Kv3 channels: voltage-gated K^+ channels designed for high-frequency repetitive firing

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Analysis of the Kv3 subfamily of K^+ channel subunits has lead to the discovery of a new class of neuronal voltage-gated K^+ channels characterized by positively shifted voltage dependencies and very fast deactivation rates. These properties are adaptations that allow these channels to produce currents that can specifically enable fast repolarization of action potentials without compromising spike initiation or height. The short spike duration and the rapid deactivation of the Kv3 currents after spike repolarization maximize the quick recovery of resting conditions after an action potential. Several neurons in the mammalian CNS have incorporated into their repertoire of voltage-dependent conductances a relatively large number of Kv3 channels to enable repetitive firing at high frequencies – an ability that crucially depends on the special properties of Kv3 channels and their impact on excitability.

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The ability to fire action potentials at high frequencies (often up to 1 kHz) or to follow high-frequency inputs are important physiological functions of numerous cells throughout the mammalian CNS. Neuronal populations within such disparate regions as the hippocampus, basal ganglia, neocortex, reticular thalamus, medial vestibular nucleus and auditory nuclei are capable of responding to afferent input with action potentials of brief duration and of firing repetitively at high frequencies. Recent evidence has demonstrated that the temporal overlap and voltage dependence of ligand- and voltage-gated conductances is essential for this so-called 'fast spiking' (FS) phenotype[1]. Moreover, a picture is emerging where several of the voltage- and ligand-gated channels expressed are peculiar to these cell populations[1] and responsible for conferring such diverse roles as precision timing of feedforward and feedback inhibition of principal cells[2], the generation and synchronization of high frequency oscillatory activity between discrete CNS areas[3], or the phase locking of cell activity within nuclei of the auditory brainstem[4,5]. The ability to fire brief action potentials in rapid succession relies on action potentials that are non-decremental, of short duration, repolarize rapidly and possess brief afterhyperpolarizations and interspike intervals. Although numerous channel types have been implicated in conferring such properties, the voltage-gated K^+ channels of the Kv3 subfamily have now been identified as major determinants of the FS phenotype. This article attempts to bring together data from seemingly unrelated structures within the mammalian CNS to illustrate that when neurons are required to fire repetitively at high frequencies, they often incorporate members of the Kv3 subfamily as an abundant component of their repertoire of voltage-gated channels.

Molecular characteristics of Kv3 subfamily members

Both rodents and humans possess four Kv3 genes: Kv3.1, Kv3.2, Kv3.3 and Kv3.4 (Refs 6,7). All four Kv3 genes generate multiple protein isoforms by alternative splicing, which produces versions with different intracellular C-terminal sequences. There are now 13 different Kv3 proteins known in mammals (Kv3.1a–Kv3.1b, Kv3.2a–Kv3.2d, Kv3.3a–Kv3.3d and Kv3.4a–Kv3.4c), yet the currents expressed in heterologous expression systems by the spliced isoforms of each Kv3 gene are virtually indistinguishable. Recent studies suggest that the alternatively spliced C termini confer isoform-specific regulation by second messenger signaling systems and targeting to distinct neuronal compartments[8–10].

Kv3 proteins express voltage-gated channels with unusual properties in heterologous expression systems and in native cells

Chinese hamster ovary (CHO) cells transfected with cDNAs of transcripts from each of the four Kv3 genes (Kv3.1b, Kv3.2a, Kv3.3d and Kv3.4a) have large voltage-dependent K^+ currents with similar voltage dependencies (Fig. 1a,b)[6,7]. The currents become apparent when the membrane is depolarized to potentials more positive than approximately –20 mV, which is more depolarized than any other known mammalian voltage-gated K^+ channel by at least 10–20 mV (Ref. 16). Another crucial property of the currents mediated by Kv3.1 and Kv3.2 channels (as well as Kv3.3 and Kv3.4 channels before they fully inactivate) is their fast rate of deactivation upon repolarization[6,11,16–20] (e.g. at –70 mV, Kv3.1b currents deactivate with a time constant of ≤1 ms at room temperature), which is significantly faster than that of any other known neuronal voltage-gated K^+ channels by about an order of magnitude[16]. The rightward shifted voltage dependence and fast deactivation rates of Kv3 channels are likely to be related properties, probably reflecting instability of the open state of the channel at voltages near the resting potential. Indeed, mutagenesis experiments suggest that both properties

