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Ion Channels and Pain: Important Steps Towards Validating a New Therapeutic Target for Neuropathic Pain

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Introduction

Acute pain is caused by noxious stimuli, and by stimuli that threaten to or cause tissue damage. Pain serves a crucial physiological function as a rapid warning system that can help prevent injury or limit the extent of damage. However, chronic pain, which is pain that persists after the initial noxious stimulus, tissue damage, and subsequent healing periods have passed, and is elicited by stimulus types or levels that do not normally elicit pain, is a major clinical challenge. Most chronic pain patients complain of a lack of complete relief from currently available drugs, and many of these drugs have adverse side effects. As such, there are intensive basic research and drug discovery efforts focused on developing better treatments for chronic pain (http://www.ninds.nih.gov/disorders/chronic_pain/chronic_pain.htm). One major form of chronic pain is neuropathic, in that it is based in the neurons involved in perception of pain, and the transduction, or processing of the resultant pain signals to the brain. Peripheral neuropathic pain is due to altered function and sensitization of neurons within the peripheral nociceptive system (*i.e.*, nociceptive neurons), the sensory system responsible for the perception of pain and the transduction of pain signals to the spinal cord (http://www.iasppainorg/AM/Template.cfm?Section=Pain_Definitions). A recent paper published in *Experimental Neurology* (Tsantoulas et al., 2014) identifies a novel contributor to peripheral neuropathic pain that may represent an attractive target for future drug discovery efforts aimed at ameliorating this form of chronic pain.

Sensory ganglia such as trigeminal ganglia, and dorsal root ganglia (DRG), contain a variety of primary sensory neurons, with distinct morphologies and functions. Sensory neurons with medium and large diameter cell bodies have myelinated axons or A fibers that transduce signals from a variety of sensory modalities, and while those with small diameter cell bodies give rise to unmyelinated C fibers that transduce painful stimuli [reviewed in (Lawson, 2002)]. Within these major classes of neurons are those with a diversity of morphologies and functions, allowing for the selective discrimination of a wide variety of painful and non-painful stimuli. Within these neurons are the ion channels that underlie the fundamental steps of sensory transduction, namely the initiation of a propagating electrical signal at the

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sensory nerve endings in tissues, its conduction along the axon, and its transmission from the central terminals to neurons in the spinal cord [reviewed in (Gold and Gebhart, 2010)]. There are also a number of regulatory ion channels that shape diverse aspects of each of these events. When dysregulated, these ion channels that underlie the normal function of nociceptive neurons can contribute to the pathological states of neuropathic pain [reviewed in (Gold and Gebhart, 2010)]. The recent paper by Tsantoulas et al., (Tsantoulas et al., 2014) focuses on the role of a specific family of ion channels that serves as a critical component of the sensory transduction machinery in DRG neurons. The expression of these channels is reduced in an animal model of peripheral neuropathic pain. The authors were able to link this downregulation to a crucial aspect of peripheral neuropathic pain, the hyperexcitability of the nociceptive neurons that leads to ectopic transduction of pain signals to the spinal cord in the absence of a painful stimulus. This study not only identifies an important role for this particular class of ion channels, but also suggests that enhancing the activity of the remaining channels represents an attractive approach for future drug discovery efforts targeting neuropathic pain.

Ion channels and nociceptor excitability

A wealth of information has been obtained as to the functional contribution of specific classes of ion channels with particular biophysical and pharmacological properties to normal nociceptive signaling, and how they are altered in response to conditions resulting in hyperexcitability of nociceptive neurons and peripheral neuropathic pain [reviewed in (Gold and Gebhart, 2010)]. Among these, voltage-sensitive Na⁺ or Nav channels mediate the initiation and rising or depolarizing phase of the action potential, and voltage-gated K⁺ or Kv channels mediate the falling or repolarizing phase, and also contribute in diverse ways to determining whether action potentials are initiated, and if so their duration, amplitude and frequency (Hille, 2001). Nav channels are excitatory, such that Nav channel inhibitors are effective at decreasing or eliminating electrical excitability, and are in common use in neurology as anti-epileptic drugs, and as local anesthetics [reviewed in (Savio-Galimberti et al., 2012)]. In general, Kv channels are inhibitory, such that drugs that enhance Kv channel function should in principle have effects similar to Nav channel blockers. Consistent with this paradigm, retigabine, a Kv channel opener, has been recently approved as a first in class anti-epileptic drug [reviewed in (Gunthorpe et al., 2012)].

