# Kv5, Kv6, Kv8, and Kv9 subunits: No simple silent bystanders

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Members of the electrically silent voltage-gated $K^+$ (Kv) subfamilies (Kv5, Kv6, Kv8, and Kv9, collectively identified as electrically silent voltage-gated $K^+$ channel [KvS] subunits) do not form functional homotetrameric channels but assemble with Kv2 subunits into heterotetrameric Kv2/KvS channels with unique biophysical properties. Unlike the ubiquitously expressed Kv2 subunits, KvS subunits show a more restricted expression. This raises the possibility that Kv2/KvS heterotetramers have tissue-specific functions, making them potential targets for the development of novel therapeutic strategies. Here, I provide an overview of the expression of KvS subunits in different tissues and discuss their proposed role in various physiological and pathophysiological processes. This overview demonstrates the importance of KvS subunits and Kv2/KvS heterotetramers in vivo and the importance of considering KvS subunits and Kv2/KvS heterotetramers in the development of novel treatments.

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## Kv2 channels

The voltage-gated $K^+$ (Kv) channel Kv2.1 plays a crucial role in many cell types, with its contribution to various processes depending on both its conductive and nonconductive properties. For example, Kv2.1 channels control the action potential width in hippocampal neurons during high-frequency stimulation (Du et al., 2000), and they carry the augmented $K^+$ current that triggers the apoptotic cascade in cortical neurons (Pal et al., 2003). These two functions depend on Kv2.1-mediated $K^+$ current. In contrast, Kv2.1 channels help control the glucose-stimulated insulin secretion in human pancreatic β-cells by means of their interaction with the soluble *N*-ethylmaleimide–sensitive factor (NSF) attachment receptor protein syntaxin 1A (Dai et al., 2012). They also induce the formation of junctions between the ER and the plasma membrane in cultured hippocampal neurons; an enhanced Kv2.1 expression increases the amount of cortical ER that is in close proximity to the plasma membrane, and Kv2.1 cluster formation remodels the cortical ER from tubules into large, planar structures (Fox et al., 2015). In both of these processes, the role of Kv2.1 channels is independent of their ability to conduct ions.

Kv2.1 channels are homotetramers of α-subunits that are arranged around a central ion-conducting pore (Long et al., 2005). The ability to mediate their disparate functions in a host of different excitable and nonexcitable cells depends on Kv2.1 channel diversity, which is achieved through various mechanisms. These include posttranslational modifications of the Kv2.1 α-subunits (e.g., phosphorylation and SUMOylation; Mohapatra et al., 2007; Dai et al., 2009), association with auxiliary β-subunits (e.g., KChAP, AMIGO, and KCNE1-5 proteins; Kuryshev et al., 2000; McCrossan and Abbott, 2004; David et al., 2015), and heterotetramerization with modulatory α-subunits of the Kv5, Kv6, Kv8, and Kv9 subfamilies (Bocksteins and Snyders, 2012). Kv2.1 channel diversity can be further increased through a combination of these different mechanisms. For example, Kv2.1 subunits assemble with both the modulatory α-subunit Kv6.4 and the auxiliary β-subunit KCNE5 to generate functional Kv2.1/Kv6.4/KCNE5 channels with biophysical properties distinct from those of Kv2.1, Kv2.1/KCNE5, or Kv2.1/Kv6.4 channels (David et al., 2015).

## Kv5, Kv6, Kv8, and Kv9 subunits: The electrically silent Kv subunits

The members of the Kv5, Kv6, Kv8, and Kv9 subfamilies are electrically silent; they are collectively referred to as the electrically silent Kv (KvS) channel subunits. The first KvS subunits were cloned in 1992 by Drewe et al. (1992), and in the following years additional KvS subunits were discovered and characterized. A more historical perspective on the discovery of KvS subunits, and the developments made in the subsequent years, is given in a previous review (Bocksteins and Snyders, 2012). 10 KvS subunits have been identified to date:

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Abbreviations used in this paper: 4-AP, 4-aminopyridine; AVCN, anterior ventral cochlear nucleus; BAFME, benign adult familial myoclonic epilepsy; CDSR, cone dystrophy with supernormal rod electroretinogram; DCN, dorsal cochlear nucleus; DRG, dorsal root ganglion; DSM, detrusor smooth muscle; FR, fast ripple; GABA, γ-aminobutyric acid; HIF, hypoxia-inducible factor; Kv, voltage-gated $K^+$; KvS, electrically silent Kv; PASMC, pulmonary artery smooth muscle cell; PVCN, posterior ventral cochlear nucleus; RMCA, rat middle cerebral artery; RT, reverse transcription; ScTx-1, stromatoxin 1; SNP, single nucleotide polymorphism; VSMC, vascular smooth muscle cell.



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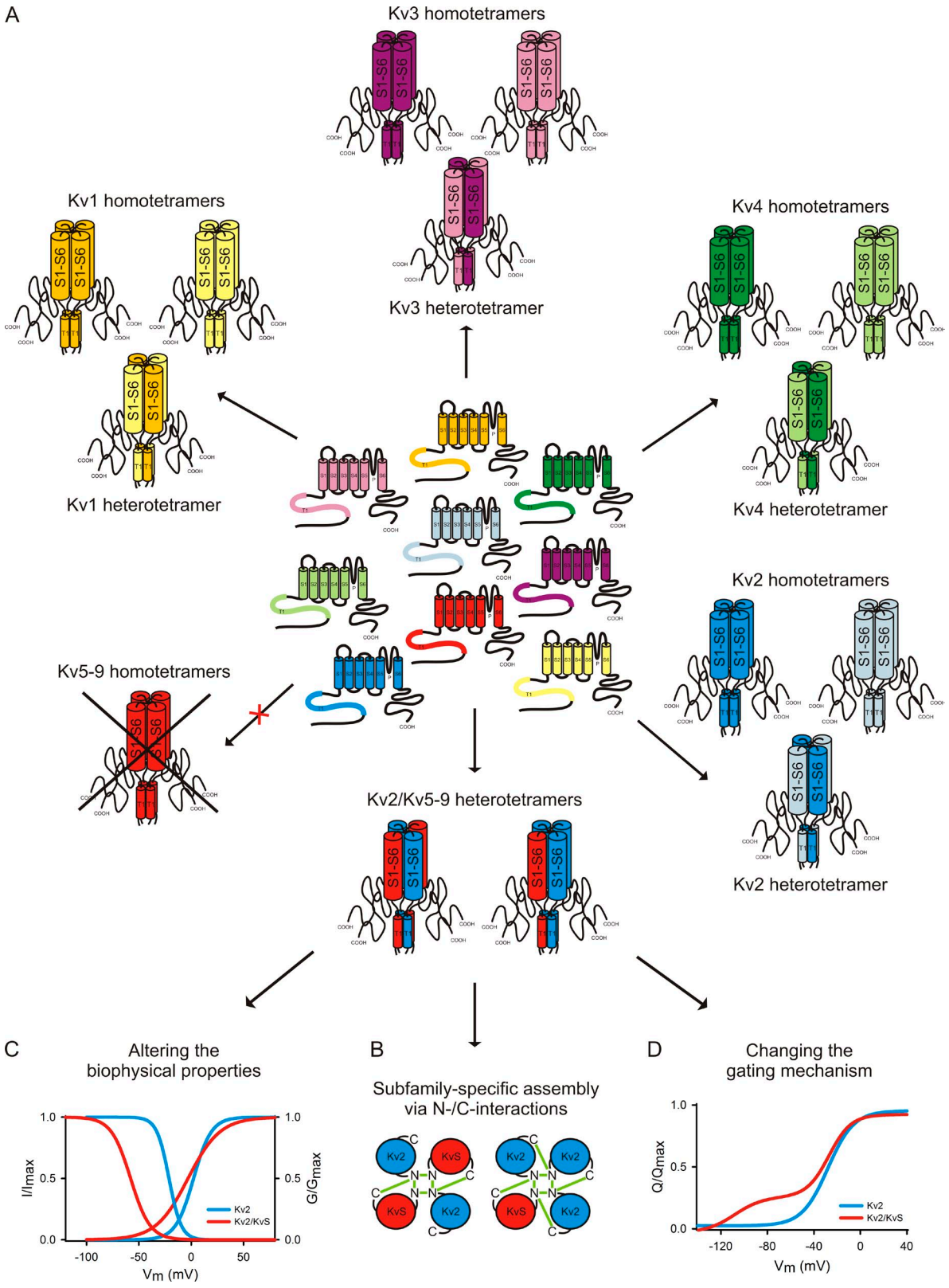


**Figure 1.** Subfamily-specific assembly into functional Kv channels. (A) Each subfamily is represented in a different color, with the lighter and darker shades representing a different subunit within the same subfamily. The Kv1–Kv4 subunits form functional homo- and heterotetrameric channels within their own subfamilies. Members of the electrically silent Kv subfamilies (Kv5, Kv6, Kv8, and Kv9) do not form functional homotetramers but heterotetramerize with Kv2 subunits to form functional channels. (B) Subfamily-specific Kv2/KvS assembly is determined by interactions (represented in green) that involve both the N and C termini: an interaction between the