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depend on the presence of specific amino acid residues in the fifth membrane-spanning domain (S5) of Kv3 proteins[21,22]. Interestingly, the S4–S5 sequence of Kv3 proteins is 100% identical in teleost fish, birds, frogs and mammals, including humans. This remarkable degree of conservation suggests strong selection for the unusual properties of Kv3 channels during vertebrate evolution, implying that these properties are closely linked to the special roles of these channels.

Currents with properties remarkably similar to those mediated by Kv3 channels in heterologous expression systems have now been recorded in several neuronal populations that express Kv3 subunits[5,18–20,23–28]. In some cells, Kv3 channels might exist as heteromultimers of different Kv3 proteins, because, as in the other Kv subfamilies, in heterologous expression systems Kv3 subunits can form heteromeric channels with other members of the

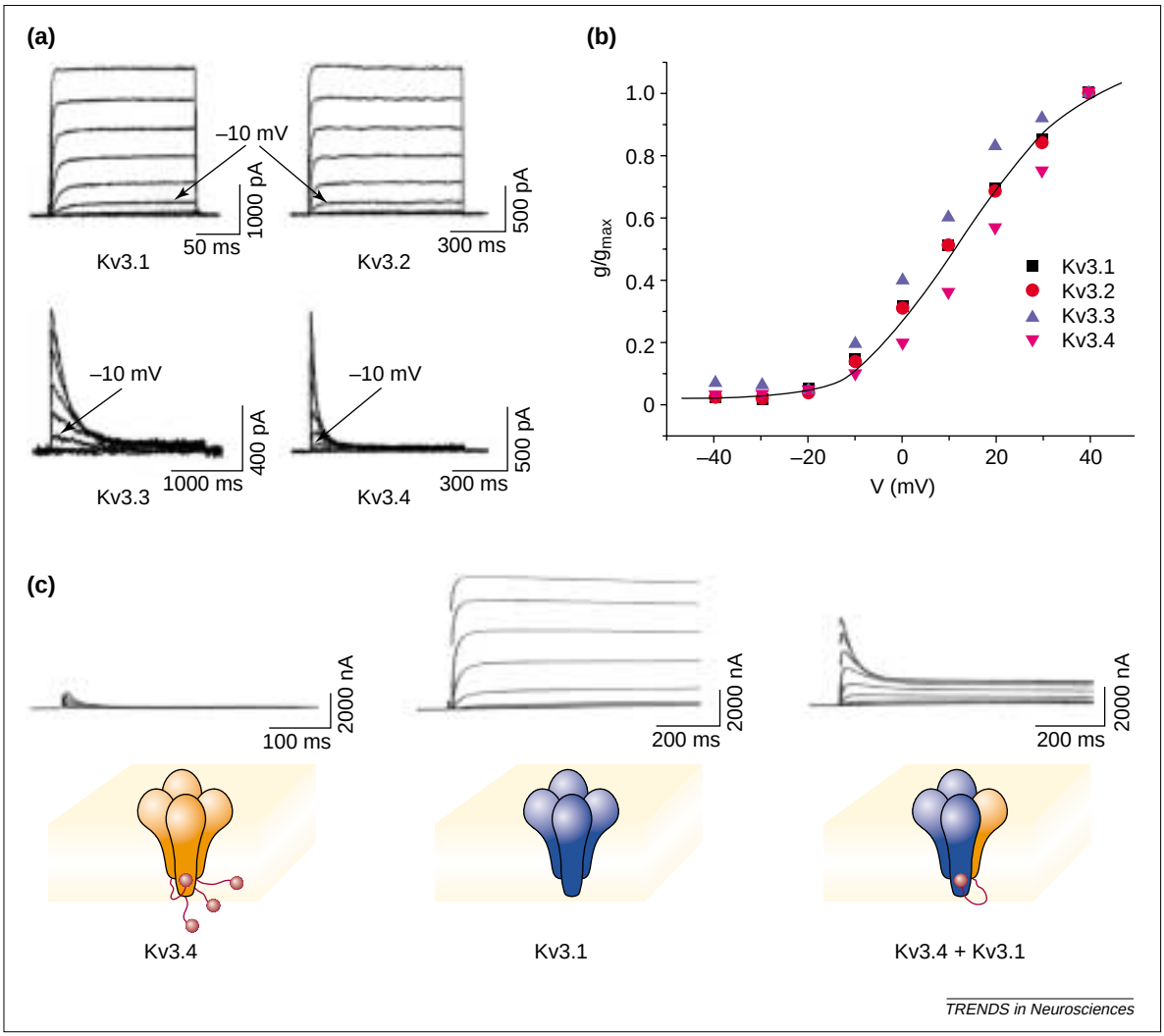

Fig. 1. (a) Kv3 currents in transfected Chinese hamster ovary (CHO) cells. Families of K^+ currents recorded in a Kv3.1b, Kv3.2a, Kv3.3d or Kv3.4b transfected cell during depolarizing pulses from –40 mV to +40 mV (in 10 mV increments, from a holding potential of –80mV). Kv3.1 and Kv3.2 currents are of the delayed-rectifier type. Upon depolarization, currents rise relatively quickly (10–90% rise time of 3–4 and 5–7 ms at +20 mV at 20°C for Kv3.1b and Kv3.2a, respectively) to a maximum level that is maintained for pulse durations lasting a few hundred ms. A slow inactivation becomes evident with pulses of longer duration[11], which is faster for Kv3.1 than for Kv3.2 channels (H. Moreno and B. Rudy, unpublished observations). Kv3.3 and Kv3.4 currents are of the A-type, although Kv3.3 currents inactivate slowly whereas Kv3.4 currents inactivate relatively quickly (note different calibration scales). The inactivation of Kv3.3 and Kv3.4 channels can be suppressed by phosphorylation by protein kinase C (PKC)[12–14]. These or other modulations could explain the variability in