The number of Nav channel voltage-sensing and pore-forming principal or α subunits encoded in the human genome is relatively small, in that there are ten different SCNA genes, as are the structural and functional differences between the different Nav channel subtypes [reviewed in (Catterall, 2012)]. This limited diversity could be considered a drawback for drug discovery, making the development of subtype-selective inhibitors problematic. Moreover, the limited size of the Nav channel gene family means that any individual Nav channel subtype might have a broad cellular and/or tissue expression, confounding development of drugs that exhibit precise cell- or tissue-specific targeting. However, Nav channel inhibitors are used successfully in the clinic for a variety of indications [reviewed in (Savio-Galimberti et al., 2012)]. Numerous drug discovery programs are focused on specific Nav channel subtypes expressed in nociceptive neurons as targets for novel therapeutics for chronic pain [reviewed in (Dib-Hajj et al., 2010; Liu and Wood, 2011)].

Compared to Nav channels, there exists a huge complexity of Kv channels that differ widely in their structural and functional characteristics [reviewed in (Jan and Jan, 2012)]. The human Kv channel superfamily contains 30 genes encoding canonical Kv channel α subunits (Yu et al., 2005). Moreover, unlike Nav channels that are formed by a single α subunit, Kv channels arise from the combinatorial assembly of four α subunits, yielding a huge repertoire of homomeric and heteromeric channel complexes with distinct properties [reviewed in (Jan and Jan, 2012)]. As different Kv channel α subunits differ in their temporal and spatial patterns of expression [reviewed in (Vacher et al., 2008)], any given α subunit can participate in a variety of distinct channel types depending on the extent and nature of its coexpression and resultant coassembly with other α subunits. Certain Kv channels contain electrically silent modulatory α -like subunits (of which 10 are encoded in the human genome), which do not form functional Kv channels themselves but that when incorporated into complexes with functional Kv channel α subunits alter the properties of the resultant channels (Bocksteins and Snyders, 2012). Both Nav and Kv channel complexes also contain auxiliary subunits, which for Kv channels are more numerous and diverse, and also more variable in their inclusion in Kv channel complexes, than are those for Nav channels [reviewed in (Brueggemann et al., 2013)]. The overall α , α -like, and auxiliary subunit composition of a particular Kv channel complex dictates not only its functional characteristics, including its pharmacology, but also the subcellular localization, and modulation by various signaling pathways [reviewed in (Vacher and Trimmer, 2011)]. The structural diversity of Kv channels offers a broad potential for drug discovery, as it may be possible to develop modulators that preferentially act on Kv channel complexes of a specific subunit composition [reviewed in (Castle, 2010; Maljevic and Lerche, 2013)]. Moreover, given the huge number of possible Kv channel complexes that can be assembled from the available α , α -like, and auxiliary subunits, a Kv channel with a particular subunit composition and stoichiometry may be present in only small subset of cell types within a tissue, offering the potential that specific modulators of that particular Kv channel would have highly selective action. However, it remains a challenge to link the native Kv currents present in nociceptive neurons with molecularly defined complexes comprising specific Kv channel subunits. The recent paper by Tsantoulas *et al.*, (Tsantoulas et al., 2014), is an important step in this process, as it combines classical biophysical and pharmacological characterization of native nociceptor Kv currents, with targeted molecular level expression and pharmacological inhibition studies that define the two members (Kv2.1, Kv2.2) of the of the Kv2 subfamily of α subunits as the basis for a native Kv delayed rectifier current important in the normal function and pathological plasticity of nociceptive neurons.

Kv2 channels in brain neurons

Kv2.1 and Kv2.2 have widespread expression in brain neurons by *in situ* hybridization (Drewe et al., 1992; Hwang et al., 1992) and by immunohistochemistry (Kihira et al., 2010; Trimmer, 1991). There appears to be distinct spatiotemporal patterns of their expression in mammalian brain neurons (Hermansteyne et al., 2010; Kihira et al., 2010), suggesting that although these Kv2 family members share a high degree of amino acid sequence similarity, and have very similar functional properties, they may have distinct physiological functions. Kv2.1 is unusual in being highly posttranslationally modified by phosphorylation, with 34 *in*

vivo phosphorylation sites identified to date from an array of mass spectrometry based proteomics analyses of Kv2.1 from rat and mouse brain [reviewed in (Trimmer and Misonou, 2014)]. Kv2.1 is also modified by SUMOylation (Plant et al., 2011). These modifications have profound effects on Kv2.1 expression, localization and function [reviewed in (Cerdeira and Trimmer, 2010; Mandikian et al., 2011)]. Less is known of the role of posttranslational modification and modulation of Kv2.2, although many fewer phosphorylation sites have been identified on Kv2.2 (11) than on Kv2.1, suggesting they may have distinct sensitivities to modulation [reviewed in (Trimmer and Misonou, 2014)].

