Driving With No Brakes: Molecular Pathophysiology of Kv7 Potassium Channels

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Kv7 potassium channels regulate excitability in neuronal, sensory, and muscular cells. Here, we describe their molecular architecture, physiological roles, and involvement in genetically determined channelopathies highlighting their relevance as targets for pharmacological treatment of several human disorders.

The crucial role of potassium (K^+) channels as primary regulators of intrinsic electrical properties in excitable cells is well established; moreover, in excitable and nonexcitable cells, K^+ channels control cell volume, proliferation, differentiation, and survival. Such an extraordinary heterogeneity of functions in each cell type at various differentiation stages is accomplished through the expression of a specific pattern of K^+ currents, each with distinct subcellular localization, biophysical properties, modulation, and pharmacological profile. In fact, electrophysiological studies in a variety of cellular models have revealed an astonishing functional diversity of the K^+ current family, often anticipating the remarkable genetic and structural heterogeneity of K^+ channels (112).

Among voltage-gated K^+ channel (Kv) genes, the Kv7 (KCNQ) family comprises five members (Kv7.1–5); Kv7.1 is mainly expressed in cardiac cells, whereas Kv7.2, Kv7.3, Kv7.4, and Kv7.5 give rise to K^+ currents widely distributed in neuronal and primary sensory cells (FIGURE 1). The spectrum of functional roles attributed to Kv7 subunits has widened further following their description in smooth muscle cells of vascular and visceral tissues and in skeletal muscle cells. The aim of the present review will be to summarize the current knowledge about the overall architecture, tissue distribution, biophysical properties, regulation and pathophysiological roles of Kv7 channels, thereby setting the basis for their increasingly important pharmacological role.

Topological Arrangement of Kv7 Subunits and Main Regulatory Sites

Similar to other Kvs, Kv7 channels are tetramers of identical or compatible subunits; each Kv7 subunit shows a topological arrangement with six transmembrane segments (S1–S6), and intracellular NH_2 and COOH termini (FIGURE 2). The region encompassing segments S1–S4 forms the voltage-sensing domain (VSD), whereas the S5–S6 region forms the ion-selective pore. As in other Kvs, in Kv7 channels, the S4 segment contains from four to six positively charged arginines (Rs) separated by two to three uncharged residues; unlike other Kv channels, the third R is replaced by a neutral glutamine residue. The K^+ selectivity filter has the canonical GYGD sequence. Kv7 channel function at various cellular sites is influenced by accessory subunits characterized by a single membrane-spanning domain and encoded by the KCNE gene family (65, 104), similar to other Kv channels (1, 2). A long COOH-terminal region is characteristic in Kv7 subunits; at this location, sites determining heteromeric and homomeric assembly, interaction with regulatory molecules, subcellular localization, and binding of accessory proteins have been identified (31).

Calmodulin

The regulatory protein calmodulin (CaM) appears to be constitutively tethered to the COOH-terminal region of Kv7.2/3 channels, irrespective of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (111, 118). Secondary structure analysis of this region predicts four α-helices (A, B, C, and D) conserved in all Kv7 family members. Of these, *helices A and B* contain CaM-binding sequences, an IQ-like sequence in *helix A*, and two overlapping 1-5-10 motifs in *helix B*. The precise mechanism by which CaM regulates Kv7 channel function is still highly debated. In general, CaM is believed to play a key role for Kv7 channel folding and trafficking; mutations that affect CaM binding profoundly impair channel maturation and plasmamembrane expression (27). In addition to this "structural" function of CaM, it has also been shown that Kv7 currents are highly sensitive to $[Ca^{2+}]_i$ and that CaM acts as their Ca^{2+} sensor; such a mechanism would mediate channel modulation by $[Ca^{2+}]_i$-mobilizing receptors (34).

Syntaxin 1A

Helix-A appears to mediate Kv7.2 direct interaction with the plasma membrane protein syntaxin 1A, possibly a crucial phenomenon for Kv7.2 subunits

targeting at presynaptic sites (FIGURE 1; Ref. 74) where they regulate neurotransmitter release (62, 63).

Phosphatidylinositol-(4,5)-Bisphosphate

A decrease in phosphatidylinositol-(4,5)-bisphosphate (PIP$_2$) membrane concentration is the main biochemical signal by which agonists acting at G$_{q/11}$-coupled metabotropic receptors regulate the activity of Kv7 channels in native and recombinant sytems. In addition, the differences in the single channel open probability (p$_o$) among channels formed by Kv7.2, Kv7.3, Kv7.4, and Kv7.5 subunits appear to be dependent on their intrinsic affinities for intracellular PIP$_2$ (55). Indeed, higher PIP$_2$ concentrations increase the p$_o$ of homomeric and heteromeric channels formed by Kv7 subunits, thus stabilizing their open state. In support of this hypothesis is the higher sensitivity of "low p$_o$" Kv7.2, Kv7.4, and Kv7.5 channels to receptor-dependent PIP$_2$ depletion compared with "high p$_o$" Kv7.3 channels (Table 1; Ref. 35). Although initial studies identified a histidine residue in the proximal COOH terminus in Kv7.2 (121) as the receptor site for PIP$_2$-induced modulation of Kv7 channels, more recent evidence also suggests involvement of a region rich in positively charged residues located between *helix A* and *B* of the COOH-terminal domain (35).