inactivation rates in different cells expressing Kv3.3 or Kv3.4 channels. (b) Normalized conductance–voltage relationship of Kv3 currents expressed in CHO cells. The conductance at the indicated voltage [$g = I_V/(V-V_K)$], divided by the maximum conductance (g_{max}) for the currents shown in (a), is plotted as a function of membrane potential. (c) Heteromultimeric Kv3 channels in *Xenopus* oocytes. On the left are currents recorded in a representative oocyte injected with Kv3.4b cRNA alone. In the middle are currents recorded in an oocyte injected with Kv3.1b cRNA alone. On the right are currents in an oocyte injected with the same amount of Kv3.4b and Kv3.1b, as in the previous panels. The inactivating currents are several fold larger than those seen with the same amount of Kv3.4 cRNA alone, contrary to what might be expected from the algebraic sum of two independent currents and consistent with the formation of heteromultimeric channels. Similar results have been obtained with Kv3.3 cRNA. There is amplification of the transient current because a single inactivating subunit is sufficient to impart fast inactivating properties to the channel complex[15], as illustrated in the schematic lower panels. The Kv3.4 subunits are shown in orange and Kv3.1 subunits are shown in blue. The red lines with filled circles are the amino-terminal domains of Kv3.4 subunits forming inactivating 'ball and chain' domains. The currents produced by co-injection of Kv3.1 and Kv3.2 cRNAs, which produce similar currents in homomultimeric channels, are, not surprisingly, similar to Kv3.1 or Kv3.2 currents alone[15]. (a) currents recorded by Y. Amarillo. (c) modified, with permission, from Ref. 15.

same subfamily, but not with subunits of other Kv subfamilies[7,15,29] (however, see Ref. 30). Moreover, co-immunoprecipitation studies of neocortical, hippocampal, pallidal and cerebellar membranes provide evidence that Kv3 proteins exist as heteromeric complexes in the CNS (Refs 19,31,32). The functional consequences of heteromultimer formation in the Kv3 subfamily are illustrated in Fig. 1c.

