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Physiological roles of heteromerization: focus on the two-pore domain potassium channels

Lamyaa Khoubza¹, Franck C. Chatelain¹, Sylvain Feliciangeli^{1,2}, Florian Lesage^{1,2}, Delphine Bichet¹

¹Université côte d'Azur, IPMC CNRS UMR7275, Laboratory of Excellence ICST, 660 route des Lucioles 06650 Valbonne, France.

²Inserm

Keywords

K⁺ channels, heteromerization, diversity, gating, trafficking

Abstract

Potassium channels form the largest family of ion channels with more than 80 members involved in cell excitability and signaling. Most of them exist as homomeric channels, whereas specific conditions are required to obtain heteromeric channels. It is well established that heteromerization of voltage-gated and inward rectifier potassium channels affects their function, increasing the diversity of the native potassium currents. For potassium channels with two-pore-domain (K_{2P}), homomerization has long been considered the rule, their polymodal regulation by a wide diversity of physical and chemical stimuli being responsible for the adaptation of the leak potassium currents to cellular needs. This view has recently evolved with the accumulation of evidence of heteromerization between different K_{2P} subunits. Several functional intragroup and intergroup heteromers have recently been identified, which contribute to the functional heterogeneity of this family. K_{2P} heteromerization is involved in the modulation of channel expression and trafficking, promoting functional and signaling diversity. As illustrated in the Abstract Figure, heteromerization of TREK1 and TRAAK provides the cell with more possibilities of regulations. It is becoming increasingly evident that K_{2P} heteromers contribute to important physiological functions including neuronal and cardiac excitability. Since heteromerization also affects the pharmacology of K_{2P} channels, this understanding helps to establish K_{2P} heteromers as new therapeutic targets for physiopathological conditions.

Abstract Figure Legend

TREK1 and TRAAK, two K_{2P} channel subunits, assemble to form functional homodimers and heterodimers. Characterization of their regulations by protein kinase A and C, phospholipase D, intracellular and extracellular protons and chemical compounds such as ML67 and fluoxetine has shown that the TREK1/TRAAK heteromers have unique properties compared to TREK1 and TRAAK homomers.

Abbreviations

K_{IR}: Inwardly rectified K⁺ channel; K_{2P}: two-pore domain K⁺ channels; K_V: voltage-dependent K⁺ channel; K_{Ca/Na}: Ca²⁺/Na⁺-dependent K⁺ channel; TWIK: two pore-forming domain in a

weakly inward rectifying K⁺ channel; TREK: TWIK-related K⁺ channel; TASK: TWIK-related acid-sensitive K⁺ channel; TALK: TWIK-related alkaline-sensitive K⁺ channel; THIK: tandem pore domain halothane-inhibited K⁺ channel; TRESK: TWIK-related spinal cord K⁺ channel; ER: endoplasmic reticulum; GPCR: G-protein coupled receptor; DGGC: dente gyrus granular cells.

Author profiles

Lamyaa Khoubza is a PhD student in biological science from University of Nice Côte-d'Azur. She's studying heteromerization of K_{2P} channels under the supervision of Dr Bichet. **Franck Chatelain** is a CNRS researcher specialized in ion channel structure and function. **Sylvain Feliciangeli** is an INSERM researcher specialized in trafficking of ion channels. **Florian Lesage** is an INSERM director of research (PI), team leader, deputy director of the Institute of Cellular and Molecular Pharmacology (IPMC) and coordinator of the laboratory of excellence Ion Channel Science and Therapeutics (LabEx ICST); **Delphine Bichet** is a CNRS researcher interested in molecular and cellular aspects of ion channel functions. She obtained her PhD from the University of Aix-Marseille working on calcium channel trafficking with Dr Michel DeWaard. She did her post-doctoral training in the laboratory of Pr Lily Yeh Jan at UCSF where she worked on selectivity and gating of potassium channel. She is now studying intracellular K_{2P} channels and heteromerization. All authors are part of Dr Lesage's team at the IPMC.

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Introduction

Ion channels consist of a central hydrophobic core containing pore-forming subunits. This core forms the ionic pore, through which ions pass through the biological membranes. With more than 78 members, potassium channels constitute a large class of pore-forming subunits. Different subclasses are identified based on the basis of structural and functional similarities (Coetzee *et al.*, 1999). 40 voltage-gated channels (K_V) are distributed into 12 subfamilies (K_V1 to K_V12) while Ca^{2+} and Na^+ -sensitive channels ($K_{Ca/Na}$) have 8 members. 15 inward rectifiers (K_{IR} channels) are divided into 7 subgroups (K_{IR1} to K_{IR7}), and 15 two-pore domain K^+ (K_{2P}) channels into 6 subgroups (Figure 1) (Coetzee *et al.*, 1999; Enyedi & Czirjak, 2010; Hibino *et al.*, 2010; Renigunta *et al.*, 2015; Niemeyer *et al.*, 2016). All these subunits form homomeric channels, some of them being not functional (Bocksteins, 2016). Heteromerization is a more selective mechanism with a limited number of heteromeric channels identified to date (Clarke & Gulbis, 2012; Jan & Jan, 2012; Feliciangeli *et al.*, 2015; Capera *et al.*, 2019).

Among the potassium channels, K_{2P} channels assemble as dimers rather than tetramers (Lesage *et al.*, 1996). Two subunits, each comprising 4 membrane-spanning helices (M1 to M4) and two pore-forming domains (P1 and P2), interact and constitute the active channel. A conformational feature, called domain swapping, is the overlap of the 2 subunits at the top of their extracellular domain M1P1 and a permutation of the membrane spanning helices (Brohawn *et al.*, 2013), the external helix of one subunit (M1A) interacting with the internal helix of the other subunit (M2B). This domain swapping suggests that oligomerization occurs very early on, probably during biosynthesis when the polypeptide chains are still attached to the ribosomes and translocated across the ER membrane.

K_{2P} channels produce a background conductance that is essential for maintaining the negative membrane potential of many cells, thus reducing the excitability of excitable cells (Enyedi & Czirjak, 2010; Feliciangeli *et al.*, 2015). They are sensitive to a variety of stimuli and molecules, including internal and external pH, phospholipids, voltage, temperature, membrane stretching, volatile anesthetics, cytoskeleton, and intracellular signals from, for example, activation of G protein-coupled receptors (Noel *et al.*, 2011; Feliciangeli *et al.*, 2015). The diversity of K_{2P} channels, their regulation and their involvement in various pathophysiological conditions have been extensively studied. Here we will focus on their ability to form active heteromers, and compare it to heteromerization in other families of potassium channels.

Heteromers in the K_V and K_{IR} families

A number of heteromeric K_V and K_{IR} channels have been described (Hibino *et al.*, 2010; Sepulveda *et al.*, 2015; Bocksteins, 2016; Capera *et al.*, 2019). Heteromerization can occur between subunits of the same subfamily producing “intragroup heteromers”, or between subunits from different subfamilies producing “intergroup heteromers”.