Neurons also express a family of Kv8 and Kv9 electrically silent Kv channel α subunits that specifically associate with and modulate the function of Kv2 channel α subunits [reviewed in (Bocksteins and Snyders, 2012)]. Although resembling Kv channel α subunits overall, Kv8 and Kv9 α subunits have the property of true modulatory subunits, in that when expressed alone in heterologous cells do not generate functional channels [reviewed in (Bocksteins and Snyders, 2012)]. However, when coexpressed with functional Kv2 channel α subunits, these modulatory subunits form heteromeric channels with functional properties distinct from channels formed from Kv2 α subunits alone (Otschytsch et al., 2002; Salinas et al., 1997). Recently, an additional Kv2 interacting protein, AMIGO-1, was identified as an auxiliary subunit of brain Kv2 channels (Peltola et al., 2011). There exist three highly related AMIGO family members, although the association of AMIGO-2 and AMIGO-3 with Kv2 channel complexes has not been studied. Thus, native Kv2 channel complexes may exist as diverse assemblies of functional Kv2.1 and Kv2.2 α subunits, modulatory electrically silent Kv8 and Kv9 α subunits, and auxiliary AMIGO subunits. Kv2 channels formed from distinct combinations of these subunits would exhibit a wide array of biophysical and pharmacological properties, and sensitivity to modulation. This structural and functional complexity presents a challenge for those interested in defining the contributions of specific Kv channels, in this case Kv2 channels, to the function of specific types of neurons, including nociceptors. However, this same daunting complexity also enhances the potential of these channels as targets for neuropathic pain.

Kv2 channels and nociceptive neurons

Previous studies have demonstrated the expression of Kv2 channel subunit mRNA and/or polypeptides in rodent DRG neurons, in both cell culture (Ishikawa et al., 1999) and in intact DRG (Kim et al., 2002). Expression of Kv2 subunits is decreased in models of neuropathic pain, in DRG neurons cultured from animals subjected to sciatic nerve axotomy (Ishikawa et al., 1999), and in DRGs from animals subjected to chronic constriction injury (Kim et al., 2002). Bocksteins and colleagues (Bocksteins et al., 2009) showed that Kv2 family-associated electrically silent subunits are also expressed in DRG neurons, which contain a delayed rectifier Kv current inhibited by stromatoxin, an inhibitor of Kv channels containing Kv2 and Kv4 family α subunits (Escoubas et al., 2002), but whose action here is presumably on Kv2 family members as Kv4 α subunits form transient A-type channels [reviewed in (Gutman et al., 2005)]. This study also showed that the biophysical properties of the delayed rectifier Kv channels in DRG neurons most closely resembled those in heterologous cells expressing Kv2.1 together with Kv9.3, and not cells expressing Kv2.1 alone (Bocksteins et al., 2009). Interestingly, the gating of the current obtained from expression of recombinant

Kv2.1/Kv9.3 channels in heterologous cells, and the stomatoxin-sensitive delayed rectifier Kv current in DRG neurons, were similarly regulated by constitutive phosphorylation (Bocksteins et al., 2009). A subsequent study from this same group combined electrophysiological recordings and single cell RT-PCR to show that the quantity of Kv2.1 mRNA and the amplitude of stomatoxin-sensitive delayed rectifier current in cultured DRG neurons were highly correlated, suggesting that Kv2.1 was a fundamental component of the Kv channels underlying this current (Veys et al., 2012).

Expression of Kv9.1 mRNA and protein has been found in medium to large diameter DRG neurons that correspond to cell bodies of sensory neurons with A-type myelinated fibers (Tsantoulas et al., 2012). Double label immunohistochemistry for Kv9.1 with either Kv2.1 or Kv2.2 revealed that medium to large diameter cells have overlapping expression of these subunits, while small diameter cells had labeling for Kv2 subunits in the absence of Kv9.1 (Tsantoulas et al., 2012). This study also revealed that expression of Kv9.1 mRNA and protein was dramatically decreased/eliminated in DRG neurons in an animal model of neuropathic pain (spinal nerve transection). Kv9.1 was also rapidly lost after placing DRG neurons in culture, which, like spinal nerve transection, results in *de facto* axotomy of the DRG neurons (Tsantoulas et al., 2012). Importantly, a four day intrathecal application of a Kv9.1-specific silencing RNA led to decreased Kv9.1 mRNA and protein expression in DRG, behavioral hyperalgesia to mechanical stimulation, and hyperexcitability of myelinated A fibers, suggesting a crucial role for this electrically silent modulatory subunit in nociceptive neuronal function (Tsantoulas et al., 2012).