Kv3 currents are activated specifically during action potential repolarization

The electrophysiological properties of Kv3 channels suggest a specific role in action potential repolarization[6,7,11,15,18,33–38]. Given their voltage dependence, it is unlikely that Kv3 channels will be significantly activated other than during action potentials, and only once these have reached their peak. Confirmation of this hypothesis has come from recording currents from HEK293 cells transfected with Kv3.1 or Kv3.2 cDNAs voltage clamped to a waveform in the shape of two sequential brief action potentials (Fig. 2a). This experiment clearly illustrates the specific activation of Kv3.1 and Kv3.2 currents during the repolarizing phase of the action potential and their quick deactivation following the spike. These properties suggest that, when present in sufficient amounts, Kv3 channels can facilitate action potential repolarization and dictate action potential duration, without compromising action potential threshold, rise time or magnitude, and, more importantly, without contributing current that would increase the refractory period duration. This is in contrast to voltage-gated K^+ channels that activate significantly at more negative potentials and deactivate more slowly[7,11,15,18,33,36–39] (Fig. 2a; see also Box 1).

Evidence that Kv3 channels play a role in action potential repolarization has now been obtained in several neurons that express Kv3 proteins[5,18,23–25,40–43]. Moreover, Kv3-like currents have been recorded by voltage clamping hippocampal FS GABAergic interneurons to a high-frequency spike train waveform[24] (Fig. 2b).

Kv3 channels might also be activated during receptor-mediated depolarizations that depolarize the membrane to potentials more positive than approximately –10 mV. This probably happens rarely in the CNS, although it has been suggested to occur in the large end bulb synapses of the auditory nerve fibers in the ventral cochlear nucleus, where, similar to their role in action potentials, Kv3 channels could shorten the duration of the EPSPs without compromising their magnitude[39].

Kv3 channels are prominently expressed in neurons that fire at high frequency

In rodents, three of the four known Kv3 genes (Kv3.1–Kv3.3) are prominently expressed in the CNS. Kv3.4 transcripts are abundant in skeletal muscle and sympathetic neurons, but are only weakly expressed in a few neuronal types in the brain, often in neurons that also express other Kv3 genes[6,15,19,44,45]. However, as a single Kv3.4 subunit is capable of enabling fast inactivating properties to a Kv3 tetrameric channel[15] (Fig. 1c), they might be