The first example is the intragroup heterotetramer $K_V1.2/K_V1.4$, which produces a fast-inactivating potassium current (IK_A). This current is present at the neuronal presynaptic terminations where it plays a predominant role in the control of the neurotransmission (Sheng *et al.*, 1993). $K_V1.2$ can also assemble with $K_V1.1$ in the initial segment of axons (AIS) and at the nodes of Ranvier (NR) where the $K_V1.2/K_V1.1$ heteromers play an essential role in the initiation and propagation of action potentials (Wang *et al.*, 1993; Robbins & Tempel, 2012). The multimerization of K_V7 channel subunits has also been intensively studied. The intragroup heterotetramers between $K_V7.2$ (KCNQ3) and $K_V7.3$ (KCNQ3) are responsible for the M current (IK_M) while the homomers of $K_V7.2$ are the basis of the nodal S current (IK_S) (Cooper, 2011). The M current, inhibited by the application of agonists of the muscarinic acetylcholine receptors, is particularly important for the control of neuronal excitability in many neuronal circuits, especially those involved in learning and memory (Wang *et al.*, 1998). The nodal S current, which activates slowly (S stands for "slow") and does not inactivate, is essential for the repolarization of motor neurons (Devaux *et al.*, 2004). In the cerebral arteries, the $K_V7.4$ and $K_V7.5$ subunits exist mainly as part of a functional heteromer, which regulates intrinsic myogenicity and vasodilation (Chadha *et al.*, 2014). For K_V1 and K_V7 , heteromerization increases functional diversity but can also cause cellular rerouting of K_V channels. $K_V1.1$ is mainly localized in the ER, $K_V1.4$ in the plasma membrane, and $K_V1.2$ is found at both locations. When coassembled with $K_V1.4$, $K_V1.2$ is expressed at the cell surface, whereas co-assembly with $K_V1.1$ has a dominant-negative effect on the surface expression of $K_V1.2$ (Manganas & Trimmer, 2000). Another example concerns the intragroup heteromerization of $K_V1.3$ and $K_V1.5$ in immune cells. While homomeric $K_V1.5$ does not address to lipid raft domains, heteromerization with $K_V1.3$ redirects $K_V1.5$ to the caveolar domains (Vicente *et al.*, 2008). The last example concerns the K_V7 subfamily. Together with $K_V7.2$, $K_V7.3$ forms functional intragroup heteromers, a mechanism that overcome its inefficiency to reach the plasma membrane. This is due to a specific residue in the selectivity filter (T315) with a strong impact on channel trafficking and stability. $K_V7.3$ needs to associate with $K_V7.2$ to escape the ER, reach the cell surface and localize to the axon initial segment by an ankyrin-G interaction (Rasmussen *et al.*, 2007; Gomez-Posada *et al.*, 2010). For the K_{Ca2} subfamily a preferential heteromeric association between $K_{Ca2.1}$ and $K_{Ca2.2}$ has been demonstrated based on the differential sensibility of the two subunits to the apamine toxin (Church *et al.*, 2015).

Intergroup heteromerization has also been widely described in the K_V family which include subunits that form electrically silent homomers (Bocksteins & Snyders, 2012). To generate a current, these subunits, named K_S (S for "silent"), must associate with K_V2 , a member of another K_V subfamily (Hugnot *et al.*, 1996; Salinas *et al.*, 1997a; Salinas *et al.*, 1997b). 10 different K_VS subunits of the K_V5 , K_V6 , K_V8 and K_V9 groups, have been identified so far, which can associate with K_V2 and modulate its biophysical properties. These modulations include a change in the voltage dependence of activation and/or inactivation, and changes in the activation, deactivation or inactivation kinetics (Bocksteins, 2016). It is interesting to note that $K_V2.1/K_V8.2$ heteromers, rather than homomeric $K_V2.1$, have been found to support the voltage-gated K^+ current described in the retinal rods of amphibians and mammals contributing to photoreception (Wu *et al.*, 2006; Czirjak *et al.*, 2007). Subsequently,

mutations in the $K_V8.2$ subunit preventing its assembly with $K_V2.1$ were found to cause retinal dysfunction (Wissinger *et al.*, 2011).

In the K_{IR} channel family, heterotetramers with major roles have also been identified. $K_{IR3.1}$, which does not produce K^+ current as a homomer, plays an important role in heteromers by modifying sensitivity to G-proteins (Kofuji *et al.*, 1995). The intragroup heteromer $K_{IR3.1}/K_{IR3.2}$ is responsible for the main neuronal G-protein-sensitive current (Lesage *et al.*, 1996; Liao *et al.*, 1996), while $K_{IR3.1}/K_{IR3.4}$ produces the $I_{K_{ACh}}$ current in the heart (Krapivinsky *et al.*, 1995). $K_{IR2.1}$, whose mutations are associated with Andersen-Tawil syndrome, also forms intragroup heteromers with $K_{IR2.2}$ and $K_{IR2.3}$ in the heart. Since most mutations have dominant-negative effects, multimerization in this disease suppresses not only the activity of $K_{IR2.1}$ homomers, but also those of $K_{IR2.1}/K_{IR2.2}$ and $K_{IR2.1}/K_{IR2.3}$ heteromers, thus contributing to the complex phenotype of the Andersen-Tawil syndrome (Preisig-Muller *et al.*, 2002). An example of intergroup heteromers is found between $K_{IR4.1}$ and $K_{IR5.1}$. Coexpression of the two subunits leads to the formation of $K_{IR4.1}/K_{IR5.1}$ heteromers with a conductance and sensitivity to pH different from that of the $K_{IR4.1}$ or $K_{IR5.1}$ homomers. Expressed on the basolateral membrane of renal epithelial cells, this intergroup heteromer contributes to finely tune NaCl uptake in the distal tubule (Tanemoto *et al.*, 2000; Pessia *et al.*, 2001). In astrocytes, the subcellular distribution of $K_{IR4.1}/K_{IR5.1}$ heteromers is different from that of the homomers, which allows specific control of K^+ homeostasis (Hibino *et al.*, 2010).

Heteromers in the K_{2P} channel family

In contrast to K_V and K_{IR} channels, the standard nomenclature for K_{2P} channels does not differentiate between different subgroups (channels are numbered K_{2P1} to K_{2P18}). In this review on heteromerization, it is more appropriate to use their original names that identify subgroups (Figure 1). On the basis of their primary sequences and functional properties, these subunits are classified into 6 subgroups: TWIK (two pore-forming domain in a weakly inward rectifying K^+ channel); TREK (TWIK-related K^+ channel); TASK (TWIK-related acid-sensitive K^+ channel); TALK (TWIK-related alkaline-sensitive K^+ channel); THIK (Tandem pore domain halothane-inhibited K^+ channel); and TRESK (TWIK-related spinal cord K^+ channel).

Heteromerization affects channel expression and trafficking

An important role of heteromerization is to allow a silent subunit to be part of active channels (Bocksteins, 2016). In the K_{2P} family, three subunits, THIK2, TASK5 and TWIK3, fail to produce K^+ currents when expressed alone (Bichet *et al.*, 2015). THIK2 has been found to inhibit THIK1 currents in oocytes in a dose-dependent manner, suggesting an interaction between THIK1 and THIK2 (Blin *et al.*, 2014). A non-conducting subunit was constructed by introducing a single mutation in the selectivity filter of THIK2. This dominant negative subunit, THIK2^{DN}, renders non-functional any channel in which it assembles. The coexpression of THIK2^{DN} results in a dose-dependent decrease in THIK1 currents, showing that THIK2 is capable to of specifically associating with THIK1 (Blin *et al.*, 2014). THIK2 is

exclusively localized in the endoplasmic reticulum (ER) when expressed alone. For multimeric membrane proteins such as channels and receptors, ER represents a crucial step in the control of assembly. An example of such control is the assembly of 4 K_{IR6} subunits with 4 SUR subunits of the sulfonyleurea receptor to form an ATP-sensitive K^+ channel (Zerangue *et al.*, 2000). Each K_{IR6} and SUR subunit contains an arginine rich retention signal (RxR) that prevents export of the subunits from the ER to the Golgi and cell surface. Until the multimer is properly folded, with all retention signals masked, complexes are not exported to the plasma membrane. The THIK2 subunit contains in its N-ter domain a signal for ER retention (RRxRRR) (Chatelain *et al.*, 2013). Exposure of this signal prevents THIK2 subunit to exit from the ER and expression of the homomer at the plasma membrane. THIK2 assembly with THIK1 leads to the inactivation of this signal and the targeting of THIK1/THIK2 heterodimers to the plasma membrane.