Phosphorylation

The COOH-terminus region of Kv7.2 subunits contains the binding site for the anchoring protein AKAP79/150 that forms a trimeric complex with protein kinase C (PKC; Ref. 36). Although the direct regulation of Kv7 channels by PKC and how this
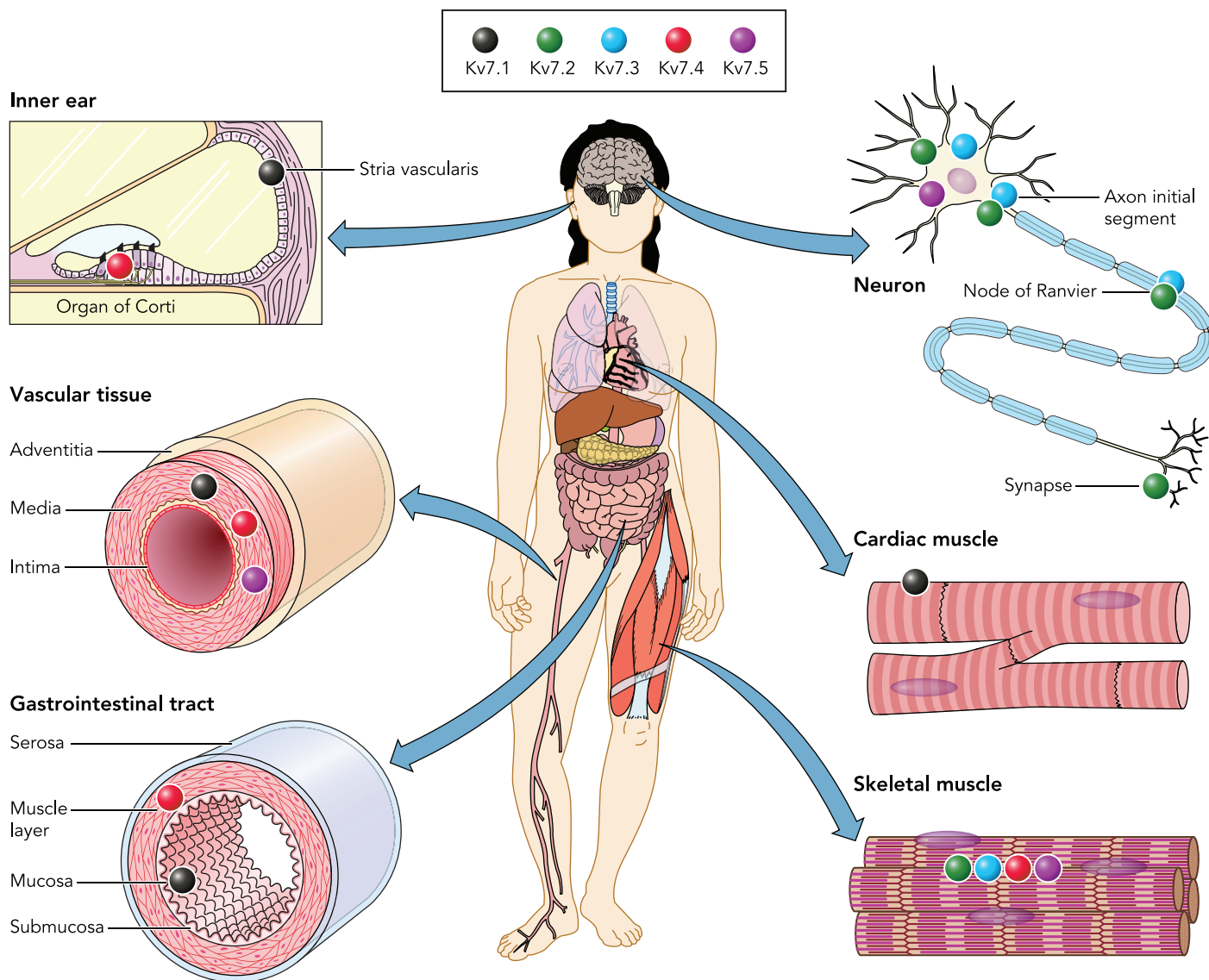

FIGURE 1. Tissue distribution of Kv7 subunits
The different panels in the figure report the principal sites of expression for each Kv7 channel subunit. In addition to those shown, it should be noted that Kv7.1 subunit expression has been detected in epithelial cells from various tissues not shown in the figure (lungs, pancreas, liver, thymus, kidneys, adrenal glands, and testis).

interferes with the CaM- and PIP_2-induced channel regulation is still controversial, the removal of two putative PKC phosphorylation sites in *helix B* largely prevents the inhibition of Kv7.2 currents by agonists acting at $G_{q/11}$-coupled metabotropic receptors (36). Kv7.1 does not form complexes with AKAP79/150; instead, it interacts with the anchoring protein yotiao, which binds protein kinase A (64). It has been recently shown that Ca^{2+}-bound CaM disrupts the functional interactions between AKAP79 and Kv7 channels, likely due to CaM binding to the channels at an overlapping site with AKAP79/150 (5). The Kv7 COOH-terminal region also contains tyrosine residues that can be phosphorylated via receptor and nonreceptor tyrosine kinases. Among the latter, Src phosphorylates Kv7.3, Kv7.4, and Kv7.5, leading to current suppression (29). In Kv7.3, Src recognizes two tyrosine residues, one in the NH_2 terminus (Y67) and the other in the COOH terminus at *helix A* (Y349) (56). Moreover, activation of EGF receptors inhibits Kv7.2/3 currents by activating PIP_2 hydrolysis and phosphorylation of the same tyrosine residues involved in Src-dependent modulation (43). Additional residues phosphorylated by endogenous kinases on intact channel proteins in living mammalian cells have been identified in the S4-S5 linker using mass spectrometry (102).

Subunit Interaction Domain

In Kv7 COOH terminus, further down to CaM-binding *helices A* and *B*, regions involved in multimerization and subunit-specific heteromerization have been identified, which correspond to *helices C* and *D* (90). At this location, coiled-coil interactions among subunits occur, as also recently revealed by structural analysis of the D helix in Kv7.4 channels (38), and it has also been suggested that this subunit interaction domain (*sid*) also acts as a platform for Kv7 interactions with accessory KCNE subunits (32), although additional sites (in S6 and in the VSD) also have been implicated (57).

Nedd4–2

The ubiquitin-protein ligase Nedd4–2 regulates membrane expression of Kv7.2/3 and Kv7.3/5 channels (26), as well as of Kv7.1/KCNE1 heteromultimers (42). Nedd4–2 downregulates the current carried by these channels, possibly by promoting ubiquitination, internalization, and degradation; a PY motif located at the COOH-terminal end of the Kv7.1 subunit has been identified as crucial in this process, whereas the role of the same region in Kv7.2/3 subunits is less defined.

