British Journal of Pharmacology

British Journal of Pharmacology (2019) **176** 256–266 256

INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY MINI-REVIEW

Two-pore domain potassium channels: emerging targets for novel analgesic drugs: IUPHAR Review 26

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Received 6 June 2018; **Revised** 23 July 2018; **Accepted** 18 August 2018

This article, contributed by members of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) subcommittee for the Two-pore domain potassium (K2P) channels, confirms the existing nomenclature for these channels, and reviews our current understanding of their structure, pharmacology and functions, and likely physiological roles in health and disease. More information on these channels can be found in the Concise Guide to PHARMACOLOGY (http://onlinelibrary.wiley.com/doi/10.1111/bph.13884/full), and in the corresponding open access IUPHAR/BPS Guide to PHARMACOLOGY database (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79).

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Chronic pain is a debilitating and increasingly common medical problem with few effective treatments. In addition to the direct and indirect economic burden of pain syndromes, the concomitant increase in prescriptions for narcotics has contributed to a sharp rise in deaths associated with drug misuse – the 'opioid crisis'. Together, these issues highlight the unmet clinical and social need for a new generation of safe, efficacious analgesics. The detection and transmission of pain stimuli is largely mediated by somatosensory afferent fibres of the dorsal root ganglia. These nociceptive cells express an array of membrane proteins that have received significant attention as attractive targets for new pain medications. Among these, a growing body of evidence supports a role for the two-pore domain potassium (K2P) family of K^+ channels. Here, we provide a concise review of the K2P channels, their role in pain biology and their potential as targets for novel analgesic agents.

Abbreviations

CFA, complete Freund's adjuvant; DRG, dorsal root ganglia; SNP, single-nucleotide polymorphisms; TRG, trigeminal root ganglia

Introduction

More than a third of all adults in the USA require medical treatment for pain, at a cost that exceeds US $130 billion per year (Johannes *et al.,* 2010; Volkow and McLellan, 2016). For this reason, opioids are among the most commonly prescribed drugs in the USA, with more than 245 million prescriptions dispensed during 2014 alone (Volkow and McLellan, 2016). Although opioids are effective analgesics, particularly for moderate to severe pain, their use is associated with deleterious side effects, including respiratory depression, tolerance and dependence. The prevalence and misuse of these drugs has fuelled the current 'opioid crisis', which is characterized by 750 000 emergency medical cases and ~19 000 deaths annually, in the USA alone (Compton *et al.,* 2016). Despite intense and sustained biomedical research and drug-development efforts, safe, efficacious alternatives to opioids are yet to succeed in clinical trials. This deficit reflects the heterogeneous nature of the disease processes that elicit pain, the complex physiology of pain signalling and the challenges associated with translating laboratory findings into clinically efficacious pharmaceuticals (Hughes *et al.,* 2012; von Hehn *et al.,* 2012). Many new candidate analgesics in development target membrane

DOI:10.1111/bph.14518

proteins, particularly GPCRs or ion channels in nociceptive sensory neurons, involved in pain signalling (Waxman and Zamponi, 2014; Yekkirala *et al.,* 2017). Among these, the two-pore domain potassium (**K2P**) channels are perhaps the least well explored. Multiple lines of evidence implicate K2P channels in the regulation of multiple types of pain, such as inflammatory, neuropathic, mechanical and thermal, migraine and cancer pain.

A brief introduction to K2P channels

The K2P channels are a diverse family of K^+ selective ion channels that contribute to background or leak currents in excitable and non-excitable tissues (Enyedi and Czirjak, 2010). Although the importance of background K^+ flux to cellular physiology had been appreciated for more than a century (Bernstein, 1902), specific proteins that mediate background K^+ currents were not identified until the mid-1990s (Goldstein *et al.,* 1996; Lesage *et al.,* 1996).

In mammals, K2P subunits are encoded by 15 KCNK-genes and are stratified into six subgroups based on structural and functional similarity (Table 1) (Czirjak and Enyedi, 2010; Plant 2012). Although K2P channels pass currents across the physiological voltage range, their gating is highly regulated (for detailed reviews, please see Goldstein *et al.,* 2001; Honore, 2007; Enyedi and Czirjak, 2010). Physicochemical factors or drugs that inhibit K2P channels, including pH, oxygen-tension, specific cellular signalling pathways and membrane lipids, increase cellular excitability. In contrast, factors that potentiate currents, such as mechanical stretch, heat, anaesthetics and phosphoinositides, dampen excitability (Chemin *et al.,* 2007a,b). This basic operational paradigm is true for heterologous cells. However, it might not reflect the action of drugs on native cells, tissue or whole animal phenotypes, which has bearing on the development of pharmaceuticals that target K2P channels. In native cells, depolarization might decrease excitability if the magnitude and timescale is sufficient to inactivate voltage-gated ion channels. At the systems level, overall excitability is determined by the interplay of inhibitory and excitatory circuits, which are in turn dependent on the relative expression levels of a panoply of ion channels and regulatory proteins.