The recent paper by Tsantoulas et al., (Tsantoulas et al., 2014) focuses on the latter class of sensory neurons, from the DRG of rats, using a model of neuropathic pain as induced by peripheral axotomy. This model yields DRG neuron hyperexcitability and sensitization of the animal and nociceptive system to types and levels of stimuli that would not normally be sensed as noxious. The authors report a set of important findings that suggest a critical role for Kv2 family channels in regulating excitability of these neurons, under both normal conditions, where pharmacological inhibition of these channels yields neuronal hyperexcitability, and in a model of neuropathic pain where downregulation of these channels yields downregulation of Kv2 channel expression, and patterns of neuronal hyperexcitability similar to what is observed upon the pharmacological inhibition in control animals. That the downregulation of Kv2 channels upon axotomy yields effects on neuronal excitability that mimic and occlude the impact of Kv2 channel inhibitors on neuronal excitability supports a key role for Kv2 channels in mediating the changes in excitability associated with neuropathic pain.

Exploring the potential for Kv2 channels as drug targets for chronic pain

It is clear from the recent study of Tsantoulas et al., (Tsantoulas et al., 2014) that Kv2 channel modulators represent an attractive new candidate for targeting neuropathic pain. The most prominent drug discovery programs to date that have been focused on Kv2 channels have been those that aim to develop Kv2 channel inhibitors as a new class of diabetes drugs. One prominent program at Merck Research Laboratories not only led to the discovery of a novel peptide neurotoxin called Guangxitoxin or GxTx-1 (Herrington et al., 2006), but also

small molecule inhibitors (RY785 and RY796) that are selective for Kv2 channels (Herrington et al., 2011). However, as these compounds do not distinguish between Kv2.1 and Kv2.2, the desired effects on Kv2.1 to enhance glucose-stimulated insulin secretion from pancreatic β cells were offset somewhat by triggering Kv2.2 regulated somatostatin secretion from pancreatic δ cells (Herrington et al., 2011). There are no compounds or neurotoxins that act as selective inhibitors for Kv2.1 or Kv2.2 that would allow for a pharmacological dissection of their specific roles in the Kv2 family-dependent development of neuropathic pain identified in the recent study highlighted here (Tsantoulas et al., 2014). However, anti-Kv2.1 antibodies have been developed as selective inhibitors of channels containing these subunits, although with the drawback that they bind to intracellular epitopes and need to be introduced into cells via microinjection or patch pipet (Murakoshi and Trimmer, 1999).

The studies by Tsantoulas and colleagues (Tsantoulas et al., 2014) suggest that Kv2 channels are attractive targets for the development of novel therapeutics for chronic pain, in that the changes in action potential waveform seen in neuropathic pain models could be directly attributed to these channels. One would presume that enhancing the activity of residual Kv2 channels in these neurons offers therapeutic potential to restore normal function. This would require the development of Kv2 channel openers, whose action would be analogous to that of the anti-epileptic drug retigabine on Kv7 channels (Orhan et al., 2012; Passmore et al., 2003). Importantly, in addition to its efficacy as an anti-epileptic drug, retigabine is also effective at suppressing painful transmission (Blackburn-Munro and Jensen, 2003; Passmore et al., 2012; Passmore et al., 2003), providing proof of concept for targeting Kv channels for pain medications. Retigabine acts by shifting the voltage-dependence of activation of Kv7 channels to more hyperpolarizing membrane potentials, acting as an allosteric modulator that enhances channel opening [reviewed in (Gunthorpe et al., 2012)], somewhat analogous to the effects of benzodiazepines in enhancing opening of GABA-A receptors. A number of other Kv7 openers have been developed and are at various stages of clinical trials, with newer generation compounds exhibiting preferential actions on channel complexes of certain combinations of Kv7 α subunits [reviewed in (Castle, 2010)]. One would speculate that such positive allosteric modifiers of channel activity could be developed for other classes of Kv channels, including those of the Kv2 family that Tsantoulas and colleagues have shown are critical to both normal nociceptive function and neuropathic chronic pain (Tsantoulas et al., 2012), and that these would have great potential as novel treatments for chronic pain.

Future studies to further define the role of Kv channels in nociceptive neurons

One important question that emerges from the study of Tsantoulas and colleagues (Tsantoulas et al., 2012) is whether genetic ablation of Kv2 channel expression in nociceptive neurons would cause neuropathic pain *in vivo*. A global constitutive Kv2.1 knockout mouse has been used to study role of Kv2.1 in metabolism (Jacobson et al., 2007), based on the high level expression of Kv2.1 in pancreatic beta cells (MacDonald et al., 2001) where it regulates glucose-induced insulin secretion (Li et al., 2013). A global

constitutive Kv2.2 knockout mouse has disrupted sleep-wake cycles (Hermansteyne et al., 2013), presumably due to high-level expression of Kv2.2 in basal forebrain GABAergic neurons (Hermansteyne et al., 2010). Future studies should evaluate whether these mice have enhanced sensitivity to thermal, tactile or other sensory stimuli, as would be predicted from the studies detailed above. It will also be important to pursue studies employing conditional elimination of Kv2.1 and/or Kv2.2 expression in adult nociceptive neurons, for example through the generation of inducible nociceptor-specific knockout animals. These animal models will also be important in characterizing the mode of action of any candidate Kv2 channel openers.