Ankyrin-G

In the distal end of Kv7.2 and Kv7.3 COOH termini (but not in other Kv7 subunits), an interaction domain for the adaptor protein Ankyrin-G has been identified (71), which allows heteromeric Kv7.2/3 channels to localize at the axonal initial segment and Ranvier nodes, crucial sites for action potential initiation and propagation (FIGURE 1).

Molecular Identification of the Kv7 Genes: Role in Human Diseases

The identification of the molecular defects responsible for human genetically transmitted channelopathies has
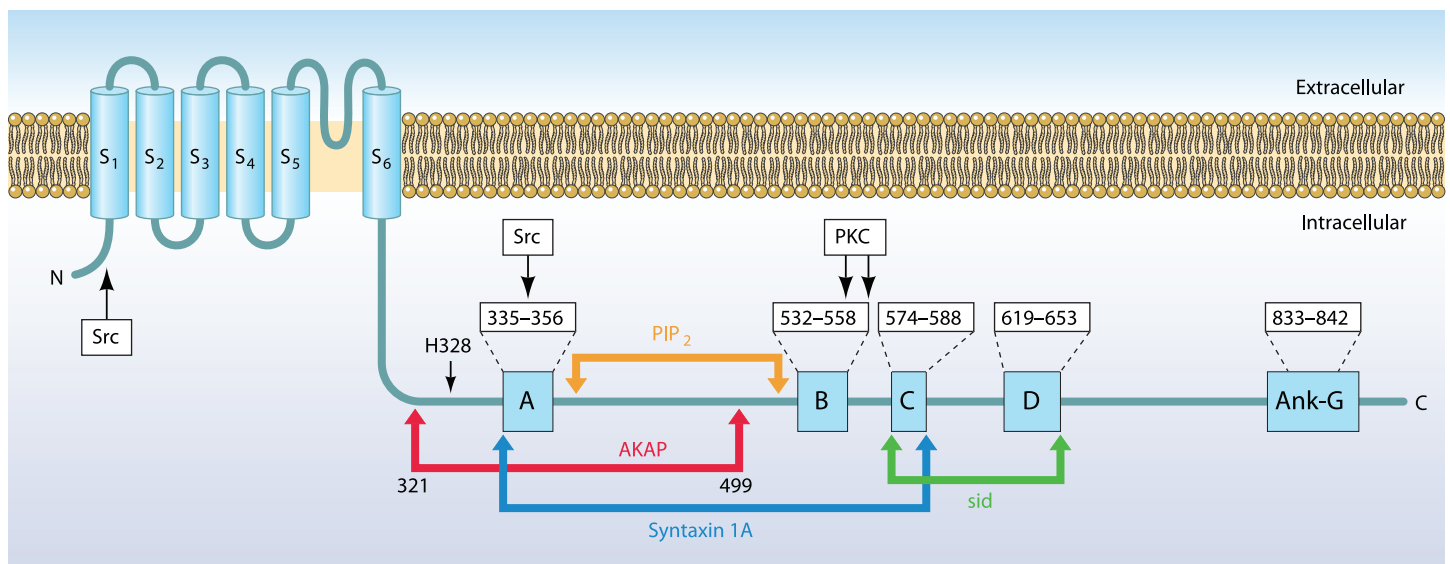

FIGURE 2. Schematic representation of a single Kv7.2 subunit
The approximate position of the binding domain for PIP_2 in the COOH terminus is indicated. A, B, C, and D correspond to the four hypothetical α-helical regions identified in Ref. 118. sid, Subunit interaction domain; CaM, calmodulin; AKAP, A-kinase-anchoring protein; PKC, protein kinase C; Ank-G, ankyrin-G; PIP_2, phosphatidylinositol-(4,5)-bisphosphate.

Table 1. *Kv7 family genes and proteins*

Kv7 Member	Locus	Disease	Exons and Splice Variants	Biophysical Parameters		
				$V_{1/2}$, mV	k, mV/e-fold	τ act, ms at +40 mV
Kv7.1	11p15.5	LQTS1 JLNS1 SQTS FAF	Six splice variants (isoforms 0–5): isoform 1 is the primary isoform and is composed of 16 exons coding for a protein of 676 amino acids	~−20[a]	~12[a]	~90[b]
Kv7.2	20q13.3	BFNS	Five splice variants; the longest isoform A is composed of 17 exons that code for a protein of 872 amino acids	~−30[f]	~8[f]	~130[b]
Kv7.3	8q24	BFNS	No splice variants; 15 exons coding for a protein of 872 amino acids	~−40[f]	~7[f]	~60[b]
Kv7.4	1p34	DFNA2	Two isoforms: the longest A consists of 14 exons encoding for a protein of 695 amino acids	~−20[f]	~13[f]	~160[b]
Kv7.5	6q14		Five splice variants. Isoform 4 is the longest, with 14 exons that translate in a protein of 951 amino acids. Isoform 1 is predominantly expressed in the brain, while isoforms 2 and 3 are mainly expressed in skeletal muscle	~−40[f]	~7[f]	~150[b]

Data in table are adapted from Refs. [a]104a, [b]29, [c]77a, [d]75a, [e]103, [f]67, [g]54, [h]98, [i]116a. LQTS1, long QT syndrome type 1; JLNS1, Jervell and Lange-Nielsen syndrome type 1; SQTS, short QT syndrome; FAF, familial atrial fibrillation; BFNS, Benign Familial Neonatal Seizures; DFNA2, autosomal dominant type 2 deafness. *Low = p_o <20%; high = p_o >80%; **Data from Kv7.2/Kv7.3 heteromeric channels (Ref. 108).

been instrumental for the cloning of the Kv7 genes and for the identification of their protein products as major molecular determinants of several currents at specific cellular sites. Most of these genetic defects impair Kv7 channel regulation of cell intrinsic electrical properties; therefore, Kv7 channelopathies are often associated with an enhanced cellular excitability unopposed by the "braking effect" exerted by Kv7 channels, hence, the title of this review.

