19 Two-pore domain potassium channels

Leigh D. Plant and Steve A.N. Goldstein

Contents

19.1 Introduction 261
19.2 Restless membrane potential 262
19.3 K2P potassium currents shape excitability 263
19.4 Fungal two-pore domain K^+ channels 263
19.5 K2P channels in invertebrates pass background currents 264
19.6 Two-pore domain K^+ channel subunits in mammals 264
19.7 Structure of K2P channels 264
19.8 K2P channel families in humans: Normal operation and pathophysiology 266
19.8.1 K2P1, K2P6, and K2P7 channels: SUMO regulation of ion channels 266
19.8.2 K2P2, K2P4, and K2P10 channels: Polymodal rheostats 267
19.8.3 The acid-sensitive channels, K2P3 and K2P9: Control of surface expression 268
19.8.4 K2P5, K2P16, and K2P17: Alkaline-activated channels 269
19.8.5 K2P12 and K2P13 channels 269
19.8.6 K2P18 channels 269
19.9 Future of K2P channel research 269
References 270

19.1 INTRODUCTION

The ability of the central nervous system (CNS) to receive, integrate, and process information is dependent on the operation of a finely tuned orchestra of receptors and ion channels that reside at the surface of excitable cells. Among the ensemble at the neuronal plasma membrane are many types of K^+ channels that act together to control some of the most fundamental determinants of excitable behavior, in particular, the frequency and duration of action potentials and the resting membrane potential (V_M). It is valuable therefore to understand the structural basis for K^+ channel function, the role of K^+ channels in physiology, and the factors that regulate K^+ channel activity in health and disease. In this chapter, we consider the most recently discovered family of mammalian K^+ channels, the predominant contributors to background (leak) K^+ currents, the two-pore domain K^+ (K2P) channels.

Encoded by 15 *kcnk* genes in humans (Figure 19.1a), K2P channels open and close (gate) in response to a variety of stimuli, including neurotransmitters, volatile anesthetics, and changes in pH. K2P channels are recognized by their distinct body plan of four transmembrane domains (TMD) and two-pore (P)-forming loops in each subunit (Figure 19.1b). Another distinction of the K2P channels is that they operate across the physiological voltage range (Figure 19.1c) and therefore influence both the shape of excitable events at depolarized voltages and the stability of V_M at hyperpolarized voltages below the firing threshold for action potentials.

The *kcnk* genes were identified in the late 1990s, advancing the study of background K^+ currents onto a molecular footing. Now, nearly two decades on, a wealth of research and discovery spanning the natural kingdom from yeast to man has propelled K^+ leak currents from insightful predictions by Hodgkin et al. required to model neuronal biophysics (Goldman 1943; Hodgkin et al. 1949) to K2P channels as identified mediators of the resting state from which neuronal action potentials rise and return.

Here, we present an overview of K2P channels and the regulatory pathways that modulate their roles in physiology; by necessity, this is an image in time, because our understanding of the mechanistic basis for their operation and the extent of their responsibilities in vivo are still rapidly expanding. As we consider individual K2P channels, it will become clear that a great deal of work has focused on the nervous system, but this should not mislead the reader; K2P channels are being discovered to be important to normal functions and in disease pathogenesis throughout the body, notably, in other excitable tissues like the heart and skeletal muscle, organs such as the kidney and pancreas, and even in circulating immune cells.

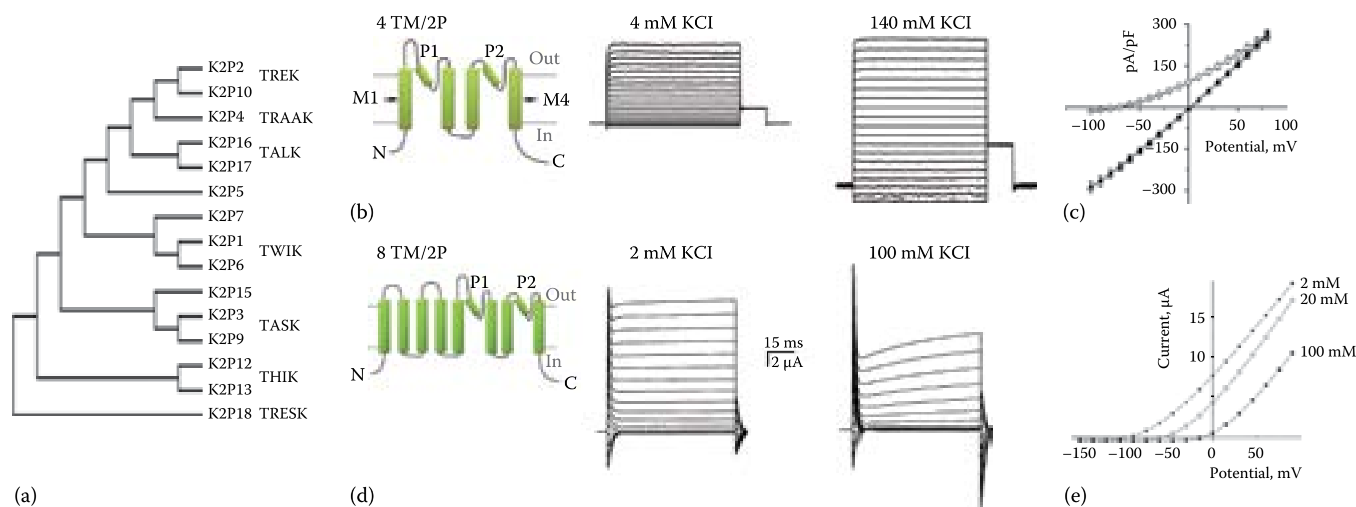

Figure 19.1 Classification and operation of two-P domain channels. (a) A phylogenetic tree calculated to show the relatedness of the 15 K2P subunits found in humans based on ClustalW alignments of the IUPHAR accession numbers for each clone (see http://www.iuphar-db.org/DATABASE/VoltageGatedSubunitListForward). To date, functional expression has not been observed for K2P7, K2P12, and K2P15 (b). *Left*: K2P subunits are integral membrane proteins with internal amino (N) and carboxy (C) termini, four TMDs (M1–M4), and two-pore-forming (P)-loops. *Middle*: Current recorded from a Chinese hamster ovary cell heterologously expressing active K2P1 channels (in whole cell mode with de-SUMOylating enzyme in the pipette). (Adapted from Plant, L.D., *Proc. Natl. Acad. Sci. U.S.A.*, 107, 10743, 2010.) The inside of the cell contains 140 mM K^+ and the external solution contains 4 mM K^+. *Right*: The same cell recorded with 140 mM K^+ on both sides of the membrane. (c). Mean current–voltage relationships for cells studied as in (b). Active K2P1 channels show open (GHK) rectification passing more outward current under quasiphysiologic conditions (Δ, 4 mM external K^+) and a linear current–voltage relationship with symmetrical 140 mM K^+ (▲). (d) *Left*: Fungal two-P domain subunits have eight TMDs. *Middle*: Current recorded from *Xenopus laevis* oocytes expressing TOK1 channels studied in 2 mM (*Middle*) or 100 mM external K^+ (*Right*). (Adapted from Ketchum, K.A. et al., *Nature*, 376, 690, 1995.) (e) Current–voltage relationships for the cell studied in panel D show outward rectification, that is, a shift of the potential where outward current is measured with changes in the external concentrations of K^+ that accord with the Nernst potential for K^+ (E_K, as described in Table 19.1).

19.2 RESTLESS MEMBRANE POTENTIAL

Even when neurons are electrically quiescent, the V_M is never truly at rest. Active transport proteins consume ATP to maintain ionic imbalance across the plasma membrane so that the concentration of Na^+ is low and the concentration of K^+ is high in the cytoplasm relative to the extracellular solution (Table 19.1). These ionic gradients are a repository of electrochemical energy that is harnessed when excitable cells respond to physicochemical stimuli. Thus, depolarization, temperature change, deformation of the surface membrane, and increases in the local concentrations of neurotransmitters are among the many stimuli that activate (or inactivate) ion channels and use the energy stored in the transmembrane ionic gradients to do the work of living cells.

When activation opens a channel conduction pore, large numbers of select ions diffuse down their electrochemical gradient to produce changes in cell behavior. Hence, in neurons, action potentials are triggered when V_M is depolarized to the firing threshold. At threshold, voltage-gated Na^+ (Na_V) channels are activated and this allows Na^+ to rush into the cell. The influx of Na^+ instigates greater depolarization, because additional Na_V channels become active. This rising phase of the action potential is regenerative and explosive. The phase is rapidly followed by fast inactivation of the channels, a process that stops Na^+ flow and allows the neuron to recover (repolarize) in preparation for the next firing event.

Outward flow of K^+ repolarizes the neuron. Thus, voltage-gated K^+ (K_V) channels activate in response to membrane depolarization, like Na_V channels, but with slower kinetics. K_V channels therefore allow the rapid rise of the action potential and then contribute to restoring V_M to resting levels until they inactivate or close with repolarization. Since K2P channels remain open during firing and recovery, net K^+ efflux continues through these pores until the magnitude of negative countercharge inside the cell is sufficient to balance the chemical and electrostatic driving force favoring K^+ outflow.

The V_M where there is no net flux of K^+ through open K^+-selective channels is called the Nernst potential for K^+ (E_K) and is usually around −95 mV in mammalian cells (Table 19.1). Typical Nernst potentials in human cells for Na^+ (E_{Na}), Ca^{2+} (E_{Ca}), and Cl^- (E_{Cl}) are 65, 120, and −90 mV, respectively, values that depend on the concentration of the ion on either side of the membrane and temperature in a manner described by the Nernst equation (Table 19.1). The V_M of cells at rest is determined by the sum of the Nernst potentials for the major ions scaled to reflect their relative permeability across the membrane, as described by the Goldman–Hodgkin–Katz (GHK) equation (Table 19.1). Typically, the resting V_M of mammalian cells is close to E_K, because K^+ channels populate most surface membranes densely and K^+ currents flow via K2P channels across the physiological voltage range.

Because different cells in the body express distinct types of ion channels and because regulators modify the level of expression and operation of ion channels, membrane permeability is dynamic. Thus, the resting V_M of neurons in dorsal root ganglia ranges from ~−45 to −75 mV, depending on the experimental condition (Baccaglini 1978; Puil et al. 1987; Wang et al. 1994; Baumann et al. 1996). In addition to the background K^+ currents

Table 19.1 Concentrations of free ions in mammalian skeletal muscle

ION	EXTRACELLULAR CONCENTRATION (mm)	INTRACELLULAR CONCENTRATION (mm)	$\frac{[Ion]_o}{[Ion]_i}$	EQUILIBRIUM POTENTIAL (mV)
Na^+	145	12	12	67
K^+	4	155	0.026	–98
Ca^{2+}	1.5	10^{-7} M	15,000	129
Cl^-	123	4.2	29	–90

Cellular membrane potential is dependent on the internal and external concentration of ions and the relative permeability of the membrane to each ion (Hille 2001). In many cells, resting V_M is strongly influenced by the magnitude of background K^+ currents. Equilibrium potentials for each ion (*Ex*) are calculated using the Nernst equation:

$$Ex = \frac{RT}{zF} \cdot \ln \frac{[X]_o}{[X]_i},$$

where

R is the Gas constant (1.987 cal K^{-1} mol^{-1})
T is temperature in Kelvin
z is ionic charge
F is Faraday's constant (9.648 × 10^4 C mol^{-1})
$[X]_o$ and $[X]_i$ are the external and internal concentrations of *X*, respectively

At physiological temperature (37°C), *RT*/*zF* is ~27 mV. The GHK equation shows that V_M is determined by the relative concentrations and permeabilities (*P*) of each ion, for example, *X* and *Y*:

$$V_M = \frac{RT}{F} \cdot ln \frac{Px[X]_o + Py[Y]_o}{Px[X]_i + Py[Y]_i}.$$

passed by K2P channels, V_M is also influenced by the activity of other channels that are open at resting potentials. These include K^+ currents passed by inwardly rectifying K^+ channels, for example, in guinea pig ventricular myocytes (Sakmann and Trube 1984), M-currents mediated by $K_V7.2$ and $K_V7.3$ subunits in central and peripheral neurons such as those in frog sympathetic neurons (Brown and Adams 1980) and rat dorsal root ganglia cells (Delmas and Brown 2005), and chloride currents observed in frog (Hodgkin and Horowicz 1959) and rat (Blatz and Magleby 1985) skeletal muscle.