Beyond sequence diversity, the correlation of specific K2P channels with currents in native tissues has been hampered by the broad range of transcript processing and post-translational modification mechanisms that diversify channel function. Modifications include phosphorylation, SUMOylation, glycosylation and alternative translation

Table 1

The 15 K2P channels expressed in mammals

Channel name	Gene name	Common name	Expression in DRG neurons
K2P1	KCNK1	TWIK1	Large/medium fibres, IB4- C-fibres[e]
K2P2	KCNK2	TREK1	TRPV1+, C and medium fibres[a,e]
K2P3	KCNK3	TASK1	TRPV1+ fibres[a,e]
K2P4	KCNK4	TRAAK	Highly expressed[a,e]
K2P5	KCNK5	TASK2	Highly expressed[a]
K2P6	KCNK6	TWIK2	Highly expressed[a]
K2P7	KCNK7	Kcnk8	N/A
K2P9	KCNK9	TASK3	TRPV1-, IB4- C-fibres[a,e]
K2P10	KCNK10	TREK2	IB4+ C-fibres[a,d]
K2P12	KCNK12	THIK2	Small > medium, large fibres[a,b,e]
K2P13	KCNK13	THIK1	Highly expressed[a,b]
K2P15	KCNK15	TASK5	N/A
K2P16	KCNK16	TALK1	N/A
K2P17	KCNK17	TALK2	N/A
K2P18	KCNK18	TRESK	Highly expressed in C-fibres of DRG and TRG[a,c,e]

Mammals express 15 distinct K2P channels. Channel names are designated by IUPHAR (K2P*X*); however, the gene names (KCNK*X*) and historic names based on biophysical or pharmacological properties remain in common use. Abbreviations: TWIK, tandem of P-domains in a weak inward rectifying K+ channel; TREK, TWIK-related K+ channel; TASK, two-pore domain, acid-sensitive K+ channel; TRAAK, two-pore domain-related arachidonic acid-activated K+ channel; THIK, two-pore domain halothane-inhibited K+ channel; TALK, two-pore domain alkaline-activated K+ channel; and TRESK, TWIK-related spinal cord potassium channel. N/A is not applicable. See Figure 2. K2P6, 7, 12 and 15 channels pass little or no current when expressed in experimental cells. For more information, see the IUPHAR Guide to Pharmacology: http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=79. The expression pattern of K2P channels in DRG and TRG is summarized from the following:
[a]Marsh *et al.* (2012);
[b]Haskins *et al.* (2017);
[c]Lafrenière and Rouleau (2011);
[d]Acosta *et al.* (2014);
[e]Pollema-Mays *et al.* (2013).

initiation (Goldstein *et al.,* 2001; Lopes *et al.,* 2005; Thomas *et al.,* 2008; Plant *et al.,* 2012). Further, a growing body of evidence shows that the functional attributes of K2P channels can be diversified by heterodimerization of specific pairs of subunits to form channels with distinct physiological and pharmacological properties (Czirjak and Enyedi, 2002; Plant *et al.,* 2012; Blin *et al.,* 2014, 2016). Thus, the multiscale nature of physiology can hamper the prediction of how pharmaceuticals designed to act at K2P channels might affect pathophysiological processes or cause off-target effects.

There are currently no approved drugs that have a selective action at K2P channels. This deficit reflects several factors that have stymied the development of K2P-selective pharmacophores. Until crystal structures of **K2P1** and **K2P4** were solved in 2012 (Brohawn *et al.,* 2012; Miller and Long, 2012), the most prominent bottleneck in the development of drugs that act at K2P channels was a lack of structural data. The unique secondary structure of K2P subunits prohibited easy extrapolation from existing data available for voltage-gated (**K_V**), inwardly rectifying (**K_{IR}**) K^+ channels, or the canonical K^+ channel, KcsA (Doyle *et al.,* 1998; Long *et al.,* 2005; Nishida *et al.,* 2007). KcsA, K_{IR} and K_V subunits have a single pore-forming P-loop with two, two and six transmembrane domains, respectively, while K2P subunits have 4-transmembrane domains (M1–M4) and two pore-forming loops – thus, 'two-pore domain' (Goldstein *et al.,* 2001). While KcsA, K_{IR} and K_V are tetramers, K2P channels are formed by two subunits that come together to create a single, central K^+ selective pore (Figure 1B) (Plant *et al.,* 2017).

The most prominent structural motif observed in K2P channels is an extracellular cap-domain formed by the first external loop of each subunit that extends above the outer leaflet of the plasma membrane, bifurcating the K^+ permeation pathway at the outer mouth of the pore (Figure 1C, D). First described in the structures of K2P1 and K2P4 channels (Brohawn *et al.,* 2012; Miller and Long, 2012), the cap-domain is also apparent in the structures of K2P2 and K2P10 (Dong *et al.,* 2015; Lolicato *et al.,* 2017) and is thus expected to be observed throughout the K2P-family. The position of the cap-domain is proposed to create steric hindrance that renders K2P channels and background K^+ currents in native cells largely insensitive to classical toxin peptides and pore blocking molecules like tetraethylammonium ions that act by blocking the pore of delayed-rectifier K_V channels (Niemeyer *et al.,* 2016).