Kv7.1, Arrhythmias, and I_{Ks}

The first human channelopathy in which a Kv7 gene defect was discovered is the Long QT Syndrome (LQTS), an arrhythmic disorder characterized by the lengthening of the QT interval of the electrocardiogram, indicative of a delayed cardiac action potential repolarization. Most patients affected by LQTS are asymptomatic until some event (strenuous exercise, stress, drugs, etc.) leads to torsades de pointes, a distinctive re-entrant ventricular tachycardia in which the amplitude of the QRS complex varies around the isoelectric line. Torsades de pointes can self-terminate or degenerate into ventricular fibrillation. In the most common form (LQTS-1), accounting for over 50% of the LQTS cases, mutations in the gene encoding for the K^+ channel subunit Kv7.1 (previously known as KvLQT1 or KCNQ1) have been found (109). LQTS caused by Kv7.1 mutations can be transmitted in a dominant or recessive manner; in the first case (the Romano-Ward syndrome), only cardiac symptoms

are present in heterozygous affected individuals, whereas in homozygous carriers of the recessive form (the Jervell and Lange-Nielsen syndrome), bilateral deafness accompanies the severe cardiac dysfunction. Kv7.1 subunits, when expressed in heterologous systems together with the accessory β-subunit KCNE1, form channels that give rise to voltage-gated currents whose biophysical and pharmacological properties closely resembled those of the I_{Ks} component of the cardiac delayed rectifier I_K (FIGURE 3; Refs. 77a, 84, 104a). In fact, in guinea-pig atrial and ventricular myocytes (82, 83), similar to human atrial and ventricular myocytes, canine ventricular cells, and rabbit ventricular cells, two prominent components of I_K, referred to as I_{Kr} ($I_{K,rapid}$) and I_{Ks} ($I_{K,slow}$), can be defined on the basis of their differential sensitivity to blockade by class III anti-arrhythmics, including dofetilide, E-4031, and sotalol. Drug-sensitive I_{Kr} activates and inactivates rapidly, displaying marked inward rectification; on the other hand, drug-resistant I_{Ks} activates and deactivates slowly during membrane depolarization and hyperpolarization, respectively, shows very little rectification, and fails to inactivate significantly (FIGURE 3; Table 1). I_{Kr} and I_{Ks} also differ in their single-channel conductances (10–13 pS for I_{Kr}, 2–8 pS for I_{Ks}) (93, 107, 114). The functional role of I_{Ks} for cardiac repolarization in different species has been a matter of extensive debate; in human ventricular cells, I_{Ks} contributes to the repolarization process only to a minimal extent under normal circumstances, but

Biophysical Parameters		Pharmacology				
τ deact, ms at −60 mV	P_o	g, pS	TEA (IC$_{50}$, mM)	Lino (IC$_{50}$, μM)	XE-991 (IC$_{50}$, μM)	RET (EC$_{50}$, μM)
~450[b]	low[c]*	~8[c]	5[d]	9[d]	1[d]	>100[e]
~150[b]	low[g]	~6[g]	0.3[d]	4[d]	1[d]	2[e]
~100[b]	high[g]	~8[g]	>30[d]	5[d]	~1**	1[e]
~100[b]	low[g]	~2[g]	3[d]	>10[d,h]	>5[h]	5[e]
~120[b]	low[g]	~2[g]	>30[d]	>10[d]	>25[d]	6[i]

it provides an important safety mechanism to prevent excessive and dangerous repolarization lengthening when repolarization is prolonged beyond normal and/or sympathetic tone is elevated (45, 46). In fact, downregulation of I_{Ks}, either as a consequence of diseases such as heart failure, diabetes, or cardiac hypertrophy, or of mutation-induced loss of function (as in LQTS-1), or of its pharmacological inhibition, does not produce a marked repolarization lengthening but makes the repolarization less stable and the heart vulnerable toward repolarization abnormalities and torsades de pointes arrhythmias. Modeling studies confirm a crucial role for I_{Ks} in providing a "repolarization reserve" when other repolarizing currents (e.g., I_{Kr}) are compromised (79). As recently reviewed (72), almost 300 mutations have been identified in Kv7.1 in LQTS-1 patients; several others have been found in the KCNE1 gene (LQTS-5) (106). In both LQTS-1 and LQTS-5, the disease pathogenetic mechanism is an impairment of I_{Ks} function (loss-of-function mutations). Noticeably, a few gain-of-function mutations in both Kv7.1 and KCNE1 have been linked to familial atrial fibrillation (FAF; Ref. 18) or to short QT syndrome (SQTS; Ref. 10).

In addition to cardiomyocytes, Kv7.1 mRNA is widely distributed in epithelial tissues, often in association with various members of the KCNE gene family (FIGURE 1). In particular, voltage-dependent Kv7.1 channels can be converted to voltage-independent, constitutively open K$^+$ leak channels by co-assembly with the KCNE2 or KCNE3 ancillary subunits (86). In gastric parietal cells, Kv7.1-KCNE2 channels provide an apical K$^+$ recycling pathway required for acidification by the H$^+$-K$^+$-ATPase (33, 88); consistent with this, a severe gastric phenotype with impaired acid secretion and gastric mucosal hyperplasia has been observed in Kv7.1 (52) or KCNE2 (76) knockout mice. In the colon, basolateral Kv7.1-KCNE3 channels provide a driving force for cAMP-stimulated Cl$^−$ secretion (86). Noticeably, in murine gastrointestinal tissue, despite abundant expression in enterocytes, no evidence for Kv7.1 expression was found in smooth muscle cells (Ref. 41; see below). Kv7.1 and KCNE2 also form constitutively active K$^+$ channels in thyrocytes, and Kv7.1-KCNE2 channels regulate normal thyroid hormone biosynthesis (77). Kv7.1 is also expressed in pancreatic β-cells, where it regulates insulin secretion; Kv7.1 has been recently identified as a strong susceptibility gene for Type 2 diabetes in Japanese (105) and Scandinavians (44). Finally, delayed-rectifier K$^+$ currents in murine portal vein myocytes are inhibited by the selective Kv7 channel blocker linopirdine, providing the first functional evidence for Kv7 channels in vascular smooth muscle (69); in addition to Kv7.1, expression of other Kv7 members likely contributes to this pharmacological sensitivity (Refs. 58, 116; see below). Recently, Kv7.1 transcripts and proteins have been shown to be expressed in brain regions involved in epileptogenesis and in

brain stem nuclei involved in autonomic control of cardiac function (30).