In addition to THIK2, other K_{2P} channel subunits have intracellular localization. TWIK1 and TWIK2 are preferentially localized in the endo-lysosomal compartments whereas TALK1, TASK1, TASK2 and TRAAK are expressed at the plasma membrane level but with a large pool present in the ER compartment (Feliciangeli *et al.*, 2010; Bobak *et al.*, 2017; Vierra *et al.*, 2017). The question whether heteromerization plays a specific role in the cellular localizations of K_{2P} channels remains open.

Heteromerization promotes functional, pharmacological and signaling diversity

Functional diversity

In the family of K_{2P} channels, the heteromerization of TASK1 and TASK3 was the first to be reported (Czirjak & Enyedi, 2002) (Table 1). In *Xenopus* oocytes, their co-expression produces a K^+ current with electrophysiological characteristics that are distinct from the currents produced by a combination of the TASK1 and TASK3 homomers. Heteromerization has also been demonstrated in mammalian cells by the expression of tandems in which TASK1 and TASK3 are covalently bound (Czirjak & Enyedi, 2002; Talley & Bayliss, 2002; Kang *et al.*, 2004; Musset *et al.*, 2006; Rinne *et al.*, 2015). Compared to the TASK1 or TASK3 homodimers, the electrophysiological properties of the tandem display intermediate unitary conductance and sensibility to external pH, and insensitivity to ruthenium red (RR) (Table 2). The TASK1/TASK3 and TASK1 currents are also differentially inhibited by extracellular Ca^{2+} and Mg^{2+} (Table 2) (Kang *et al.*, 2004).

Unlike TASK1/TASK3, we have shown that the properties of the THIK1/THIK2 current are very similar to those of THIK1 and THIK2 in terms of extracellular pH and halothane sensitivity (Chatelain *et al.*, 2013; Blin *et al.*, 2014). The activation kinetics and conductances of the THIK1/THIK2 tandem are slightly different but not enough to allow their identification in native tissues. The THIK1 current is inhibited by cold (10°C) and enhanced by the co-expression of $G\beta\gamma$ subunits (Kang *et al.*, 2004; Ishii *et al.*, 2010). A caspase 8 cleavage motif has also been identified in the cytoplasmic C-ter of THIK1. Interestingly, the motifs

implicated in some of those regulations are not retained in THIK2 suggesting that there may be specific regulations that may influence THIK1, THIK2 and THIK1/THIK2 differentially.

Three research groups, including ours, have used complementary approaches to demonstrate heteromerization of the TREK and TRAAK subunits (Table 1) (Blin *et al.*, 2016; Lengyel *et al.*, 2016; Levitz *et al.*, 2016). We and Lengyel *et al.* performed reciprocal co-IP of TREK1 with TREK2, and TREK1 with TRAAK from mammalian MDCK cells or from *Xenopus* oocytes, while Levitz *et al.* used single-molecule pull-down (SiMPull) assay from HEK cells. Using in situ proximity ligation assay (PLA) and transfer of energy between fluorescent molecules (FRET) in transfected cells, Blin and collaborators, demonstrated that all isoforms of TREK1 and TREK2, produced by alternative splicing or alternative translation initiation, form heterodimers but do not assemble with the K_{2P} subunits of other groups. Using “single-molecule subunit counting” Levitz *et al.* measured the number of heteromers at the plasma membrane of *Xenopus* oocytes expressing TREK1-GFP (green) and TREK2-RFP (red). Fluorescent dots on the surface can be quenched by successive photobleaching at specific excitation wavelengths for RFP and GFP allowing the proportion of TREK1/TREK1 (green) and TREK2/TREK2 (red) homomers versus TREK1/TREK2 (yellow) heteromers to be quantified. According to this study, the affinities of TREK1 for itself or for TREK2 are similar, while the association with TRAAK seems less effective (Levitz *et al.*, 2016). A comparison of the electrophysiological properties of TREK1/TREK2 and TREK1/TRAAK heteromers with their respective homomers had shown that single channel conductance and rectification of TREK1/TREK2 are closer to those of TREK1, and those of TREK1/TRAAK are closer to those of TRAAK (Blin *et al.*, 2016) (Table 2).

Signaling and pharmacological diversification

TASK1 and TASK3 have distinct sensitivities to RR and to external pH variations (Czirjak & Enyedi, 2002). While TASK3 is strongly inhibited by RR, TASK1 is almost insensitive. The acidification of the extracellular pH from 7.5 to 6.5 causes an inhibition of more than 50% of TASK1 current but has practically no effect on TASK3. It is interesting to note that the TASK1/TASK3 heteromer has the same insensitivity to RR as TASK1, and an intermediate pH-sensitivity compared to the two homomers (Table 2). Thus, from two subunits, three channels with distinct properties were detectable (Berg *et al.*, 2004). These differential profiles contributed to the identification of native TASK currents in several cell populations (see below).

Despite a high level of homology between TREK1 and TREK2, the two homomeric channels show a different sensitivity to RR and opposite sensitivity to extracellular pH (Sandoz *et al.*, 2009; Braun *et al.*, 2015). TREK2 is inhibited by external application of RR but not TREK1. This difference is mainly due to the presence in TREK2 of negative charges at the base of the extracellular M1P1 loop near the outer mouth of the pore. We found that the TREK1/TREK2 tandem is not sensitive to RR and that a single mutation in TREK1 allows heteromers to acquire RR inhibition (Blin *et al.*, 2016). In this case, as for TASK1/TASK3, the presence of the two RR binding sites, one on each subunit, is essential to confer RR sensitivity to the channel

(Czirjak & Enyedi, 2002; Blin *et al.*, 2016). Of note, the Enyedi group found that the TREK1/TREK2 heteromer has an intermediate sensitivity to RR, supporting a model of inhibition in which a single binding site is sufficient to confer partial RR sensitivity, but two sites confers maximal sensitivity (Lengyel *et al.*, 2016) (Table 2). A recent study shows that a pair of aspartate residues introduced into TREK1 (I110D mutation on each subunit) creates a polycation binding site, referred to as “keystone inhibitor site”. The binding of a RR molecule to this site is enough to block the exit of ions from the selectivity filter by a proposed “finger in the dam” mechanism (Pope *et al.*, 2020). However, it is still not clear whether one residue (like in TREK1/TREK2) or two residues (like in TREK1_{I110D} or TREK2) are necessary for RR binding. With regard to the extracellular pH sensitivity of the TREK1/TREK2 heteromers, the mechanism is also not fully elucidated. According to the Sandoz group the TREK1/TREK2 tandem has a pH-sensitivity between those of TREK1 and TREK2 (Levitz *et al.*, 2016). Acidification (pH 7.4 to pH 6.5) activates TREK1/TREK2 in the same way as TREK2 while it inhibits TREK1, whereas alkalinization (pH 7.4 to pH 8.5) activates TREK1/TREK2 in a same way as TREK1 while it inhibits TREK2 (Table 2). Each subunit of the heteromer participates in pH regulation in a non-linear manner. Different results obtained by the Enyedi group showed that the TREK1/TREK2 tandem behaves more like TREK1 in terms of external acidification and alkalinization (Lengyel *et al.*, 2016), suggesting that the inhibitory mechanism responsible for the pH sensitivity of TREK1 is dominant over the activating effect on TREK2. Further data will be needed to decipher the mechanisms of RR and pH-sensitivity.

