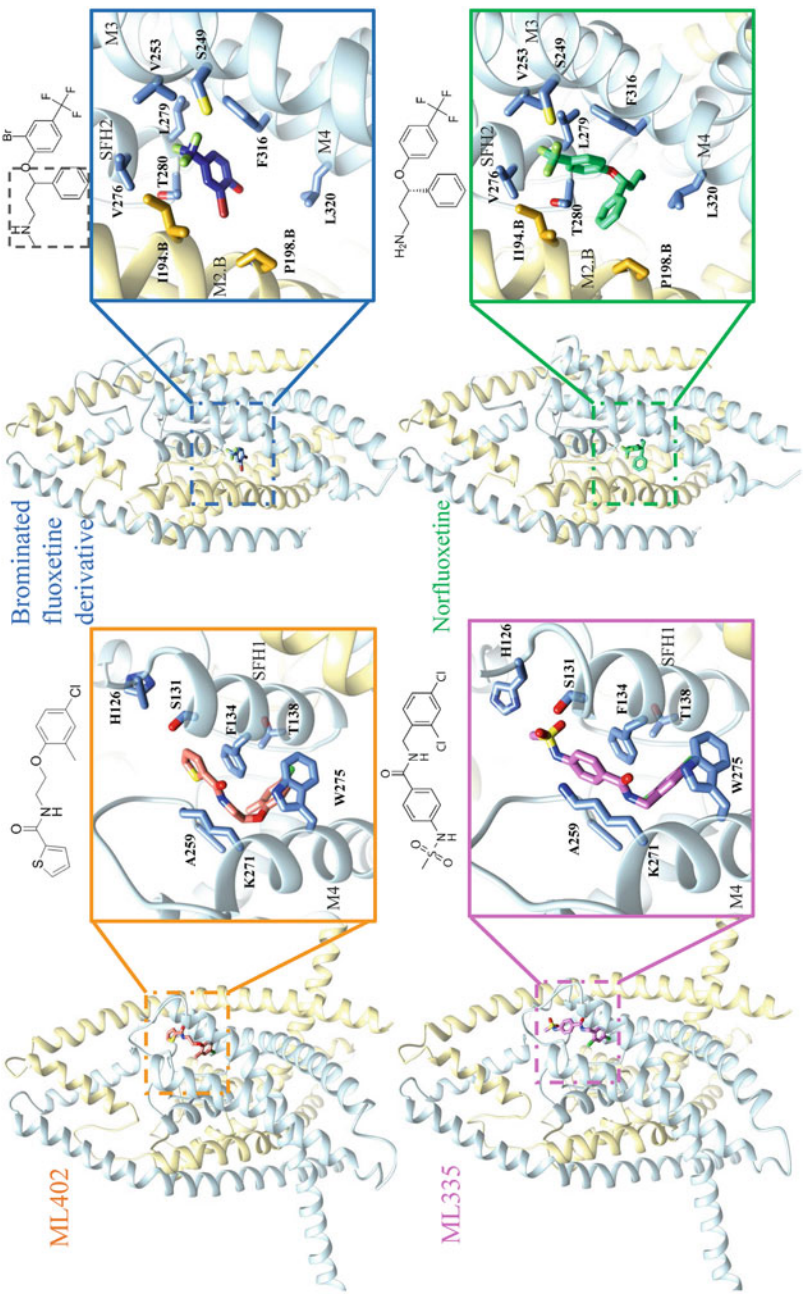


**Fig. 3** Interactions between $K_{2P}$ channels and pharmacophores. A comparison of four pharmacophores that have been captured in complex with K2P channels. For each channel, one subunit is shown in light blue and the adjacent subunit is shown in yellow. In each case, the structure of the pharmacophore is given above