K2P-structures also revealed two lateral portals that expose the channel's pore to the interior of the membrane lipid, an observation that led to a proposed mechanism for the mechanosensitivity of **K2P2** and K2P4 (Brohawn *et al.,* 2014) (Figure 1C,D). In addition to visualizing channel architecture, these detailed snapshots elucidate how K2P channels interact with drugs. The structure of **K2P10** was solved in complex with **norfluoxetine**, the active metabolite of the **selective serotonin reuptake (SERT**) inhibitor **fluoxetine**, bound within the lateral portal (Dong *et al.,* 2015). Recent work by Lolicato *et al.* (2017) revealed a novel druggable site, dubbed the 'modulator pocket', located between the first pore loop and the M4-helix of each subunit of K2P2 and K2P10 (Figure 1C,D).

K2P channels are expressed throughout the body, with each channel type having a distinct expression profile. The observation that several KCNK mRNA transcripts are highly expressed in somatosensory fibres (Marsh *et al.,* 2012; Pollema-Mays *et al.,* 2013) led to the proposal that K2P channels regulate pain signals by determining the excitability of specific nerve fibres of dorsal and trigeminal root ganglia (DRG and TRG) (Plant, 2012).

Expression of K2P channels in dorsal root ganglia neurons

DRG comprise a heterologous population of primary afferent, somatosensory nociceptive neurons that are broadly divided into Aδ-, Aβ- and C-fibres. Aδ-fibres are lightly myelinated, have intermediate cell body sizes and respond to acute, localized pain. Aβ-fibres have larger cell bodies with a small nociceptive population. C-fibres are unmyelinated, have the smallest cell bodies in DRG and respond to diffuse pain and itch (Tsunozaki and Bautista, 2009). C-fibres are designated as peptidergic, based on the expression of **CGRP** or **Substance P**, and non-peptidergic, based on the binding of isolectin B4 (Le Pichon and Chesler, 2014).

Numerous studies (Table 1) have identified mRNA transcripts for several members of the K2P channel family in DRG and TRG neurons with distinct expression patterns in Aδ, Aβ and C-fibres (Figure 2) (Lafrenière and Rouleau, 2011; Marsh *et al.,* 2012; Pollema-Mays *et al.,* 2013; Acosta *et al.,* 2014; Mathie and Veale, 2015; Haskins *et al.,* 2017).

Role of K2P channels in inflammatory pain

K2P channels appear to be important in the mitigation of inflammatory pain, perhaps because transcript levels of several K2P subunits are significantly altered during inflammation. In an animal model of induced inflammation using complete Freund's adjuvant (CFA), significant changes in mRNA encoding **K2P3**, **K2P9**, **K2P12**, **K2P16** and **K2P18** subunits were correlated with a decrease in spontaneous foot lifting behaviour (Marsh *et al.,* 2012). Pain responses also correlate with changes in K2P12/**13** channel expression in animal models of inflammatory injury. Thus, siRNA-mediated knockdown of K2P12/13 channels in mouse models of CFA-induced inflammation resulted in increased nocifensive behaviour (Haskins *et al.,* 2017). K2P10 channels are involved in **PGE_2**-mediated hyperalgesia with $K2P10^{-/-}$ mice showing an absence of nocifensive behaviour in response to hypertonic saline injections after PGE_2 sensitization (Pereira *et al.,* 2014).

K2P18 channels in migraine

Genetic analysis of *KCNK18*, the gene that encodes for the K2P18 channel, identified several mutations, some of which were associated with migraine (Lafrenière *et al.,* 2010). Rainero *et al.* (2014) identified several variants of KCNK18