Kv7.2, Kv7.3, Epilepsy, and I_{KM}

Kv7.2 and Kv7.3 genes were characterized upon identification of the molecular defects responsible for Benign Familial Neonatal Seizures (BFNS), a rare autosomal-dominant idiopathic epilepsy of the newborn characterized by the occurrence of focal, multifocal, or generalized tonic-clonic convulsions starting around *day 3* of postnatal life and

spontaneously disappearing after a few weeks or months (73). Using positional cloning strategies, the genes altered in both EBN1 (11, 96) and EBN2 (16), two chromosomal loci previously linked to BFNS, were identified and found to belong to the same gene family of Kv7.1; they were therefore named KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3), respectively. Mutations in $K_v7.2$ are 10 times more frequent than in $K_v7.3$; all $K_v7.3$ mutations described to date are missense, whereas $K_v7.2$ mutations consist of truncations, splice site defects,
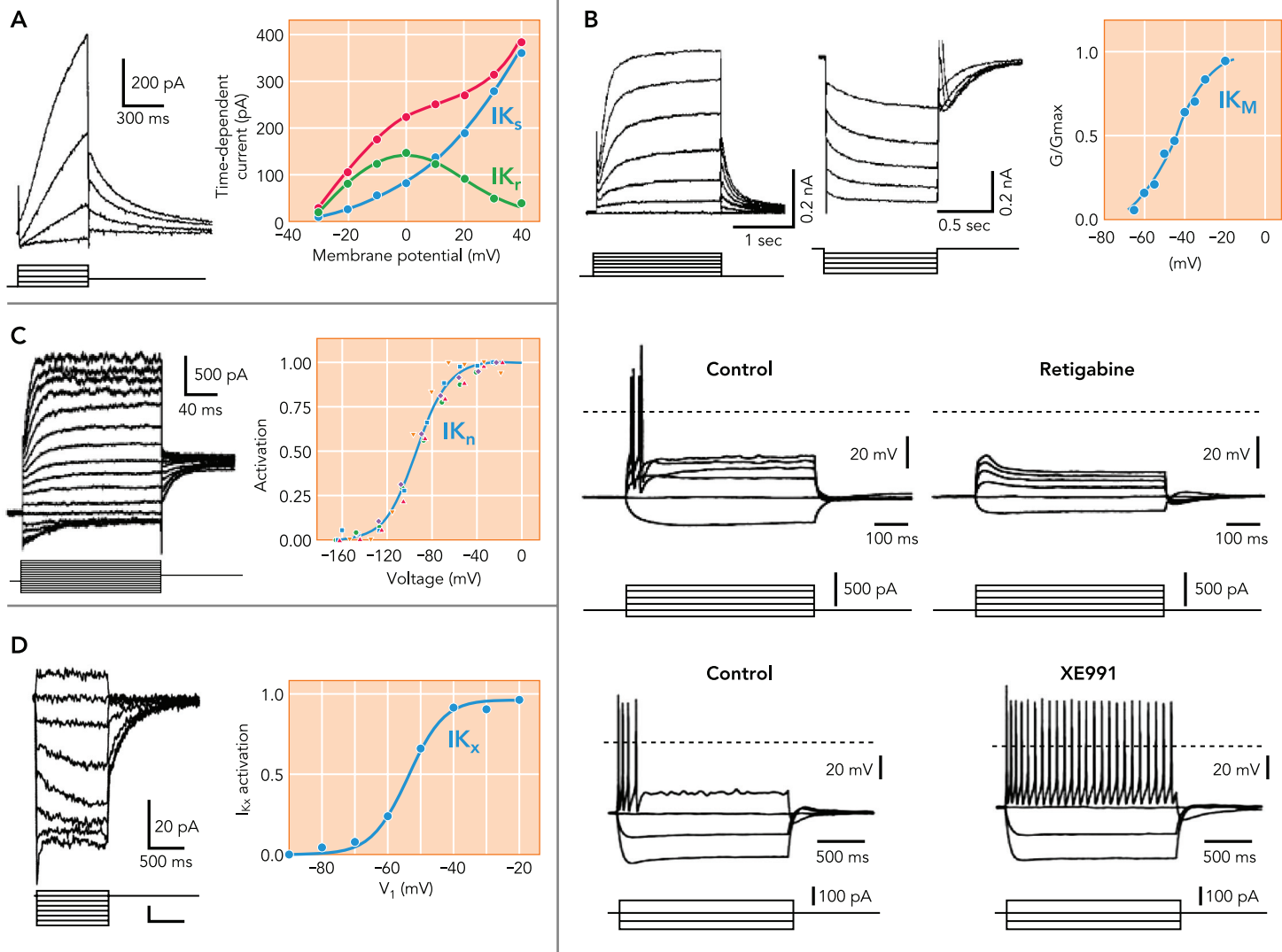

FIGURE 3. **A summary of voltage-gated K^+ currents possibly underlined by Kv7 subunits**
A: an example of IK_s (data from Ref. 82). *Left*: outward K^+ currents recorded in guinea-pig ventricular myocytes after exposure to 5 µM E-4031. Currents were recorded during 550-ms pulses to −20, 0, +20, and +40 mV from a holding potential of −40 mV. *Right*: the I/V relation for the total I_K and the two components I_{Kr} (E-4031-sensitive) and I_{Ks} (E-4031-insensitive). *B*: an example of IK_M (data taken from Ref. 108). In rat sympathetic neurons of the cervical ganglia, currents were activated by holding the cells at −60 mV and depolarizing to 0 mV (10-mV increments; *top left*), and deactivated with hyperpolarizing pulses up to −80 mV from an holding potential of −30 mV (−10-mV increments; *bottom middle*). *Top right*: a conductance-voltage curve fitted with a single Boltzmann function ($V_{1/2}$ = −44 mV; k = −8.8 mV/e-fold). *Middle* and *bottom*: the effects of retigabine (3 µM), an IK_M activator, and XE-991 (10 µM), an IK_M blocker, respectively, on somatic excitability of sympathetic neurons from the superior cervical ganglion (data from Refs. 103 and 120, respectively). *C*: an example of $IK_{(n)}$ [data taken from Ref. 61 (*left*) and Ref. 37 (*right*)]. $IK_{(n)}$ was identified in mouse cochlear outer hair cells at P12 as the voltage-dependent membrane current remaining after subtraction from the control current of the linopiridine (200 µM)-sensitive component (holding voltage: −84 mV; test pulses from −124 mV to +34 mV, in 10-mV increments). *Right*: a conductance-voltage curve fitted with a single Boltzmann function ($V_{1/2}$ = −86 mV; k = −17 mV/e-fold). *D*: an example of IK_x (data taken from Ref. 51). IK_x was recorded in rod photoreceptors from tiger salamander held at −30 mV at voltages between −90 and 0 mV, as indicated. The activation curve shown at *right* resulted in a $V_{1/2}$ of −54 mV and a slope factor (k) of 5.4 mV/e-fold.