TREK1, TREK2 and TRAAK have distinct regulations by phosphorylation (Table 2). TREK1 and TREK2, but not TRAAK, are inhibited by protein kinase A (PKA) and protein kinase C (PKC) (Fink *et al.*, 1998). If sensitivity to phosphorylation is transmitted by the subunits, the TREK1/TRAAK tandem should be sensitive to both PKA and PKC activation through its TREK1 subunit. This is the case for PKA with an intermediate sensitivity of the TREK1/TRAAK heteromer to PKA activators (Blin *et al.*, 2016). However, TREK1/TRAAK current, like the TRAAK current, is insensitive to PKC activators. A number of G-protein-coupled receptors (GPCRs) for neurotransmitters and hormones regulate PKA and PKC activities. In native cells co-expressing both TREK1 and TRAAK subunits, a controlled change in the TREK1: TRAAK ratio can be expected to influence the proportion of homo- versus heteromeric channels, and thus the degree of cellular response to GPCR stimulation, without the need to alter to other cellular parameters. To our knowledge such a mechanism has never been reported before and should be studied *in vivo*.

Several pharmacological agents that target the TASK and TREK channels have been tested on heteromers (Table 2). The TASK1 blockers A293 and A1899 were shown to differentially block the two tandem channels constructed by fusing TASK1 and TASK3. Surprisingly, TASK1/TASK3 tandem behaves more like TASK3 while TASK3/TASK1 reacts more like TASK1, suggesting that the order of the subunits in the tandem influences the behavior and functionality of the channel, probably by introducing physical constraints in the assembly and gating. This effect, reported only for tandems containing TASK1 and TASK3 so far, has to

be taken into account for future experiment using tandem or chimeric constructs (Rinne *et al.*, 2015). The effects of ML67 and fluoxetine were studied on TREK1/TRAAK while the effect of spadin was examined on TREK1/TREK2 (Blin *et al.*, 2016; Lengyel *et al.*, 2016). ML67 produces a much greater activation of TRAAK (~11 fold) than TREK1 (~3 fold) and had intermediate level of activation on TREK1/TRAAK (~8 fold). Intermediate inhibitory effects were obtained with fluoxetine (Blin *et al.*, 2016) (Table 2). This molecule, which inhibits more than 50% of TREK1 and has no effect on TRAAK, moderately inhibits the current generated by TREK1/TRAAK (~24%). More surprisingly, spadin, which decreases TREK1 opening probability by 60% and has no effect on TREK2, strongly inhibits TREK1/TREK2 (85%) (Lengyel *et al.*, 2016) (Table 2). While these differences cannot be easily used to discriminate between the various combinations of TREK and TRAAK subunits in native tissues, they allow the study of the mechanisms of action of these molecules, and the identification of the regions responsible for the differential effect on homomeric and heteromeric channels.

Intergroup heterodimerization of K_{2P} channels

TWIK1/TASK1 and TWIK1/TASK3

In 2012, a study changed our view on the heteromerization of K_{2P} channels by proposing that K_{2P} subunits could heteromerize with subunits of another group (Plant *et al.*, 2012). The interaction of TWIK1 with TASK1 or TASK3 has been proposed to generate intergroup heteromers that would underlie the main component of the acid-sensitive outward potassium current that regulates the excitability of the cerebellar granule neurons. More recently, the Park group found that TWIK1/TASK3 heteromers could also contribute to hippocampal excitability by regulating firing frequency of dentate gyrus granule cells (DGGC) (Choi *et al.*, 2018). The physical association between the two subunits was evaluated in a heterologous expression system using BIFC and co-IP and in native dentate gyrus granule cells (DGGC) combining the PLA assay and interference RNA tools (Table 1). It is interesting to note that the TWIK1/TASK3 heteromers retain the sensitivity of TASK3 to neurotensin (NT), this regulation being important for the control of DGGC firing frequency (Table 2). The TWIK1 and TASK3 currents are highly regulated. TASK3 is modulated by extracellular pH, anesthetics, zinc, RR activation of receptor coupled to Gq, binding proteins such as 14-3-3, β COP, p11 and phosphorylation, while TWIK1 is affected by endocytosis, extracellular pH and K^+ (for review (Bichet *et al.*, 2015)). A better characterization of the regulatory properties of TWIK1/TASK3 is needed to distinguish them from the TASK1/TASK3 intragroup heteromers and TASK1 and TASK3 homomers. Another issue is related to the subcellular localization of these heteromers because both TWIK1 and TASK3 have traffic signals controlling their targeting to different cellular compartments. TWIK1 contains a di-isoleucine motif in the C-ter domain responsible for the rapid endocytosis of the homomeric channel whereas the addressing of TASK3 to the plasma membrane is highly regulated by 14-3-3, β COP and phosphorylation (O'Kelly *et al.*, 2002; Rajan *et al.*, 2002; Zuzarte *et al.*, 2009;

Kilisch *et al.*, 2016). Additional studies are also needed to ensure complete loss of the heteromeric current in double or triple KO mice.

TWIK1/TREK1 and TWIK1/TREK2

TWIK1/TREK1 is the second report of intergroup heteromerization (Hwang *et al.*, 2014). The evidence for this interaction in heterologous expression systems is quite convincing, although other research groups, including our own, have not found such interaction using similar approaches (Plant *et al.*, 2012). It remains to be determined whether these differences reflect a limitation of the experimental approaches. TWIK1/TREK1 heteromerization requires a disulphide bridge between cysteine residues located in the M1P1 loops of TWIK1 and TREK1. However, the role of this disulfide bond in assembly, dimerization or stability of K_{2P} is still unclear (Lesage *et al.*, 1996). Heteromeric TWIK1/TREK2 and TWIK1/TRAAK channels were also reported using biochemical approaches but without electrophysiological characterization (Table 1). As TWIK1 and TREK1 have distinct subcellular localizations, the impact of TREK1 on the trafficking of endosomal TWIK1 has been studied in heterologous expression systems and astrocytes (Hwang *et al.*, 2014). Results showed that both subunits are detected at the plasma membrane when they are co-expressed. This observation suggests that the 2 subunits only exist as heteromeric channels and that heteromerization is favored over homomerization, a phenomenon that is contradicted by other studies showing that TWIK1 and TREK1 contribute independently to the passive conductance of astrocytes (Zhou *et al.*, 2009) or do not even participate in the basic electrophysiological properties of mature hippocampal astrocytes (Du *et al.*, 2016). It has also been reported that the TWIK1/TREK1 channels are responsible for the transport of glutamate in astrocytes. The TWIK1/TREK1 tandem expressed in mammalian cells generates a current with substantial permeability to Cl^- and glutamate upon $G\beta\gamma$ binding and the inhibition of TWIK1 with shRNA eliminated the rapid release of glutamate induced by activation of the cannabinoid receptor CB1 in astrocytes (Hwang *et al.*, 2014). It has also been shown that the expression and membrane localization of TWIK1 in astrocytes is regulated by glutamate receptor 3 (mGluR3) (Wang *et al.*, 2015).

TALK2 and TASK1

Using TIRF microscopy, the TALK2 and TASK1 subunits were detected together at the cell surface of a pancreatic cell line overexpressing both subunits (Suzuki *et al.*, 2017) (Table 1). A tandem construct of TASK1 and TALK2 was generated, and channel regulation compared to each homomeric channels (Table 2). As previously shown for TASK1/TASK3, TASK1/TALK2 exhibits an intermediate pH sensitivity but in a wider range (7.4 to 9.4) than the TASK1 (7.4 to 8.4) and TALK2 (8.4 to 9.4) homomers, with major differences in the shape of the fitting curves. While the pH-dependent curve for TASK1/TASK3 could be fitted by a single Hill's equation, the curve for TASK1/TALK2 is better fitted by the sum of two equations (Czirjak & Enyedi, 2002). This difference is probably due to the large difference in pKa between the pH-sensing histidine in TASK1 and TASK3 and lysine in TALK2 (Suzuki *et al.*, 2017).