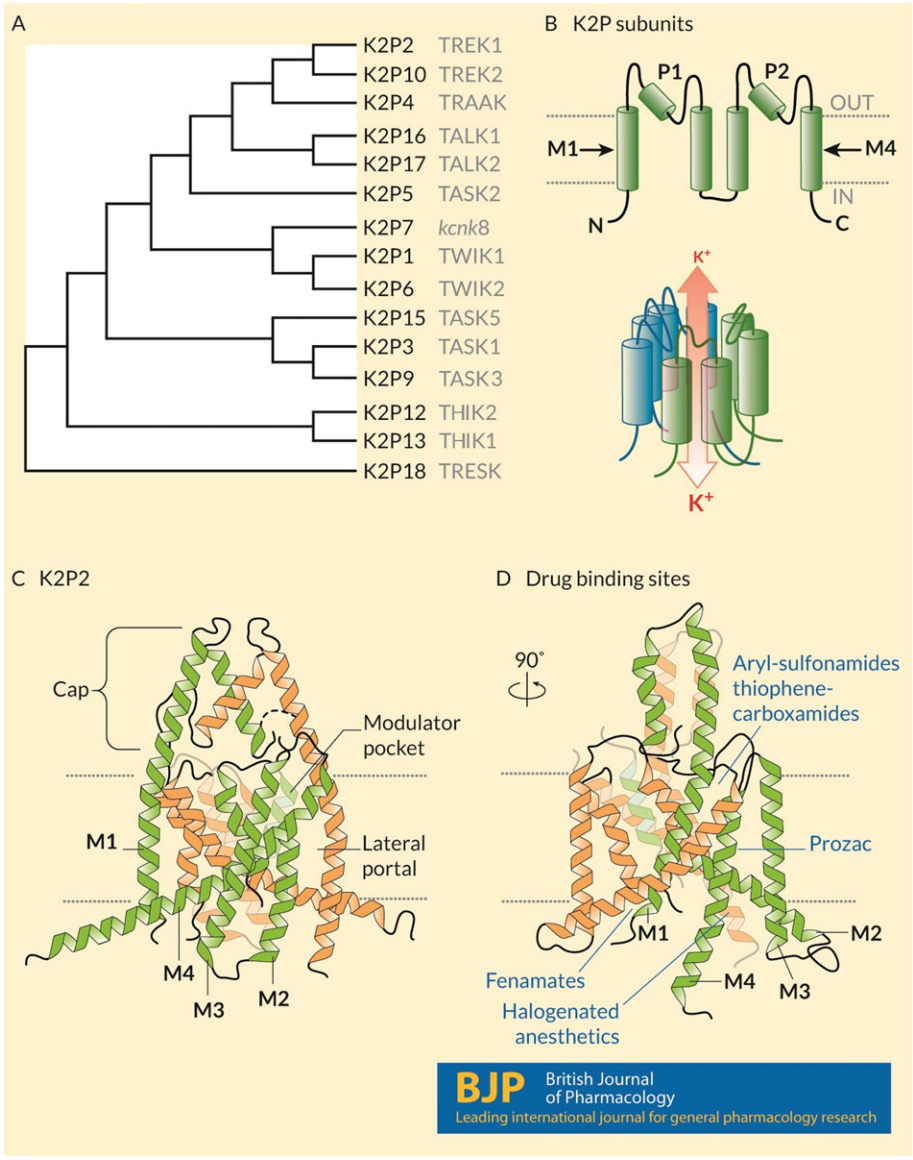

Figure 1

Structure and known drug interaction sites on K2P channels. (A) A phylogenetic tree calculated based on the primary sequences of the 15 K2P subunits expressed in humans. Common names appear in grey text. (B) (Upper) Humans express 15 K2P subunits with similar topologies: Intracellular N- and C-termini, four-transmembrane domains (M1–M4) and two re-entrant pore loops (P1 and P2) that contribute to the K^+ selective pore. (Lower) Two subunits come together as homodimers, and in some cases heterodimers, to form a K^+ selective channel. Under physiological conditions, K^+ ions move through the channel down their electrochemical gradients from the inside to the outside of the cell. (C) The crystal structure of mouse K2P2 (Lolicato *et al.,* 2017) showing the architecture of the channel including the arrangement of the transmembrane domains, the extracellular cap above the plane of the membrane that bifurcates the K^+ permeation pathway, the lateral portal and the modulator pocket. (D) A 90° rotation in the pose of K2P2 showing the location of four distinct drug binding sites: fenamates bind to the N-terminus; aryl-sulfonamides and thiophene-carboxamides interact with the modulator pocket; fluoxetine (Prozac) interacts at the lateral port; and halogenated anaesthetics require the proximal C-terminus of the channel and interact in a manner that involves Gαq proteins.

in an Italian cohort of patients suffering from migraine with and without aura. Using *in silico* models, they predict that the C110R, S178T, S231P and F372L mutations in K2P18 may have deleterious effects on channel function and contribute to migraine pathogenesis (Rainero *et al.,* 2014). Some mutations, like S231P, identified in both control samples and from unrelated migraine probands, were subsequently shown to have no overt effect on channel function (Lafrenière *et al.,* 2010; Andres-Enguix *et al.,* 2012). In contrast, A34V and C110R mutations result in decreased channel currents, possibly due to their proximity to the channel pore (Andres-Enguix *et al.,* 2012). K2P18-A34V channels are linked to the development of typical migraines while a frameshift mutation, F139WfsX24, that truncates K2P18 at M2, is implicated in familial migraine with aura (Lafrenière *et al.,* 2010). Mutant/truncated K2P18 subunits