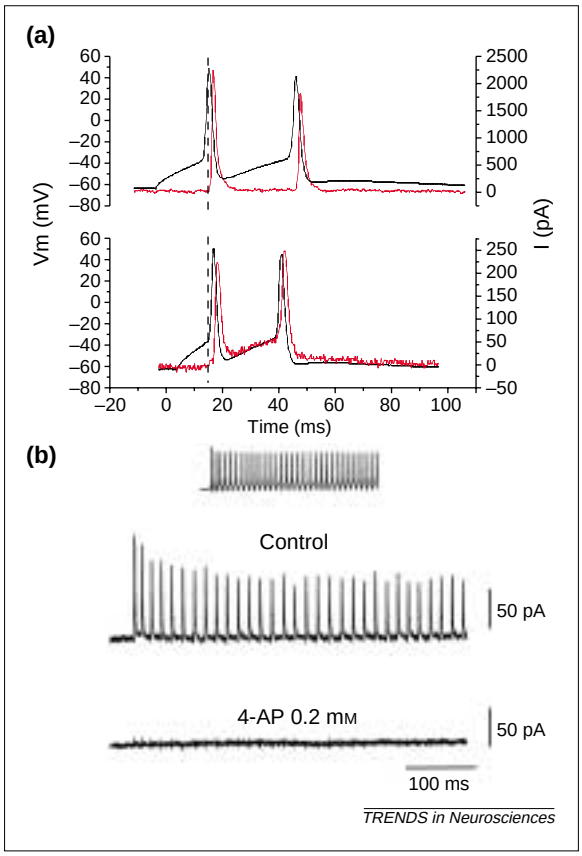

Fig. 2. (a) Currents evoked in cells that express Kv3.1b or Kv1.1 during fast action potential waveforms. HEK293 cells were transfected with Kv3.1b cDNA (top) or Kv1.1 cDNA (bottom), and voltage-clamped to a waveform corresponding to a pair of action potentials (AP) recorded from a neocortical neuron. The currents, indicated by red lines, have been superimposed on the command voltage trace (black). A perpendicular broken line has been drawn at the time that the current begins to rise (shown only during the first spike) to illustrate the temporal relationship between current activation and the action potential. Kv3.1b current is not seen until after the action potential has reached its peak, achieving a maximum value during the repolarizing phase of the spike (similar results were obtained with Kv3.2). Furthermore, because of the fast deactivation rates of the channels, the Kv3 current is quickly eliminated during the after-hyperpolarization and negligible current remains during the interspike interval. When the next action potential command arrives, the current is again activated after the peak of the action potential and is similar to that during the first spike. In cells transfected with Kv1.1 channels, the K^+ current is activated during the rising phase of the action potential and it remains active during the interspike interval. During the second action potential command, there is more current and it is activated earlier than during the first spike. After the second spike, the Kv1.1 current deactivates slowly. (b) K^+ currents recorded in hippocampal basket cells during a high-frequency spike train. The experimentally recorded action potentials were applied as voltage-clamp command (top). The resulting currents (in the presence of 0.3 μM extracellular tetrodotoxin) are shown before (middle traces, control) and after (lower traces) the application of 0.2 mM 4-aminopyridine (4-AP). The outward currents activated rapidly at the action potential peak, deactivated rapidly at the resting potential and were almost completely blocked by 0.2 mM 4-AP – all hallmark features of Kv3 channels (see Box 2). (a) records obtained by M. Saganich; modified, with permission, from Ref. 6. (b) modified, with permission, from Ref. 24.

Box 1. Positive-shifted conductance–voltage relationship: an efficient strategy to activate K^+ channels specifically during action potential repolarization

The evidence discussed in this article suggests that the voltage dependence and deactivation rates of Kv3 channels represent an adaptation that specifically enables fast and complete repolarization of action potentials, and allows short interspike intervals. Shifting the midpoint ($V_{1/2}$) of activation in the depolarized direction, as occurs on Kv3 channels, not only minimizes the current during rest and during the spike upstroke but it also produces fast closing rates because, for channels obeying Boltzmann kinetics, the rates of transition between states become faster as the voltage moves away from $V_{1/2}$ – the equilibrium point. In Kv3 channels, the resting voltage (approx. –70 mV) is far (about 85 mV) away from $V_{1/2}$ (approx. +15 mV), so the rate of closure is maximized.

However, during brief action potentials the membrane potential remains at voltages positive to –10 mV for only short periods, raising the question of whether sufficient channels can open and generate the current necessary to powerfully repolarize the action potential. In fact, during the cloning of Kv3.2 cDNAs, it was argued that these channels might not be activated during brief action potentials[a]. Perney and Kaczmarek reached a similar conclusion, based on computer modeling of the firing of cochlear nucleus bushy cells[b]. A simple solution is to have Kv3 channels occur at relatively high densities. This will also tend to increase the Kv3 current at more negative potentials, however, given the value of the $V_{1/2}$ of Kv3 channels, the maximum proportion of channels that could open at negative voltages is miniscule {~0.001 of the total number of channels at –40 mV, according to the Boltzmann function [$G/G_{max} = 1/1 + \exp(V - V_{1/2})/k$ with $V_{1/2}$ = 15 mV and k = –8]}. Compare this with the percentage of channels open (~0.2) at the same voltage if $V_{1/2}$ is –20 mV and k = –7 (in the range for many Kv1 channels).

Neurons with brief spikes that use Kv3 channels to repolarize the action potential seem to have employed the strategy of using high channel densities. The 'fast spiking' (FS) interneurons in the neocortex and hippocampus have much larger K^+ currents (about twofold larger) than pyramidal cells, the majority of which (>75% in the neocortex) have Kv3-like properties[c–e]. Similarly, principal neurons in the medial nucleus of the trapezoid body have large K^+ currents (typically more than 15 nA at +60 mV), of which the Kv3-like component accounts for >70% (Ref. f).