TREK1/TREK2 and TRESK

The last and most recent heteromers identified in the K_{2P} channel family contain the TRESK subunit. TRESK, the sole member of its group, shares a relatively low homology with the other K_{2P} s (<20%). Originally described in the spinal cord and named accordingly, TRESK is particularly abundant in DRG neurons, in which it modulates nociception (for review (Enyedi *et al.*, 2012)). TREK1, TREK2 and TRAAK, and to a lesser extent TASK and THIK, are also expressed in DRG neurons. Two research groups have independently shown that TRESK assembles with TREK1 and TREK2 (Royal *et al.*, 2019; Lengyel *et al.*, 2020) (Table 1). Using dominant negative assay, the Enyedi group reported an inhibitory effect of a TRESK pore-mutant on TREK2 current in transfected cells (Lengyel *et al.*, 2020). Using a more refined approach, the Sandoz group showed that co-expression of a ER-localized and light-gated TREK1 channel (TREK1-PCS) with TRESK produces a heteromeric channel that is addressed to the plasma membrane and produces a photoswitchable current (Royal *et al.*, 2019).

The TRESK/TREK1 and TRESK/TREK2 heteromers have distinct biophysical, pharmacological and regulatory properties compared to the TRESK and TREK homomers (Table 2). While TREK1 is sensitive to arachidonic acid (AA), TRESK is resistant, and the TRESK/TREK1 tandem has an intermediate sensitivity (Royal *et al.*, 2019). TRESK has a unitary conductance of 14 pS, for TRESK/TREK-2 it is about 40 pS, while TREK2 has a high conductance of more than 140 pS (Czirjak *et al.*, 2004; Lengyel *et al.*, 2020). In the absence of other co-expressed K_{2P} subunit, this clear distinction can be used to determine the contribution of the TRESK and TREK2 homomers *versus* the TRESK/TREK2 heteromer in any cells. TRESK channels are constitutively activated by calcineurin, a calcium-dependent protein phosphatase (Czirjak *et al.*, 2004). Application of the Ca^{2+} ionophore ionomycin increases the activity of TRESK, allowing pharmacologists to test TRESK activators and inhibitors on pre-activated currents. Under these conditions, the TRESK/TREK2 heteromer, like TRESK, was activated by cloxyquine and inhibited by A2764, suggesting that the sensitivity of TRESK to these compounds is transmitted to the heteromer. T2A3, a selective activator of TREK2, was also tested on TRESK/TREK2. TREK2 transmits a partial T2A3 sensitivity to the tandem (Lengyel *et al.*, 2020).

In humans, dominant-negative mutations in TRESK are associated with a genetic form of migraine with aura, while TRESK KO mice develop allodynia (Lafreniere *et al.*, 2010; Tulleuda *et al.*, 2011; Zhou *et al.*, 2016; Guo *et al.*, 2019). The frameshift mutations identified in migraine patients result in two non-functional TRESK fragments (MT1 and MT2) with different effects on the TRESK and TREK channels (Royal *et al.*, 2019). Through this new mechanism called frameshift mutation-induced alternative translation initiation (fsATI), the N-ter fragment of TRESK (MT1) exerts a dominant-negative effect on TRESK, while the MT2 fragment inhibits exclusively the TREK1 or TREK2 channels. While further evidence is needed to confirm that TRESK/TREK heteromers are formed in trigeminal neurons and are the major actor in the control of migraine pain, specific pharmacological tools can already be considered to target heteromers more specifically.

Ongoing and future challenges

Five functional intragroup heteromers have been identified (TASK1/TASK3, THIK1/THIK2, TREK1/TREK2, TREK1/TRAAK and TREK2/TRAAK) involving three of six K_{2P} subfamilies (Figure 1). Several intergroup heteromers have also been described, some confirmed by independent studies (TRESK/TREK1/2), and others requiring more investigation as they were found negative by other studies (TWIK1/TREK1 and TWIK1/TASK1/3).

The studies described above reported physical interaction and investigate the functional impact of heteromerization in heterologous expression systems where the amount of expressed proteins far exceeds the endogenous levels in physiological conditions. A difficult task is to study these heteromeric channels *in vivo*. In many cases, there is evidence of cellular co-expression of the subunits but the respective quantities of homomers and heteromers, as well as their respective physiological roles, remain open questions.

The first step toward this goal is to verify that heteromers are formed in native tissues. Some studies have reported proximity or physical associations of K_{2P} subunits using co-IP, co-localization or Duolink® PLA assay (Table 1). However, the relevance of these approaches *in vivo* depends primarily on the specificity of the anti- K_{2P} antibodies used. Anti-TWIK1, anti-TREK1 and anti-TASK antibodies have been validated by knocking down channel expression using shRNA and by showing that the labeling is absent under these conditions. However, it would be more appropriate to use control tissues prepared from KO animals for one subunit or from native tissues expressing only one of the partners. In addition, given the propensity of K_{2P} channels to aggregate, special caution should be taken when interpreting data obtained from Western blot analysis. The generation of heteromer-specific monoclonal antibodies can also be considered by subtractive immunization, although the production of antibodies against K_{2P} channels had already proved difficult. Bivalent ligands, capable of bridging two adjacent subunits, can also be considered to reveal physical proximity and thus heteromerization *in vivo*. Native heteromers will likely to be discovered in the near future, but this will probably require the development of tools to efficiently demonstrate proximity or interaction *in vivo*.

The second step is to study the contribution of the identified heteromers to the endogenous currents. The distinctive properties of the TASK1, TASK3 and TASK1/TASK3 currents were used for the identification of native heteromeric current in specific cell types. In cerebellar granular cells and in hypoglossal motoneurons, 44% and 52% respectively of the total TASK current were attributed to the TASK1/TASK3 heteromers (Berg *et al.*, 2004; Kang *et al.*, 2004). These values are consistent with the theoretical quantity of heterodimers expected if identical quantities of TASK1 and TASK3 subunits were expressed in these cells and if TASK1 had the same affinity for itself as for TASK3 and vice versa. In the nervous system, TASK1/TASK3 current has also been reported to be predominant in facial motoneurons, thalamocortical relay neurons and in hippocampal interneurons (Larkman & Perkins, 2005; Musset *et al.*, 2006; Torborg *et al.*, 2006). In contrast, in glomus cells of the carotid

corpuscle, 75% of a TASK-like conductance was attributed to the TASK1/TASK3 heteromer (Kim *et al.*, 2009). A similar observation was made in human atrial cardiomyocytes expressing TASK1 and TASK1/TASK3 currents, and almost no TASK3 currents (Rinne *et al.*, 2015). A similar study was conducted in the NRs of trigeminal afferent nerves where TREK1, TRAAK and TRESK are co-expressed. The biophysical and pharmacological features of the endogenous leak K^+ current suggest that functional K_{2P} channels are more likely to be TREK1/TRAAK heteromers than TREK1, TRAAK or TREK1/TRESK channels (Kanda *et al.*, 2019).

Another approach is to use KO animals to probe the function of heteromers. Once again, studies on TASK heteromers are the most advanced to date. In cerebellar granule cells (CGC) and in carotid body type-1 cells of the TASK1 or TASK3 KO mice, a current present in WT mice with characteristics of the heteromeric currents was systematically eliminated demonstrating the role of TASK1/TASK3 heteromers in those cells. In cerebellar granule cells, the absence of TASK1 was compensated by an increased contribution of TASK3 (Aller *et al.*, 2005). This compensatory mechanism allows the cells to support high frequency firing even in the absence of TASK1 or TASK1/TASK3 heteromers. This compensatory mechanism is not at work in the TASK3 KO mice suggesting that a significant fraction of the TASK3 subunit functions independently of TASK1 (Brickley *et al.*, 2007). In carotid body type-1 cells, comparison between TASK1 KO, TASK3 KO and TASK1/TASK3 KO shows that TASK1 and TASK1/TASK3 rather than TASK3 play a key role in the control of ventilation by peripheral chemoreceptors (Trapp *et al.*, 2008; Turner & Buckler, 2013). A similar approach was used for TWIK1/TREK1 heteromers but here the results were in contradiction with the contribution of the heteromeric current in hippocampal astrocytes, as no detectable changes in the passive conductance of astrocytes was observed in TWIK1 KO or double TWIK1/TREK1 KO (Du *et al.*, 2016). Conditional KO mice, in which a K_{2P} channel is selectively removed from the cells studied, will provide a better system for assessing tissue-specific function of heteromers by limiting any compensation.