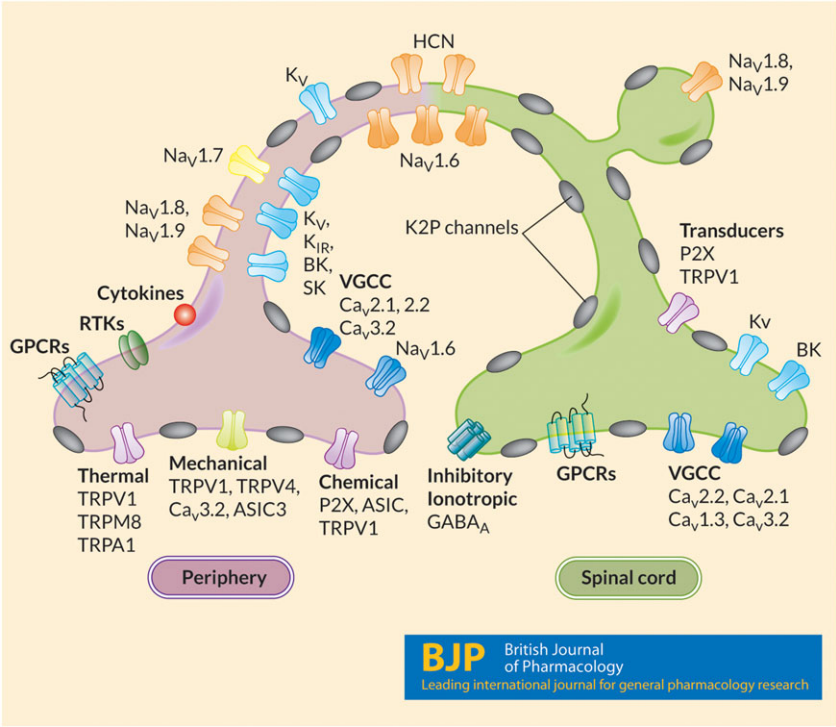

Figure 2

Somatosensory nociceptive fibres express several K2P channels. Somatosensory fibres in DRG and TRG are a heterogenous population of nociceptive cells that detect, integrate and transmit sensory information and pain signals. The excitability and sensory properties of peptidergic and non-peptidergic C-fibres as well as Aβ and Aδ-fibre primary afferent cells are, in part, dependent on the expression patterns of numerous types of ion channels. Several K2P channels, including K2P1–6, K2P9–13 and K2P18 are highly expressed in populations of DRG and TRG somatosensory neurons, where they regulate excitability and responses to numerous pain stimuli.

have a dominant-negative phenotype, suppressing the activity of wild-type K2P18 channels in heterologous cell systems (Lafrenière and Rouleau, 2011; Andres-Enguix *et al.*, 2012) and cultured trigeminal neurons (Liu *et al.*, 2013). In support of these reports, the overexpression of K2P18 in small TRG fibres inhibits action potential firing (Guo and Cao, 2014), presumably through K2P18-mediated currents. A growing body of evidence suggests that many cases of migraine are polygenic in nature (Anttila *et al.*, 2018; Gormley *et al.*, 2018), leading to the notion that genetic variants in KCNK18 might act as risk factors, rather than as a single, penetrant 'channelopathy'. However, the notion that activators of K2P18 channels could act as anti-migraine medications is intriguing and worthy of further investigation.

K2P channels in neuropathic pain

K2P18 channels mediate the largest component of the background K^+ current in DRG neurons (Tulleuda *et al.*, 2011; Plant, 2012), but the channel is down-regulated in animals with spinal cord injury. This phenotype is associated with significantly decreased thresholds for withstanding mechanical pain (allodynia), activation of astrocytes and microglia and up-regulation of **connexin**-36 and connexin-43, components of neuronal and astrocyte-oligodendrocyte gap junctions respectively (Zhou *et al.*, 2017). Down-regulation of K2P18 in nerve injury occurs in combination with an increase in MAPK, **ERK** and **p38**, which are also implicated in the pathogenesis of neuropathic pain. This phenotype can be reproduced *in vivo* by shRNA knockdown of K2P18 channels and is rescued when K2P18 channels are overexpressed using adenoviral vectors (Zhou *et al.*, 2017). Similarly, increased nocifensive behaviour is observed after complete axotomy of the sciatic nerve and in K2P18$^{-/-}$ animals (Tulleuda *et al.*, 2011). Further support for the role of decreased K2P18 channel current activity in neuropathic pain and nerve injury comes from the observation that activation of K2P18 balances depolarizing stimuli through **TRPV1** channels during nerve injury through lysophosphatidic acid signalling in DRG neurons (Kollert *et al.*, 2015).

Changes in the activity of other K2P channels are also involved in the pathology of nerve injury. In spared sciatic nerve injury, K2P9 and K2P1 channels were down-regulated in L4-L5 DRG's ipsilateral to the nerve lesion while K2P3 expression remained constant. The K2P9 channels returned to homeostatic levels within weeks, although the K2P1 channels remained depleted for months after the initial injury (Pollema-Mays *et al.*, 2013). In a similar model, K2P13 channel knockdown increased the response to inflammation in the form of longer spontaneous foot lifting times. Of note, K2P2$^{-/-}$ and K2P2/4/10$^{-/-}$ triple knockout mice showed significantly increased sensitivity to mechanical stimuli

compared to wild-type animals but with no difference between knockout models, suggesting that K2P10 channels might play a protective role against mechanical allodynia (Pereira *et al.,* 2014).