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important functional determinants, even when expressed at low levels. The distribution and developmental regulation of Kv3 gene transcripts and protein products in the rat and mouse CNS are reasonably well described[10,15,19,31,32,34–39,46,47]. Each Kv3 gene has a unique pattern of expression in the CNS (Fig. 3a), although transcripts of two or more Kv3 genes overlap in many neuronal populations, suggesting a large potential for heteromultimer formation.

It was the observation that Kv3.1 transcripts might be expressed in neurons that fire at high frequencies that first led to the suggestion of a role for Kv3 channels in high-frequency firing[34]. Localization studies of the products of the other Kv3 genes confirmed and extended the association between Kv3 transcript expression and the ability to fire at high frequencies[15]. The association is clearly illustrated in the neocortex and hippocampus, where both Kv3.1 and Kv3.2 are specifically expressed[18,31,36,38] in subsets of GABAergic interneurons that fire long trains of action potentials at high frequency and are known as FS neurons[2,32,50,51] (Fig. 3b,c). Kv3 proteins are also prominently expressed in central auditory nuclei, where neurons can follow stimuli of unusually high frequency[36,39,52].

FS interneurons and auditory principal neurons have largely been the focus of studies investigating the functional role of Kv3 channels and their impact in high-frequency firing. However, the coincidence of high-frequency firing and Kv3 channel expression is seen throughout the brain[10,15,18,19,31,32,34–36,38,39,46]. In fact, we are unaware of any neuron type in brain capable of sustained or repetitive high-frequency firing that does not express at least one of the Kv3.1–Kv3.4 genes. One possible exception is the neocortical pyramidal cell type with 'chattering' properties[53–55], because, in rat and mouse, Kv3 proteins have not been detected in cortical pyramidal neurons[31,36,38]. However, chattering cells have not been observed in rodents and it is not known whether Kv3 proteins are expressed in cortical pyramidal cells in species that possess chattering neurons.

Kv3 channels are necessary for high-frequency repetitive firing

How do the unique electrophysiological properties of Kv3 channels lend themselves to facilitating high frequency repetitive firing? As discussed, neurons can use large numbers of Kv3 channels to accelerate action potential repolarization with a minimum risk of compromising action potential generation (Box 1). By increasing the rate of spike repolarization and thus keeping action potentials short, Kv3 currents can reduce the amount of Na^+ channel inactivation occurring during the action potential. Moreover, the