19.3 K2P POTASSIUM CURRENTS SHAPE EXCITABILITY

Whereas the variety of Na_V and Ca_V channels in individual excitable cells is limited, the repertoire of K^+ channels that shape electrical activity is broad. Channels that pass background K^+ currents stabilize cells at hyperpolarized membrane voltages, below the firing threshold (Goldman 1943; Hodgkin et al. 1949). Regulatory stimuli that augment the magnitude of background K^+ currents decrease excitability, because V_M moves closer to E_K and larger depolarizations are required to initiate action potentials. In contrast, inhibition of background K^+ currents depolarizes V_M, allowing smaller stimuli to initiate action potentials. In this manner, background K^+ channels tune not only the magnitude of the stimulation required to initiate an action potential but modify the shape, frequency, and the magnitude of each spike (Hodgkin and Huxley 1952; Jones 1989).

Many distinct stimuli regulate the activity of background K^+ currents, including neurotransmitters (Siegelbaum et al. 1982; Shen et al. 1992; Watkins and Mathie 1996), changes in pO_2 (Buckler 1997; Plant et al. 2002), extracellular pH (Nattel et al. 1981), and pharmaceuticals such as volatile anesthetics (Nicoll and Madison 1982; Plant et al. 2012). Although the physiological significance of background K^+ currents had been appreciated since the 1940s (Goldman 1943; Hodgkin et al. 1949), the question of whether they were mediated by dedicated pathways or accumulated through the operation of other processes and membrane damage was not resolved until the mid-1990s.

19.4 FUNGAL TWO-PORE DOMAIN K^+ CHANNELS

Two-P domain K^+ subunits were identified in the genomes of the budding yeast *Saccharomyces cerevisiae* and nematode *Caenorhabditis elegans* (Ketchum et al. 1995). Each subunit of the yeast channel, called TOK1 (for two-P domain, outwardly rectifying, K^+ channel 1), has two reentrant P-loops and eight TMDs, whereas subunits in the roundworm have two P-loops and four TMDs like their congeners in higher organisms (Figure 19.1). Subunits with two P-loops and four TMDs were described shortly thereafter in *Drosophila melanogaster* (Goldstein et al. 1996) and in mammals (Lesage et al. 1996; Plant et al. 2005).

To date, TOK-like channels with eight TMDs have been identified only in fungi—*S. cerevisiae* (Ketchum et al. 1995), the bread mold *Neurospora crassa* (Roberts 2003), and the opportunistic pathogen *Candida albicans* (Baev et al. 2003). In addition to their unique subunit architecture, TOK channels are novel for passing large outward K^+ currents when the membrane is depolarized above E_K but little or no inward currents when the V_M is below E_K (Ketchum et al. 1995) (Figure 19.1d).

Even though the V_M of *S. cerevisiae* is determined primarily by the transmembrane gradient of protons rather than K^+, an important role for TOK1 in fungal physiology was highlighted by a surprising view into biological competition between yeast populations. Yeast cells infected with RNA killer virus release K1 killer toxin, a peptide that activates TOK1 channels, causing fatal efflux of K^+ from virus-free neighbors yeast (Ahmed et al. 1999). In contrast, virus-positive cells are immune because internal K1 killer toxin blocks TOK1 channels, suppressing the action of external toxin (Sesti et al. 2001). Consistent with these observations, excess K^+ efflux is toxic for yeast cells engineered to overexpress the TOK1 channel (Loukin et al. 1997).

19.5 K2P CHANNELS IN INVERTEBRATES PASS BACKGROUND CURRENTS

Two-P domain K^+ subunits with four TMDs are now called K2P channel subunits. K2PØ, encoded by *kcnkØ*, was cloned from a *D. melanogaster* neuromuscular gene library and shown to exhibit the functional properties expected for background K^+ channels (Goldstein et al. 1996; Zilberberg et al. 2000, 2001; Ilan and Goldstein 2001). At first called dORK channels, to reflect their identification in *D. melanogaster* and operation as openly rectifying and K^+-selective leak pores, both the cloned channels and native background currents show GHK (or open) rectification—that is, the simple property of an ion-selective pore to pass permeant ions more readily across the membrane from the side of higher ion concentration to the side of lower concentration (Figure 19.1). Thus, intracellular K^+ is higher than extracellular and this favors K^+ efflux. Also like native background currents, cloned K2PØ channels expressed in experimental cells open and close with little or no dependence on voltage and time. *D. melanogaster* is now known to have 11 *kcnk* genes (Adams et al. 2000; Littleton and Ganetzky 2000) and K2PØ channels have been described to play a role in the circadian locomotor rhythms of fruit flies (Park and Griffith 2006) as well as in control of their cardiac rhythms by determining the slow diastolic depolarization phase of the heartbeat (Lalevee et al. 2006).

The nematode *C. elegans* has 47 genes that encode K2P subunits (Salkoff et al. 2005; Yook et al. 2012). This remarkable inventory is posited to represent a large number of rapidly evolving, nonconserved channels unique to *C. elegans*. This hypothesis is supported by the observation that there is poor conservation between the genes in *C. elegans* and the closely related roundworm *C. briggsae* (Salkoff et al. 2005). Genetic studies of *C. elegans* have determined that many of the channels are expressed in one neuron, or just a few, and that expression can be dynamic, allowing for fine, responsive control of excitability (Salkoff et al. 2001).

19.6 TWO-PORE DOMAIN K+ CHANNEL SUBUNITS IN MAMMALS

In the decade that followed the identification of two-P domain channels in yeast, worms, and fruit flies, 15 *kcnk* genes for K2P channel subunits were identified in the genomes of human, rat, and mouse (Figure 19.1a). The mammalian channels were initially named according to the biophysical, physiological, or pharmacological attributes of the currents that they passed (Table 19.2). However, discrepant observations in different species and experimental systems encouraged adoption of a formal nomenclature in 2005 (Plant et al. 2005). While proteins encoded by *kcnk* genes are now called K2P subunits (and numbered to match their encoding gene, thus *kcnk1* and K2P1), original descriptive names remain useful in ongoing efforts to correlate cloned channels and the background K^+ currents they mediate in native cells.

K2P channels fall into seven subfamilies based on sequence homology, and the channels in each subfamily are discussed later. To date, no currents have been reported to pass through three of the channels when they are expressed in experimental cells, K2P7, K2P12, and K2P15.

Consistent with studies of native leak currents, the operation of K2P channels is tightly controlled by a plethora of regulators (Goldstein et al. 2001). Regulatory pathways that decrease K2P channel activity by decreasing the density of channels at the cells surface, by blocking the conduction pore, or by reducing current magnitude due to lowered single channel conductance, open probability or altered ion selectivity, serve to increase cellular excitability. Reciprocally, those regulators that increase the activity of K2P channels increase the permeability of the membrane to K^+ and dampen excitability.

19.7 STRUCTURE OF K2P CHANNELS

Mammalian K2P channels have the membrane topology and subunit stoichiometry demonstrated for K2PØ channels: each subunit has two P-loops and four TMDs, the amino- and carboxy-termini reside in the cytoplasm, and channels operate as homodimers (Lopes et al. 2001; Kollewe et al. 2009; Plant et al. 2010) or heterodimers (Berg et al. 2004; Plant et al. 2012).

Initial insights into the 3D structure of K2P channels came from homology models that were based on K^+ channels with known x-ray structures and constraints defined by observations of K2P channels in action. Thus, a model of K2PØ was produced using the crystal structure of the $K_V1.2$ channel and 23 pairs of residues in K2PØ channels that were inferred by serial substitution analysis to interact directly based on second-site electrostatic compensation; the model predicted K2PØ channels to employ two subunits, arranged to form a single pore, with a K^+ selectivity filter that had fourfold symmetry (even though it was lined by the nonhomologous P1 and P2 loops from each subunit), and to have a channel corpus with bilateral symmetry, as expected for a channel complex formed by two subunits (Kollewe et al. 2009).

The strategy used to develop a homology model for K2P3 utilized the crystal structure of an aracheal K^+ channel, K_VAP, and the impact of serial alanine mutagenesis of K2P3 on pore blockade

Table 19.2 **The nomenclature of mammalian K2P channels**

IUPHAR CHANNEL NAME	HUGO GENE NAME	COMMON NAME	OTHER NAMES
K2P1	*kcnk1*	TWIK1	hOHO
K2P2	*kcnk2*	TREK1	TPKC1
K2P3	*kcnk3*	TASK1	TBAK1, OAT1
K2P4	*kcnk4*	TRAAK	KT4
K2P5	*kcnk5*	TASK2	
K2P6	*kcnk6*	TWIK2	TOSS
K2P7	*kcnk7*		*kcnk8*
K2P9	*kcnk9*	TASK3	
K2P10	*kcnk10*	TREK2	
K2P12	*kcnk12*	THIK2	
K2P13	*kcnk13*	THIK1	
K2P15	*kcnk15*	TASK5	*kcnk11*, *kcnk14*
K2P16	*kcnk16*	TALK1	
K2P17	*kcnk17*	TALK2	TASK4
K2P18	*kcnk18*	TRESK1	

K2P channels are named according to IUPHAR nomenclature (see http://www.iuphar-db.org/DATABASE/VoltageGatedSubunitListForward). *HUGO* (Human Genome Organization) designations for each gene are also listed. It is customary for gene names to be in capital letters for human genes (KCNK1), in lower case for rat genes (*kcnk1*), and in italics for mouse genes (*kcnk1*). Common names refer to a biophysical or pharmacological property from an early report.

to infer residues exposed in the ion conduction pathway (Streit et al. 2011). Supporting the predictions of the models of K2PØ and K2P3, the separate studies identified pore-lining residues in common even though the two channels do not share a high degree of sequence homology (Kollewe et al. 2009; Streit et al. 2011).

In 2012, x-ray structures were reported for human K2P1 (Miller and Long 2012) and K2P4 (Brohawn et al. 2012) at ~3.8 Å and appear to show the open state of the channels. As predicted by the homology models, both crystal structures revealed a fourfold symmetric ion conduction pore within a bilaterally symmetric channel corpus. Unexpectedly, the first external loop of each subunit was observed to extend ~35 Å beyond the outer leaflet of the plasma membrane to form a *cap-domain* above the outer mouth of the pore, bifurcating the entry to the K^+ conduction pathway. The cap-domain seen in both K2P1 and K2P4 has not been described in other channels and may explain failure of pore-blocking peptide neurotoxins to act on K2P channels due to limited access.