**Table 7** Modulators of TASK subfamily of K2P channels[a]

| Channel | Activators | Inhibitors |
|---|---|---|
| K2P3 (TASK1, KCNK3) | Alkaline pHo (Duprat et al. 1997; Kang et al. 2004), Halothane and Isoflurane (Patel et al. 1999) | Acidic pHo (Duprat et al. 1997; Kim et al. 2000; Rajan et al. 2000), Gαq (Chen et al. 2006) K2P9: Ruthenium Red (Czirják and Enyedi 2002) |
| K2P9 (TASK3, KCNK9) | | |
| K2P15 (TASK5) | No known modulators | No known modulators |

[a]A regularly updated summary of the activators and inhibitors for all K2P channels is available at https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79

K2P15 channels are electrically silent when expressed alone or with other TASK channels (Ashmole et al. 2001; Bayliss and Barrett 2008; Czirják and Enyedi 2002; Duprat et al. 2007). Under physiological conditions activation of TASK1 and TASK3 channels occurs instantaneously and the channels exhibit outward rectification (Duprat et al. 2007; Kim et al. 2000).

The sine qua non of TASK channels is inhibition of the channel activity by extracellular acidification (Table 7) (Czirják and Enyedi 2002; Duprat et al. 1997; Kim et al. 2000; Rajan et al. 2000). In mutational studies of Guinea pig K2P9 (62.3% and 88.3% homology to human K2P3 and K2P9, respectively) Rajan and colleagues found that the histidine at position 98 (His98) conferred pH sensitivity to the channel (Lopes et al. 2000, 2001; Rajan et al. 2000). Similarly, Lopes and colleagues found that protonation of the equivalent residue in K2P3 conferred pH-sensitivity to that channel (Lopes et al. 2000, 2001). Of note, K2P3 and K2P9 heterodimers are also inhibited by by extracellular acidification (Czirják and Enyedi 2002).

Two groups have found that K2P9 homodimers are inhibited by Ruthenium Red (RR) while micromolar concentrations of RR were also unable to inhibit K2P3 homodimers in both *Xenopus* oocytes and COS-7 cells (Table 7). Interestingly, K2P3-K2P9 heterodimers are minimally inhibited by RR (Czirják and Enyedi 2002; Kang et al. 2004). RR appears to inhibit K2P9 homodimers by binding to Glutamate 70 (Glu70) on both subunits (Czirjak and Enyedi 2003). With K2P3-K2P9 heterodimers there is only one subunit with Glu70 for RR to bind which is likely insufficient to cause inhibition (Czirjak and Enyedi 2003).

K2P3 and K2P9 are both inhibited by $G\alpha_q$ (Chen et al. 2006) although whether this result is secondary to hydrolysis of $PIP_2$ remains an area of active debate. Both K2P3 and K2P9 are activated by volatile anesthetics (halothane and isoflurane) (Kang et al. 2004; Patel et al. 1999).

**Fig. 3** (continued) a zoomed-in view of how the molecule interacts with the channel protein. **Right-hand column: ML402** (top) or **ML335** (bottom) interacting with K2P2 channel (PDB ID: 6CQ9, 6CQ8) (Lolicato et al. 2017). **Left-hand column: Brominated fluoxetine derivative** or **Norfluoxetine** binding to K2P10 (PDB ID: 4XDL, 4XDK (Dong et al. 2015)

## 9 TOK Channels

$K^+$ channel subunits with two pore domains are not limited to expression in higher order eukaryotes but have also been identified in fungi. The transient outward current (TOK) channels were first cloned and described in *Saccharomyces cerevisiae* following a genome search that identified a P domain peptide sequence homologous to those of other $K^+$ channels (Ketchum et al. 1995). In contrast to the K2P subunits discussed above, TOK channels are dimers of subunits with eight transmembrane domains with intracellular amino- and carboxy-terminal tails (M1-M8), with two reentrant P-loops located between transmembrane domains composed with two P-loops regions located between M5 and M6 and M7 and M8 (Fig. 3) (Ketchum et al. 1995; Lesage et al. 1996a; Zhou et al. 1995). Expression of the *S. cerevisiae* TOK (ScTOK) channels in *Xenopus* oocytes revealed $K^+$-selective channels with outward rectification that were activated by depolarizing voltages (Ketchum et al. 1995; Lesage et al. 1996a; Zhou et al. 1995). Activation of ScTOK channels is coupled to the $K^+$ equilibrium potential ($E_K$) in that changes in the external concentration of $K^+$ results in loss of outward rectification (Bertl et al. 1998; Ketchum et al. 1995; Lesage et al. 1996a; Zhou et al. 1995). ScTOK currents are inhibited by barium ions, quinine, or tetraethylammonium (TEA) (Ketchum et al. 1995; Lesage et al. 1996a; Zhou et al. 1995).