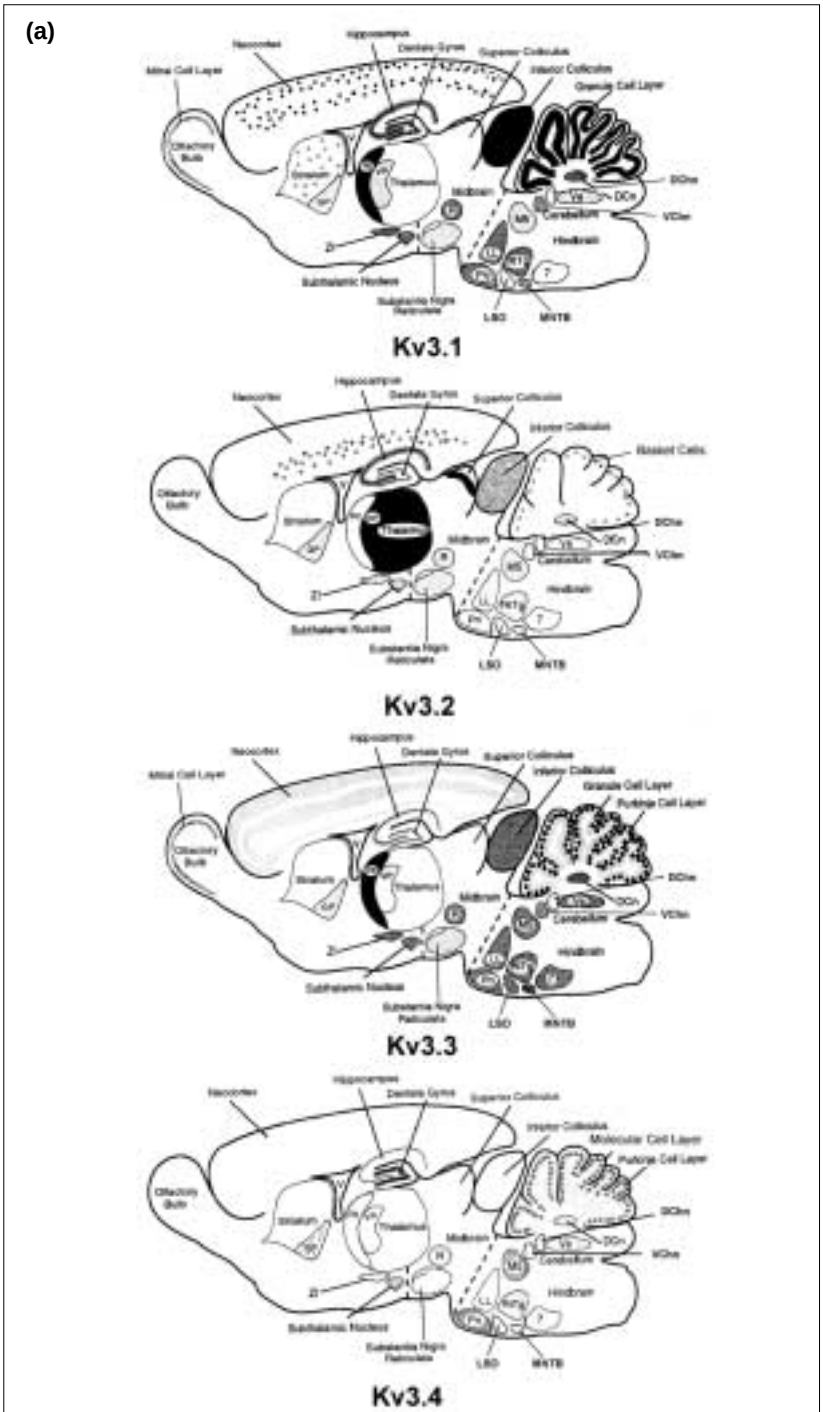

Fig. 3. (a) Distribution of Kv3 mRNAs in rodent brain. The levels of expression of Kv3 mRNA transcripts based on *in situ* hybridization studies[15] are represented in these diagrams by different grades of shading. The position of some of the structures, particularly in the brainstem, is only approximate, and structures are shown in these diagrams that might not exist in the same sagittal plane. Some important structures expressing one or more Kv3 genes are not illustrated, such as several areas of the basal forebrain. Kv3.1 and Kv3.3 mRNA transcripts overlap in many areas, particularly in the posterior part of the brain and in the spinal cord. Kv3.1 and Kv3.2 overlap in some neuronal populations, particularly in the anterior part of the brain, including the neocortex, hippocampus and globus pallidus. Kv3.1 and Kv3.3 are expressed in many neuronal populations, including most auditory central processing neurons (some of which also express Kv3.2) and many cranial nerve nuclei, whereas Kv3.2 transcripts show the most restricted pattern of expression, with >80% of Kv3.2 mRNAs being present in thalamocortical (TC) neurons throughout the dorsal thalamus[15,37,46]. Abbreviations: 7, facial nucleus; DChn, dorsal cochlear nucleus; DCn, deep cerebellar nuclei; GP, globus pallidus; LL, lateral lemniscus nuclei; LSO, superior olive; M5, trigeminal motor nucleus; MNTB, medial nucleus of the trapezoid body; Pn, pontine nuclei; R, red nucleus; Rt, reticular thalamic nucleus; RtTg, reticulo–tegmental nucleus of the pons; VChn, ventral cochlear nucleus; Ve, vestibular nucleus; VP, ventral-posterior complex of the dorsal thalamus; ZI, zona incerta. (b) Kv3 proteins are prominently expressed in somatic and axonal terminal membranes. Specific antibodies to Kv3 proteins have been raised to localize the protein products in CNS neurons[18,19,31,32,35–39,44]. Throughout the brain, Kv3 proteins are localized in somata, axons and presynaptic terminals of expressing neurons. They are also present in proximal dendrites but typically not throughout most of the dendritic arborization (however, see Ref. 26). However, the balance of somatic versus axonal terminal distribution of the protein varies in different neuronal populations and for different Kv3 proteins. For example, in TC neurons – the neuronal population that expresses the overwhelming majority of Kv3.2 transcripts in brain – the protein is predominantly axonal. In the neocortex and hippocampus, Kv3.1 and Kv3.2 proteins are expressed in specific subsets of GABAergic interneurons[18,31,32,36,38]. There is prominent expression in the soma, as well as in the axons and terminals of these cells. This figure shows a confocal immunofluorescence image of layer V somatosensory cortex labeled with antibodies to Kv3.2. There is a prominently labeled interneuron (top left) near an unstained pyramidal neuron surrounded by Kv3.2-positive puncta, presumably presynaptic boutons from nearby interneurons. (c) Specific expression of Kv3.1 and Kv3.2 proteins in fast-spiking neurons. Double-labeling