or missense, non-sense, and frame-shift mutations, as well as sub-microscopic deletions or duplications. $K_v7.2$ mutations have also been detected in sporadic cases of benign neonatal seizures (9).

Kv7.2 subunits have been heterologously expressed in *Xenopus* oocytes and in a wide range of mammalian cells. Homomeric Kv7.2 channels carry robust K^+-selective currents activated by depolarization at membrane potentials around -50 mV; these currents exhibit slow activation and deactivation kinetics and lack significant inactivation (Table 1). Currents carried by Kv7.3 homomultimers are rather small and activate at more negative potentials (approximately -60 mV); at the single-channel level, Kv7.3 channels show the highest opening probability and unitary conductance among Kv7 members (55). Expression of Kv7.2 and Kv7.3 subunits in the same cell generates currents whose amplitude is ∼10 times larger than that expected from the simple summation of the currents produced by the Kv7.2 or Kv7.3 homomultimers (108, 113). An higher opening probability of Kv7.2/Kv7.3 heteromers compared with Kv7.2 homomers, together with a two- to threefold increase in the number of channel-forming subunits expressed at the membrane (89) contributes to this phenomenon.

Several studies in heterologous expression systems have focused on the molecular mechanism by which BFNS-causing mutations affect the function of Kv7.2/Kv7.3 subunits, shedding light on disease pathogenesis (9). Although some mutations drastically decrease steady-state cellular levels of channel subunits, others affect their intracellular trafficking (99) and polarized targeting (20), or their function once normally inserted into the plasmamembrane (15, 22, 100). Based on this evidence, it has been proposed that a mild (20–30%) decrease of I_{KM} function appears sufficient to cause BFNS (85) and that haploinsufficiency is the primary pathogenetic mechanism for both familial and sporadic cases of BFNS.

More importantly, heterologous expression studies revealed that the biophysical and pharmacological properties of the Kv7.2/Kv7.3 heteromultimers recapitulate those of the native M-current (I_{KM}) (108), a voltage-gated K^+ current first isolated in sympathetic neurons of the bullfrog in the 1980s (FIGURE 3B; Ref. 12). In these cells, acetylcholine released from preganglionic cells, in addition to fast nicotininc excitatory postsynaptic potentials (fEPSPs), was known to trigger slow excitatory membrane responses (sEPSPs) (25). Compared with nicotinic fEPSPs, these slow responses showed long synaptic delays (tenths to hundreds of milliseconds), were long-lasting (enduring for many seconds) and were due to acetylcholine binding to muscarinic receptors. sEPSPs were accompanied by a decrease in membrane conductance of the postganglionic cell (48), an effect consequent to the suppression of a K^+ conductance (110), later identified as I_{KM}. Following its discovery in amphibian sympathetic neurons, I_{KM} was soon described in rat superior cervical ganglion (SCG) cells (21) and in a variety of central neurons, including mammalian hippocampal and cortical pyramidal cells (60). Kv7.2 and possibly Kv7.3 subunits also appear to mediate the slow K^+ current observed in nodes of Ranvier of mammalian peripheral myelinated fibers (24, 91). I_{KM} is a non-inactivating, voltage-dependent K^+ current that activates in a time- and voltage-dependent manner at -60 mV, close to the resting membrane potential and, in cells expressing M1, M3, or M5 muscarinic receptors, is suppressed by muscarinic receptor stimulation (hence its name). I_{KM} opposes cell depolarization by incoming stimuli, therefore inhibiting neuronal hyperexcitability and causing spike frequency adaptation during sustained depolarizations. Muscarinic receptor activation, by suppressing I_{KM}, depolarizes the cells and enhances membrane excitability, causing tonic firing. Although it was originally believed that activation of I_{KM} was too slow to influence the repolarization of solitary spikes, it has been recently shown that I_{KM} (120) activates during the spike after depolarization and limits its duration, thereby precluding its escalation to a burst (117). In addition to muscarinic receptors, agonists binding at several $G_{q/11}$-coupled receptors, by activating phospholipase Cβ and depleting membrane PIP_2 levels, can suppress I_{KM}. The existence of additional I_{KM} modulatory pathways, possibly exploited by other neurotransmitters, has also been hypothesized (23, 34).

Given the inhibitory role of I_{KM} on neuronal excitability, together with the observation that I_{KM}-forming Kv7.2 and Kv7.3 subunits are widely distributed in the hippocampus, neocortex, and cerebellar cortex at key sites for neuronal network oscillations and synchronization control, it is perhaps not too surprising that mutations in Kv7.2 or Kv7.3 genes can cause epilepsy in humans. The specific vulnerability of the neonatal brain to these mutations is likely due to the excitatory actions of GABA in early life (19), combined to a low density and more depolarized voltage range of activation of I_{KM} (81), which would render neonatal neurons more sensitive to the "epileptogenic" consequences of I_{KM} dysfunction caused by BFNS mutations. Likewise, the developmental changes in GABA's action, together with the increased expression and hyperpolarization shift in the activation threshold for I_{KM}, can explain the disappearance of spontaneous seizures in BFNS patients with brain maturation.