Kv5.1, Kv6.1–Kv6.4, Kv8.1, Kv8.2, and Kv9.1–Kv9.3. Although each KvS subunit has unique characteristics, they all have three features in common. These features are discussed in more depth in a previous review (Bocksteins and Snyders, 2012) and will therefore only be shortly discussed here. First, unlike the Kv1–Kv4 subunits, KvS subunits do not form functional homotetrameric channels when expressed alone in heterologous expression systems (Fig. 1 A). Based on sequence homology, KvS subunits appear to possess several hallmarks of a typical Kv α-subunit: six transmembrane segments (S1–S6) with several positive residues on S4 that may function as the main voltage-sensing component, the $K^+$ signature sequence GYG on the pore loop between S5 and S6, and cytoplasmic N and C termini (Bocksteins and Snyders, 2012). However, when expressed alone, KvS subunits do not generate functional channels at the plasma membrane because of their retention in the endoplasmic reticulum. Second, KvS subunits heterotetramerize with Kv2 subunits but not with members of the Kv1, Kv3, and Kv4 subfamilies to generate functional channels at the plasma membrane (Fig. 1 A). This subfamily-specific assembly depends, at least for Kv2.1 and Kv6.4 subunits, on both an interaction between the Kv2.1 and Kv6.4 N terminus and an interaction between the N terminus of Kv2.1 and the C terminus of Kv6.4 (Fig. 1 B; Bocksteins et al., 2014). In contrast, the N-terminal tetramerization domain T1 is assumed to be the only determinant of the subfamily-specific assembly of the Kv1–Kv4 subunits: tetramerization between members of the same subfamily is promoted by a compatible T1 domain, whereas heterotetramerization between subunits of different subfamilies is prevented by an incompatible T1 domain (Li et al., 1992; Shen et al., 1993; Lee et al., 1994; Shen and Pfaffinger, 1995; Xu et al., 1995). Third, KvS subunits modulate the biophysical properties of Kv2. Therefore, Kv2/KvS heterotetramers show shifts in the voltage dependence of activation and/or the voltage dependence of inactivation; changes in the activation, deactivation, and/or inactivation kinetics; and alterations in the current density compared with Kv2 homotetramers (Fig. 1 C). The different KvS subunits may affect the biophysical properties of Kv2 through various mechanisms. For example, Kv6.1 subunits may shift the Kv2.1 voltage dependence of inactivation ∼50 mV in the hyperpolarized direction compared with that of Kv2.1 homotetramers by promoting the $Ca^{2+}$/calmodulin-dependent dephosphorylation of Kv2.1 (Salinas et al., 1997b; Kramer et al., 1998) because Kv6.1 binds calmodulin (O'Connell et al., 2010). In contrast, Kv6.4 may induce a 40-mV hyperpolarizing shift in the Kv2.1 voltage dependence of inactivation compared with that of Kv2.1 homotetramers by affecting the gating mechanism of Kv2.1/Kv6.4 heterotetramers. A comparison of gating current ($I_Q$) recordings from Kv2.1 homotetramers and Kv2.1/Kv6.4 heterotetramers reveals a hyperpolarized second component in the charge (Q) versus voltage (V) distribution curve of the heterotetramers (Fig. 1 D). The voltage dependence of this hyperpolarized charge movement corresponds with the Kv6.4-induced hyperpolarizing shift in voltage-dependent inactivation (Bocksteins et al., 2012).

### Physiological role of Kv2/KvS heterotetramers in different tissues

Unlike the ubiquitously expressed Kv2.1, KvS subunits show a more restricted expression, suggesting that Kv2/KvS heterotetramers have tissue-specific functions. This is supported by the increasing number of publications in which the contribution of KvS subunits to different physiological and pathophysiological processes in various cells has been demonstrated. KvS expression in different tissues and cell types is presented in Table 1 and Fig. 2, and the proposed role of KvS subunits in these cells is summarized in Table 2 and discussed in the following paragraphs.

#### Neural tissues

*Central nervous system.* Numerous neurons in the central nervous system express Kv2.1, and several studies have suggested a role for KvS subunits in these Kv2-expressing neurons. For example, Kv2.1 contributes to the slowly inactivating $K^+$ current in rat neocortical pyramidal neurons (Guan et al., 2007) to regulate the firing rate (Guan et al., 2013). This Kv2-mediated current shows a voltage dependence of activation and inactivation that is shifted in the hyperpolarized direction compared with that of heterologously expressed Kv2 channels. Coexpression of Kv2.1 with Kv5 (Kramer et al., 1998), Kv6 (Kramer et al., 1998), or Kv9 (Salinas et al., 1997b) subunits shifts the voltage dependence of Kv2.1 activation and/or inactivation in the hyperpolarized direction. Kv5, Kv6, and Kv9 subunits are expressed in the cortex (Drewe et al., 1992; Salinas et al., 1997b; Georgiev et al., 2012, 2014), suggesting that the hyperpolarizing shift in the voltage dependence of Kv2.1 activation and inactivation seen in rat neocortical pyramidal neurons could be caused by KvS subunits. Furthermore, it has been suggested that the functional specialization of cortical networks relates as two ring-shaped networks to their spatial distribution over the cerebral cortex. The visual-sensorimotor-auditory ring includes visual, somatosensory, auditory, and motor cortices that are dedicated to sensorimotor functions. The parieto-temporo-frontal

Kv2 and KvS N terminus and an interaction between the C terminus of the KvS subunits and the N terminus of the Kv2 subunits. For clarity, both possible Kv2/KvS stoichiometries (2:2 and 3:1) are shown. (C) Kv2/KvS heterotetramerization can cause a shift in the voltage dependence of Kv2 activation and inactivation. (D) KvS subunits (partially) modulate the biophysical Kv2 properties by changing the gating mechanism. This figure is adapted from Fig. 3 in Bocksteins and Snyders (2012) and Fig. 5 in Bocksteins et al. (2014).

TABLE 1

*Tissue and cell expression of the different electrically silent Kv subunits*

| Tissue | Kv5.1 | Kv6.1 | Kv6.2 | Kv6.3 | Kv6.4 | Kv8.1 | Kv8.2 | Kv9.1 | Kv9.2 | Kv9.3 |
|---|---|---|---|---|---|---|---|---|---|---|
| Adrenal gland | | | | R (30, 40) | R (30) | | | | | |
| Brain: Whole | R (12, 13, 44, 49) | R (12, 13, 44, 49) | | R (30, 33, 40, 48) | R (30, 33) | R (6, 12, 23, 39) | R (11, 24) | R (12, 37, 41, 43) | R (12, 37) | R (12, 21, 34, 41, 43) |
| Brain: Cortex | G, R (8, 13) | G, R (8, 13) | | | | | | R (37) | R (37) | R (14, 15) |
| Brain: Hippocampus | | R (19) | | | | R (52) | G, R (3, 24) | | | |
| Brain: Pituitary | | R (54) | | R (40) | | | | | | |
| Cancer cells: Colon | R (42) | | | | | | | | | R (28, 42) |
| Cancer cells: Lung | | | | | | | | | | R (28) |
| Cancer cells: Neuronal | R (29) | | | | | | | | R (29) | |
| Cancer cells: Uterus | | | | | | | | | | R (45) |
| Colon | | | | R (30, 33) | R (30, 33) | | R (33) | R (41) | | R (41) |
| Cochlear nucleus | | | | | | R (18) | | R (18) | | R (18) |
| DRG | | R (4) | | | | R (4) | | R, P (4, 10, 47) | R, P (4) | R, P (4) |
| Ductus arteriosus | | | | | | | | | | R (21) |
| Eye: Lens epithelial | | | | | | | | R (41) | | R (41) |
| Eye: Retina | R (53) | | | | | | R, P (11, 22, 53) | R (37) | R (37) | |
| Epithelial cells: Alveolar | | | | | | | | | | R (27) |
| Epithelial cells: Intestinal | | | | | | | | | | R (36) |
| Epithelial cells: Uterus | | | | | | | | | | R (45) |
| Heart | R (5, 13, 44) | R (5, 13) | R (59) | | R (30) | | R (11) | R (41) | | R (41, 43) |
| Kidney | R (13, 44) | R (13, 44) | R (59) | R (30, 33, 48) | R (30) | | R (33) | R (41) | | R (34, 41, 43) |
| Liver | R (44) | R (13) | R (59) | R (30) | R (30, 33) | | R (33) | R (41) | | R (41) |
| Lung | | R (13) | | R (30, 33, 48) | R (30) | | R (11, 38) | R (41) | R (12) | R (21, 34, 41, 43) |
| Motor neurons | | | | | R, P (32) | | | | | |
| Ovary | | | | R (33) | | | R (33) | R (41) | | R (41) |
| Pancreas | R (44) | R (44, 55) | R (55, 59) | R (33, 48, 55) | R (55) | | R (33) | R (41, 55) | R (55) | R (41, 55) |
| Placenta | | R (44) | | | | | | R (41) | | R, P (9, 20, 21, 41, 51) |
| Prostate | | | | | | | R (33) | R (41) | | R (41) |
| Skeletal muscle | R (13, 44) | R (13, 44) | R (59) | | | | | | | R (41) |
| Small intestine | | | | R (33, 40) | R (33) | | | R (41) | | R (34, 41) |
| Smooth muscle cells: Aortic vascular | | | | | | | | | | R (31) |
| Smooth muscle cells: Cerebral artery | R (1, 58) | R (58) | R (58) | R (58) | | | | | R (58) | R, P (58) |
| Smooth muscle cells: Mesenteric vascular | | | | R, P (31) | | | | | | R (31) |
| Smooth muscle cells: Pulmonary artery | R (12, 16, 17, 35) | R (12, 16, 35) | R (16, 17, 35) | R (16, 17, 35) | | | R (16, 35) | R (12, 16, 17, 35) | R (12) | R (2, 12, 16, 17, 34, 35, 50, 57) |
| Smooth muscle cells: Urinary bladder | R (46) | R (46) | R (25, 46) | R (25) | R (46) | | R (25) | R (25) | R (25) | R (7, 25, 26) |
| Spinal cord | | | | R (40) | | | | R (37) | R (37) | |
| Spleen | | | | | | | R (33) | R (41) | | R (41, 43) |
| Stem cells | | | | | | | | | | R (38, 56) |
| Testis | | | | R (33, 40) | | | R (33) | R (41) | | R (34, 41) |
| Thymus | | | | R (33, 40) | | | R (33) | R (41) | | R (41) |

G, gene; R, mRNA; P, protein; 1, Amberg and Santana (2006); 2, Archer et al. (2004); 3, Bergren et al. (2009); 4, Bocksteins et al. (2009); 5, Brahmajothi et al. (1996); 6, Castellano et al. (1997); 7, Chen et al. (2010); 8, Cioli et al. (2014); 9, Corcoran et al. (2008); 10, Costigan et al. (2010); 11, Czirják et al. (2007); 12, Davies and Kozlowski (2001); 13, Drewe et al. (1992); 14, Georgiev et al. (2012); 15, Georgiev et al. (2014); 16, Fantozzi et al. (2006); 17, Firth et al. (2011); 18, Friedland et al. (2007); 19, Friedman et al. (2013); 20, Fyfe et al. (2012); 21, Hayama et al. (2006); 22, Hölter et al. (2012); 23, Hugnot et al. (1996); 24, Jorge et al. (2011); 25, Hristov et al. (2012b); 26, Hristov et al. (2012a); 27, Lee et al. (2003); 28, Lee et al. (2015);

ring comprises parietal, temporal, and frontal cortical regions that are involved in cognitive functions (Cioli et al., 2014). Correspondence analysis and discriminant correspondence analysis have demonstrated that the genes encoding Kv5.1 and Kv6.1 were preferentially expressed on the parieto-temporo-frontal ring (Cioli et al., 2014), suggesting that Kv5.1 and Kv6.1 may play a role in cognitive functions but not in sensorimotor functions.