The crystal structure of K2P1 predicts a *C-helix* that follows directly from the final (M4) TMD and runs close to the intracellular lipid interface in K2P1 (Miller and Long 2012). In K2P4, an analogous helix is modeled as part of the second (M2) TMD (Brohawn et al. 2012). A role for these segments in gating was proposed based on their location below the conduction pore in the structure and previous reports that mutation of residues in the domains modified K2P channel activity.

In 2013, the structure of human K2P4 was obtained at higher resolution by crystallization of the channel in complex with a Fab antibody fragment (Figure 19.2). The new structure at 2.75 Å

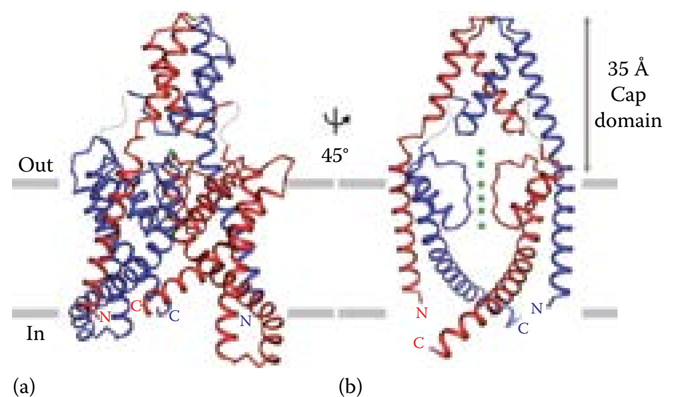

Figure 19.2 A 3D structure of K2P4 channels. (a) A ribbon representation of the x-ray structure of human K2P4 resolved at 2.75 Å. (Adapted from Brohawn, S.G. et al., *Proc. Natl. Acad. Sci. U.S.A.*, 110, 2129, 2013.) Fab fragments used to stabilize the protein have been removed for clarity. The channel is viewed from the membrane plane with one subunit in red and the other in blue, K^+ ions are shown in green, and the boundary of the membrane is in gray. Loops for which structure was not resolved are suggested by dashed lines. (b) A view of the channel rotated by ~45° demonstrating the unique domain swap wherein the outer pore helix interacts with the inner helix from the other subunit rather than its own. Note also the ~35 Å cap-domain above the outer mouth of the pore that bifurcates the entrance to the K^+ conduction pathway.

showed a novel arrangement not apparent at lower resolution: in contrast to other K^+ channels of known structure, each of the four outer pore helices of K2P4 was modeled to interact with the inner helix from the other subunit, an unexpected means for intersubunit communication (Brohawn et al. 2013). In addition,

one of two lateral portals exposing the ion conduction pore to the hydrophobic interior of the lipid bilayer in the low resolution models of both K2P1 and K2P4 appeared in the Fab complex at higher resolution to be occluded.

19.8 K2P CHANNEL FAMILIES IN HUMANS: NORMAL OPERATION AND PATHOPHYSIOLOGY

19.8.1 K2P1, K2P6, AND K2P7 CHANNELS: SUMO REGULATION OF ION CHANNELS

Studies of K2P1 channels have been exciting, at times confusing, and ultimately very informative. Encoded by the *kcnk1* gene, and initially named TWIK1, for tandem of P-domains in a weak inward rectifying K^+ channel (Lesage et al. 1996), other groups were at first unable to observe any currents when the subunits were expressed in a variety of cell types (Goldstein et al. 1998; Pountney et al. 1999). Arguing that *kcnk1* was not a pseudogene but encoded a functional protein product, transcripts were demonstrated at first in the placenta, the lungs, the kidneys (Lesage et al. 1996; Talley et al. 2001), the CNS (Talley et al. 2001), and the cardiac conduction pathway (Gaborit et al. 2007), and later, K2P1 protein was shown in cerebellar granule neurons (Talley et al. 2001; Gaborit et al. 2007; Plant et al. 2012).

The paradox was resolved when a novel mechanism for regulating the electrical activity of ion channels was revealed—K2P1 channels are silenced by the small ubiquitin-like modifier protein (SUMO) pathway (Rajan et al. 2005). Mediated by an enzyme cascade, SUMOylation is the reversible, covalent modification of the ε-amino group of specific lysine(s) in target proteins by the 97 amino acid SUMO protein (Geiss-Friedlander and Melchior 2007). The SUMO pathway, present in all mammalian cells, was first identified to modulate the activity of transcription factors and nuclear import/export (Mahajan et al. 1997). Electrical silencing of K2P1 channels by SUMO was unexpected, because the pathway had not previously been found to operate beyond the nucleus (Wilson and Rosas-Acosta 2005).

Stoichiometric analysis using total internal reflection microscopy revealed that K2P1 channels in tissue culture cells carry two SUMO monomers, one on the Lys^{274} residue in each subunit of the channel and that SUMOylation of only one subunit was sufficient to suppress the function of channels in the plasma membrane (Plant et al. 2010). Thus, K2P1 channels can be activated when de-SUMOylated by SUMO-specific proteases or by mutation of Lys^{274} since only this residue can be SUMOylated.

As expected for a background K^+ current, and like K2PØ channels, K2P1 channels activated by de-SUMOylation pass openly rectifying, K^+ currents (Rajan et al. 2005). The channels are inhibited by external acidification. Thus, when active K2P1 channels are studied in expression systems (Rajan et al. 2005; Plant et al. 2010) or in neuronal cells (Plant et al. 2012), half-block is observed at physiological levels of pH and is sensitive to external K^+ concentration (~pH 6.7 at 4 mM external K^+). Block is due to protonation of a histidine residue in the outer portion of the first P-loop of each subunit, a mechanism described for K2P3 and K2P9 channels (Lopes et al. 2000, 2001). Reciprocally, K2P1, K2P3, and K2P9 channels appear to be less selective for K^+ when external pH is below normal (Ma et al. 2012).

Since 2005, SUMOylation has been shown to modulate the operation of a growing cadre of membrane proteins including transporters (Gibb et al. 2007), G-protein-coupled receptors (Tang et al. 2005), and other ion channels in the CNS (Martin et al. 2007; Plant et al. 2011), the cardiovascular system (Benson et al. 2007; Kruse et al. 2009), and the pancreas (Dai et al. 2009). The mechanism by which SUMOylation silences K2P1 channels remains unknown. The observation that silencing and activation are reversed by enzymes applied to the inside of off-cell patches argues the impact of SUMO, once covalently linked to the channel, does not require other proteins and suggests that SUMO may directly block the pore or interfere with channel gating. Both mechanisms are consistent with the x-ray structure of human K2P1, showing Lys^{274} to be exposed on the intracellular C-helix (Miller and Long 2012), and demonstrate that K2P channels appear to open and close at the K^+ selectivity filter (Zilberberg et al. 2001). What regulates SUMOylation of K2P1 channels is also as yet unknown.

K2P channels have been posited to pass *IKso*, the standing outward, background K^+ current that determines the V_M of cerebellar granule neurons and the response of these CNS cells to changes in pH (Millar et al. 2000), pO_2 (Plant et al. 2002) and volatile anesthetics. Recently, transcripts for K2P1 were observed in individual cerebellar granule neurons, along with mRNAs that encode for K2P3 and K2P9 (Plant et al. 2012). In vivo FRET analysis showed that K2P1, K2P3, and K2P9, but not K2P2, interacted with SUMO in cerebellar granule neurons, although only K2P1 was subject to SUMOylation when the cloned subunits were expressed in tissue culture cells. The findings were reconciled by the demonstration that K2P1 subunits assembled with K2P3 or K2P9 to form heterodimeric channels and that the mixed subunit channels were silenced by a single SUMO monomer on K2P1 Lys^{274} and reactivated by de-SUMOylation (Plant et al. 2012). This also explained why de-SUMOylation augmented *IKso* to decrease neuronal excitability. Because K2P1, K2P3, and K2P9 are expressed together throughout the brain, heart, and somatosensory systems, and the SUMO pathway is ubiquitous, regulation of K2P1 heterodimeric channels is expected to be commonplace.

SUMO regulation of K2P1 heterodimer channels was found to be important in the response of cerebellar granule neurons to volatile anesthetics, including halothane (Plant et al. 2012). Halogenated anesthetics act to hyperpolarize neurons via effects on multiple targets including the ligand-gated ion channel $GABA_A$ (Franks and Lieb 1994) and K^+ leak currents (Nicoll and Madison 1982; Sirois et al. 1998). Roles for K2P channels in the effects of volatile anesthetics implicate K2P3, K2P9 (Sirois et al. 2000; Talley and Bayliss 2002; Lazarenko et al. 2010b), K2P2, K2P10, (Patel et al. 1999), and K2P18 channels (Liu et al. 2004). Halogenated-volatile anesthetic agents such as halothane, isoflurane, and sevoflurane increase the activity of all these K2P channels, thereby decreasing neuronal excitability. In contrast, the activity of K2P13 (also known as THIK1 for two-P domain halothane-inhibited K^+ channel) is suppressed by volatile anesthetics, including halothane. While the effects of volatile anesthetics in humans are believed to be the most pronounced on K2P18 channels, marked species

differences have been reported (Keshavaprasad et al. 2005). Of note, halothane has little impact on homomeric K2P1 channels and increases K2P9 channel currents by just ~30%, but it augments the current through heterodimeric channels formed of K2P1 and K2P9 by ~300% (Plant et al. 2012).

Volatile anesthetics appear to interact with a domain on the proximal C-terminus of K2P2, K2P3 (Patel et al. 1999), and K2P9 channels (Talley and Bayliss 2002). The same portion of these channels is implicated in binding of Gαq signaling molecules, leading to inhibition of channel activity. These observations suggest that volatile anesthetics augment the activity of K2P channels by suppressing the constitutive activity of Gαq proteins (Conway and Cotten 2011).

Recent studies have provided evidence that K2P1 channels contribute to the resting V_M of cardiac myocytes and mediate paradoxical depolarization, a pro-arrhythmic condition observed when the serum concentration of K^+ falls below 3 mM (hypokalemia) (Ma et al. 2011). Hypokalemic depolarization is paradoxical because low levels of serum K^+ are expected to hyperpolarize V_M (Table 19.1). The explanation appears to be that hypokalemia leads K2P1 channels to loosen their specificity for K^+ and to pass a sufficient amount of Na^+ to depolarize the cells. Still to be explained, the phenomenon is observed in cardiac myocytes but not in neurons even though both cell types express the channel.

Although the physiological role of K2P1 has been best explored in cerebellar granule neurons (Plant et al. 2012) and cardiac myocytes (Ma et al. 2011), the channel is also expressed in many other tissues and with reduced mRNA transcript levels in a variety of tumors, including melanoma, glioblastomas, and ovarian cancers (Beitzinger et al. 2008). Transcripts for *kcnk1* have also been detected throughout the renal nephron, leading to the suggestion that K2P1 is involved in K^+ recycling (Cluzeaud et al. 1998). Thus, knockout of the *kcnk1* gene in mice led to hyperpolarization of the principal cells of the cortical collecting duct and altered phosphate handling (Millar et al. 2006). This correlated with altered levels of the sodium–phosphate cotransporter in the proximal tubule and inappropriate internalization of aquaporin 2 in the collecting duct (Nie et al. 2005).