Through the use of tandems, data on the pharmacological properties of heteromers are accumulating (Table 2). The subunits forming the heteromers most often transmit their properties unevenly. This is why the biophysical, pharmacological and regulatory properties of the heteromers must be determined experimentally. The physical constraints introduced by the covalent bonding of the subunits in tandem constructs can influence the pharmacological properties. When using these tools, specific controls should be systematically included, including the expression of homomeric tandems and inverted tandems.

Because K_{2P} channels are implicated in a growing list of diseases (atrial fibrillation, depression, stroke) or pathophysiological conditions (ischemia, hypoxia, pain, development, anesthesia, mood) they represent an important family of therapeutic targets. The identification of K_{2P} channels heteromers has complicated the search of pharmacological drugs by allowing a combinatorial increase in the number of targets, but it could also represent an opportunity for the development of drugs with increased therapeutic efficacy

and reduced side effects. The roles of the TREK/TRESK heteromers in analgesia suggest that the heteromers will be increasingly important as targets for future pain killers. Studies of TASK channels in neuronal population have shown that TASK1 and TASK3 primarily form heteromeric channels at the cell surface. The same phenomenon has been reported in atrial cardiomyocytes, making the TASK channel a drug targets for modulation of brain excitability but also for atrial fibrillation.

Conclusion

The next step in the study of K_{2P} channel assembly is to establish a complete mapping of the homo and heteromers. The complex mosaic of K_{2P} family gene expression in different tissues will partly determine the potential K_{2P} partnerships that may form. Other factors, such as chaperones, may also control assembly and heteromerization of K_{2P} channels in the cellular environment in a temporal and more dynamic manner. The other challenge is to identify the molecular and structural determinants responsible for recognition and assembly. This knowledge will undoubtedly open a new area for the modulation of important physiological functions influenced by the K_{2P} channels.

Abstract Figure

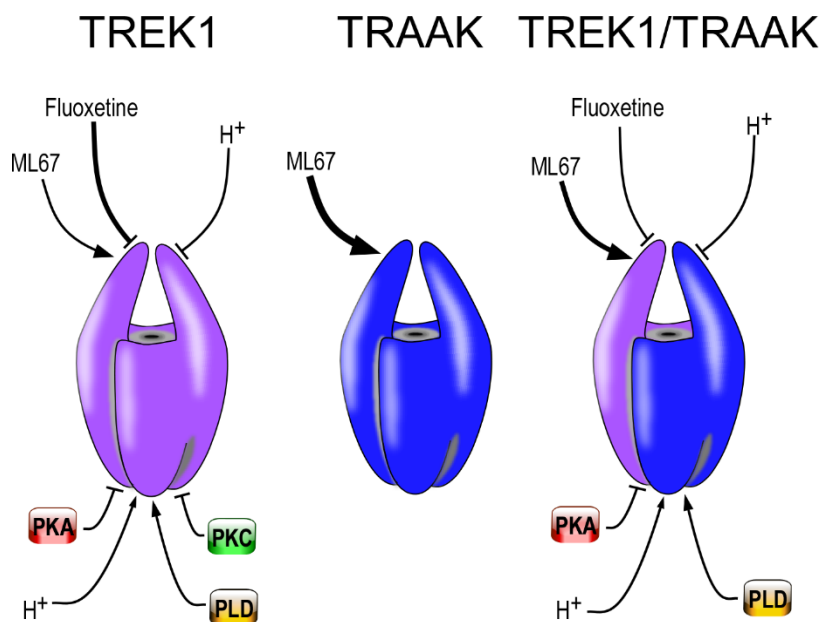


Figure 1: Homodimers and heterodimers in the K_{2P} channel family

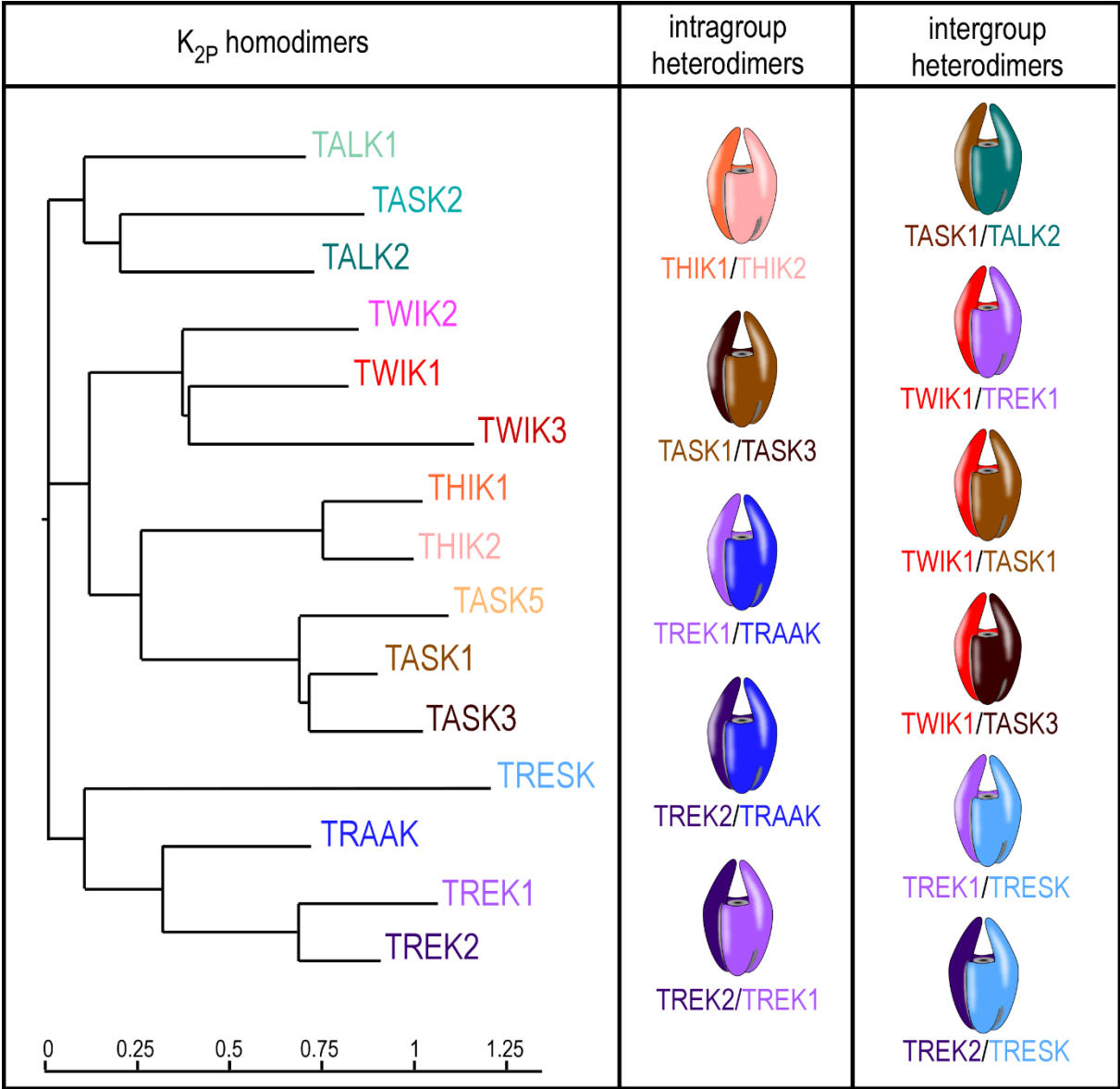


Table 1: Identification of K_{2P} heteromers and potential pathophysiological functions