Another question raised by this paper is the subcellular localization of the Kv2.1 and Kv2.2 proteins in nociceptive neurons. Regardless of modality, nociceptive neurons have peripheral sensory endings in somatic or visceral tissues, have axons (either myelinated or unmyelinated) that carry the signals from the tissues through the dorsal roots toward the nociceptor cell bodies in the sensory ganglia (trigeminal or DRG), and that continue along the axons to the central terminals that synapses onto their spinal cord targets (Feirabend and Marani, 2003). The targeting of Kv channels to distinct subcellular domains determines their functional contribution, in that a Kv channel localized in a central nerve ending would have a function distinct from the same channel located in a DRG cell body, or in a peripheral nerve ending [reviewed in (Gold and Gebhart, 2010)]. High quality antibodies are available for both Kv2.1 and Kv2.2, and limited multiple labeling experiments have been performed to analyze the extent of their coexpression and colocalization in mammalian brain neurons (Hermansteyne et al., 2010; Kihira et al., 2010). Determining the localization specific Kv2 channel α and auxiliary subunits within nociceptive neurons would provide important insights into the potential of Kv2 channel complexes with specific subunit composition to distinct steps in the transduction of pain signals. At best, these analyses would include samples from animal models, as well as postmortem human samples.

Another question that arises from the current study is the subunit composition of Kv2 channels in nociceptive neurons. As discussed above, the ratio of Kv2.1, Kv2.2 and modulatory/electrically silent subunits in a channel tetramer, and the nature and number of auxiliary AMIGO subunits, should define the biophysical and pharmacological properties, subcellular localization, and sensitivity to modulation of the native Kv2 channel complexes. Biochemical studies of the subunit composition of Kv2 channels, by co-immunoprecipitation with subunit-specific antibodies, followed by immunoblot analyses for each of these candidate component subunits, may allow for determination of the nature of native Kv2 channels in DRG neurons, and the specific form of Kv2 channel complex that is most relevant to drug discovery and that could be recapitulated in heterologous cells for high throughput pharmaceutical screening. It is attractive to consider performing these subpopulations of DRG neurons that have been separated based on cell diameter or molecular markers, to determine whether cell-type differences exist. Proteomic analyses employing mass spectrometry may allow for the identification of the extent of interaction with both known components of Kv2 channels, such as modulatory and auxiliary subunits, as well as novel DRG neuron-specific interacting proteins. This approach would also allow for analyses of overall extent and nature of phosphorylation of the channel subunits, under both normal conditions, and under conditions where sensory neurons are subjected to acute

painful stimuli, or to conditions that lead to chronic neuropathic pain. As above, studies on samples from animal models should be followed by analyses in human samples.

Lastly, one aspect of Kv2.1 function that is exceptional is its extent of regulation by phosphorylation (Cerdeira and Trimmer, 2010). Whether DRG neurons have the capacity to regulate Kv2.1 in this manner, for example through stimuli that trigger acute or sustained hyperactivity of DRG neurons, is as yet unknown and may provide another route to regulating Kv2 channel activity in nociceptive neurons. A recent study has revealed that peripheral nerve injury leads to dephosphorylation of Kv2.1 in spinal α motoneurons, as judged by antibody labeling experiments that showed rapid (within 20 min of nerve injury, the shortest time point examined) changes in phosphorylation-dependent Kv2.1 clustering (Romer et al., 2013). It will be important to determine whether phosphorylation of Kv2.1 expressed in DRG neurons is also regulated by nerve injury, through immunohistochemical analyses aimed at determining the extent of Kv2.1 clustering, or utilizing phosphospecific antibodies specific for individual Kv2.1 phosphorylation sites, or through biochemical analyses of Kv2.1 phosphorylation, including those employing mass spectrometry-based proteomics. Identification of specific phosphorylation sites may allow for links to specific protein kinase and protein phosphatases that could serve as targets for therapeutic drugs that could prevent the changes in channel function leading to altered neuronal excitability, or provide enhancement of function that could offset decreased expression. For example, stimulation of calcineurin, which leads to decreased Kv2.1 phosphorylation and its enhanced activity (Misonou et al., 2004; Park et al., 2006), would be expected to be beneficial in enhancing the activity of remaining Kv2.1 channels, and to rescue neurons from the hyperexcitability resulting from downregulation of Kv2.1 expression that can occur in peripheral neuropathic pain, as shown by Tsantoulas and colleagues (Tsantoulas et al., 2014). It is interesting to note that the use of calcineurin inhibitors, for example as immunosuppressives, has been associated with development of a disabling pain syndrome (Grotz et al., 2001). While a link between calcineurin inhibition and pain has been proposed to be based in effects on increased phosphorylation of another class of K^+ channels (Prommer, 2012), it is possible that enhanced phosphorylation of Kv2.1 in DRG neurons upon calcineurin inhibition, and the resulting change in its voltage-dependence of gating making channel opening less likely, also contributes to the generation of hyperexcitability and pain.