K2P6 and K2P7 show sequence similarity to K2P1 (Figure 19.1) and because they generate small currents on heterologous expression, clarity about their operation and roles has been slow to develop (Chavez et al. 1999; Salinas et al. 1999; Bockenhauer et al. 2000). K2P6 (previously TWIK2) was cloned from mouse where it is expressed in the eye, lung, and stomach (Salinas et al. 1999) and appears to play a role in regulating arterial blood pressure by determining the V_M of vascular smooth muscle cells (Lloyd et al. 2011). Transcript analysis shows that human and rat K2P6 are highly expressed in the aorta, esophagus, stomach, and spleen (Patel et al. 2000) as well as in isolated articular chondrocytes (Clark et al. 2011). Transcripts from aorta were posited to represent K2P6 channels in vascular smooth muscle, because blood vessels were denuded of endothelial cells (Patel et al. 2000).

Both human and rat K2P6 channels pass openly rectifying currents when heterologously expressed in tissue culture cells, although the rat isolate passes K^+-selective currents that are 15 times larger human K2P6 channels and therefore better characterized. Transcript analysis predicts that a splice variant of K2P6 lacking the first TMD is present in both human and rat with a similar expression pattern to the full-length channel (Patel et al. 2000).

K2P7 is expressed in the eye, lung, and stomach (Salinas et al. 1999; Bockenhauer et al. 2000) but does not appear to pass currents on heterologous expression. Sequence analysis predicts that K2P7 contains an EF-hand motif and four potential SH3-binding motifs in the C-terminal domain of each subunit. In common with K2P1, K2P3, K2P9, and K2P15, K2P7 has a histidine residue in the first P-domain (Gly-Tyr-Gly-His), that has been shown to mediate proton block of K2P1, K2P3, and K2P9 channels (Lopes et al. 2000; Rajan et al. 2000; Plant et al. 2010). In contrast, K2P7 is notable for an unconventional sequence in the second P-domain (Gly-Leu-Glu) not found in other human K2P subunits that carry instead Gly-Tyr-Gly, Gly-Leu-Gly, or Gly-Phe-Gly. The presence of a large, negatively charged glutamate residue in the second P-domain, rather than a small glycine, has led some to suggest that K2P7 may not function as a K^+ channel (Enyedi and Czirjak 2010).

19.8.2 K2P2, K2P4, AND K2P10 CHANNELS: POLYMODAL RHEOSTATS

K2P2 (or TREK1 for TWIK-related K^+ channel 1) and K2P4 (or TRAAK for TWIK-related arachidonic acid activated K^+ channel) are widely expressed throughout the CNS (Fink et al. 1996, 1998; Medhurst et al. 2001; Talley et al. 2001; Heurteaux et al. 2006) including in striatal neurons (Lauritzen et al. 2005), hippocampal neurons (Thomas et al. 2008) and cerebellar granule neurons (Plant et al. 2012). Both K2P2 and K2P4 have also been observed in the peripheral nervous system, trafficking along somatosensory neurons (Bearzatto et al. 2000). However, there is disagreement about the expression level of these channels in the periphery. This highlights that linking native background K^+ currents to the activity of specific K2P channels is challenging. In this case, discrepancies may reflect the expression of variants of K2P2 (Thomas et al. 2008) and K2P4 (Ozaita and Vega-Saenzde Miera 2002) that have altered function, native channels with similar phenotypes, such as K2P10 (Bang et al. 2000; Lesage et al. 2000; Gu et al. 2002; Simkin et al. 2008), and/or differences between species, experimental conditions and even among individual neurons.

In addition to wide expression in nervous tissue, transcript for K2P2 has also been observed in both atrial and ventricular myocytes isolated from rat heart (Terrenoire et al. 2001). Expression appears to increase with age (Liu and Saint 2004) and is stronger in subendocardium compared to subepicardium where the protein appears to be arranged in longitudinal stripes across cardiac myocytes (Tan et al. 2004).

The functional properties of neuronal K2P2 channels are subject to a novel type of regulation that leads altered excitability due to Na^+ permeation through the channels. Because the *kcnk2* gene has a weak Kozak initiation sequence, translation can also start at a second codon, to generate K2P2Δ, a channel variant lacking the first 56 residues (Thomas et al. 2008). Thus, alternative translation initiation (ATI) of *kcnk2* mRNA transcripts is a process that is regulated across brain regions over development. Although K2P2Δ channels have a truncated intracellular N-terminus, it is operation of the K^+ conduction pathway that is

altered so that Na^+ ions pass under physiological conditions with a relative permeability of 0.18 compared to K^+, nearly an order of magnitude greater than for full-length channels (0.02) (Thomas et al. 2008). ATI has also been reported for rat *kcnk10* mRNA transcripts studied in experimental cells (Simkin et al. 2008).

The activities of K2P2, K2P4, and K2P10 channels are regulated by a diverse array of lipids including arachidonic acid, neurotransmitter-activated G-protein-coupled receptor pathways, anesthetics, and drugs (Honore 2007; Enyedi and Czirjak 2010). Several studies have reported on modulation of this family of K2P channels by changes in temperature (Maingret et al. 2000; Alloui et al. 2006), and by membrane stretch (Maingret et al. 1999a,b; Brohawn et al. 2012). Because a number of these stimuli often occur simultaneously, K2P2 channels have been posited to act as a polymodal signal integrators (Alloui et al. 2006; Honore 2007).

The temperature sensitivity of K2P2 and K2P4 channels has been reported to contribute to thermal regulation of nociceptive neurons. The activities of these channels increase as body temperature rises above normal (37°C) but falls again when temperature reaches the threshold for noxious heat (>~43°C) (Noel et al. 2009). Thus, increased activity of this group of channels is expected to dampen changes in the excitability of nociceptors up to the threshold for the detection of noxious heat, beyond which decreased background K^+ currents would facilitate excitability.

Background K^+ currents have similarly been implicated in the detection of cold. Temperature decrease to below ~18°C was observed to depolarize a subset of mouse trigeminal ganglion neurons (Viana et al. 2002) and rat dorsal root ganglion cells (Reid and Flonta 2001) by inhibiting a background K^+ conductance. Consistent with this observation, knockout mice engineered to lack the *kcnk2* and *kcnk4* genes are reported to have altered responses to temperature change (Noel et al. 2009). These findings suggest that K2P2 and K2P4 channels contribute as thermal rheostats, tuning the responsiveness of neurons to changes in temperatures. Consistent with this notion, K2P2 and K2P4 channels have been demonstrated to colocalize with various TRP channels in temperature-sensitive neurons (Yamamoto et al. 2009).

The Q_{10} for K2P2 and K2P4 channels increases by approximately sixfold for a 10°C rise. In contrast, the vanilloid family of transient receptor potential channels (TRPVs) has a Q_{10} close to 20. TRPV1 opens in response to noxious heat, while TRPV3 and TRPV4 channels are activated by more moderate, warm temperatures, >~30°C (Peier et al. 2002; Nilius et al. 2007). It remains unclear if temperature modulation of K2P2 and K2P4 channels is a direct effect or due to effects of temperature on second messenger pathways, phosphorylation cascades, or free fatty acid concentrations that act secondarily on the channels to modify their activity.

The operation of K2P2 channels is subject to regulation by protein kinase A–dependent phosphorylation of Ser^{348}. This regulatory change is rapid and notable because it transforms K2P2 channels from openly rectifying K^+ currents in hippocampal cells to currents that manifest voltage dependence (Bockenhauer et al. 2001). Furthermore, it appears that increased intracellular concentrations of phosphatidylinositol-4,5-bisphosphate (PIP2) shift the voltage dependence of K2P2 channels to hyperpolarized potentials, augmenting current magnitude across the physiological voltage range (Chemin et al. 2005; Lopes et al. 2005).

19.8.3 THE ACID-SENSITIVE CHANNELS, K2P3 AND K2P9: CONTROL OF SURFACE EXPRESSION

Originally called TASK1 and TASK3, for TWIK-related, acid-sensitive K^+ channel 1 and 3, K2P3 (Duprat et al. 1997) and K2P9 (Kim et al. 2000; Rajan et al. 2000) pass K^+-selective currents that are blocked by protonation of a histidine residue in the outer mouth of the pore in the P1 loop (Gly-Tyr-Gly-His) (Lopes et al. 2000, 2001), a mechanism of pH regulation shared with K2P1 (Rajan et al. 2005; Plant et al. 2010). Acid-sensitive background K^+ currents in tissues that express K2P1, K2P3, or K2P9 currents appear to be those identified as *IKso* in cerebellar granule neurons (Millar et al. 2000; Plant et al. 2002, 2012).

K2P3 and K2P9 channels are expressed throughout the central and peripheral nervous systems and have been proposed to mediate currents that depolarize neurons during acidification (Talley et al. 2000, 2001; Cooper et al. 2004; Rau et al. 2006; Plant et al. 2012). K2P3 is also expressed in the kidney, in adrenal glomerulosa cells, in the cardiac conduction pathway (Duprat et al. 1997; Gaborit et al. 2007), and in the carotid body; K2P3 channels are posited to depolarize type 1 glomus cells of the carotid body in response to acidosis and hypoxia (Peers 1990; Buckler 1997; Buckler et al. 2000). While the TASKs are not the only channels modulated by pH, changes in the magnitude of background K^+ currents correlate well with the slow kinetics of depolarization in somatosensory neurons in response to acidification. In contrast, other pH-modulated channels, such as the acid-sensing ion channels (ASICs), typically pass transient currents in response to acidification (Gold and Gebhart 2010).

K2P3 and K2P9 subunits are also notable because they form heterodimeric TASK channels (Czirjak and Enyedi 2002) with distinct sensitivities to acidification and pungent stimuli such as hydroxy-α-sanshool, the active ingredient in *Xanthoxylum* Szechuan peppercorns (Bautista et al. 2008). As noted earlier, K2P3 and K2P9 also coassemble with K2P1 subunits to form heterodimeric, SUMO-regulated channels in central neurons (Plant et al. 2012).

Surfaces levels of K2P3 and K2P9 are tightly regulated by the opposing action of signaling motifs that determine retention of the channels in the endoplasmic reticulum (ER) (O'Kelly et al. 2002). Thus, forward trafficking of K2P3 to the cell membrane requires the phosphorylation-dependent binding of the ubiquitous, soluble adapter protein, 14-3-3β. Binding of 14-3-3 suppresses the interaction between K2P3 and βCOP, a vesicular transport protein that promotes retention of the channel in the ER (O'Kelly et al. 2002). βCOP binding is mediated by separate, basic motifs on the N- and C-termini of K2P3 subunits and disruption of either site promotes forward trafficking of the channel to the cell membrane (O'Kelly and Goldstein 2008).

Competitive interaction between 14-3-3 and βCOP also regulates the forward trafficking of other membrane proteins including K2P9 (O'Kelly et al. 2002; Rajan et al. 2002; Zuzarte et al. 2009) and nicotinic acetylcholine receptors (O'Kelly et al. 2002). Forward trafficking of K2P9 was shown to require a di-acidic motif (EDE) located on the proximal C-terminus of the channel (Zuzarte et al. 2007).