Kv2.1 channels also underlie a major component of the delayed rectifier $K^+$ current in cultured rat hippocampal neurons (Murakoshi and Trimmer, 1999) and control the action potential width in hippocampal CA1 neurons during high-frequency stimulation (Du et al., 2000). Not only Kv2.1 homotetramers but also heterotetrameric Kv2/KvS channels may contribute to hippocampal physiology. Hippocampal CA3 pyramidal cells express a current, $I_{K(slow)}$, with properties that resemble those of the currents seen when Kv2 and Kv8.1 subunits are coexpressed in mammalian cells (Lüthi et al., 1996; Castellano et al., 1997; Salinas et al., 1997a), suggesting that Kv8.1 subunits play a role in these neurons. A recent study demonstrated that Kv8.1 mRNA was present in neurons in the dentate gyrus of the hippocampus (Winden et al., 2015). This Kv8.1 mRNA expression is lower in small clusters of neurons in the dentate gyrus that display abnormal high-frequency electrophysiologic oscillations, termed fast ripples (FRs), compared with non-FR regions in the epileptogenic regions of subjects suffering from temporal lobe epilepsy (Köhling and Staley, 2011; Winden et al., 2015). Furthermore, the concordance of gene expression between Kv8.1 and other genes associated with several processes essential in both pre- and postsynaptic functions is higher in non-FR regions than in FR regions, and this connectivity is induced by seizure activity (Winden et al., 2015). In the hippocampus, FR activity is uniquely associated with epileptic activity, and FR can appear before the onset of epilepsy, suggesting that Kv8.1 contributes to the pathological mechanisms involved in epileptogenesis. Assembly with Kv8.1 reduces the current density of Kv2.1 (Hugnot et al., 1996; Castellano et al., 1997), and hippocampal neurons lacking Kv2.1-mediated currents demonstrate broadened action potentials (Du et al., 2000), which leads to hypersynchronized firing at high frequencies, as observed in FR regions during epileptic activity. A proposed role for Kv8.1 in epilepsy is strengthened by gene mapping of Kv8.1 and the gene locus responsible for the autosomal dominant idiopathic epileptic syndrome benign adult familial myoclonic epilepsy (BAFME): the human Kv8.1 gene mapped onto the chromosome 8q22.3–8q24.1 (Hugnot et al., 1996; Sano et al., 2002a; Ebihara et al., 2004), whereas the gene locus responsible for BAFME is assigned to chromosome 8q23.3–q24.11 (Mikami et al., 1999), implying that there is a higher correlation between Kv8.1 and other genes associated with BAFME.

Kv8.2 is also proposed to be involved in epilepsy. Linkage analysis identified the Kv8.2 gene as a strong candidate for the modifier of epilepsy 2 locus, which influences the epilepsy phenotype of the transgenic mouse model $Scn2a^{Q54}$ (Bergren et al., 2009). *Scn2a* encodes the voltage-gated sodium channel Nav1.2, and the Q54 mutation slows channel inactivation, resulting in an elevated persistent sodium current that is predicted to increase neuronal excitability and precede the onset of seizure activity (Kearney et al., 2001). The $Scn2a^{Q54}$ mouse has a less severe phenotype in the C57BL/6J background compared with the SJL/J background (Bergren et al., 2005). Two nonsynonymous coding variants of Kv8.2 have been identified that are differentially expressed in these two genetic backgrounds, suggesting that Kv8.2 contributes to the difference in epilepsy phenotype; the SJL/J strain contains the amino acids H205 and R252, whereas the C57BL/6J background contains the amino acids R205 and Q252 (Bergren et al., 2009). Although differences in the functional effects of these two Kv8.2 coding variants on Kv2.1/Kv8.2 currents in transfected CHO cells have been observed, these differences cannot explain the difference in phenotype severity (Jorge et al., 2011). However, whereas whole brain Kv8.2 mRNA level was comparable in the C57BL/6J and SJL/J strains (Bergren et al., 2009), Kv8.2 mRNA level was threefold higher in the hippocampus of the susceptible SJL/J strain than in that of the resistant C57BL/6J strain (Jorge et al., 2011). These findings suggest that higher expression of Kv8.2 in the hippocampus rather than changes in the biophysical properties of the Kv2.1/Kv8.2 channel itself is associated with greater severity of the epilepsy phenotype. Kv8.2 reduces the current density of Kv2.1, and an increased abundance of Kv8.2 would lead to an increased suppression of Kv2.1 currents, resulting in reduced neuronal delayed rectifier current as observed in epilepsy. This was confirmed by analysis of transgenic mice expressing either the C57BL/6J-derived or SJL/J-derived

*Table 1 (Continued)*

29, Li et al. (2015); 30, Mederos y Schnitzler et al. (2009); 31, Moreno-Domínguez et al. (2009); 32, Müller et al. (2014); 33, Ottschytsch et al. (2002); 34, Patel et al. (1997); 35, Platoshyn et al. (2004); 36, Rao et al. (2002); 37, Salinas et al. (1997b); 38, Sandberg et al. (2014); 39, Sano et al. (2002a); 40, Sano et al. (2002b); 41, Shepard and Rae (1999); 42, Spitzner et al. (2007); 43, Stocker and Kerschensteiner (1998); 44, Su et al. (1997); 45, Suzuki and Takimoto (2004); 46, Thorneloe and Nelson (2003); 47, Tsantoulas et al. (2012); 48, Vega-Saenz de Miera (2004); 49, Verma-Kurvari et al. (1997); 50, Wang et al. (2005); 51, Wareing et al. (2006); 52, Winden et al. (2015); 53, Wu et al. (2006); 54, Wulfsen et al. (2000); 55, Yan et al. (2004); 56, You et al. (2013); 57, Yuan et al. (1998); 58, Zhong et al. (2010); 59, Zhu et al. (1999).

Kv8.2 transgenes in the C57BL/6J background: both double-transgenic lines (with higher transgene expression) showed more frequent seizures and accelerated mortality, whereas both single-transgenic lines (with basal transgene expression) failed to show an increase in phenotype severity (Jorge et al., 2011). Screening of 209 pediatric epilepsy subjects for variants in Kv8.2 revealed two unique nonsynonymous Kv8.2 coding

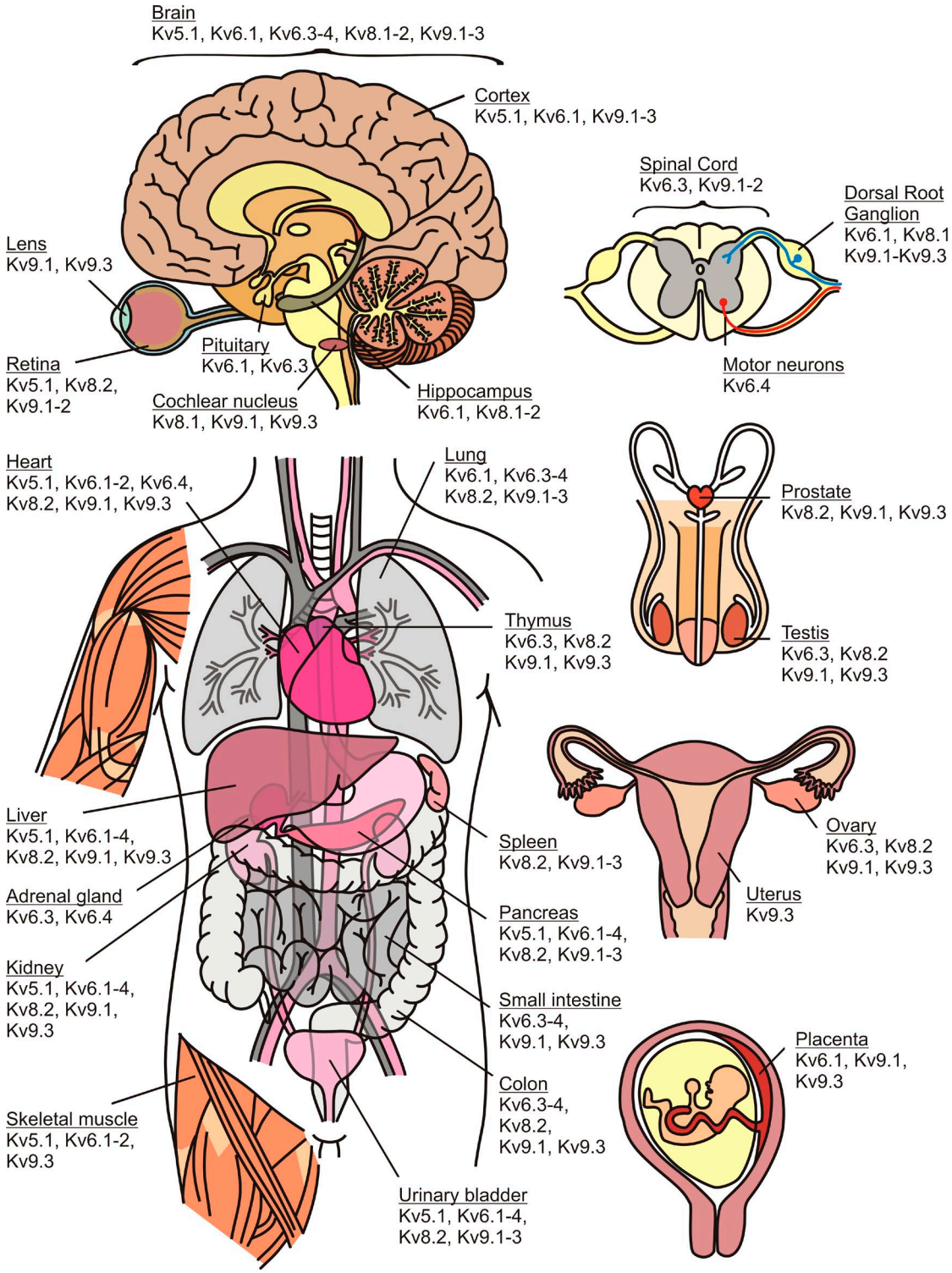


**Figure 2.** Tissue expression of the different electrically silent Kv subunits. Cartoon representation of the brain (top left), internal organs (bottom left), spinal cord (top right), male reproductive system (second right), and female reproductive system before (third right) and after (bottom right) fertilization.