References

- Blackburn-Munro G, Jensen BS. The anticonvulsant retigabine attenuates nociceptive behaviours in rat models of persistent and neuropathic pain. *European journal of pharmacology*. 2003; 460:109–116. [PubMed: 12559370]
- Bocksteins E, Raes AL, Van de Vijver G, Bruyns T, Van Bogaert PP, Snyders DJ. Kv2.1 and silent Kv subunits underlie the delayed rectifier K^+ current in cultured small mouse DRG neurons. *Am J Physiol Cell Physiol*. 2009; 296:C1271–1278. [PubMed: 19357235]
- Bocksteins E, Snyders DJ. Electrically silent kv subunits: their molecular and functional characteristics. *Physiology (Bethesda)*. 2012; 27:73–84. [PubMed: 22505664]
- Brueggemann LI, Gentile S, Byron KL. Social networking among voltage-activated potassium channels. *Progress in molecular biology and translational science*. 2013; 117:269–302. [PubMed: 23663972]

- Castle NA. Pharmacological modulation of voltage-gated potassium channels as a therapeutic strategy. Expert opinion on therapeutic patents. 2010; 20:1471–1503. [PubMed: 20726689]
- Catterall WA. Voltage-gated sodium channels at 60: structure, function and pathophysiology. J Physiol. 2012; 590:2577–2589. [PubMed: 22473783]
- Cerda O, Trimmer JS. Analysis and functional implications of phosphorylation of neuronal voltage-gated potassium channels. Neurosci Lett. 2010; 486:60–67. [PubMed: 20600597]
- Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. Annual review of neuroscience. 2010; 33:325–347.
- Drewe JA, Verma S, Frech G, Joho RH. Distinct spatial and temporal expression patterns of K⁺ channel mRNAs from different subfamilies. J Neurosci. 1992; 12:538–548. [PubMed: 1740690]
- Escoubas P, Diochot S, Celerier ML, Nakajima T, Lazdunski M. Novel tarantula toxins for subtypes of voltage-dependent potassium channels in the Kv2 and Kv4 subfamilies. Mol Pharmacol. 2002; 62:48–57. [PubMed: 12065754]
- Feirabend, HKP.; Marani, E. Dorsal root ganglion. In: Aminoff, MJ.; Daroff, RB., editors. Encyclopedia of the Neurological Sciences. Academic Press, Amsterdam; Boston: 2003. p. 28-33.
- Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med. 2010; 16:1248–1257. [PubMed: 20948530]
- Grotz WH, Breitenfeldt MK, Braune SW, Allmann KH, Krause TM, Rump JA, Schollmeyer PJ. Calcineurin-inhibitor induced pain syndrome (CIPS): a severe disabling complication after organ transplantation. Transplant international : official journal of the European Society for Organ Transplantation. 2001; 14:16–23. [PubMed: 11263551]
- Gunthorpe MJ, Large CH, Sankar R. The mechanism of action of retigabine (ezogabine), a first-in-class K⁺ channel opener for the treatment of epilepsy. Epilepsia. 2012; 53:412–424. [PubMed: 22220513]
- Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stuhmer W, Wang X. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol Rev. 2005; 57:473–508. [PubMed: 16382104]
- Hermansteyne TO, Kihira Y, Misono K, Deitchler A, Yanagawa Y, Misonou H. Immunolocalization of the voltage-gated potassium channel Kv2.2 in GABAergic neurons in the basal forebrain of rats and mice. J Comp Neurol. 2010; 518:4298–4310. [PubMed: 20853508]
- Hermansteyne TO, Subedi K, Le WW, Hoffman GE, Meredith AL, Mong JA, Misonou H. Kv2.2: a novel molecular target to study the role of basal forebrain GABAergic neurons in the sleep-wake cycle. Sleep. 2013; 36:1839–1848. [PubMed: 24293758]
- Herrington J, Solly K, Ratliff KS, Li N, Zhou YP, Howard A, Kiss L, Garcia ML, McManus OB, Deng Q, Desai R, Xiong Y, Kaczorowski GJ. Identification of novel and selective Kv2 channel inhibitors. Mol Pharmacol. 2011; 80:959–964. [PubMed: 21948463]
- Herrington J, Zhou YP, Bugianesi RM, Dulski PM, Feng Y, Warren VA, Smith MM, Kohler MG, Garsky VM, Sanchez M, Wagner M, Raphaelli K, Banerjee P, Ahaghotu C, Wunderler D, Priest BT, Mehl JT, Garcia ML, McManus OB, Kaczorowski GJ, Slaughter RS. Blockers of the delayed-rectifier potassium current in pancreatic beta-cells enhance glucose-dependent insulin secretion. Diabetes. 2006; 55:1034–1042. [PubMed: 16567526]
- Hille, B. Ionic channels of excitable membranes. 3. Sinauer; Sunderland, MA: 2001.
- Hwang PM, Glatt CE, Brecht DS, Yellen G, Snyder SH. A novel K⁺ channel with unique localizations in mammalian brain: molecular cloning and characterization. Neuron. 1992; 8:473–481. [PubMed: 1550672]
- Ishikawa K, Tanaka M, Black JA, Waxman SG. Changes in expression of voltage-gated potassium channels in dorsal root ganglion neurons following axotomy. Muscle & nerve. 1999; 22:502–507. [PubMed: 10204786]
- Jacobson DA, Kuznetsov A, Lopez JP, Kash S, Ammala CE, Philipson LH. Kv2.1 ablation alters glucose-induced islet electrical activity, enhancing insulin secretion. Cell Metab. 2007; 6:229–235. [PubMed: 17767909]
- Jan LY, Jan YN. Voltage-gated potassium channels and the diversity of electrical signalling. J Physiol. 2012; 590:2591–2599. [PubMed: 22431339]