The TREK-family of channels in thermal and mechanical pain

In line with the role of leak K^+ channels in reducing cellular excitability, expression of TREK-family channels (K2P2, K2P4 and K2P10) decreased the thermal sensitivity of DRG neurons. In a model of unilateral peripheral mononeuropathy, DRG from K2P2$^{-/-}$ mice showed increased excitability when exposed to temperatures in the 30–45°C range (Alloui *et al.,* 2006). In a similar animal model of neuropathy, K2P2/4$^{-/-}$ mice showed increased cold sensitivity at 15–20°C, compared with K2P4$^{-/-}$ and wild-type animals (Noël *et al.,* 2009). C-fibres taken from K2P2$^{-/-}$ and K2P2/4/10$^{-/-}$ animals fire action potentials at lower temperatures than wild-type mice and display increased firing frequencies in the 30–50°C temperature range. K2P10$^{-/-}$ mice also showed increased sensitivity to temperatures (40–45°C) that were agreeable to wild-type animals (Pereira *et al.,* 2014). K2P10 channels are also implicated in the mediation of cold hypersensitivity following treatment with **oxaliplatin,** a drug commonly used to treat colon cancer (Pereira *et al.,* 2014). K2P4$^{-/-}$ animals have a decreased heat threshold in comparison to wild-type animals. Heat-induced hyperalgesia in K2P2$^{-/-}$ and K2P2/4/10$^{-/-}$ mice is congruent with inflammation and neuropathy-induced hyperalgesia in wild-type mice (Noël *et al.,* 2009), probably indicating a decrease in the expression of K2P2 and K2P4 channels after nerve injury. Cold sensing C-fibres have bimodal thresholds with one population responding to temperatures above 17°C while the other group of fibres is pain-sensing and only fires at temperatures below 17°C (Reid, 2005). In K2P2/4$^{-/-}$ mice, the latter population of fibres is depleted and the higher cold threshold fibres predominate (Noël *et al.,* 2009).

K2P9 channels in cancer

The expression of several K2P channels is altered in different cancers (Williams *et al.,* 2013). *KCNK9*, the gene encoding K2P9, is reportedly a proto-oncogene, promoting tumour growth and hypoxia resistance (Dookeran and Auer, 2017). Conversely, down-regulation of K2P9 channels through PKC activation, increased cancer metastasis by enhancing cell migration (Lee *et al.,* 2012). K2P9 channels appear to have a dual role as drug targets in decreasing breast cancer pain as well as arresting cancer metastasis. Two single-nucleotide polymorphisms (SNP) in the K2P9 gene (KCNK9-rs3780039 and rs11166921) have been linked to an increase in the incidence of pre-operative breast pain, in breast cancer patients. Individuals homozygous for KCNK9-rs11166921 were more than twice as likely to report breast pain before surgery while individuals heterozygous and homozygous for KCNK9-rs3780039 were twice as likely to experience breast pain (Langford *et al.,* 2014). It is not known if this SNP affects K2P9 channel function or whether the SNP results in a dominant negative phenotype. While the mechanistic association between K2P9 and breast cancer pain is unclear, it is possible that variant K2P9 channels in somatosensory fibres are more responsive to signalling inflammatory factors in the penumbra of the tumour (Langford *et al.,* 2014). Further, it is possible that these intronic SNPs may affect channel expression and warrant further investigation.

Pain management through K2P channels?

Drugs and small molecules

The number of drugs and small molecules recognized to interact with K2P channels is steadily increasing (Figure 1D). Halogenated volatile anaesthetics such as **isoflurane** and **sevoflurane** activate several K2P channels, including K2P2, K2P3, K2P4, K2P9 and K2P18, perhaps *via* the disruption of an inhibitory interaction between Gαq and the proximal C-terminus of the channel (Chen *et al.,* 2006). Recent data suggest that DAG produced following activation of Gq-coupled receptors is the active inhibitory factor, rather than Gαq itself (Wilke *et al.,* 2014). An interaction between K2P channels and DAG, however, does not preclude an interaction with Gq and remains an open question in the field. Volatile anaesthetics are proposed to promote membrane hyperpolarization *via* an increase in channel open probability and K^+ flux (Patel *et al.,* 1999; Plant, 2012). Thus, activation of K2P channels, in addition to **$GABA_A$** receptors, is proposed to play an important role in the mediation of anaesthesia and analgesia by halothane-like agents.

TREK-family channels are inhibited by the SERT inhibitors, fluoxetine and norfluoxetine, *via* an interaction in the lateral-portal – see Introduction (Dong *et al.,* 2015). Clinical evidence supports the analgesic activity of numerous anti-depressants (Obata, 2017), whereas inhibition of K2P channels is expected to augment pain signalling (Kennard *et al.,* 2005). Hence, further studies will be required to determine the clinical pharmacology of K2P channel-antidepressant interactions.