TOK channels have now been identified in a range of fungi, including strains that are pathogenic to humans. A comparative study of four pathogenic fungi, *Aspergillus fumigatus* (AfTOK1), *Candida albicans* (CaTOK), and two strains of *Cryptococcus neoformans* (CnTOK and H99TOK), by Lewis and colleagues revealed that the TOK subfamily of $K^+$ channels share similar biophysical characteristics as ScTOK (Lewis et al. 2020). Their unique distribution in only fungi suggests that these TOK channels could be important therapeutic targets for anti-fungal pharmaceutics. This intriguing proposal is supported by data showing that extracellular K1 killer toxin kills *Saccharomyces* yeast by increasing the open probability of ScTOK and perturbing $K^+$ homeostasis (Ahmed et al. 1999). In contrast, infection with killer toxin virus protects against the effects of the external toxin, allowing virus-positive cells to propagate (Sesti et al. 2001). Thus, selective, small molecule activators of TOK channels are potential anti-fungal agents.

## 10 Conclusion and Future Perspectives

The $K^+$ channels comprise a large, diverse, and ubiquitous superfamily of membrane proteins that regulate various biological processes in both excitable and non-excitable cells (Kuang et al. 2015; Tian et al. 2014). The two-pore domain $K^+$ channels constitute a subfamily of $K^+$ channels that are categorized based on structural and sequence similarity. Since the discovery of these channels more than 20 years ago much has been revealed about these channel's physiology and pharmacology. The expression of $K_{2P}$ channels is widespread across various tissues and organ systems. This broad distribution and expression highlight their importance in

the biology of many tissues and suggest that $K_{2P}$ channels will continue to emerge as important potential druggable targets for the treatment of diverse diseases. Given the fundamental role that $K_{2P}$ channels play in physiology, it is not surprising that their activity is tightly regulated and modulated by diverse physicochemical and mechanical stimuli including temperature, mechanical stress, $pH_i$, $pH_o$, second-messenger pathways, PUFAs, and phosphoinositides.

Despite the growing body of work which has implicated $K_{2P}$ channels in various cardiac and neuronal diseases, there is much that is yet to be learned about $K_{2P}$ physiology and its role in pathophysiology. A present obstacle in attaining this knowledge is the lack of channel selective pharmacophores although this landscape is starting to evolve, particularly for the TREK subfamily of $K_{2P}$ channels. Following the elucidation of several $K_{2P}$ structures we now appreciate that a part of the delay in identifying selective pharmacophores comes from the cap-domain of the $K_{2P}$ channels. This structural feature, seemingly unique amongst $K^+$ channels, protects the outer mouth of the channel pore from infiltration by classical $K^+$ channel blockers, particularly protein toxins. However, the same structural revolution that identified the problems has also helped to initiate solutions. Using computational approaches to understand the dynamics of K2P channels, researchers have started to identify druggable pockets and binding sites within the channel corpus. Of note, Lolicato and colleagues identified a cryptic binding pocket behind the pore of the K2P2 channel that can co-ordinate the newly identified channel activators ML335 and ML402 (Lolicato et al. 2017). Bagriantsev and colleagues demonstrated that selective and potent compounds of $K_{2P}$ channels can also be identified using high-throughput screens (Bagriantsev et al. 2013). These powerful approaches promise to break the gridlock in the development of selective new $K_{2P}$ channel modulators in the future.

**Conflict of Interest** The authors report no competing financial interests.

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