variants involved in human epilepsy: R7K and M285R (Jorge et al., 2011). Both Kv8.2 coding variants suppress Kv2.1 current more than wild-type Kv8.2, consistent with reduced neuronal delayed rectifier current in epilepsy. In addition, M285R shifted the Kv2.1/Kv8.2 voltage dependence of activation almost 10 mV in the depolarizing direction and slowed the activation kinetics compared with wild-type Kv2.1/Kv8.2, which will be expected to broaden the action potential waveform, leading to hypersynchronized firing at high frequencies as observed during epileptic activity.

Kv6.1 may also be involved in epilepsy. Juvenile rats on postnatal day 20 (P20) are sensitive to injury in the hippocampal CA1 region after a single kainic acid–induced epileptic seizure, but they are resistant to this injury when they have two prior sustained neonatal seizures on P6 and P9. Microarray analysis demonstrated that the expression of Kv6.1 increases in the hippocampal CA1 region after a single kainic acid–induced epileptic seizure in P20 rats (Friedman et al., 2013). However, this up-regulation was similar to the up-regulation observed in P20 rats with two prior neonatal seizures on P6 and P9, suggesting that Kv6.1 contributes to epileptic seizures but not to the spatially protective effects produced by early life conditioning seizures (Friedman et al., 2013).

KvS subunits may also be involved in other diseases involving the central nervous system, such as schizophrenia. In the cortex of schizophrenic subjects, γ-aminobutyric acid (GABA)–mediated inhibitory neurotransmission appears to be altered in the subsets of GABA neurons that express either parvalbumin or somatostatin (Gonzalez-Burgos et al., 2010). Kv9.3 mRNA is expressed in parvalbumin but not in somatostatin-expressing GABA neurons of human prefrontal cortex; ∼91% of the Kv9.3 mRNA-positive neurons in the prefrontal cortex were labeled for parvalbumin, suggesting that the majority of prefrontal cortical neurons in which Kv9.3 is found are parvalbumin-expressing GABA neurons (Georgiev et al., 2012). Activity of parvalbumin-expressing GABA neurons has been linked to the generation of cortical gamma oscillations, which are 30–80-Hz waves essential for cortical information transmission and processing, and it has been suggested that alterations in these gamma oscillations contribute to the cognitive dysfunction in individuals with schizophrenia (Sohal et al., 2009; Gonzalez-Burgos et al., 2010). In situ hybridization experiments revealed lower Kv9.3 mRNA levels in schizophrenic subjects compared with controls (Georgiev et al., 2014). This was because of both a decreased number of Kv9.3-expressing neurons and reduced Kv9.3 mRNA abundance per neuron. This selective Kv9.3 expression may be useful for the development of novel treatments targeting Kv2.1/Kv9.3 channels in cortical parvalbumin-expressing GABA neurons in individuals with schizophrenia, because there are currently no selective pharmacologic modulators available for Kv2.1 (Gutman et al., 2005), which is ubiquitously expressed in cortical neurons (Du et al., 1998; Sarmiere et al., 2008).

Migraine, another disease involving the central nervous system, is a complex episodic disorder characterized by headaches and is usually accompanied by nausea, vomiting, photophobia, and/or phonophobia. Several recent studies have linked proteins involved in ion homeostasis, such as ion channels, to migraine (Yan and Dussor, 2014). This seems to be the case for KvS subunits. An extensive screening of 150 brain-expressed genes encoding ion channels, transporters, exchangers, and their associated regulatory subunits revealed that the Kv6.4 gene was potentially linked to migraine: the L360P missense mutation in the Kv6.4 gene was found only in migraine patients (compared with nonmigraine control patients; Lafrenière and Rouleau, 2012).

Apoptosis is crucial to the normal development of several tissues and is also involved in various neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease (Favaloro et al., 2012). An up-regulated $K^+$ current through Kv2.1 channels resulting in a disturbed $K^+$ homeostasis is a key factor in the early apoptotic cascade (Pal et al., 2003). This augmented Kv2.1 current is mainly caused by an increase in the abundance of Kv2.1 channels at the plasma membrane (Redman et al., 2007; Yao et al., 2009; McCord and Aizenman, 2013; McCord et al., 2014). This increase in Kv2.1 abundance is induced by p38-dependent phosphorylation of Kv2.1 subunits, which leads to their increased interaction with the soluble NSF attachment receptor proteins syntaxin 1A (McCord and Aizenman, 2013) and SNAP25 (Yao et al., 2009) and thereby to enhanced incorporation of Kv2.1 channels in the plasma membrane. These findings indicate that the degree of phosphorylation of Kv2.1 subunits is a key factor in the apoptotic cascade. KvS subunits may play a role in this apoptotic cascade by affecting the Kv2.1 phosphorylation status. Kv2.1 dephosphorylation is mediated by the $Ca^{2+}$/calmodulin-dependent protein phosphatase calcineurin (Mohapatra et al., 2007), and calmodulin binds to human Kv6.1 (O'Connell et al., 2010).

KvS subunits have also been identified in the auditory system: Kv8.1, Kv9.1, and Kv9.3 mRNA were detected in the anterior ventral cochlear nucleus (AVCN), posterior ventral cochlear nucleus (PVCN), and dorsal cochlear nucleus (DCN; Friedland et al., 2007). The cochlear nucleus, which is located on the dorsolateral side of the brainstem, receives innervation from the auditory nerve and is divided into the DCN and ventral cochlear nucleus. The auditory nerve fibers enter at the middle of the ventral cochlear nucleus, dividing it into the AVCN and PVCN. The Kv8.1, Kv9.1, and Kv9.3 mRNA levels differed in the different subdivisions of the cochlear nucleus. Kv9.3 was more highly expressed

TABLE 2

*Functions of the different electrically silent Kv subunits*

| KvS subunit | Tissue | Function |
|---|---|---|
| Kv5.1 | Cortex | Kv5.1 may cause the shift in hyperpolarized direction in the voltage dependence of inactivation that the Kv2-mediated current in neocortical pyramidal neurons shows.<br>Kv5.1 may play a role in cognitive functions but not in sensorimotor functions. |
| | Urinary bladder | Kv2/Kv5.1 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv6.1 | Cortex | Kv6.1 may cause the shift in hyperpolarized direction in the voltage dependence of inactivation that the Kv2-mediated current in neocortical pyramidal neurons shows.<br>Kv6.1 may play a role in cognitive functions but not in sensorimotor functions. |
| | DRG neurons | Kv2.1/Kv6.1 heterotetramers may contribute to the outward delayed rectifier $K^+$ current in small DRG neurons. |
| | Hippocampus | Kv6.1 may be involved in epilepsy; Kv6.1 contributes to epileptic seizures but not to the spatially protective effects produced by early life conditioning seizures. |
| | Urinary bladder | Kv2/Kv6.1 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv6.2 | Urinary bladder | Kv2/Kv6.2 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv6.3 | Urinary bladder | Kv2/Kv6.3 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| | VSMCs | Kv6.3 subunits contribute to the Kv current in mesenteric VSMCs during hypertension; de novo expression of Kv6.3 mRNA is found in mesenteric VSMCs obtained from a hypertensive mice strain. |
| Kv6.4 | Central nervous system | Kv6.4 is involved in migraine; the L360P missense mutation in the Kv6.4 gene was found only in migraine patients. |
| | Fast motor neurons | Kv6.4 expression is regulated by Notch ligand Dlk1 in fast motor neurons where an excess of Kv6.4 increased firing threshold, increased firing frequency, and shortened the duration of firing periods, shifting the neuronal biophysical properties of late-gestation chick motor neurons toward that of a typical fast motor neuron. |
| | Urinary bladder | Kv2/Kv6.4 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv8.1 | DRG neurons | Kv2.1/Kv8.1 heterotetramers may contribute to the outward delayed rectifier $K^+$ current in small DRG neurons. |
| | Hippocampus | Hippocampal CA3 pyramidal cells express a current, $I_{K(slow)}$, with properties that resemble those of the currents seen when Kv2/Kv8.1 subunits are expressed in mammalian cells.<br>Kv8.1 may contribute to the pathologic mechanisms involved in epileptogenesis. |
| Kv8.2 | Eye | Kv2.1/Kv8.2 heterotetramers contribute to $I_K$ in amphibian photoreceptors.<br>Several mutations in Kv8.2 are known to cause the retinal disorder CDSR. |
| | Hippocampus | Kv8.2 is involved in epilepsy; the Kv8.2 mRNA level was threefold higher in the hippocampus of the susceptible SJL/J mouse strain than in that of the resistant C57BL/6J mouse strain, and the two unique nonsynonymous Kv8.2 coding variants R7K and M285R are involved in human epilepsy. |
| | Urinary bladder | Kv2/Kv8.2 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv9.1 | Cortex | Kv9.1 may cause the shift in hyperpolarized direction in the voltage dependence of inactivation that the Kv2-mediated current in neocortical pyramidal neurons shows. |
| | DRG neurons | Kv2.1/Kv9.1 heterotetramers may contribute to the outward delayed rectifier $K^+$ current in small DRG neurons.<br>Kv9.1 is involved in pain sensation; Kv9.1 down-regulation reduced the mechanical pain threshold in rats, and two Kv9.1 SNPs were associated with pain. |
| | Cochlear nucleus | The preferential expression of Kv9.1 in the DCN may provide some flexibility to DCN neurons. |
| | Urinary bladder | Kv2/Kv9.1 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv9.2 | Cortex | Kv9.2 may cause the shift in hyperpolarized direction in the voltage dependence of inactivation that the Kv2-mediated current in neocortical pyramidal neurons shows. |
| | DRG neurons | Kv2.1/Kv9.2 heterotetramers may contribute to the outward delayed rectifier $K^+$ current in small DRG neurons. |