| K_{2P} Heteromers | Protein interaction identification | Heteromeric current detection | Physiological consequences |
|----------------------------------|---|--|--|
| TASK1/TASK3 | IF-colocalization ¹ , co-IP ² , DN assay ² , SiMPull ³ | cerebellar granule cells, hypoglossal and facial motoneurons, hippocampal interneurons, thalamocortical relay neurons, carotid body glomus cell, atrial cardiomyocytes | cell excitability (anesthesia), neuronal development, cardiac repolarization (atrial fibrillation) |
| THIK1/THIK2 | DN assay ⁴ , PLA ⁴ , FRET ⁴ | ND | ND |
| TREK1/TREK2 | IF-colocalization ¹¹ , co-IP ^{11,12} , FRET ¹¹ , PLA ¹¹ , DN assay ¹¹ , SiMPull ³ , SMcoLM ³ , | dorsal root ganglion neurons | cell excitability (nociception) |
| TREK1/TRAAK | IF-colocalization ¹¹ , co-IP ¹¹ , FRET ¹¹ , PLA ¹¹ , DN assay ¹¹ , SiMPull ³ , SmcoLM ³ , | trigeminal afferent nerves | cell excitability (nerve conduction) |
| TREK2/TRAAK | FRET ¹¹ , PLA ¹¹ , DN assay ¹¹ , SiMPull ³ , SMcoLM ³ , | ND | ND |
| TWIK1/TREK1 | yeast two-hybrid ⁵ , co-IP ⁵ , BiFC ⁵ , PLA ⁵ , IF-colocalization^{5*}, co-IP^{5*}, PLA^{5*} | hippocampal astrocytes (?) | passive conductance (glutamate release) |
| TWIK1/TREK2 | co-IP ⁵ | ND | ND |
| TWIK1/TRAAK | co-IP ⁵ | ND | ND |
| TWIK1/TASK1 | FRET^{6*} , co-IP ⁶ | cerebellar granule neurons | cell excitability (anesthesia) |
| TWIK1/TASK3 | FRET ⁶ , co-IP ⁶ , BiFC ⁷ , PLA^{7*} | dentate gyrus granule cells | neuronal excitability (learning and memory) |
| TALK2/TASK1 | TIRF-colocalization ⁸ , DN assay ⁸ , BiFC ⁸ , FRET ⁸ , co-IP ⁸ | ND | ND |
| TRESK/TREK1 | SiMPull ⁹ , photoswitchable partner ⁹ | trigeminal neurons | neuronal excitability (nociception, migraine) |
| TRESK/TREK2 | SiMPull ⁹ , DN assay ¹⁰ | primary somatosensory neurons | cell excitability (nociception) |

*from native protein (cerebellar granule neurons⁶, astrocytes⁵ or dentate gyrus granule cells⁷)

Abbreviations: IF: immunofluorescence, co-IP: coimmunoprecipitation, DN: dominant negative TIRF: total internal reflection fluorescence, SiMPull: single-molecule pull-down, PLA: *proximity ligation assay*, FRET: *fluorescence resonance energy transfer*, SMcoLM: single-molecule co-localization microscopy, BiFC: bimolecular fluorescence complementation, photoswitchable partner.

Superscripts are follow : (1) (Czirjak & Enyedi, 2002) (2) (Berg *et al.*, 2004) (3) (Levitz *et al.*, 2016) (4) (Blin *et al.*, 2014) (5) (Hwang *et al.*, 2014) (6) (Plant *et al.*, 2012) (7) (Choi *et al.*, 2018) (8) (Suzuki *et al.*, 2017) (9) (Royal *et al.*, 2019) (10) (Lengyel *et al.*, 2020) (11) (Blin *et al.*, 2016) (12) (Lengyel *et al.*, 2016)

Table 2: Biophysical and pharmacological properties of the K_{2P} heteromers

| Parameters/properties | Homomer 1 | Homomer 2 | Tandem 1/2 |
|--|--|---|--|
| Intragroup heteromer | TASK1 | TASK3 | TASK1/TASK3 |
| Unitary conductance (pS) • -100 mV ¹ • -60 mV ² • -80 mV ³ | 20 ± 3 pS [†] 14.2 ± 0.4 pS [†] ND | 122 ± 14 pS [†] 37.8 ± 0.7 pS [†] ND | ~40 pS [†] (T ₃ /T ₁ : ~66 pS) 38.1 ± 0.7 pS [†] (T ₃ /T ₁ : 38.1 ± 0.6 pS) 38 ± 6 pS [†] (T ₃ /T ₁ : 65.7 ± 15.3 pS) |
| External Ca ²⁺ (1 mM) ² | No effect | ↓ 31 ± 1 % [†] | ↓ 32 ± 2 % [†] |
| External Zn ²⁺ (100 μM) ⁴ | No effect | ↓ ~70 % [‡] | ↓ ~20 % [‡] |
| Ruthenium Red • RR (5 μM) ⁵ • RR (5 μM) ² | ↓ 4.5 ± 1.2 % [‡] ↓ 8 ± 7 % [†] | ↓ 77.6 ± 2.3 % [‡] ↓ 78 ± 7 % [‡] | ↓ 4.5 ± 1.5 % [‡] ↓ 3 ± 3 % [†] (T ₃ /T ₁ : ↓ 3 ± 4 %) |
| External acidification • pH 8.3 to pH 7.3 ² (whole-cell) • pH 8.3 to pH 7.3 ² (single-channel) • pH 7.5 to pH 6.5 ⁵ | ↓ ~45 % [†] ↓ 56 ± 8 % [†] ↓ 70 ± 1.9 % [‡] | ↓ ~10 % [†] ↓ 26 ± 4 % [†] ↓ 19.3 ± 1.9 % [‡] | ↓ 25 ± 4 % [†] (T ₃ /T ₁ : ↓ 24 ± 5 %) ↓ 36 ± 2 % [†] (T ₃ /T ₁ : ↓ 35 ± 2 %) ↓ 36.4 ± 2.5 % [‡] |
| External alkalization • pH 7.3 to pH 8.4 ⁶ | ↑ 69.6 ± 2 % [†] | ↑ 9.4 ± 1.7 % [†] | ↑ 48.4 ± 2.4 % [†] |
| Various compounds • angiotensin II (10 nM) ⁵ • A293 (200 nM) ³ • A1899 (40 nM) ³ • TRH (0.2 μM) ⁷ • halothane (0.3 mM) ⁷ • isoflurane (0.8 mM) ⁶ • CHET3 (10 μM) ⁸ | ↓ ~85 % [‡] ↓ 49.3 ± 0.6 % [‡] ↓ 45.8 ± 1.0 % [‡] ↓ 94.2 ± 1.5 % [†] ↑ 52.5 ± 3.6 % [†] ↓ 15 ± 2 % [†] No effect | ↓ ~25 % [‡] ↓ 16.1 ± 0.4 % [‡] ↓ 9.13 ± 0.9 % [‡] ↓ 86.4 ± 1.6 % [†] ↑ 134.6 ± 20 % [†] ↑ 138 ± 25 % [†] ↑ ~4 fold [†] | ↓ ~60 % [‡] ↓ 7.0 ± 1.5 % [‡] (T ₃ /T ₁ : ↓ 44 ± 3.7 %) ↓ 8.6 ± 1.0 % [‡] (T ₃ /T ₁ : ↓ 34.5 ± 2 %) ↓ 82.4 ± 5.4 % [†] ↑ 150.8 ± 12.6 % [†] ↑ 106 ± 7 % [†] ↑ ~2 fold [†] (T ₃ /T ₁) |
| Intragroup heteromer | THIK1 | THIK2 | THIK1/THIK2 |
| Unitary conductance (-100 mV) (pS) ⁹ | ~ 5 pS [†] | 2.5 ± 1.0 pS [†] | 3.5 ± 0.8 pS [†] |
| Intragroup heteromer | TREK1 | TREK2 | TREK1/TREK2 |
| Unitary conductance (pS) • +100 mV ¹⁰ • -100 mV ¹⁰ • +60 mV ¹¹ | 88.5 ± 6.2 pS [†] 55.6 ± 0.6 pS [†] 39.2 ± 2.7 pS [‡] | 39.8 ± 2.8 pS [†] 140.9 ± 2 pS [†] 148.8 ± 4.2 pS [‡] | 71.6 ± 2 pS [†] 78.9 ± 1.3 pS [†] 83.5 ± 5 pS [‡] |
| Rectification (I _{-80mV} /I _{+80mV}) ¹² | 0.68 ± 0.05 [†] | 0.58 ± 0.02 [†] | 0.31 ± 0.05 [†] |
| Internal acidification • pH 7.4 to pH 5.5 ¹² | ↑ 8 ± 3 fold [†] | ↑ 27 ± 5 fold [†] | ↑ 6 ± 0.8 fold [†] |
| External acidification • pH 8.5 to pH 7.4 ¹² • pH 7.4 to pH 6.5 ¹² • pH 8.5 to pH 6.5 ¹¹ | ↓ 71 ± 6 % [†] ↓ (no value) ↓ 49.7 ± 4.3 % [‡] | ↑ (no value) ↑ 49 ± 11 % [†] ↑ 243.3 ± 17.5 % [‡] | ↓ 51 ± 6 % [†] ↑ 34 ± 9 % [†] ↓ 32.7 ± 1.4 % [‡] |
| Ruthenium Red • 30 μM, pH 6.5 ¹¹ • 10 μM, pH 7.4 ¹⁰ | ↓ 9.6 ± 2.3 % [‡] ↑ 11.3 ± 5.6 % [‡] | ↓ 85.9 ± 2.3 % [‡] ↓ 70.4 ± 5 % [‡] | ↓ 49.4 ± 4.3 % [‡] ↑ 7.3 ± 2.6 % [‡] |
| Spadin ¹¹ | ↓ 60 ± 11.2 % [†] | No effect | ↓ 85 ± 12 % [†] |
| Intragroup heteromer | TREK1 | TRAAK | TREK1/TRAAK |
| Unitary conductance (pS) ¹⁰ | | | |