Forward trafficking of K2P3 channels is also regulated by the annexin II subunit p11 (Girard et al. 2002; Renigunta et al. 2006;

O'Kelly and Goldstein 2008). The role of p11 in K2P3 channel trafficking appears to be via interaction with 14-3-3 and is specific to only some tissues, such as brain and lung where p11 is expressed prominently. In contrast, p11 is poorly expressed in the heart where expression of K2P3 is high (O'Kelly and Goldstein 2008).

K2P15 shows sequence similarity to K2P3 and K2P9, and *kcnk15* transcripts are identified in the pancreas, liver, lung, ovary, testis, and heart; nonetheless, the operation of the channel and its role in physiology remains unclear because it has thus far failed to pass currents in heterologous expression systems (Ashmole et al. 2001). Functional expression of K2P15 has been postulated to require an as-yet unidentified subunit or regulator.

19.8.4 K2P5, K2P16, AND K2P17: ALKALINE-ACTIVATED CHANNELS

K2P5 (previously TASK2) was initially considered to be a member of the TASK subfamily of K2P channels because it passed background K^+ currents regulated by changes in extracellular pH (Reyes et al. 1998). However, further characterization demonstrated that the channel passed currents only when the extracellular solution exceeded pH 7.5. K2P16 and K2P17 (also known as TALK1 and TALK2, respectively, for TWIK-related alkaline-activated K^+ channel) are also activated by extracellular alkalization above normal levels and, along with K2P5 are expressed at high levels in the pancreas (Decher et al. 2001; Girard et al. 2001). All three channels have been implicated in mediating the apical K^+ conductance that facilitates the secretion of bicarbonate from the epithelial cells in the tubular lumen of the exocrine pancreas (Fong et al. 2003).

K2P5 has been associated with bicarbonate handling in proximal tubule cells and the papillary collecting ducts of the kidney (Warth et al. 2004). Thus, K2P5 knockout mice show impaired bicarbonate reabsorption, metabolic acidosis, hyponatremia, and hypotension. Based on these findings, a role was suggested for K2P5 in renal acidosis syndromes.

Gating of K2P5, K2P16, and K2P17 by alkaline extracellular pH is proposed to be due to the neutralization of basic residues (arginine in K2P5 and K2P16, lysine in K2P17) that reside near the second P-loop and influence properties of the K^+ selectivity filter. A recent study employing concatemers of cloned K2P5 subunits demonstrated that both of these putative pH sensors must be neutralized for dimeric channels to conduct (Niemeyer et al. 2007).

19.8.5 K2P12 AND K2P13 CHANNELS

K2P12 and K2P13 (called THIK2 and THIK1, respectively, for TWIK-related halothane-inhibited K^+ channel 2 and 1) are expressed in the heart, skeletal muscle, and pancreas (Girard et al. 2001; Rajan et al. 2001). In situ hybridization suggests that both channels are expressed also in proximal tubules, thick ascending limbs and cortical collecting ducts of human kidney (Theilig et al. 2008), and in the CNS, including in the retrotrapezoid nucleus (Lazarenko et al. 2010a).

In common with other K2P clones, K2P13 channels pass K^+-selective leak currents in experimental cells. The currents are insensitive to physiological changes in pH, temperature, and free fatty acids. In contrast to many K2P channels, K2P13 currents are inhibited by the volatile halogenated anesthetics, such as halothane with a K_i of 2.8 mM (Rajan et al. 2001). Inhibition of a K2P13-like conductance has been proposed to activate central respiratory chemoreceptor neurons in the retrotrapezoid nucleus, and this mechanism is posited to preserve adequate respiratory motor activity and ventilation during anesthesia (Lazarenko et al. 2010a).

K2P12 clones do not pass currents in experimental systems despite being trafficked to the plasma membrane (Rajan et al. 2001), nor does coexpression of K2P12 appear to impact the magnitude of currents passed by K2P13, suggesting that these channel subunits do not form heterodimers (Rajan et al. 2001).

19.8.6 K2P18 CHANNELS

The final K2P channel identified in the human genome, K2P18 (also called TRESK for TWIK-related, spinal cord K^+ channel), has been found at the transcript level in human spinal cord (Sano et al. 2003), mouse cerebellum (Czirjak et al. 2004) and mouse testis (Kang et al. 2004). In common with other K2P channels, K2P18 channels pass K^+-selective currents and functions as an open rectifier (Keshavaprasad et al. 2005; Kang and Kim 2006; Dobler et al. 2007; Bautista et al. 2008).

Rodent clones of K2P18 are sensitive to external acidification because like K2P1, K2P3, and K2P9 channels, they are inhibited by protonation of a histidine in the first P-loop (pK_a ~6.8) (Dobler et al. 2007). In contrast to the rodent isoform, human K2P18 channels are insensitive to acidification, because the critical histidine residue is instead a tyrosine; the human channels can be endowed with sensitivity by mutation of the tyrosine to histidine (Dobler et al. 2007).

The activity of K2P18 channels is regulated by the intracellular concentration of Ca^{2+}. An increase in cytosolic Ca^{2+} activates the calmodulin-dependent protein phosphatase calcineurin and this leads to dephosphorylation of an intracellular serine (Ser^{264}) precluding the binding of 14-3-3 proteins that are observed to impact the activity of surface channels (Czirjak et al. 2004; Czirjak and Enyedi 2006). Channel activity is inhibited by the binding of the η and γ 14-3-3 but not by the β, ζ, ε, ς, or τ isoforms (Czirjak et al. 2008).

K2P18 is notable for its expression in the spinal cord, trigeminal and dorsal root ganglia, where it has been suggested to pass a significant portion of the background K^+ current that determines the excitability of somatosensory nociceptive fibers. K2P18 channels were further implicated in the normal operation of somatosensory nervous system by the observation that surgical denervation (axiotomy) of peripheral nociceptor neurons produced a decrease of ~50% in *kcnk18* mRNA (Tulleuda et al. 2011).

Formal correlation of K2P18 channels and pain was provided by linkage of a frame-shift mutation (F139WfsX24) in the *kcnk18* gene associated with migraine with aura in a large, multigenerational family (Lafreniere et al. 2010). The F139WfsX24 variant of K2P18 is truncated at 162 residues and appears to act as a dominant-negative subunit to suppress the function of wild type K2P18 channels.

19.9 FUTURE OF K2P CHANNEL RESEARCH

Over the last two decades, identification and study of the channels passing background K^+ currents has advanced our understanding of their roles in physiology and disease. K2P channels are now

appreciated to operate in all phases of excitability, establishing resting V_M in many types of cells and influencing the rise to activation threshold, the shape of action potentials, the rate of recovery from firing (Plant 2012), and even the slow after-hyperpolarizations observed in developing starburst amacrine cells (Ford et al. 2013). The channels are now recognized to respond to anesthetics, changes in pH, temperature, membrane stretch, and regulatory pathways that include SUMO, kinases and phosphatases, GPCRs, lipids, and those that mediate trafficking (Plant et al. 2005; Honore 2007; Enyedi and Czirjak 2010). K2P channels are now recognized to have roles in clinical disorders including cardiac arrhythmia associated with hypokalemia (K2P1) (Ma et al. 2011), oncogenesis (K2P9) (Mu et al. 2003), and pain syndromes (K2P18) (Lafreniere et al. 2010). It is clear that we are just beginning to appreciate how and where K2P channels operate and how this varies at different times in different tissues. In some cases, we have yet to discover what the channels do even though they are synthesized and expressed at the plasma membrane. We have much to learn. Nonetheless, K2P channels are already understood to calm, control, and shape electrical activity, and this offers them, despite their recent addition to the family of K^+ channels, as attractive, tissue-specific targets for pharmaceutical therapy.

REFERENCES

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, S. E. Scherer et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287 (5461):2185–2195.

Ahmed, A., F. Sesti, N. Ilan, T. M. Shih, S. L. Sturley, and S. A. Goldstein. 1999. A molecular target for viral killer toxin: TOK1 potassium channels. *Cell* 99 (3):283–291.

Alloui, A., K. Zimmermann, J. Mamet, F. Duprat, J. Noel, J. Chemin, N. Guy et al. 2006. TREK-1, a K^+ channel involved in polymodal pain perception. *EMBO J* 25 (11):2368–2376.

Ashmole, I., P. A. Goodwin, and P. R. Stanfield. 2001. TASK-5, a novel member of the tandem pore K^+ channel family. *Pflugers Arch* 442 (6):828–833.

Baccaglini, P. I. 1978. Action potentials of embryonic dorsal root ganglion neurones in *Xenopus tadpoles*. *J Physiol* 283:585–604.

Baev, D., A. Rivetta, X. S. Li, S. Vylkova, E. Bashi, C. L. Slayman, and M. Edgerton. 2003. Killing of *Candida albicans* by human salivary histatin 5 is modulated, but not determined, by the potassium channel TOK1. *Infect Immun* 71 (6):3251–3260.

Bang, H., Y. Kim, and D. Kim. 2000. TREK-2, a new member of the mechanosensitive tandem-pore K^+ channel family. *J Biol Chem* 275 (23):17412–17419.

Baumann, T. K., K. J. Burchiel, S. L. Ingram, and M. E. Martenson. 1996. Responses of adult human dorsal root ganglion neurons in culture to capsaicin and low pH. *Pain* 65 (1):31–38.

Bautista, D. M., Y. M. Sigal, A. D. Milstein, J. L. Garrison, J. A. Zorn, P. R. Tsuruda, R. A. Nicoll, and D. Julius. 2008. Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. *Nat Neurosci* 11 (7):772–779.

Bearzatto, B., F. Lesage, R. Reyes, M. Lazdunski, and P. M. Laduron. 2000. Axonal transport of TREK and TRAAK potassium channels in rat sciatic nerves. *Neuroreport* 11 (5):927–930.

Beitzinger, M., L. Hofmann, C. Oswald, R. Beinoraviciute-Kellner, M. Sauer, H. Griesmann, A. C. Bretz, C. Burek, A. Rosenwald, and T. Stiewe. 2008. p73 poses a barrier to malignant transformation by limiting anchorage-independent growth. *EMBO J* 27 (5):792–803.

Benson, M. D., Q. J. Li, K. Kieckhafer, D. Dudek, M. R. Whorton, R. K. Sunahara, J. A. Iniguez-Lluhi, and J. R. Martens. 2007. SUMO modification regulates inactivation of the voltage-gated potassium channel Kv1.5. *Proc Natl Acad Sci USA* 104 (6):1805–1810.

Berg, A. P., E. M. Talley, J. P. Manger, and D. A. Bayliss. 2004. Motoneurons express heteromeric TWIK-related acid-sensitive K^+ (TASK) channels containing TASK-1 (KCNK3) and TASK-3 (KCNK9) subunits. *J Neurosci* 24 (30):6693–6702.

Blatz, A. L. and K. L. Magleby. 1985. Single chloride-selective channels active at resting membrane potentials in cultured rat skeletal muscle. *Biophys J* 47 (1):119–123.

Bockenhauer, D., M. A. Nimmakayalu, D. C. Ward, S. A. Goldstein, and P. G. Gallagher. 2000. Genomic organization and chromosomal localization of the murine 2 P domain potassium channel gene Kcnk8: Conservation of gene structure in 2 P domain potassium channels. *Gene* 261 (2):365–372.

Bockenhauer, D., N. Zilberberg, and S. A. Goldstein. 2001. KCNK2: Reversible conversion of a hippocampal potassium leak into a voltage-dependent channel. *Nat Neurosci* 4 (5):486–491.