*Table 2 (Continued)*

| KvS subunit | Tissue | Function |
|---|---|---|
| | Urinary bladder | Kv2/Kv9.2 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |
| Kv9.3 | Cancer cells | Kv9.3 plays a role in cell-cycle progression and cell proliferation in cancer cells; stable down-regulation of Kv9.3 reduced tumor growth in mouse xenografts. |
| | Cerebral arteries | Kv2.1/Kv9.3 channels underlie the ScTx-1–sensitive current that constitutes approximately 60% of the Kv current in RMCAs.<br>Kv2.1/Kv9.3 channels contribute to controlling RMCA diameter. |
| | Cortex | Kv9.3 may cause the shift in hyperpolarized direction in the voltage dependence of inactivation that the Kv2-mediated current in neocortical pyramidal neurons shows.<br>Kv9.3 is involved in schizophrenia; Kv9.3 is found in the parvalbumin-expressing GABA neurons from which the transmission appears to be altered in schizophrenic subjects, and lower Kv9.3 mRNA levels are found in schizophrenic subjects compared with controls. |
| | Cochlear nucleus | Kv9.3 is preferentially expressed in the AVCN, indicating that Kv9.3 may play a role in spherical bushy cells that are found mainly in the AVCN and carry the information that is used to localize sounds. |
| | DRG neurons | Kv2.1/Kv9.3 heterotetramers may contribute to the outward delayed rectifier $K^+$ current in small DRG neurons. |
| | Lung | Kv2.1/Kv9.3 complexes are involved in hypoxia-induced vasoconstriction of resistance PASMCs; Kv2.1/Kv9.3 currents are reversibly inhibited by hypoxia in the voltage range of the resting membrane potential of PASMCs, and chronic hypoxia causes a decrease in Kv2.1 and Kv9.3 abundance in these cells.<br>Kv9.3 gene abundance affects lung function; SNPs of the Kv9.3 gene were associated with increased values of forced expiratory volume in the first second, one of the parameters used to determine lung function.<br>Kv9.3 has been proposed to be involved in airway hyperresponsiveness; an observed association between two SNPs of the Kv9.3 gene and airway hyperresponsiveness has been revealed. |
| | Placenta | Kv2.1/Kv9.3 channels have been proposed to be involved in hypoxic fetoplacental vasoconstriction; the U46619-induced contraction of chorionic plate arteries was enhanced by the relatively specific Kv2/Kv9.3 blocker ScTx-1, and Kv9.3 is present in the arteries and veins of the chorionic plate. |
| | Urinary bladder | Kv2/Kv9.3 heterotetramers may contribute to $I_K$ in DSMs of the urinary bladder.<br>ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs of the urinary bladder. |

in AVCN and PVCN than in DCN, with a preferential expression in AVCN over PVCN (Friedland et al., 2007). This preferential expression of Kv9.3 in AVCN suggests that Kv9.3 plays a role in spherical bushy cells that are found mainly in the AVCN and carry the information that is used to localize sounds. The expression of Kv9.1, on the other hand, was higher in DCN compared with that in the other two subdivisions (Friedland et al., 2007). Given the differences in modulation of the biophysical properties of Kv2 currents by these two Kv9 subunits, this difference in expression may reflect functional differences between AVCN, PVCN, and DCN; Kv9.3 increases Kv2 current in heterologous expression systems, whereas Kv9.1 reduces it, and Kv9.3 shifts the voltage dependence of inactivation of Kv2 to hyperpolarized potentials more substantially than Kv9.1 does (Patel et al., 1997; Salinas et al., 1997b; Richardson and Kaczmarek, 2000). The preferential expression of Kv9.1 in DCN may also provide some flexibility to DCN neurons, because computer simulations of model neurons suggest that Kv9.1 may have a different physiological effect depending on whether the neuronal firing pattern is limited by the inactivation of inward currents (Richardson and Kaczmarek, 2000). Kv2.1 was not detected in the different cochlear nuclei using serial analysis of gene expression and microarray experiments, whereas Kv2.2 was detected in all subdivisions. This suggests that heterotetrameric Kv2/KvS channels comprising Kv2.2 instead of Kv2.1 subunits may play a role in the different cochlear nuclei.

*Peripheral nervous system.* KvS subunits may also play a role in the peripheral nervous system. Kv6.4 is found in fast motor neurons, where its RNA level is regulated by the Notch ligand Delta-like homologue 1 (Dlk1), which is expressed in large fast α motor neurons, but not in smaller α motor neurons or γ motor neurons, throughout the spinal cord: Kv6.4 RNA level in Dlk1 knock-out mouse fast motor neurons was decreased compared with that in wild-type neurons (Müller et al., 2014). Furthermore, similar to an excess of Dlk1 in late-gestation chick motor neurons, an excess of Kv6.4 increased firing threshold, increased firing frequency, and shortened the duration of firing periods, shifting the neuronal biophysical properties of late-gestation chick motor neurons toward that of a typical fast motor neuron

(Müller et al., 2014). These findings indicated that Dlk1 is involved in the functional diversification of motor neurons partially by regulating the expression of the motor neuron type-specific Kv6.4 gene.

KvS subunits have also been identified in cultured small mouse dorsal root ganglion (DRG) neurons (Bocksteins et al., 2009). Using the relatively specific Kv2 channel blocker stromatoxin 1 (ScTx-1; Escoubas et al., 2002) and Kv2.1 antibodies, it was shown that both homotetrameric Kv2.1 and heterotetrameric Kv2/KvS channels contribute to the outward delayed rectifier $K^+$ current ($I_K$) in these neurons. Small DRG neurons give rise to thin myelinated Aδ and unmyelinated C fibers that are involved in nociception (Harper and Lawson, 1985). This suggests that KvS subunits may be involved in nociception. Indeed, subsequent studies in neuropathic pain models demonstrated that the Kv9.1 mRNA abundance in myelinated DRG neurons was rapidly and robustly down-regulated after peripheral nerve injury (Costigan et al., 2010; Tsantoulas et al., 2012). Using Kv9.1 small interfering RNA treatment, it has been demonstrated that this Kv9.1 down-regulation reduced the mechanical pain threshold in rats and that this was coupled to an increased A-fiber excitability (Tsantoulas et al., 2012). The suggested role of Kv9.1 in pain was strengthened by an unbiased network analysis in which Kv9.1 was used as a target and its 30 nearest coassociated neighbors were identified, characterizing Kv9.1 as a putative pain gene. This was substantiated by single nucleotide polymorphism (SNP) association studies in humans demonstrating that two Kv9.1 SNPs were associated with pain; both rs734784, in which the allele coding for valine instead of the alternative allele coding for isoleucine, and rs13043825, in which the uncommon allele possessing the codon GAA that codes for glutamic acid instead of the common allele possessing the codon GAG that codes for glutamic acid, were associated with greater pain (Costigan et al., 2010).

*Cerebral arterial smooth muscle cells.* KvS subunits are also present in cerebral arterial smooth muscle cells (Zhong et al., 2010). An increase in intravascular resistance causes depolarization in small resistance arteries, resulting in $Ca^{2+}$ influx through voltage-gated L-type $Ca^{2+}$ channels and leading to constriction in what is known as the myogenic response (Davis and Hill, 1999). This intravascular resistance-induced membrane depolarization activates several Kv channels. Experiments using the Kv2 channel blocker ScTx-1 have suggested that Kv2-containing channels contribute to the feedback regulation of the cerebral myogenic response because ScTx-1–induced Kv2 inhibition leads to constriction in rat middle cerebral arteries (RMCAs; Amberg and Santana, 2006; Zhong et al., 2010). Moreover, real-time quantitative reverse-transcription (RT) PCR analysis demonstrated that both Kv2.1 and Kv9.3 were substantially expressed in RMCAs, whereas Kv2.2, Kv5.1, Kv6.1–Kv6.3, and Kv9.2 were only detectable at very low levels (Zhong et al., 2010). These findings suggest that Kv2.1/Kv9.3 channels underlie the ScTx-1–sensitive current that constitutes ∼60% of the Kv current in RMCAs. Indeed, the biophysical properties of the ScTx-1–sensitive current in RMCAs resemble those of Kv2.1/Kv9.3 channels heterologously expressed in HEK293 cells (Zhong et al., 2010). Proximity ligation assays have also demonstrated colocalization of Kv2.1 and Kv9.3 subunits at the plasma membrane of dissociated RMCA myocytes (Zhong et al., 2010), further supporting the hypothesis that Kv2.1/Kv9.3 channels contribute to controlling RMCA diameter.

### Lung

Hypoxia causes the vasoconstriction of small resistance pulmonary arteries, providing a mechanism for diverting blood flow away from poorly ventilated alveoli (Weir and Archer, 1995). Hypoxic pulmonary vasoconstriction contributes to the development of pulmonary hypertension in patients with chronic obstructive lung diseases and people living at high altitudes (Barnes and Liu, 1995; Weir and Archer, 1995). Hypoxia leads to the inhibition of one or more Kv channels, and thereby to a decrease in the delayed rectifier $K^+$ current, in small resistance pulmonary artery smooth muscle cells (PASMCs). This $I_K$ inhibition leads to depolarization of the cells and activation of L-type voltage-gated $Ca^{2+}$ channels, which results in a cytosolic $Ca^{2+}$ rise that causes vasoconstriction (Weir and Archer, 1995). Both Kv2.1 and Kv9.3 mRNA are found in PASMCs (Patel et al., 1997), and Kv2.1 contributes significantly to the $I_K$ current in the lung (Archer et al., 1998). Furthermore, chronic hypoxia causes a decrease in Kv2.1 and Kv9.3 at both the mRNA (for Kv2.1 and Kv9.3) and protein level (for Kv2.1; Wang et al., 2005), suggesting that Kv2.1/Kv9.3 complexes are involved in hypoxia-induced vasoconstriction of resistance PASMCs. Indeed, the biophysical and pharmacologic characteristics of currents mediated by Kv2.1/Kv9.3 channels are similar to $I_K$ described in rat PASMCs (Yuan et al., 1998; Patel et al., 1999). Kv9.3 shifts the activation threshold of Kv2.1 into the range of the resting membrane potential of pulmonary artery myocytes. Furthermore, in heterologous expression systems, Kv2.1/Kv9.3 currents are reversibly inhibited by hypoxia in the voltage range of the resting membrane potential of PASMCs (Hulme et al., 1999). Collectively, this suggests that the Kv2.1/Kv9.3 complex contributes to establishment of the PASMC resting membrane potential.