The fenamate class of non-steroidal anti-inflammatory drugs are proposed to mediate their analgesic action through the inhibition of pro-excitatory ion channels while selectively activating K^+ channels, including K2P channels (Takahira *et al.,* 2005). This effect is proposed to involve an interaction with the N-terminus of K2P channels (Veale *et al.,* 2014).

Recently, Lolicato *et al*. identified a new druggable site, the K2P modulator pocket in K2P2, 4 and 10 channels. The arylsulfonamide, ML335 and the thiophene-carboxamide ML402 activate the channels *via* specific cation–π and π–π interactions with residues behind the K^+ selectivity filter (Lolicato *et al.,* 2017).

K2P2 channel activation by the **μ-opioid receptor** is a component of **morphine**-induced signalling and is integral to its analgesic effects but does not contribute to the adverse effects of morphine (Devilliers *et al.,* 2013). In keeping with this finding, a series of acrylic acid compounds that selectively activate K2P2 channels has recently been reported to show significant pain mitigation *in vivo* (Vivier

et al., 2017). Similarly, a selective activator of K2P2 and K2P10, GI-530139, has been reported to be effective in hyperpolarizing DRG neurons by increasing channel activity (Loucif *et al.,* 2018).

Natural compounds

In addition to low MW compounds, the activity of K2P channels is also modulated by several naturally occurring compounds. The analgesic effects of traditional medicines like Schezuan peppers and aristolochic acid have been attributed to their modulation of K2P channels. Schezuan peppers have been used as analgesics in folk medicine, due to their numbing ability and as flavouring agents to elicit a tingling-like pungency. Hydroxy-α-sanshool, the active component of Schezuan peppers, mediates these effects by activating nociceptive and light-touch sensory neurons; depolarization is mediated by inhibition of K2P3, 9 and 18 channels (Bautista *et al.,* 2008). *In vivo* studies with knockout animals may yield further information as to the range and selectivity of these effects. Aristolochic acid is a natural remedy used in the Balkan region for pain relief potential *via* inhibition of K2P18 and activation of K2P2 and K2P10 channels (Veale and Mathie, 2016). However, the pharmacology of aristolochic acid is complex, with links to nephritis and carcinogenesis (Chen *et al.,* 2012; Schmeiser *et al.,* 2014).

Ion channels in pain mediation

K2P channels are a relatively recent addition to a list of ion channels that serve as potential pharmaceutical targets for novel analgesics (Figure 2). The exploration of ion channels as targets for pain management has been ongoing for some time but has resulted in limited progress in producing novel analgesics. Here, we provide a concise summary of progress, but more extensive reviews can be found elsewhere (Waxman and Zamponi, 2014; Yekkirala *et al.,* 2017).

Transient receptor potential (TRP) channels. The peripheral termini of sensory neurons innervate target tissues and express several types of transducer ion channels including numerous members of the **TRP** family. Of these, TRPV1 channels are activated by acidification, temperatures greater than ~42°C, membrane depolarization, several arachidonic acid metabolites and capsaicin, the pungent chemical in hot chilli peppers (Caterina *et al.,* 1997; Julius, 2013). TRPV1 channel antagonists have been proposed as analgesic agents. However, in clinical trials, blockade of TRPV1 channels modulated core body temperature, providing a significant problem in the development of TRPV1 channel antagonists (Skerratt and West, 2015). Interestingly, TRPV1 channel agonists such as **capsaicin** can produce paradoxical analgesia by causing channel desensitization, facilitating the use of capsaicin formulations in the treatment of osteoarthritis pain (Alexander *et al.,* 2017a). Recently, a blocker of **TRPM8** channels has been described in the attenuation of cold-related pain (Andrews *et al.,* 2015). A gain of function mutation of **TRPA1** channels has been linked to familial episodic pain syndrome, and at least two candidate drugs targeting this channel are in clinical trials for the treatment of diabetic neuropathy and inflammatory pain (Skerratt and West, 2015).

Voltage-gated calcium channels. **Calcium channels** are crucial to the transmission of pain signals from the primary sensory neurons to neurons in the dorsal ganglia (Yekkirala *et al.,* 2017). Blockade of **$Ca_V2.2$** channels by ziconitide (a formulation of **ω-conotoxin MVIIA**) administered intrathecally provides potent analgesia, under conditions where opioids are counter-indicated. However, ziconitide-induced analgesia is short-lived and fraught with several side effects, leading to restricted use of this drug (Patel *et al.,* 2018). **Gabapentin** and **pregabalin** are both inhibitors of $\alpha_2\delta$ subunit-containing Ca_V channels, and the latter is currently the drug-of-choice in the treatment of pain associated with diabetic neuropathy (Skerratt and West, 2015). Several new compounds targeting calcium channels have since entered clinical trials but have failed to reproduce the efficacy displayed in preclinical models. The applications and limitations of calcium channels in pain pathophysiology are dealt with in detail by Patel *et al*. (2018).