Noteworthy, Kv7.2/3 channels appear as emerging targets for pharmacological interventions directed against human hyperexcitability diseases. As a matter

of fact, I_{KM} openers like retigabine (103) are effective in a broad range of in vitro and in vivo models predictive of anticonvulsant activity, also showing potent antiepileptic activities in humans (6, 75a). Besides epilepsy, Kv7.2/3 channel modulators may provide clinical benefit also in other neuropsychiatric diseases, ranging from pain to migraine, anxiety, dystonia, sensory deficits, and neurodegenerative diseases (66).

Kv7.4, Deafness, and M-Like Currents

Hearing loss is the most frequent inherited sensory defect in humans, with genetic factors playing a crucial role. Genetically transmitted hearing loss can be associated with other abnormalities (syndromic) or occur in isolation (nonsyndromic). Among the large number of genes associated with genetically transmitted deafness, those that encode for ion channels involved in sensory transduction in cochlear hair cells have received particular attention. In fact, the gene encoding for Kv7.4 was cloned by sequence homology to Kv7.3 and found as the causative gene for autosomal dominant type 2 deafness (DFNA2), a progressive form of sensorineural hearing loss, starting in the high frequencies and also affecting the middle and low frequencies later in life (49). Most DFNA2-causing mutations in Kv7.4 are missense alterations affecting amino acids located within or close to the channel pore (97); these mutations cause hearing loss via a dominant-negative effect as mutant subunits interfere with the assembly and/or function of the wild-type subunits in the inner ear (49). Some DFNA2-causing mutations in Kv7.4 are deletions that result in haploinsufficiency, since the mutant subunits fail to interact with the normal protein encoded by the wild-type allele; the hearing loss associated with these mutations is milder in low and mid frequencies, more severe in high frequencies, and later in onset than the hearing loss associated to missense mutations (97).

When expressed in *Xenopus* oocytes, Kv7.4 subunits yield voltage-dependent, K^+-selective currents that activate slowly upon depolarization around -40 mV (Table 1); compared with other Kv7 channels, current activation kinetics of Kv7.4 channels are significantly slower, whereas the deactivation process seems to be faster (49). Despite showing the lowest opening probability (0.07) and unitary conductance (2.1 pS) among Kv7 channels (54, 55), homomeric Kv7.4 channels give rise to very large currents, suggesting a very high density of functional Kv7.4 channels at the plasma membrane (119) and allowing one to record their gating currents (67). Kv7.4 subunits do not co-assemble with Kv7.1 or Kv7.2 subunits, but heteromeric channels composed of Kv7.3 and Kv7.4 subunits can be generated in heterologous expression systems; formation of Kv7.4/Kv7.5 heteromers in vivo has also been proposed (14).

High levels of Kv7.4 expression have been described in the basolateral membrane of cochlear outer hair cells (OHCs) (FIGURE 1), where Kv7.4 subunits are thought to provide a major contribution to the K^+ conductance defined as $I_{K,n}$, a voltage-dependent K^+ current first described in OHC of the guinea-pig organ of Corti (FIGURE 3C; Ref. 37). $I_{K,n}$ activates near normal resting potentials of -70 to -80 mV and deactivates below -90 mV; therefore, its function would be to hold the cell near the Nernst equilibrium potential for K^+ ions, thereby maximizing the driving force for K^+ entry through the apical transducer. K^+ currents having a negative activation range have been also described in mouse and rat inner hair cells (IHCs); these currents contribute to the resting conductance of the IHC, thereby maintaining low intracellular Ca^{2+} levels by favoring the closed state of Ca^{2+} channels. Also in IHCs, Kv7.4 expression has been detected by immunofluorescence (70) or in situ hybridization and RT-PCR analysis (8), although at a lower level than in OHCs; these observations, in one with the temporal overlap between Kv7.4 and $I_{K,n}$ occurrence during ontogenesis, provide solid evidence for Kv7.4 subunits contributing to $I_{K,n}$ also in IHCs (47). Similarly, in vestibular epithelia, type I hair cells express a high density of K^+ channels with an activation range at very negative voltages, overlapping the resting membrane potential; this I_{KM}-like current, described as g_{KI} (75) or $g_{K,L}$ (80) is responsible for the extremely low input resistances of type I cells compared with type II cells. Although Kv7.4 has been suggested to provide a major contribution to the described I_{KM}-like currents, significant functional and pharmacological differences exist between Kv7.4-mediated currents in heterologous expression systems and native $I_{K,n}$ or $g_{(K,L)}$ in cochlear and vestibular hair cells, respectively. In fact, $I_{K,n}$ (59) and $g_{K,L}$ (17) voltage range of activation is far more negative ($I_{K,n}$: $V_{1/2} = -92$ mV; $g_{K,L}$: $V_{1/2} = -73$ mV) than that of Kv7.4 homomultimers (49, 92); furthermore, activation kinetics are much slower in recombinant channels (49, 98). In addition, $I_{K,n}$ is blocked by linopirdine with an IC_{50} of 0.7 μM (61), whereas Kv7.4 homomers and Kv7.3/4 heteromers have an higher IC_{50} for this compound. It has been suggested that such differences might be attributable to modulation of Kv7.4 by interacting KCNE subunits; in particular, KCNE4 appears responsible for the marked leftward shift of the activation curve of $I_{K,n}$ in vivo (101).

In isolated salamander rod photoreceptors, Attwell and Wilson (3) characterized the ionic basis for the strong outward rectification observed in these cells for potentials more positive than about -35 mV and found that the voltage- and time-dependent current I_A was composed of two ionic current components,

one TEA-sensitive activated by depolarization (I_{Kx}) (4) and another Cs^+-sensitive activated by hyperpolarization (I_h). Further studies (7) revealed that I_{Kx} is a standing (non-inactivating) outward K^+ current that deactivates slowly when the cell is hyperpolarized, thus showing a voltage and time dependence similar to that of I_{KM} (FIGURE 3D). In rods, I_{Kx} is believed to contribute prominently in setting the dark resting potential and in accelerating the response to dim light (50, 51). Whether Kv7.4, whose cDNA was first isolated from a human retinal library (49), provides a significant contribution to I_{Kx} (98) has not been demonstrated.