In contrast with longer (≥6 h) hypoxia exposures, which lead to a decrease in the mRNA levels of Kv2.1 and Kv9.3 (Hong et al., 2004; Wang et al., 2005), shorter hypoxia exposures increase the expression of Kv2.1 and Kv9.3 in cultured rat PASMCs (Dong et al., 2012). Hypoxia in cultured PASMCs increases the expression

of hypoxia-inducible factor 1α (HIF-1α), which translocates to the nucleus and dimerizes with the constitutively expressed HIF-1β to form the heterodimeric transcription factor HIF-1. HIF-1 binds to many hypoxia-inducible genes, including erythropoietin; binding of HIF-1 to the erythropoietin gene enhancer increases the production of erythropoietin (Marti, 2004). Binding of HIF-1 to the erythropoietin gene enhancer also regulates the hypoxia-induced up-regulation of Kv2.1 and Kv9.3 expression in cultured PASMCs on brief exposures to hypoxia (Dong et al., 2012).

Other KvS subunits, including Kv5.1, Kv6.1–3, Kv8.2, Kv9.1, and Kv9.2, have also been detected in PAMSCs; however, their function in these cells has not been elucidated (Davies and Kozlowski, 2001; Platoshyn et al., 2004; Fantozzi et al., 2006; Firth et al., 2011). Functional variety between tissues can be achieved by expression of different KvS subunits, but functional variety within a tissue can also be achieved by changes in the abundance of one particular KvS subunit as a function of age and cell type. For example, the Kv9.3 mRNA level in the lung increases with age and with decreased pulmonary artery diameter (Coma et al., 2002; Archer et al., 2004).

Kv9.3 mRNA has also been detected in alveolar epithelial cells (Lee et al., 2003). Higher amounts of Kv9.3 mRNA have been observed in airway epithelial cells with higher serum 25-hydroxyvitamin D concentrations (Reardon et al., 2013), and higher serum 25-hydroxyvitamin D concentrations are associated with better lung function (Finklea et al., 2011). This suggests that variations in Kv9.3 gene abundance affect lung function. Indeed, SNPs of the Kv9.3 gene were associated with increased values of forced expiratory volume in the first second, one of the parameters used to determine lung function (Reardon et al., 2013).

Airway hyperresponsiveness is one of the major clinical symptoms of asthma, and linkage analysis revealed linkage between the p-terminal region of chromosome 2 (Xu et al., 2001), where the Kv9.3 gene is situated, and airway hyperresponsiveness. Therefore, genetic Kv9.3 variants have been considered as a potential candidate for asthma. This notion is strengthened by the observed associations between the rs1031771 and rs1031772 SNPs and airway hyperresponsiveness (Hao et al., 2005). Furthermore, the rs1031771 G allele and rs1031772 T allele were associated with a higher risk of airway hyperresponsiveness.

### Heart

Kv2.1 channels contribute to the noninactivating, steady-state current ($I_{ss}$) and the TEA-sensitive rapidly activating, slowly inactivating current ($I_{K,slow2}$) in rodent atrial (Bou-Abboud et al., 2000) and ventricular (Xu et al., 1999) myocytes. The amplitude of this TEA-sensitive $I_K$ current can differ markedly between individual ventricular myocytes of the same origin (Schultz et al., 2001). Kv2.1 mRNA level is heterogeneous among individual myocytes, suggesting that this variability in current could be caused by variability in Kv2.1 expression (Brahmajothi et al., 1997). However, patch clamp recordings and single-cell RT-PCR analysis of different individual myocytes indicated that the distribution pattern of Kv2.1 mRNA cannot explain $I_K$ variability among individual myocytes (Schultz et al., 2001). Therefore, differences in $I_K$ among myocytes appear to depend on another mechanism. Several KvS subunits are expressed in the heart, making it likely that heterotetrameric complexes with Kv2 exist and are responsible for fine-tuning the cardiac currents in different myocytes. The presence of such KvS subunits may also contribute to the difference in Kv2.1 expression pattern between arterial and ventricular myocytes: Kv2.1 forms large clusters on the cell soma in arterial myocytes, whereas in ventricular myocytes Kv2.1 shows a dispersed expression pattern and is mainly localized in the transverse-axial tubules and sarcolemma (O'Connell et al., 2008). Kv2.1 currents are also present in the smooth muscles of the ductus arteriosus of the heart (Michelakis et al., 2002). In fetal ductus arteriosus smooth muscle cells, hypoxia elicits an opposite reaction to that in the lung (i.e., vasodilatation instead of vasoconstriction) which could be the result of the low expression levels of Kv9.3 in these cells (Hayama et al., 2006).

### Eye

In amphibian photoreceptors, a Kv current has been described that contributes to setting the dark resting potential and underlies the transient hyperpolarizing overshoots of the membrane potential in response to rapid-onset illumination (Beech and Barnes, 1989). The biophysical and pharmacological profiles of heterologously expressed Kv2.1/Kv8.2 channels resemble those of this $I_K$ reasonably well, and both Kv8.2 and Kv2.1 are expressed in the inner segments of rods and cones of mammalian retina (Wu et al., 2006; Czirják et al., 2007), suggesting that heterotetrameric Kv2.1/Kv8.2 channels contribute to $I_K$. This possibility is supported by current-clamp experiments demonstrating that oocytes expressing Kv2.1/Kv8.2 develop transient hyperpolarizing overshoots of the membrane potential (Czirják et al., 2007). In contrast, oocytes expressing Kv2.1 alone fail to do so, further strengthening the argument that functional Kv2.1/Kv8.2 is required for proper photoreceptor function.

The expression of Kv8.2 and Kv2.1 shows a daily rhythm in which the abundance of Kv8.2 and Kv2.1 increases during the night (Hölter et al., 2012). Although Kv8.2 and Kv2.1 are expressed throughout the retina, these daily changes in expression only occur in photoreceptor cells and not in inner retinal neurons. Kv8.2 mRNA levels also undergo a daily rhythm in the pineal gland, which is phylogenetically related to the retina,

TABLE 3

*Kv8.2 variations causing the retinal disorder CDSR*

| Mutation status | | | Reference |
|---|---|---|---|
| Nucleotide | Protein | Homozygous or heterozygous | |
| Point mutations resulting in amino acid substitutions | | | |
| c.80G>A | p.R27H | Hom | Fujinami et al., 2013 |
| c.107G>A | p.R36H | Het | Wissinger et al., 2011 |
| c.190G>A | p.E64L | Het | Wissinger et al., 2011 |
| c.222G>C | p.E74D | Het | Wissinger et al., 2011 |
| c.328C>G | p.L110V | Het | Wissinger et al., 2011 |
| c.377T>A | p.L126Q | Het | Wu et al., 2006 |
| c.551A>T | p.E148V | Het | Wissinger et al., 2008 |
| c.415T>C | p.F151V | Het | Robson et al., 2010 |
| c.473T>G | p.F158C | Het | Thiagalingam et al., 2007 |
| c.491T>C | p.F164S | Het | Wissinger et al., 2011 |
| c.529T>C | p.C177R | Het | Fujinami et al., 2013 |
| c.533C>T | p.P178L | Het | Thiagalingam et al., 2007 |
| c.550G>A | p.E184K | Het | Wissinger et al., 2008 |
| c.564G>C | p.W188C | Het | Wu et al., 2006 |
| c.592T>A | p.C198S | Het | Thiagalingam et al., 2007 |
| c.617G>C | p.R206P | Hom | Fujinami et al., 2013 |
| c.638G>C | p.R213P | Het | Thiagalingam et al., 2007 |
| c.725A>G | p.Q242R | Het | Wissinger et al., 2011 |
| c.727C>T | p.R243W | Het | Wissinger et al., 2011 |
| c.782C>A | p.A261D | Hom | Wissinger et al., 2011 |
| c.853A>T | p.M285L | Het | Wissinger et al., 2011 |
| c.874G>A | p.G292S | Het | Wissinger et al., 2011 |
| c.964G>C | p.A322P | Het | Sergouniotis et al., 2012 |
| c.989T>C | p.F330S | Het | Wissinger et al., 2011 |
| c.1211T>C | p.L404P | Het | Wissinger et al., 2011; Zobor et al., 2012 |
| c.1318C>T | p.T439I | Hom | Sergouniotis et al., 2012 |
| c.1348T>G | p.W450G | Hom and Het | Thiagalingam et al., 2007 |
| c.1348T>A | p.W450R | Hom | Wissinger et al., 2011 |
| c.1376G>A | p.G459R | Hom | Wu et al., 2006 |
| c.1381G>A | p.G461R | Hom and Het | Thiagalingam et al., 2007; Ben Salah et al., 2008; Wissinger et al., 2008; Friedburg et al., 2011; Fujinami et al., 2013; Vincent et al., 2013 |
| c.1607A>G | p.N536S | Het | Wissinger et al., 2011 |
| c.1616T>C | p.L539P | Het | Wissinger et al., 2011 |
| c.1133-1141dup | p.L381-R383dup | Het | Wissinger et al., 2011 |
| Point mutations resulting in premature stop codons | | | |
| c.7A>T | p.K3X | Het | Wu et al., 2006 |
| c.217G>T | p.E73X | Het | Wissinger et al., 2008 |
| c.226C>T | p.Q76X | Hom | Wissinger et al., 2008 |
| c.238G>T | p.E80X | Hom | Vincent et al., 2013 |
| c.325C>T | p.Q109X | Het | Wu et al., 2006 |
| c.339C>A | p.C113X | Het | Zobor et al., 2012 |
| c.427G>T | p.E143X | Hom | Wu et al., 2006 |
| c.430C>T | p.Q145X | Het | Wu et al., 2006 |
| c.442G>T | p.E148X | Het | Ben Salah et al., 2008; Wissinger et al., 2008; Zobor et al., 2012 |
| c.721C>T | In cis changes resulting in p.P214X | Het | Thiagalingam et al., 2007 |
| c.722C>A | In cis changes resulting in p.P214X | Het | Thiagalingam et al., 2007 |
| c.667C>T | p.Q223X | Het | Vincent et al., 2013 |
| c.778A>T | p.K260X | Het | Thiagalingam et al., 2007; Wissinger et al., 2008 |
| Not reported | p.G416X | Het | Robson et al., 2010 |
| Point mutations resulting in later stop codons | | | |