Voltage-gated sodium channels. Significant effort is underway to identify novel **Na_V** channel blockers that could dampen the excitability of somatosensory neurons. Similarly, medications that are currently available for the treatment of other Na_V channel-related maladies are being investigated for their utility as analgesics. For example, the anti-arrhythmic agent, **ranolazine**, also decreases mechanical allodynia and cold hypersensitivity after nerve injury (Gould *et al.,* 2009).

Significant interest in the role of Na_V channels in pain signalling arises from the observation that specific mutations in the SCN9A gene that encodes the **$Na_V1.7$** channel result in congenital pain indifference syndromes (Cox *et al.,* 2006) as well as increased pain sensitivity in the case of loss-of-function mutations (Mathie, 2010). Several compounds targeting $Na_V1.7$ channels are currently undergoing clinical trials for their efficacy in managing neuropathic pain (Skerratt and West, 2015). **$Na_V1.8$** channels are under investigation for roles in inflammatory and neuropathic pain, particularly mechanical allodynia and heat hypersensitivity associated with inflammation (Joshi *et al.,* 2006; Joshi and Honore, 2006; Wang *et al.,* 2011). A ligand targeting $Na_V1.8$ channels has, however, failed to show efficacy in a post-surgical dental pain clinical trial, raising questions about the role of these channels in pain pathogenesis (Wang *et al.,* 2011). **$Na_V1.9$** channels are also expressed in peripheral neurons and has been proposed as a druggable target for the treatment of hyperalgesia following inflammation. However, low channel expression in heterologous systems is proving to be a significant hindrance to research efforts in this direction (Yekkirala *et al.,* 2017). Of note, a recent report by Osteen *et al*. (2016) revealed a role for **$Na_V1.1$** channels in mechanical pain, including in a mouse model of irritable bowel syndrome.

Purinoreceptors. ATP-activated **P2X receptors** are cation channels that contribute to pain signalling by enhancing neuronal excitation (Mathie, 2010). The **P2X4** subtype is involved in the mediation of neuropathic pain *via* the activation of microglia and the subsequent release of **BDNF** and pro-inflammatory cytokines which are known to

exacerbate pain after nerve injuries (Ulmann *et al.,* 2008; Mathie, 2010).

Acid-sensing ion channels. **Acid-sensing ion channels (ASICs)** are cation-permeable channels that are activated by extracellular acidic pH. The anti-hypertensive and diuretic drug, amiloride, can inhibit various ASIC subunits and alleviated cutaneous and migraine pain. A corpus of literature links ASIC channel blockade to analgesia and is succinctly summarized in Wemmie *et al*. (2013).

Challenges and future perspectives on the pharmacology of K2P channels

With the renewed effort to develop non-opioid pain medications, K2P channels are evolving into exciting potential targets for novel analgesics. Although K2P channels have a paucity of specific blockers and activators to guide drug development, two decades of study have provided powerful tools for understanding the pathophysiological relevance of these proteins. More recently, this nascent field has been invigorated by the opening of an exciting new frontier in K2P channel research – combining drug development with atomic resolution structures and models of specific K2P channels. We predict that this approach will lead to fruitful advances in the understanding of structure–activity relationships for K2P channels and the next generation of K2P-pharmacophores with clinical application as analgesic agents.

Despite these advances, significant questions remain unanswered. Perhaps chief among these is the safety of drugs that target K2P channels. Although the expression of K2P18 channels appears to be restricted to DRG and TRG fibres, other pain-related K2P channels are expressed in multiple tissues. Broad tissue expression could lead to off-target effects of drugs that target K2P channels. For example, K2P2 channels are highly expressed in GABAergic interneurons, the prefrontal cortex, the hippocampus and dorsal raphe nuclei neurons, as well as in the cardiovascular system and gastrointestinal tract (Fink *et al.,* 1996; Talley *et al.,* 2001; Honore, 2007; Thomas *et al.,* 2008; Plant *et al.,* 2012). Several studies using knockout animal studies have been undertaken to provide specific information about the expression and function of individual K2P channels. However, the knockdown of one channel may cause up-regulation of other K2P channels and necessitates double or triple knockout models or perhaps conditional knockout models, as well as rigorous controls to validate study results. The distribution and regulation of the different K2P channels is catalogued extensively elsewhere (Enyedi and Czirjak, 2010; Plant *et al.,* 2017).

More rigorous studies in heterologous and primary cell systems are needed to define the composition of K2P subunits in specific cell types during development and in the context of specific pathophysiological states. Concurrently, evidence is mounting to support the observation first reported by Czirjak and Enyedi (2002) that at least some K2P channels are heterodimers. Because of the reported differences in function of heterodimers versus homodimeric K2P channels, it is possible that the pain phenotype will be altered or that drug discovery efforts will require modification to accommodate the expanded role of homodimeric and heterodimeric K2P channels in the mediation of different types of pain.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.,* 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.,* 2017a,b,c,d,e).

Acknowledgements

The authors are grateful to Diomedes Logothetis and the Logothetis lab for vibrant discussion and feedback. K.G. is supported by Northeastern University, Bouvé College of Health Sciences.

Conflict of interest

The authors declare no conflicts of interest.

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