- Kihira Y, Hermanstynne TO, Misonou H. Formation of heteromeric Kv2 channels in mammalian brain neurons. *J Biol Chem*. 2010; 285:15048–15055. [PubMed: 20202934]
- Kim DS, Choi JO, Rim HD, Cho HJ. Downregulation of voltage-gated potassium channel alpha gene expression in dorsal root ganglia following chronic constriction injury of the rat sciatic nerve. *Brain Res Mol Brain Res*. 2002; 105:146–152. [PubMed: 12399117]
- Lawson SN. Phenotype and function of somatic primary afferent nociceptive neurones with C-, Delta- or Aalpha/beta-fibres. *Experimental physiology*. 2002; 87:239–244. [PubMed: 11856969]
- Li XN, Herrington J, Petrov A, Ge L, Eiermann G, Xiong Y, Jensen MV, Hohmeier HE, Newgard CB, Garcia ML, Wagner M, Zhang BB, Thornberry NA, Howard AD, Kaczorowski GJ, Zhou YP. The role of voltage-gated potassium channels Kv2.1 and Kv2.2 in the regulation of insulin and somatostatin release from pancreatic islets. *J Pharmacol Exp Ther*. 2013; 344:407–416. [PubMed: 23161216]
- Liu M, Wood JN. The roles of sodium channels in nociception: implications for mechanisms of neuropathic pain. *Pain medicine*. 2011; 12(Suppl 3):S93–99. [PubMed: 21752183]
- MacDonald PE, Ha XF, Wang J, Smukler SR, Sun AM, Gaisano HY, Salapatek AM, Backx PH, Wheeler MB. Members of the Kv1 and Kv2 voltage-dependent K(+) channel families regulate insulin secretion. *Molecular endocrinology*. 2001; 15:1423–1435. [PubMed: 11463864]
- Maljevic S, Lerche H. Potassium channels: a review of broadening therapeutic possibilities for neurological diseases. *Journal of neurology*. 2013; 260:2201–2211. [PubMed: 23142946]
- Mandikian D, Cerda O, Sack JT, Trimmer JS. A SUMO-Phospho tag team for wrestling with potassium channel gating. *J Gen Physiol*. 2011; 137:435–439. [PubMed: 21518832]
- Misonou H, Mohapatra DP, Park EW, Leung V, Zhen D, Misonou K, Anderson AE, Trimmer JS. Regulation of ion channel localization and phosphorylation by neuronal activity. *Nat Neurosci*. 2004; 7:711–718. [PubMed: 15195093]
- Murakoshi H, Trimmer JS. Identification of the Kv2.1 K⁺ channel as a major component of the delayed rectifier K⁺ current in rat hippocampal neurons. *J Neurosci*. 1999; 19:1728–1735. [PubMed: 10024359]
- Orhan G, Wuttke TV, Nies AT, Schwab M, Lerche H. Retigabine/Ezogabine, a KCNQ/K(V)7 channel opener: pharmacological and clinical data. *Expert opinion on pharmacotherapy*. 2012; 13:1807–1816. [PubMed: 22783830]
- Otschytch N, Raes A, Van Hoorick D, Snyders DJ. Obligatory heterotetramerization of three previously uncharacterized Kv channel alpha-subunits identified in the human genome. *Proc Natl Acad Sci U S A*. 2002; 99:7986–7991. [PubMed: 12060745]
- Park KS, Mohapatra DP, Misonou H, Trimmer JS. Graded regulation of the Kv2.1 potassium channel by variable phosphorylation. *Science*. 2006; 313:976–979. [PubMed: 16917065]
- Passmore GM, Reilly JM, Thakur M, Keasberry VN, Marsh SJ, Dickenson AH, Brown DA. Functional significance of M-type potassium channels in nociceptive cutaneous sensory endings. *Frontiers in molecular neuroscience*. 2012; 5:63. [PubMed: 22593734]
- Passmore GM, Selyanko AA, Mistry M, Al-Qatari M, Marsh SJ, Matthews EA, Dickenson AH, Brown TA, Burbidge SA, Main M, Brown DA. KCNQ/M currents in sensory neurons: significance for pain therapy. *J Neurosci*. 2003; 23:7227–7236. [PubMed: 12904483]
- Peltola MA, Kuja-Panula J, Lauri SE, Taira T, Rauvala H. AMIGO is an auxiliary subunit of the Kv2.1 potassium channel. *EMBO reports*. 2011; 12:1293–1299. [PubMed: 22056818]
- Plant LD, Dowdell EJ, Dementieva IS, Marks JD, Goldstein SA. SUMO modification of cell surface Kv2.1 potassium channels regulates the activity of rat hippocampal neurons. *J Gen Physiol*. 2011; 137:441–454. [PubMed: 21518833]
- Prommer E. Calcineurin-inhibitor pain syndrome. *The Clinical journal of pain*. 2012; 28:556–559. [PubMed: 22673490]
- Romer SH, Dominguez KM, Gelpi MW, Deardorff AS, Tracy RC, Fyffe RE. Redistribution of Kv2.1 ion channels on spinal motoneurons following peripheral nerve injury. *Brain Res*. 2013
- Salinas M, Duprat F, Heurteaux C, Hugnot JP, Lazdunski M. New modulatory alpha subunits for mammalian Shab K⁺ channels. *J Biol Chem*. 1997; 272:24371–24379. [PubMed: 9305895]