Beside shaping the responses to auditory, vestibular, and visual inputs in primary sensory cells, M-like currents have been long known to play important roles in smooth muscle cells from vascular and nonvascular organs. Perhaps the first demonstration of a K^+ current resembling I_{KM} in the smooth muscle was in amphibian gastric smooth muscle cells (95); in these cells, membrane depolarization at rather negative values (less than −70 mV) triggered the slow activation of a K^+-selective current that was suppressed by cholinergic agonists. In vascular smooth muscle cells, currents having similar biophysical and pharmacological profiles were later found in all branches of the rabbit pulmonary arterial tree and named $I_{K(N)}$ to highlight their negative activation voltage range (28). The first hints into the molecular identification of $I_{K(N)}$ in vascular smooth muscle cells came from pharmacological studies: the Kv7 channel-blockers XE-991 and linopirdine (which discriminate only poorly among channels formed by different Kv7 subunits) inhibited a sustained membrane conductance and depolarized the resting membrane potential of portal vein myocytes (115). Both drugs also increased the spontaneous contractile activity of whole portal veins, suggesting that in smooth muscle cells, similar to neurons, Kv7 channels provide membrane hyperpolarization under resting conditions, thereby suppressing membrane excitability. Consistent with this, the increased membrane excitability of smooth muscle cells in vitro produced by arginine-vasopressin depends on the inhibition of constitutively active Kv7 channels (13). Retigabine and flupirtine, two Kv7 activators that do not act on Kv7 channels composed of Kv7.1 subunits, are effective as vasorelaxants, suggesting that Kv7.1-containing channels are not involved. In fact, later experiments revealed that, among Kv7s, Kv7.4 is the most abundant mRNA in mouse aorta, carotid artery, femoral artery, and mesenteric artery (116). Also in visceral adipose tissue and mesenteric human arteries, Kv7.4 mRNA is consistently expressed, whereas expression of Kv7.1, Kv7.3, and Kv7.5 mRNAs is more variable and Kv7.2 mRNA is undetectable

(FIGURE 1). In these tissues, Kv7 subunits are located in the smooth muscle layer (68). Kv7.4 and Kv7.5 genes are also expressed in different regions of the murine gastrointestinal tract (colon, jeuneum, stomach). In the distal colon, the Kv7 activator retigabine inhibited spontaneous contractile activity by a dual action on myenteric nerves and on the smooth muscle; by contrast, Kv7 channel blockers augment inherent contractile activity, thereby appearing as promising therapeutics for the treatment of motility disorders such as constipation associated with irritable bowel syndrome (41).

Finally, it is worth noticing that in C_2C_{12} cells, a widely used in vitro model of skeletal muscle differentiation, Kv7.2, Kv7.3, and Kv7.4 mRNAs have been detected (40), in addition to Kv7.1 and Kv7.5 transcripts (53, 78, 87). Moreover, Kv7.1, Kv7.3, and Kv7.4 transcripts, but not those for Kv7.2 and Kv7.5, were upregulated upon in vitro myotube formation, with Kv7.4 showing the largest increase. Pharmacological experiments revealed that Kv7 channels, and Kv7.4 in particular, regulate skeletal muscle proliferation, differentiation, and responses to drug-induced myotoxic effects, thereby expanding the potential pathophysiological and pharmacological role of Kv7 channels.

Kv7.5

Kv7.5 was the last member of the Kv7 gene family to be identified (53, 87). Kv7.5 transcripts are expressed in the brain and in sympathetic ganglia, showing a distribution largely overlapping those for Kv7.2 and Kv7.3 (87). In *Xenopus* oocytes, homomeric Kv7.5 channels generate depolarization-activated K^+-selective currents with very slow activation kinetics, requiring several seconds to fully activate. Kv7.5 subunits do not co-assemble with Kv7.2 subunits but do so with Kv7.3 and Kv7.4 subunits (14). Interestingly, Kv7.5 can reduce heteromeric currents produced by the simultaneous expression of Kv7.2 and Kv7.3 subunits, possibly by decreasing the amount of Kv7.3 subunits available to form the more "efficient" Kv7.2/Kv7.3 heteromers. Since all three Kv7 subunits are broadly expressed in the brain and show an overlapping cellular pattern of expression (94), it has been proposed that Kv7.5 subunits contribute to the functional variety of I_{KM} in distinct neuronal population. In addition to neuronal cells, Kv7.5 transcripts and/or proteins have also been identified in nonneuronal tissues, such as skeletal (40, 78, 116a) and smooth muscle cells (116). Kv7.5 transcripts and proteins have been identified in the tunica media from various blood vessels, where they participate in vascular tone regulation (13) (FIGURE 1). One major limitation in investigating the pathophysiological role of Kv7.5 channels (and of Kv7 channels in general) is the lack of pharmacological

tools selectively targeting distinct Kv7 subunits; in this scenario, the recently described ability of the well known anti-inflammatory drug diclofenac to suppress Kv7.5 while activating Kv7.4 channels (14) may allow a more precise definition of the specific pathophysiological role of Kv7.5 subunits at each cellular site of expression.

Conclusions

In 2011, we will celebrate 15 years since the molecular identification of Kv7.1, the first member of the Kv7 potassium channel gene family. Since then, our understanding of the molecular pathogenesis of Kv7 channelopathies and of the physiological role played by distinct Kv7 subunits underlying cardiac I_{Ks}, neural I_{KM}, and I_{KM}-like K$^+$ currents in several cell types has grown exponentially. Research on Kv7 channels has always proceeded with a strong translational emphasis; the benefits of this approach are likely to be seen in the near future, where Kv7-based pharmacological strategies are likely going to be available for the treatment of human diseases. The Kv7 activator retigabine has been recently approved as an anticonvulsant in Europe and awaits US approval (6, 67a). Additional indications for Kv7 modulators range from pain to arrhythmias, hypertension, and primary sensory dysfunctions, whose common pathogenetic theme is the alteration in cellular excitability controlled by this unique class of voltage-gated K$^+$ channels. ∎

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