| | | | |
|---|--------------------------------|--------------------------------|-------------------------------|
| • +100 mV | 88.5 ± 6.2 pS [†] | 65.4 ± 3.2 pS [†] | 80.7 ± 1.9 pS [†] |
| • -100 mV | 55.6 ± 0.6 pS [†] | 110.9 ± 1.5 pS [†] | 98.7 ± 3.9 pS [†] |
| Various compounds | | | |
| • PKA activator (forskolin/IBMX) ¹⁰ | ↓ 65.8 ± 7.9% [†] | No effect | ↓ 25.9 ± 3.2% [†] |
| • PKC activator (PMA) ¹⁰ | ↓ 39.8 ± 6.2 % [†] | No effect | ↑ 9.6 ± 3.7 % [†] |
| • ML67 ¹⁰ | ↑ 3.3 ± 0.4 fold [†] | ↑ 11.3 ± 1.4 fold [†] | ↑ 7.8 ± 1 fold [†] |
| • Fluoxetine ¹⁰ | ↓ 53.6 ± 1.3 % [†] | No effect | ↓ 24.4 ± 3.5 % [†] |
| • PLD2 ¹² | ↑ ~4 fold [†] | No effect | ↑ ~4 fold [†] |
| Intergroup heteromer | TWIK1 | TASK3 | TWIK1/TASK3 |
| Neurotensin (10 μM) ¹³ | No effect | ↓ ~60 % [†] | ↓ ~60 % [†] |
| Intergroup heteromer | TALK2 | TASK1 | TALK2/TASK1 |
| External acidification (pK _{1/2}) ¹⁴ | 9.56 ± 0.01 [†] | 7.7 ± 0.1 [†] | 8.8 ± 0.1 [†] |
| Hill coef. | 3.0 ± 0.1 | 2.3 ± 0.3 | 0.83 ± 0.04 |
| Halothane (0.3 mM, pH 9.4) ¹⁴ | | | |
| I/ I _{control} | ↓ ~0.6 [†] | ↑ 1.65 [†] | ↑ 1.43 [†] |
| Intergroup heteromer | TREK1 | TRESK | TREK1/TRESK |
| Arachidonic acid (10 μM) ¹⁵ | ↑ ~5 fold [†] | No effect | ↑ ~2 fold [†] |
| Ionomycin (0.5 μM) ¹⁵ | No effect | ↑ ~2 fold [‡] | ↑ ~3 fold [‡] |
| Intergroup heteromer | TREK2 | TRESK | TREK2/TRESK |
| Unitary conductance (pS) ¹⁶ | | | |
| • basal (+60 mV) | 144.0 ± 33.6 pS [†] | 14.4 ± 2.1 pS [†] | 40.0 ± 13.0 pS [†] |
| • ionomycin (0.5 μM) | ND | 15.1 ± 2.8 pS [‡] | 39.7 ± 1 1.5 pS [‡] |
| • cyclosporin A (1 μM) | ND | 13.8 ± 0.7 pS [‡] | 40.3 ± 15.3 pS [‡] |
| Open probability (+60 mV) ¹⁶ | | | |
| • ionomycin (0.5 μM) | ND | 0.24 ± 0.08 [‡] | 0.146 ± 0.063 [‡] |
| • cyclosporin A (1 μM) | ND | 0.008 ± 0.007 [‡] | 0.03 0.001 [‡] |
| Various compounds ^{16,17} | | | |
| • ionomycin (0.5 μM) | ND | ↑ 6.2 ± 0.4 fold [‡] | ↑ 8.7 ± 3.2 fold [‡] |
| • T2A3 (10 μM) | ↑ 15.5 ± 7.2 fold [‡] | No effect | ↑ 8.2 ± 6.2 fold [‡] |
| • cloxyquin (100 μM) | No effect | ↑ 4.4 ± 0.3 fold [‡] | ↑ 4.8 ± 1.1 fold [‡] |

Abbreviations: RR: ruthenium red, TRH: thyrotropin-releasing hormone, PKA/C: protein kinase A/C, IBMX: 3-isobutyl-1-methylxanthine, PMA: phorbol myristate acetate, PLD2: phospholipase D2.

Superscripts are follow : [†]: mammalian cells (HEK, COS); [‡]: *Xenopus* oocytes; (1) (Musset *et al.*, 2006) (2) (Kang *et al.*, 2004) (3) (Rinne *et al.*, 2015) (4) (Clarke *et al.*, 2004) (5) (Czirjak & Enyedi, 2002) (6) (Berg *et al.*, 2004) (7) (Talley & Bayliss, 2002) (8) (Liao *et al.*, 2019) (9) (Blin *et al.*, 2014) (10) (Blin *et al.*, 2016) (11) (Lengyel *et al.*, 2016) (12) (Levitz *et al.*, 2016) (13) (Choi *et al.*, 2018) (14) (Suzuki *et al.*, 2017) (15) (Royal *et al.*, 2019) (16) (Lengyel *et al.*, 2020) (17) (Lengyel *et al.*, 2017).