Brohawn, S. G., E. B. Campbell, and R. Mackinnon. 2013. Domain-swapped chain connectivity and gated membrane access in a Fab-mediated crystal of the human TRAAK K^+ channel. *Proc Natl Acad Sci USA* 110 (6):2129–2134.

Brohawn, S. G., J. del Marmol, and R. MacKinnon. 2012. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K^+ ion channel. *Science* 335 (6067):436–441.

Brown, D. A. and P. R. Adams. 1980. Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature* 283 (5748):673–676.

Buckler, K. J. 1997. A novel oxygen-sensitive potassium current in rat carotid body type I cells. *J Physiol* 498 (Pt 3):649–662.

Buckler, K. J., B. A. Williams, and E. Honore. 2000. An oxygen-sensitive TASK-like potassium channel in rat carotid body type-1 cells. *J Physiol-Lond* 525:66pp.

Chavez, R. A., A. T. Gray, B. B. Zhao, C. H. Kindler, M. J. Mazurek, Y. Mehta, J. R. Forsayeth, and C. S. Yost. 1999. TWIK-2, a new weak inward rectifying member of the tandem pore domain potassium channel family. *J Biol Chem* 274 (12):7887–7892.

Chemin, J., A. J. Patel, F. Duprat, I. Lauritzen, M. Lazdunski, and E. Honore. 2005. A phospholipid sensor controls mechanogating of the K^+ channel TREK-1. *EMBO J* 24 (1):44–53.

Clark, R. B., C. Kondo, D. D. Belke, and W. R. Giles. 2011. Two-pore domain K(+) channels regulate membrane potential of isolated human articular chondrocytes. *J Physiol* 589 (Pt 21):5071–5089.

Cluzeaud, F., R. Reyes, B. Escoubet, M. Fay, M. Lazdunski, J. P. Bonvalet, F. Lesage, and N. Farman. 1998. Expression of TWIK-1, a novel weakly inward rectifying potassium channel in rat kidney. *Am J Physiol* 275 (6 Pt 1):C1602–C1609.

Conway, K. E. and J. F. Cotten. 2011. Covalent modification of a volatile anesthetic regulatory site activates TASK-3 (KCNK9) tandem pore potassium channels. *Mol Pharmacol* 81 (3):393–400.

Cooper, B. Y., R. D. Johnson, and K. K. Rau. 2004. Characterization and function of twik-related acid sensing K^+ channels in a rat nociceptive cell. *Neuroscience* 129 (1):209–224.

Czirjak, B., Z. E. Toth, and P. Enyedi. 2004. The two-pore domain K^+ channel, TRESK, is activated by the cytoplasmic calcium signal through calcineurin. *J Biol Chem* 279 (18):18550–18558.

Czirjak, G. and P. Enyedi. 2002. Formation of functional heterodimers between the TASK-1 and TASK-3 two-pore domain potassium channel subunits. *J Biol Chem* 277 (7):5426–5432.

Czirjak, G. and P. Enyedi. 2006. Targeting of calcineurin to an NFAT-like docking site is required for the calcium-dependent activation of the background K(+)channel, TRESK. *J Biol Chem* 281 (21):14677–14682.

Czirjak, G., D. Vuity, and P. Enyedi. 2008. Phosphorylation-dependent binding of 14-3-3 proteins controls TRESK regulation. *J Biol Chem* 283 (23):15672–15680.

Dai, X. Q., J. Kolic, P. Marchi, S. Sipione, and P. E. Macdonald. 2009. SUMOylation regulates Kv2.1 and modulates pancreatic beta-cell excitability. *J Cell Sci* 122 (Pt 6):775–779.

Decher, N., M. Maier, W. Dittrich, J. Gassenhuber, A. Bruggemann, A. E. Busch, and K. Steinmeyer. 2001. Characterization of TASK-4, a novel member of the pH-sensitive, two-pore domain potassium channel family. *FEBS Lett* 492 (1–2):84–89.

Delmas, P. and D. A. Brown. 2005. Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat Rev Neurosci* 6 (11):850–862.

Dobler, T., A. Springauf, S. Tovornik, M. Weber, A. Schmitt, R. Sedlmeier, E. Wischmeyer, and F. Doring. 2007. TRESK two-pore-domain K(+) channels constitute a significant component of background potassium currents in murine dorsal root ganglion neurones. *J Physiol-Lond* 585 (3):867–879.

Duprat, F., F. Lesage, M. Fink, R. Reyes, C. Heurteaux, and M. Lazdunski. 1997. TASK, a human background K^+ channel to sense external pH variations near physiological pH. *EMBO J* 16 (17):5464–5471.

Enyedi, P. and G. Czirjak. 2010. Molecular background of leak K^+ currents: Two-pore domain potassium channels. *Physiol Rev* 90 (2):559–605.

Fink, M., F. Duprat, F. Lesage, R. Reyes, G. Romey, C. Heurteaux, and M. Lazdunski. 1996. Cloning, functional expression and brain localization of a novel unconventional outward rectifier K^+ channel. *EMBO J* 15 (24):6854–6862.

Fink, M., F. Lesage, F. Duprat, C. Heurteaux, R. Reyes, M. Fosset, and M. Lazdunski. 1998. A neuronal two P domain K^+ channel stimulated by arachidonic acid and polyunsaturated fatty acids. *EMBO J* 17 (12):3297–3308.

Fong, P., B. E. Argent, W. B. Guggino, and M. A. Gray. 2003. Characterization of vectorial chloride transport pathways in the human pancreatic duct adenocarcinoma cell line HPAF. *Am J Physiol Cell Physiol* 285 (2):C433–C445.

Ford, K. J., D. Arroyo, J. Kay, E. E. Lloyd, R. M. Bryan, Jr., J. Sanes, and M. B. Feller. 2013. A role for TREK1 in generating the slow after hyperpolarization in developing starburst amacrine cells. *J Neurophysiol* 109 (9):2250–2259.

Franks, N. P. and W. R. Lieb. 1994. Molecular and cellular mechanisms of general anaesthesia. *Nature* 367 (6464):607–614.

Gaborit, N., S. Le Bouter, V. Szuts, A. Varro, D. Escande, S. Nattel, and S. Demolombe. 2007. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J Physiol* 582 (Pt 2):675–693.

Geiss-Friedlander, R. and F. Melchior. 2007. Concepts in sumoylation: A decade on. *Nat Rev Mol Cell Biol* 8 (12):947–956.

Gibb, S. L., W. Boston-Howes, Z. S. Lavina, S. Gustincich, R. H. Brown, Jr., P. Pasinelli, and D. Trotti. 2007. A caspase-3-cleaved fragment of the glial glutamate transporter EAAT2 is sumoylated and targeted to promyelocytic leukemia nuclear bodies in mutant SOD1-linked amyotrophic lateral sclerosis. *J Biol Chem* 282 (44):32480–32490.

Girard, C., F. Duprat, C. Terrenoire, N. Tinel, M. Fosset, G. Romey, M. Lazdunski, and F. Lesage. 2001. Genomic and functional characteristics of novel human pancreatic 2P domain K(+) channels. *Biochem Biophys Res Commun* 282 (1):249–256.

Girard, C., N. Tinel, C. Terrenoire, G. Romey, M. Lazdunski, and M. Borsotto. 2002. p11, an annexin II subunit, an auxiliary protein associated with the background K^+ channel, TASK-1. *EMBO J* 21 (17):4439–4448.

Gold, M. S. and G. F. Gebhart. 2010. Nociceptor sensitization in pain pathogenesis. *Nat Med* 16 (11):1248–1257.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J Gen Physiol* 27 (1):37–60.

Goldstein, S. A., D. Bockenhauer, I. O'Kelly, and N. Zilberberg. 2001. Potassium leak channels and the KCNK family of two-P-domain subunits. *Nat Rev Neurosci* 2 (3):175–184.

Goldstein, S. A., L. A. Price, D. N. Rosenthal, and M. H. Pausch. 1996. ORK1, a potassium-selective leak channel with two pore domains cloned from *Drosophila melanogaster* by expression in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 93 (23):13256–13261.

Goldstein, S. A., K. W. Wang, N. Ilan, and M. H. Pausch. 1998. Sequence and function of the two P domain potassium channels: Implications of an emerging superfamily. *J Mol Med (Berl)* 76 (1):13–20.

Gu, W., G. Schlichthorl, J. R. Hirsch, H. Engels, C. Karschin, A. Karschin, C. Derst, O. K. Steinlein, and J. Daut. 2002. Expression pattern and functional characteristics of two novel splice variants of the two-pore-domain potassium channel TREK-2. *J Physiol* 539 (Pt 3):657–668.

Heurteaux, C., G. Lucas, N. Guy, M. El Yacoubi, S. Thummler, X. D. Peng, F. Noble et al. 2006. Deletion of the background potassium channel TREK-1 results in a depression-resistant phenotype. *Nat Neurosci* 9 (9):1134–1141.

Hille, B. 2001. *Ion Channels of Excitable Membranes*. 3rd ed. Sunderland, MA: Sinauer.

Hodgkin, A. L. and P. Horowicz. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J Physiol* 148:127–160.

Hodgkin, A. L. and A. F. Huxley. 1952. The components of membrane conductance in the giant axon of *Loligo*. *J Physiol-Lond* 116 (4):473–496.

Hodgkin, A. L., A. F. Huxley, and B. Katz. 1949. Ionic currents underlying activity in the giant axon of the *Squid*. *Arch Des Sci Physiologiq* 3 (2):129–150.

Honore, E. 2007. The neuronal background K2P channels: Focus on TREK1. *Nat Rev Neurosci* 8 (4):251–261.

Ilan, N. and S. A. Goldstein. 2001. Kcnko: Single, cloned potassium leak channels are multi-ion pores. *Biophys J* 80 (1):241–253.

Jones, S. W. 1989. On the resting potential of isolated frog sympathetic neurons. *Neuron* 3 (2):153–161.

Kang, D. and D. Kim. 2006. TREK-2 (K2P10.1) and TRESK (K2P18.1) are major background K^+ channels in dorsal root ganglion neurons. *Am J Physiol Cell Physiol* 291 (1):C138–C146.

Kang, D., E. Mariash, and D. Kim. 2004. Functional expression of TRESK-2, a new member of the tandem-pore K^+ channel family. *J Biol Chem* 279 (27):28063–28070.

Keshavaprasad, B., C. Liu, J. D. Au, C. H. Kindler, J. F. Cotten, and C. S. Yost. 2005. Species-specific differences in response to anesthetics and other modulators by the K2P channel TRESK. *Anesth Analg* 101 (4):1042–1049, table of contents.

Ketchum, K. A., W. J. Joiner, A. J. Sellers, L. K. Kaczmarek, and S. A. Goldstein. 1995. A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. *Nature* 376 (6542):690–695.

Kim, Y., H. Bang, and D. Kim. 2000. TASK-3, a new member of the tandem pore K(+) channel family. *J Biol Chem* 275 (13):9340–9347.

Kollewe, A., A. Y. Lau, A. Sullivan, B. Roux, and S. A. Goldstein. 2009. A structural model for K2P potassium channels based on 23 pairs of interacting sites and continuum electrostatics. *J Gen Physiol* 134 (1):53–68.

Kruse, M., E. Schulze-Bahr, V. Corfield, A. Beckmann, B. Stallmeyer, G. Kurtbay, I. Ohmert, P. Brink, and O. Pongs. 2009. Impaired endocytosis of the ion channel TRPM4 is associated with human progressive familial heart block type I. *J Clin Invest* 119 (9):2737–2744.