- Savio-Galimberti E, Gollob MH, Darbar D. Voltage-gated sodium channels: biophysics, pharmacology, and related channelopathies. *Frontiers in pharmacology*. 2012; 3:124. [PubMed: 22798951]
- Trimmer JS. Immunological identification and characterization of a delayed rectifier K⁺ channel polypeptide in rat brain. *Proc Natl Acad Sci U S A*. 1991; 88:10764–10768. [PubMed: 1961744]
- Trimmer, JS.; Misonou, H. Phosphorylation of voltage-gated ion channels. In: Zheng, J.; Trudeau, MC., editors. *Handbook of Ion Channels*. CRC Press; Boca Raton, FL: 2014.
- Tsantoulas C, Zhu L, Shaifta Y, Grist J, Ward JP, Raouf R, Michael GJ, McMahon SB. Sensory neuron downregulation of the Kv9.1 potassium channel subunit mediates neuropathic pain following nerve injury. *J Neurosci*. 2012; 32:17502–17513. [PubMed: 23197740]
- Tsantoulas C, Zhu L, Yip P, Grist J, Michael GJ, McMahon SB. Kv2 dysfunction after peripheral axotomy enhances sensory neuron responsiveness to sustained input. *Exp Neurol*. 2014; 251:115–126. [PubMed: 24252178]
- Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol Rev*. 2008; 88:1407–1447. [PubMed: 18923186]
- Vacher H, Trimmer JS. Diverse roles for auxiliary subunits in phosphorylation-dependent regulation of mammalian brain voltage-gated potassium channels. *Pflugers Arch*. 2011; 462:631–643. [PubMed: 21822597]
- Veys K, Labro AJ, De Schutter E, Snyders DJ. Quantitative single-cell ion-channel gene expression profiling through an improved qRT-PCR technique combined with whole cell patch clamp. *J Neurosci Methods*. 2012; 209:227–234. [PubMed: 22728251]
- Yu F, Yarov-Yarovoy V, Gutman G, Catterall W. Overview of molecular relationships in the voltage-gated ion channel superfamily. *Pharmacol Rev*. 2005; 57:387–395. [PubMed: 16382097]