*Table 3 (Continued)*

| Mutation status | | | Reference |
|---|---|---|---|
| Nucleotide | Protein | Homozygous or heterozygous | |
| c.1637T>C | p.X546YextX61 | Het | Thiagalingam et al., 2007; Robson et al., 2010 |
| Deletion resulting in in-frame deletions | | | |
| c.447-449del | p.F150del | Het | Zobor et al., 2012 |
| c.1015-1024delACCTGGTGG | p.D339-V341del | Het | Wu et al., 2006; Wissinger et al., 2008; Vincent et al., 2013 |
| Insertions resulting in in-frame insertions | | | |
| c.775-795dup | p.A259-A265dup | Het | Wissinger et al., 2011 |
| Frame shift mutations resulting in premature stop codons | | | |
| c.8-11delAACA | p.K3fsX93 | Het | Sergouniotis et al., 2012; Zobor et al., 2012 |
| c.19-1356del + 9571insCATTTG | p.R7HfsX57 | Hom | Wissinger et al., 2011; Zobor et al., 2012 |
| c.323_329del7 | p.T108WfsX14 | Het | Wissinger et al., 2011 |
| c.357-358insC | p.K120fsX371 | Het | Wissinger et al., 2008 |
| c.434-*30+154del | p.E145LfsX4 | Hom | Wissinger et al., 2011 |
| c.460-461insCG | p.D154AfsX58 | Hom | Vincent et al., 2013 |
| c.568delG | p.G189fsX21 | Hom | Sergouniotis et al., 2012 |
| c.794-795dupCC | p.S266PfsX57 | Het | Wissinger et al., 2011 |
| c.867delC | p.S289fs | | Thiagalingam et al., 2007 |
| c.1001delC | p.A334fsX453 | Hom | Ben Salah et al., 2008 |
| c.1199delT | p.F400fsX53 | Het | Sergouniotis et al., 2012 |
| c.1404delC | p.H468fsX503 | Hom | Ben Salah et al., 2008 |
| Gene loss | | | |
| g.2570596-2807413del | Loss | Het | Wissinger et al., 2011 |
| g.2657638-2737340del | Loss | Het | Wissinger et al., 2011; Zobor et al., 2012 |
| g.2696639-2713626del | Loss | Het | Wissinger et al., 2011 |

Het, heterozygous; Hom, homozygous.

but such a daily rhythm of Kv8.2 mRNA levels could not be detected in the hippocampus. In contrast, daily changes in Kv2.1 mRNA levels were not apparent in either the pineal gland or the hippocampus. The daily changes in retinal Kv8.2 and Kv2.1 expression persisted under constant darkness, suggesting that they were driven by an intrinsic circadian clock in the retina itself (Hölter et al., 2012).

Several mutations in Kv8.2 are known to cause the retinal disorder cone dystrophy with supernormal rod electroretinogram (CDSR). This autosomal recessive hereditary disorder is characterized by photoaversion, reduced visual acuity, abnormal color vision, and night blindness (Gouras et al., 1983). CDSR-causing Kv8.2 mutations include (a) nucleotide changes that result in amino acid substitutions and protein truncations, (b) small in-frame insertions and deletions, (c) nucleotide insertions and deletions generating frameshifts that lead to protein truncations, and (d) whole gene loss (Wu et al., 2006; Thiagalingam et al., 2007; Ben Salah et al., 2008; Wissinger et al., 2008, 2011; Robson et al., 2010; Friedburg et al., 2011; Sergouniotis et al., 2012; Zobor et al., 2012; Fujinami et al., 2013; Vincent et al., 2013). Several of the N-terminal amino acid substitutions implicated in CDSR prevent the interaction of Kv8.2 with Kv2.1 in heterotetrameric channels (Wissinger et al., 2011; Smith et al., 2012). Moreover, amino acid substitutions in the Kv8.2 pore region led to the generation of nonconducting Kv2.1/Kv8.2 heterotetramers (Smith et al., 2012). These findings suggest that the CDSR phenotype arises through the loss of functional Kv2.1/Kv8.2 heterotetramers reached by different mechanisms. To date, no mutations in another KvS subunit have been described to be directly linked to a disease or pathology; an overview of the known CDSR-causing *Kcnv2* mutations is given in Table 3.

Other KvS subunits have also been detected in the eye. Wu et al. (2006) detected Kv5.1 in human retinal cDNA using in situ hybridization, Kv9.1 and Kv9.2 mRNA have been detected in rat retina (Salinas et al., 1997b), and human Kv9.1 and Kv9.3 were first cloned from lens epithelium (Shepard and Rae, 1999). However, their function in the eye has not been elucidated.

### Urinary bladder

The biophysical and pharmacological properties of the Kv current in detrusor smooth muscles (DSMs) of the urinary bladder resemble those of the currents seen when Kv2 subunits are coexpressed with KvS subunits in heterologous expression systems (Thorneloe and Nelson, 2003). Moreover, Kv2.1, Kv2.2, Kv5.1, Kv6.1, Kv6.2, and Kv6.4 mRNA has been demonstrated in

these mouse DSMs. Later studies reported the presence of Kv2 and KvS mRNA in isolated DSMs of other species: Kv2.1, Kv6.2, Kv6.3, Kv8.2, and Kv9.1–Kv9.3 mRNA, but not that of Kv2.2 and the other KvS subunits, could be observed in isolated guinea pig DSM cells (Hristov et al., 2012b), whereas Kv2.1, Kv2.2, and Kv9.3 mRNA was present in rat and human isolated DSMs (Chen et al., 2010; Hristov et al., 2012a). These findings suggest that heterotetramers composed of Kv2 and KvS subunits contribute to $I_K$ in DSMs; application of 100 nM ScTx-1 reduced $I_K$ current amplitude in isolated DSM, supporting this possibility (Chen et al., 2010; Hristov et al., 2012a,b). Furthermore, application of 100 nM ScTx-1 to isolated DSM strips enhanced the spontaneous phasic contraction amplitude and muscle force (representing myogenic contractions) and increased the amplitude of electrical field stimulation-induced contractions (representing neurogenic contractions). These results indicated that ScTx-1–sensitive Kv2-containing channels are key regulators of the excitability and contractility of rat, guinea pig, and human DSMs, in which their action seems to oppose myogenic and neurogenic contractions (Chen et al., 2010; Hristov et al., 2012a,b).

### Placenta

During normal pregnancies, placental vascular resistance decreases by means of such adaptations as angiogenesis and vasodilation (Sala et al., 1995; Chaddha et al., 2004). This decrease in vascular resistance does not occur in intrauterine growth restriction (also known as fetal growth restriction) pregnancies in which the unborn baby does not grow at the expected rate inside the womb (Kingdom and Kaufmann, 1997; Chaddha et al., 2004). It has been commonly assumed that this pathological condition arises from hypoxia-induced vasoconstriction of the fetoplacental vessels, resulting in placental hypoperfusion and consequently fetal malnutrition (Hampl and Jakoubek, 2009). Hypoxic fetoplacental vasoconstriction is induced by the hypoxic inhibition of oxygen-sensitive Kv channels, as indicated by mimicry of the hypoxia-induced down-regulation of $I_K$ in in vitro–perfused placental cotyledons, which are placental lobules consisting of a mass of chorionic villi, by the application of the Kv channel blocker 4-aminopyridine (4-AP; Hampl et al., 2002). Both Kv2.1 and Kv9.3 are present in the arteries and veins of the chorionic plate, which forms the fetal side of the placenta, and in whole placenta homogenates, suggesting that Kv2.1 and Kv9.3 subunits are involved in hypoxic fetoplacental vasoconstriction (Wareing et al., 2006). The proposed role of Kv2.1- and Kv9.3-containing channels in hypoxic fetoplacental vasoconstriction is supported by a study demonstrating that the U46619-induced contraction of chorionic plate arteries was enhanced by 10–20 nM ScTx-1 (Kiernan et al., 2010). Furthermore, real-time quantitative RT-PCR analysis demonstrated that Kv2.1 gene expression in chorionic plate veins and Kv9.3 gene expression in placental homogenates of intrauterine growth restriction pregnancies were increased compared with normal pregnancies (Corcoran et al., 2008). Immunohistochemical experiments revealed that Kv9.3 was expressed in syncytiotrophoblast microvillus membrane, endothelial cells in intermediate stem villi and chorionic plate blood vessels, and vascular smooth muscle cells (VSMCs) of large-diameter vessels (Fyfe et al., 2012). This Kv9.3 expression pattern was unchanged in tissues from women with small-for-gestational age babies, suggesting that the expression of K9.3 does not change in this pregnancy pathology (Fyfe et al., 2012).