Lafreniere, R. G., M. Z. Cader, J. F. Poulin, I. Andres-Enguix, M. Simoneau, N. Gupta, K. Boisvert et al. 2010. A dominant-negative mutation in the TRESK potassium channel is linked to familial migraine with aura. *Nat Med* 16 (10):1157–U1501.

Lalevee, N., B. Monier, S. Senatore, L. Perrin, and M. Semeriva. 2006. Control of cardiac rhythm by ORK1, a *Drosophila* two-pore domain potassium channel. *Curr Biol* 16 (15):1502–1508.

Lauritzen, I., J. Chemin, E. Honore, M. Jodar, N. Guy, M. Lazdunski, and A. Jane Patel. 2005. Cross-talk between the mechano-gated K2P channel TREK-1 and the actin cytoskeleton. *EMBO Rep* 6 (7):642–648.

Lazarenko, R. M., M. G. Fortuna, Y. Shi, D. K. Mulkey, A. C. Takakura, T. S. Moreira, P. G. Guyenet, and D. A. Bayliss. 2010a. Anesthetic activation of central respiratory chemoreceptor neurons involves inhibition of a THIK-1-like background K(+) current. *J Neurosci* 30 (27):9324–9334.

Lazarenko, R. M., S. C. Willcox, S. Shu, A. P. Berg, V. Jevtovic-Todorovic, E. M. Talley, X. Chen, and D. A. Bayliss. 2010b. Motoneuronal TASK channels contribute to immobilizing effects of inhalational general anesthetics. *J Neurosci* 30 (22):7691–7704.

Lesage, F., E. Guillemare, M. Fink, F. Duprat, M. Lazdunski, G. Romey, and J. Barhanin. 1996. TWIK-1, a ubiquitous human weakly inward rectifying K^+ channel with a novel structure. *EMBO J* 15 (5):1004–1011.

Lesage, F., C. Terrenoire, G. Romey, and M. Lazdunski. 2000. Human TREK2, a 2P domain mechano-sensitive K^+ channel with multiple regulations by polyunsaturated fatty acids, lysophospholipids, and Gs, Gi, and Gq protein-coupled receptors. *J Biol Chem* 275 (37):28398–28405.

Littleton, J. T. and B. Ganetzky. 2000. Ion channels and synaptic organization: Analysis of the *Drosophila* genome. *Neuron* 26 (1):35–43.

Liu, C., J. D. Au, H. L. Zou, J. F. Cotten, and C. S. Yost. 2004. Potent activation of the human tandem pore domain K channel TRESK with clinical concentrations of volatile anesthetics. *Anesth Analg* 99 (6):1715–1722, table of contents.

Liu, W. and D. A. Saint. 2004. Heterogeneous expression of tandem-pore K^+ channel genes in adult and embryonic rat heart quantified by real-time polymerase chain reaction. *Clin Exp Pharmacol Physiol* 31 (3):174–178.

Lloyd, E. E., R. F. Crossland, S. C. Phillips, S. P. Marrelli, A. K. Reddy, G. E. Taffet, C. J. Hartley, and R. M. Bryan, Jr. 2011. Disruption of K(2P)6.1 produces vascular dysfunction and hypertension in mice. *Hypertension* 58 (4):672–678.

Lopes, C. M., P. G. Gallagher, M. E. Buck, M. H. Butler, and S. A. Goldstein. 2000. Proton block and voltage gating are potassium-dependent in the cardiac leak channel Kcnk3. *J Biol Chem* 275 (22):16969–16978.

Lopes, C. M., T. Rohacs, G. Czirjak, T. Balla, P. Enyedi, and D. E. Logothetis. 2005. PIP2 hydrolysis underlies agonist-induced inhibition and regulates voltage gating of two-pore domain K^+ channels. *J Physiol* 564 (Pt 1):117–129.

Lopes, C. M., N. Zilberberg, and S. A. Goldstein. 2001. Block of Kcnk3 by protons. Evidence that 2-P-domain potassium channel subunits function as homodimers. *J Biol Chem* 276 (27):24449–24452.

Loukin, S. H., B. Vaillant, X. L. Zhou, E. P. Spalding, C. Kung, and Y. Saimi. 1997. Random mutagenesis reveals a region important for gating of the yeast K^+ channel Ykc1. *EMBO J* 16 (16):4817–4825.

Ma, L., X. Zhang, and H. Chen. 2011. TWIK-1 two-pore domain potassium channels change ion selectivity and conduct inward leak sodium currents in hypokalemia. *Sci Signal* 4 (176):ra37.

Ma, L., X. Zhang, M. Zhou, and H. Chen. 2012. Acid-sensitive TWIK and TASK two-pore domain potassium channels change ion selectivity and become permeable to sodium in extracellular acidification. *J Biol Chem* 287 (44):37145–37153.

Mahajan, R., C. Delphin, T. Guan, L. Gerace, and F. Melchior. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 88 (1):97–107.

Maingret, F., M. Fosset, F. Lesage, M. Lazdunski, and E. Honore. 1999a. TRAAK is a mammalian neuronal mechano-gated K^+ channel. *J Biol Chem* 274 (3):1381–1387.

Maingret, F., I. Lauritzen, A. J. Patel, C. Heurteaux, R. Reyes, F. Lesage, M. Lazdunski, and E. Honore. 2000. TREK-1 is a heat-activated background K(+) channel. *EMBO J* 19 (11):2483–2491.

Maingret, F., A. J. Patel, F. Lesage, M. Lazdunski, and E. Honore. 1999b. Mechano- or acid stimulation, two interactive modes of activation of the TREK-1 potassium channel. *J Biol Chem* 274 (38):26691–26696.

Martin, S., A. Nishimune, J. R. Mellor, and J. M. Henley. 2007. SUMOylation regulates kainate-receptor-mediated synaptic transmission. *Nature* 447 (7142):321–325.

Medhurst, A. D., G. Rennie, C. G. Chapman, H. Meadows, M. D. Duckworth, R. E. Kelsell, Gloger, II, and M. N. Pangalos. 2001. Distribution analysis of human two pore domain potassium channels in tissues of the central nervous system and periphery. *Brain Res Mol Brain Res* 86 (1–2):101–114.

Millar, J. A., L. Barratt, A. P. Southan, K. M. Page, R. E. Fyffe, B. Robertson, and A. Mathie. 2000. A functional role for the two-pore domain potassium channel TASK-1 in cerebellar granule neurons. *Proc Natl Acad Sci USA* 97 (7):3614–3618.

Millar, I. D., H. C. Taylor, G. J. Cooper, J. D. Kibble, J. Barhanin, and L. Robson. 2006. Adaptive downregulation of a quinidine-sensitive cation conductance in renal principal cells of TWIK-1 knockout mice. *Pflugers Arch* 453 (1):107–116.

Miller, A. N. and S. B. Long. 2012. Crystal structure of the human two-pore domain potassium channel K2P1. *Science* 335 (6067):432–436.

Mu, D., L. Chen, X. Zhang, L. H. See, C. M. Koch, C. Yen, J. J. Tong et al. 2003. Genomic amplification and oncogenic properties of the KCNK9 potassium channel gene. *Cancer Cell* 3 (3):297–302.

Nattel, S., V. Elharrar, D. P. Zipes, and J. C. Bailey. 1981. pH-dependent electrophysiological effects of quinidine and lidocaine on canine cardiac purkinje fibers. *Circ Res* 48 (1):55–61.

Nicoll, R. A. and D. V. Madison. 1982. General anesthetics hyperpolarize neurons in the vertebrate central nervous system. *Science* 217 (4564):1055–1057.

Nie, X., I. Arrighi, B. Kaissling, I. Pfaff, J. Mann, J. Barhanin, and V. Vallon. 2005. Expression and insights on function of potassium channel TWIK-1 in mouse kidney. *Pflugers Arch* 451 (3):479–488.

Niemeyer, M. I., F. D. Gonzalez-Nilo, L. Zuniga, W. Gonzalez, L. P. Cid, and F. V. Sepulveda. 2007. Neutralization of a single arginine residue gates open a two-pore domain, alkali-activated K^+ channel. *Proc Natl Acad Sci USA* 104 (2):666–671.

Nilius, B., G. Owsianik, T. Voets, and J. A. Peters. 2007. Transient receptor potential cation channels in disease. *Physiol Rev* 87 (1):165–217.

Noel, J., K. Zimmermann, J. Busserolles, E. Deval, A. Alloui, S. Diochot, N. Guy, M. Borsotto, P. Reeh, A. Eschalier, and M. Lazdunski, 2009. The mechano-activated K^+ channels TRAAK and TREK-1 control both warm and cold perception. *EMBO J* 28 (9):1308–1318.

O'Kelly, I., M. H. Butler, N. Zilberberg, and S. A. Goldstein. 2002. Forward transport. 14-3-3 binding overcomes retention in endoplasmic reticulum by dibasic signals. *Cell* 111 (4):577–588.

O'Kelly, I. and S. A. Goldstein. 2008. Forward transport of K2p3.1: Mediation by 14-3-3 and COPI, modulation by p11. *Traffic* 9 (1):72–78.

Ozaita, A. and E. Vega-Saenz de Miera. 2002. Cloning of two transcripts, HKT4.1a and HKT4.1b, from the human two-pore K^+ channel gene KCNK4. Chromosomal localization, tissue distribution and functional expression. *Brain Res Mol Brain Res* 102 (1–2):18–27.

Park, D. and L. C. Griffith. 2006. Electrophysiological and anatomical characterization of PDF-positive clock neurons in the intact adult *Drosophila* brain. *J Neurophysiol* 95 (6):3955–3960.

Patel, A. J., E. Honore, F. Lesage, M. Fink, G. Romey, and M. Lazdunski. 1999. Inhalational anesthetics activate two-pore-domain background K+ channels. *Nat Neurosci* 2 (5):422–426.

Patel, A. J., F. Maingret, V. Magnone, M. Fosset, M. Lazdunski, and E. Honore. 2000. TWIK-2, an inactivating 2P domain K+ channel. *J Biol Chem* 275 (37):28722–28730.

Peers, C. 1990. Hypoxic suppression of K+ currents in type I carotid body cells: Selective effect on the Ca2(+)-activated K+ current. *Neurosci Lett* 119 (2):253–256.

Peier, A. M., A. J. Reeve, D. A. Andersson, A. Moqrich, T. J. Earley, A. C. Hergarden, G. M. Story et al. 2002. A heat-sensitive TRP channel expressed in keratinocytes. *Science* 296 (5575):2046–2049.

Plant, L. D. 2012. A role for K2P channels in the operation of somatosensory nociceptors. *Front Mol Neurosci* 5 (21):1–5.

Plant, L. D., D. A. Bayliss, D. Kim, F. Lesage, and S. A. Goldstein. 2005. International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-P potassium channels. *Pharmacol Rev* 57 (4):527–540.

Plant, L. D., I. S. Dementieva, A. Kollewe, S. Olikara, J. D. Marks, and S. A. Goldstein. 2010. One SUMO is sufficient to silence the dimeric potassium channel K2P1. *Proc Natl Acad Sci USA* 107 (23):10743–10748.