### Mesenteric arteries

Under physiological conditions, Kv channels contribute to the feedback regulation of the myogenic response in resistance VSMCs (Amberg and Santana, 2006; Chen et al., 2006). The myogenic response consists of small depolarizations induced by increases in blood pressure in the resistance vessels. Electrophysiologic experiments revealed a decreased functional expression of Kv channels in hypertensive animals (Cox and Rusch, 2002). Real-time PCR and Western blot analysis revealed the de novo expression of Kv6.3 mRNA in mesenteric VSMCs obtained from a hypertensive mouse strain (BPH; Moreno-Domínguez et al., 2009), suggesting that Kv6.3 subunits contribute to the Kv current in mesenteric VSMCs during hypertension. Indeed, the biophysical properties of the Kv2-containing current in these BPH VSMCs resemble those of heterotetrameric Kv2.1/Kv6.3 channels in heterologous expression systems (Moreno-Domínguez et al., 2009). Furthermore, intracellular application of Kv6.3 antibodies did not change the Kv current amplitude in mesenteric VSMCs from normotensive mice strains but decreased Kv current amplitude in BPH VSMCs, supporting the notion that Kv6.3 contributes to the Kv current in BPH VSMCs (Moreno-Domínguez et al., 2009). This observed de novo expression and function of Kv6.3 in BPH VSMCs may partially explain the observed decrease of Kv currents in hypertensive animals; Kv2-containing channels contribute to the Kv current in mesenteric VSMCs from both normotensive and BPH mice, and Kv6.3 reduces the Kv2.1 current density in heterologous expression systems (Vega-Saenz de Miera, 2004; Moreno-Domínguez et al., 2009). Kv2.1 and Kv9.3 mRNA could also be detected in mesenteric VSMCs; however, their expression did not differ in VSMCs from normotensive and BPH mice (Moreno-Domínguez et al., 2009).

### Pancreas

A dominant-negative knockout strategy using isolated rat β-cells and HIT-T15 insulinoma cells showed that Kv2 channels represent ∼60–70% of the delayed rectifier current in these insulin-secreting cells. Moreover,

elimination of Kv2.1 enhances glucose-dependent insulin secretion in rat islets (MacDonald et al., 2001). Similarly, pharmacologic blockade of Kv2.1 channels increases glucose-stimulated insulin secretion from perfused mouse pancreas (MacDonald et al., 2002). These findings suggest that Kv2.1 likely regulates insulin secretion in rodent pancreatic β-cells by controlling their electrical activity. Although Kv2.1 channels are abundantly expressed in human β-cells, where they mediate large $K^+$ currents, the contribution of Kv2.1 to insulin secretion by regulating the electrical activity of these cells appears to be minimal: inhibition of Kv2.1 had no effect on the electrical responses of human β-cells (Braun et al., 2008). In addition, expression of the dominant-negative pore mutant $Kv2.1^{W365C/Y380T}$ to reduce Kv2-containing currents in pancreatic β-cells had similar effects on insulin secretion as wild-type Kv2.1 (Dai et al., 2012). In contrast, expression of a Kv2.1 mutant lacking the syntaxin 1A binding site impaired the glucose-stimulated insulin secretion in these cells (Dai et al., 2012). These findings suggest that interaction between Kv2.1 and syntaxin 1A regulates insulin secretion from pancreatic β-cells independently of its conductive function. Irrespective of the mechanism, Kv2.1 channels clearly are important in both rodent and human pancreatic β-cells, and KvS subunits could have additional regulatory functions in glucose-stimulated insulin secretion through their interaction with Kv2 subunits. Should Kv2 channels regulate insulin secretion by controlling β-cell electrical activity, KvS subunits could exert a role by affecting the biophysical properties of Kv2.1 (Bocksteins and Snyders, 2012). On the other hand, KvS subunits could influence pancreatic β-cell function by influencing Kv2.1–syntaxin 1A interaction. This interaction is partially regulated by Kv2.1 phosphorylation status (McCord and Aizenman, 2013), and KvS subunits may affect the degree of phosphorylation of Kv2.1 by binding calmodulin as demonstrated for Kv6.1 (O'Connell et al., 2010), leading to $Ca^{2+}$/calmodulin-dependent Kv2.1 dephosphorylation. RT-PCR and Northern blot analysis have revealed that Kv5, Kv6, Kv8, and Kv9 subunits are expressed in the pancreas (Su et al., 1997; Shepard and Rae, 1999; Zhu et al., 1999; Ottschytsch et al., 2002; Vega-Saenz de Miera, 2004; Yan et al., 2004). Therefore, the question is not whether KvS subunits are present, but which of the different ones is relevant.

### Role of Kv2/KvS heterotetramers in cancer cells

Kv channels have been implicated in several processes in nonexcitable cells, including cell-cycle progression and cell proliferation, two processes that are dysregulated in cancer cells (Urrego et al., 2014). The role of Kv channels in these processes depends on both ion-conducting and nonconducting properties (Cidad et al., 2012; Urrego et al., 2014). Kv9.3 mRNA is present in both human HCT15 colon carcinoma cells and A549 lung adenocarcinoma cells, model systems representing colon and lung cancers, respectively (Lee et al., 2015). Both Kv9.3 and Kv5.1 mRNA are also found in T84 human colonic carcinoma cells (Spitzner et al., 2007). Using Kv9.3 small interfering RNA to transiently knock down Kv9.3, without altering the Kv2.1 mRNA level, led to a decrease in the viability of these carcinoma cells. This decreased cell viability was caused by an induced cell cycle arrest in the $G_0/G_1$ phase without any change in the fraction of apoptotic cells (Lee et al., 2015). Furthermore, stable downregulation of Kv9.3 in both HCT15 and A549 cells using Kv9.3 short hairpin RNA reduced tumor growth in mouse xenografts (Lee et al., 2015). These findings suggest that Kv9.3 alone (without Kv2.1) plays a role in cell-cycle progression and cell proliferation. However, the observed effects on cell-cycle progression and cell proliferation may also be caused by a changed ratio in available Kv2.1/Kv9.3 hetero- and Kv2.1 homotetramers secondary to the reduced Kv9.3 expression.

Kv2.1 and Kv9.3 mRNA have also been detected in cervical adenocarcinoma and squamous and endometrial carcinoma cell lines that are models for various uterine cancers (Suzuki and Takimoto, 2004). Using hanatoxin 1, Suzuki and Takimoto (2004) showed that the growth of Kv2-expressing cells was suppressed compared with that of non–Kv2-expressing cells, suggesting that heterotetrameric Kv2.1/Kv9.3 channels are involved in the proliferation of various uterine cancer cells. However, in two cervical adenocarcinoma cell lines (HT-3 and MS-751) and one endometrial carcinoma cell line (Ishikawa), Kv9.3 mRNA but not Kv2.1 mRNA (Suzuki and Takimoto, 2004) could be detected, favoring the notion that Kv9.3 might also have an independent role that does not involve Kv2.1 subunits.

In contrast with studies reporting that the pharmacologic or RNA interference–mediated reduction in Kv2.1-mediated currents resulted in decreased cell proliferation (Suzuki and Takimoto, 2004; Deng et al., 2007), Li et al. (2015) reported that decreasing Kv2.1 expression and current, obtained by an overexpression of the microtubule-associated protein tau, enhanced cell proliferation in HEK293 cells. Similarly, overexpression of tau in neuroblastoma N2A cells reduced outward Kv current and increased N2A cell proliferation. Expression of Kv2.1, Kv5.1, and Kv9.2 was detected by PCR in these mouse neuroblastoma cells, and overexpression of tau reduced the expression of Kv2.1 and Kv9.2 without affecting that of Kv5.1 (Li et al., 2015). These findings suggest that Kv2.1- and Kv9.2-containing channels contribute to the tau-induced modulation of N2A cell proliferation.

### Role of Kv2/KvS heterotetramers in stem cells

Stem cells retain the remarkable potential to develop into many different cell types and consequently to repair various damaged tissues. A recent study reported that expression of the Kv9.3 gene in adult human neuronal stem cells isolated from the subventricular zone of the

lateral ventricles was greater than that in adult human cortex (Sandberg et al., 2014). Immunofluorescent experiments revealed heterogeneous expression of Kv9.3 in adult human neuronal stem cell neurospheres, which are nonadherent spherical clusters of stem cells providing a method to investigate the differentiation of these cells in vitro. Kv9.3 mRNA abundance was also increased in fetal human neuronal stem cells but to a lower extent than in adult human neuronal stem cells. These findings suggest that Kv9.3 may provide a marker to discriminate between different neuronal stem and progenitor cells.

Kv2.1 and Kv9.3 mRNA are also expressed in human mesenchymal stem cells (You et al., 2013), which can differentiate into different cell types both in vitro and in vivo (Sundelacruz et al., 2008). Differentiation of human mesenchymal stem cells into adipocytes was accompanied by an increase in Kv2.1 mRNA level but a decrease in Kv9.3 mRNA level (You et al., 2013). The relatively specific Kv2.1 blocker guangxitoxin 1E (Herrington et al., 2006) suppressed mesenchymal stem cell differentiation into adipocytes, suggesting that Kv2.1 is involved in this process.

### Conclusion

Kv2/KvS heterotetramers contribute to distinct physiological and pathophysiological processes in various tissues, supporting their role and importance in vivo. Their more tissue-specific expression pattern, combined with the lack of available specific pharmacological modulators (Gutman et al., 2005) for the ubiquitously expressed Kv2.1 homotetramers, make Kv2/KvS heterotetramers desirable pharmacologic and therapeutic targets for the development of novel treatments. This is supported by the increasing number of studies demonstrating that KvS subunits diversify the Kv2.1 channel pharmacologically. For example, Kv2.1/Kv6.3 heterotetramers demonstrated a reduced sensitivity for guangxitoxin 1E compared with Kv2.1 homotetramers, whereas the effects of ScTx-1E and TEA are comparable for both channels (Moreno-Domínguez et al., 2009). In contrast, Kv2.1/Kv6.2 heterotetramers are more sensitive to TEA and 4-AP than are Kv2.1 homotetramers (Zhu et al., 1999). Moreover, 4-AP actually potentiates Kv2.1/Kv6.4 currents rather than blocking them (Stas et al., 2015). Therefore, even members of the same KvS subfamily diversify the pharmacological profile of Kv2 channels. This further strengthens the importance of considering Kv2/KvS heterotetramers in the development of novel treatments.

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