Plant, L. D., E. J. Dowdell, I. S. Dementieva, J. D. Marks, and S. A. Goldstein. 2011. SUMO modification of cell surface Kv2.1 potassium channels regulates the activity of rat hippocampal neurons. *J Gen Physiol* 137 (5):441–454.

Plant, L. D., P. J. Kemp, C. Peers, Z. Henderson, and H. A. Pearson. 2002. Hypoxic depolarization of cerebellar granule neurons by specific inhibition of TASK-1. *Stroke* 33 (9):2324–2328.

Plant, L. D., L. Zuniga, D. Araki, J. D. Marks, and S. A. Goldstein. 2012. SUMOylation silences heterodimeric TASK potassium channels containing K2P1 subunits in cerebellar granule neurons. *Sci Signal* 5 (251):ra84.

Pountney, D. J., I. Gulkarov, E. Vega-Saenz de Miera, D. Holmes, M. Saganich, B. Rudy, M. Artman, and W. A. Coetzee. 1999. Identification and cloning of TWIK-originated similarity sequence (TOSS): A novel human 2-pore K+ channel principal subunit. *FEBS Lett* 450 (3):191–196.

Puil, E., B. Gimbarzevsky, and R. M. Miura. 1987. Voltage dependence of membrane properties of trigeminal root ganglion neurons. *J Neurophysiol* 58 (1):66–86.

Rajan, S., L. D. Plant, M. L. Rabin, M. H. Butler, and S. A. Goldstein. 2005. Sumoylation silences the plasma membrane leak K+ channel K2P1. *Cell* 121 (1):37–47.

Rajan, S., R. Preisig-Muller, E. Wischmeyer, R. Nehring, P. J. Hanley, V. Renigunta, B. Musset et al. 2002. Interaction with 14-3-3 proteins promotes functional expression of the potassium channels TASK-1 and TASK-3. *J Physiol* 545 (Pt 1):13–26.

Rajan, S., E. Wischmeyer, C. Karschin, R. Preisig-Muller, K. H. Grzeschik, J. Daut, A. Karschin, and C. Derst. 2001. THIK-1 and THIK-2, a novel subfamily of tandem pore domain K+ channels. *J Biol Chem* 276 (10):7302–7311.

Rajan, S., E. Wischmeyer, G. Xin Liu, R. Preisig-Muller, J. Daut, A. Karschin, and C. Derst. 2000. TASK-3, a novel tandem pore domain acid-sensitive K+ channel. An extracellular histiding as pH sensor. *J Biol Chem* 275 (22):16650–16657.

Rau, K. K., B. Y. Cooper, and R. D. Johnson. 2006. Expression of TWIK-related acid sensitive K+ channels in capsaicin sensitive and insensitive cells of rat dorsal root ganglia. *Neuroscience* 141 (2):955–963.

Reid, G. and M. Flonta. 2001. Cold transduction by inhibition of a background potassium conductance in rat primary sensory neurones. *Neurosci Lett* 297 (3):171–174.

Renigunta, V., H. Yuan, M. Zuzarte, S. Rinne, A. Koch, E. Wischmeyer, G. Schlichthorl et al. 2006. The retention factor p11 confers an endoplasmic reticulum-localization signal to the potassium channel TASK-1. *Traffic* 7 (2):168–181.

Reyes, R., F. Duprat, F. Lesage, M. Fink, M. Salinas, N. Farman, and M. Lazdunski. 1998. Cloning and expression of a novel pH-sensitive two pore domain K+ channel from human kidney. *J Biol Chem* 273 (47):30863–30869.

Roberts, S. K. 2003. TOK homologue in *Neurospora crassa*: First cloning and functional characterization of an ion channel in a filamentous fungus. *Eukaryot Cell* 2 (1):181–190.

Sakmann, B. and G. Trube. 1984. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J Physiol* 347:641–657.

Salinas, M., R. Reyes, F. Lesage, M. Fosset, C. Heurteaux, G. Romey, and M. Lazdunski. 1999. Cloning of a new mouse two-P domain channel subunit and a human homologue with a unique pore structure. *J Biol Chem* 274 (17):11751–11760.

Salkoff, L., A. Butler, G. Fawcett, M. Kunkel, C. McArdle, G. Paz-y-Mino, M. Nonet et al. 2001. Evolution tunes the excitability of individual neurons. *Neuroscience* 103 (4):853–859.

Salkoff, L., A. D. Wei, B. Baban, A. Butler, G. Fawcett, G. Ferreira, and C. M. Santi. 2005. Potassium channels in *C. elegans*. *WormBook*:1–15.

Sano, Y., K. Inamura, A. Miyake, S. Mochizuki, C. Kitada, H. Yokoi, K. Nozawa, H. Okada, H. Matsushime, and K. Furuichi. 2003. A novel two-pore domain K(+) channel, TRESK, is localized in the spinal cord. *J Biol Chem* 278 (30):27406–27412.

Sesti, F., T. M. Shih, N. Nikolaeva, and S. A. Goldstein. 2001. Immunity to K1 killer toxin: Internal TOK1 blockade. *Cell* 105 (5):637–644.

Shen, K. Z., R. A. North, and A. Surprenant. 1992. Potassium channels opened by noradrenaline and other transmitters in excised membrane patches of guinea-pig submucosal neurones. *J Physiol* 445:581–599.

Siegelbaum, S. A., J. S. Camardo, and E. R. Kandel. 1982. Serotonin and cyclic AMP close single K+ channels in Aplysia sensory neurones. *Nature* 299 (5882):413–417.

Simkin, D., E. J. Cavanaugh, and D. Kim. 2008. Control of the single channel conductance of K2P10.1 (TREK-2) by the amino-terminus: Role of alternative translation initiation. *J Physiol* 586 (Pt 23):5651–5663.

Sirois, J. E., Q. Lei, E. M. Talley, C. Lynch, 3rd, and D. A. Bayliss. 2000. The TASK-1 two-pore domain K+ channel is a molecular substrate for neuronal effects of inhalation anesthetics. *J Neurosci* 20 (17):6347–6354.

Sirois, J. E., J. J. Pancrazio, C. L. Iii, and D. A. Bayliss. 1998. Multiple ionic mechanisms mediate inhibition of rat motoneurones by inhalation anaesthetics. *J Physiol* 512 (Pt 3):851–862.

Streit, A. K., M. F. Netter, F. Kempf, M. Walecki, S. Rinne, M. K. Bollepalli, R. Preisig-Muller et al. 2011. A specific two-pore domain potassium channel blocker defines the structure of the TASK-1 open pore. *J Biol Chem* 286 (16):13977–13984.

Talley, E. M. and D. A. Bayliss. 2002. Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: Volatile anesthetics and neurotransmitters share a molecular site of action. *J Biol Chem* 277 (20):17733–17742.

Talley, E. M., Q. Lei, J. E. Sirois, and D. A. Bayliss. 2000. TASK-1, a two-pore domain K+ channel, is modulated by multiple neurotransmitters in motoneurons. *Neuron* 25 (2):399–410.

Talley, E. M., G. Solorzano, Q. B. Lei, D. Kim, and D. A. Bayliss. 2001. CNS distribution of members of the two-pore-domain (KCNK) potassium channel family. *J Neurosci* 21 (19):7491–7505.

Tan, J. H., W. Liu, and D. A. Saint. 2004. Differential expression of the mechanosensitive potassium channel TREK-1 in epicardial and endocardial myocytes in rat ventricle. *Exp Physiol* 89 (3):237–242.

Tang, Z., O. El Far, H. Betz, and A. Scheschonka. 2005. Pias1 interaction and sumoylation of metabotropic glutamate receptor 8. *J Biol Chem* 280 (46):38153–38159.

Terrenoire, C., I. Lauritzen, F. Lesage, G. Romey, and M. Lazdunski. 2001. A TREK-1-like potassium channel in atrial cells inhibited by beta-adrenergic stimulation and activated by volatile anesthetics. *Circ Res* 89 (4):336–342.

Theilig, F., I. Goranova, J. R. Hirsch, M. Wieske, S. Unsal, S. Bachmann, R. W. Veh, and C. Derst. 2008. Cellular localization of THIK-1 (K(2P)13.1) and THIK-2 (K(2P)12.1) K channels in the mammalian kidney. *Cell Physiol Biochem* 21 (1–3):63–74.

Thomas, D., L. D. Plant, C. M. Wilkens, Z. A. McCrossan, and S. A. Goldstein. 2008. Alternative translation initiation in rat brain yields K2P2.1 potassium channels permeable to sodium. *Neuron* 58 (6):859–870.

Tulleuda, A., B. Cokic, G. Callejo, B. Saiani, J. Serra, and X. Gasull. 2011. TRESK channel contribution to nociceptive sensory neurons excitability: Modulation by nerve injury. *Mol Pain* 7:30.

Viana, F., E. de la Pena, and C. Belmonte. 2002. Specificity of cold thermotransduction is determined by differential ionic channel expression. *Nat Neurosci* 5 (3):254–260.

Wang, Z., R. J. Van den Berg, and D. L. Ypey. 1994. Resting membrane potentials and excitability at different regions of rat dorsal root ganglion neurons in culture. *Neuroscience* 60 (1):245–254.

Warth, R., H. Barriere, P. Meneton, M. Bloch, J. Thomas, M. Tauc, D. Heitzmann et al. 2004. Proximal renal tubular acidosis in TASK2 K^+ channel-deficient mice reveals a mechanism for stabilizing bicarbonate transport. *Proc Natl Acad Sci USA* 101 (21):8215–8220.

Watkins, C. S. and A. Mathie. 1996. A non-inactivating K^+ current sensitive to muscarinic receptor activation in rat cultured cerebellar granule neurons. *J Physiol* 491 (Pt 2):401–412.

Wilson, V. G. and G. Rosas-Acosta. 2005. Wrestling with SUMO in a new arena. *Sci STKE* 2005 (290):pe32.

Yamamoto, Y., T. Hatakeyama, and K. Taniguchi. 2009. Immunohistochemical colocalization of TREK-1, TREK-2 and TRAAK with TRP channels in the trigeminal ganglion cells. *Neurosci Lett* 454 (2):129–133.

Yook, K., T. W. Harris, T. Bieri, A. Cabunoc, J. Chan, W. J. Chen, P. Davis et al. 2012. WormBase 2012: More genomes, more data, new website. *Nucleic Acids Res* 40 (Database issue):D735–D741.

Zilberberg, N., N. Ilan, and S. A. Goldstein. 2001. KCNKO: Opening and closing the 2-P-domain potassium leak channel entails "C-type" gating of the outer pore. *Neuron* 32 (4):635–648.

Zilberberg, N., N. Ilan, R. Gonzalez-Colaso, and S. A. Goldstein. 2000. Opening and closing of KCNKO potassium leak channels is tightly regulated. *J Gen Physiol* 116 (5):721–734.

Zuzarte, M., K. Heusser, V. Renigunta, G. Schlichthorl, S. Rinne, E. Wischmeyer, J. Daut, B. Schwappach, and R. Preisig-Muller. 2009. Intracellular traffic of the K^+ channels TASK-1 and TASK-3: Role of N- and C-terminal sorting signals and interaction with 14-3-3 proteins. *J Physiol* 587 (Pt 5):929–952.

Zuzarte, M., S. Rinne, G. Schlichthorl, A. Schubert, J. Daut, and R. Preisig-Muller. 2007. A di-acidic sequence motif enhances the surface expression of the potassium channel TASK-3. *Traffic* 8 (8):1093–1100.