immunofluorescence with markers of specific neurons has been used to identify the cells expressing Kv3.1 and Kv3.2 proteins in the neocortex and hippocampus. Virtually all the neurons containing the Ca^{2+}-binding protein parvalbumin (PV) expressed both Kv3.1b and Kv3.2 proteins[18,31,32,36,38], although in the neocortex PV-containing neurons in superficial layers expressed Kv3.2 weakly, while those in deep-layers expressed Kv3.2 strongly[31]. PV is expressed in about half of the GABAergic-interneurons in the cortex, and the presence of this marker has been closely associated with the expression of the 'fast spiking' (FS) firing pattern[2,48]. In addition, in the hippocampus and neocortex, Kv3.2 (but not Kv3.1) proteins are prominently expressed in a fraction of somatostatin-expressing GABAergic interneurons[31,32,49]. In the hippocampus (but not in the neocortex), some somatostatin-containing neurons are FS (Refs 2,48). Kv3.1 and Kv3.2 proteins are apparently not expressed in glutamatergic cortical neurons in rodents[18,31,32,36,38]. This figure shows the co-expression of Kv3.2 (red fluorescence) in PV- and somatostatin (SOM)-containing (green fluorescence) interneurons in the hippocampus. (a) modified, with permission, from Ref. 6. (b) modified, with permission, from Ref. 31. (c) Image provided by E. Phillips-Tansey. Scale bars, 7.5 μm in (b), and 25 μm (PV) and 15 μm (SOM) in (c).

complete repolarization, followed by a large and fast afterhyperpolarization (fAHP) produced by the Kv3 current, facilitates both recovery from Na^+ channel inactivation and Kv3 channel deactivation. This acts to restore resting conditions (including membrane impedance) rapidly and to prepare the membrane for a subsequent spike, thus minimizing the duration of

Box 2. Pharmacological properties of Kv3 channels in heterologous expression systems

The scarcity of currently available K^+ channel blockers with high specificity complicates the task of correlating native channels with identified molecular components. However, as the examples discussed in this article illustrate, the pharmacological profile of heterologously expressed Kv3 channels, together with other data, have been useful in identifying native channels. Kv3 currents in heterologous systems are highly sensitive to external tetraethylammonium (TEA) or 4-aminopyridine (4-AP) (IC_{50} values are ~200 μM and 0.02–0.5 mM, respectively). TEA concentrations that block Kv3 currents nearly completely (~1 mM) produce significant inhibition of only a few other known K^+ channels, including large-conductance Ca^{2+}-activated K^+ channels that contain proteins of the slo family (K_d = 80–330 μM), Kv1.1 channels (K_d ~0.3 mM) and KCNQ2 (90% blocked by 1 mM). These can be distinguished from Kv3 channels by other biophysical and pharmacological properties[a,b]. 4-AP is less specific; owing to this and other factors it has been argued that, in most cases, TEA might be a better blocker than 4-AP in discriminating between Kv3 channels and other K^+ channels[a]. Kv3 currents are not affected by scorpion or snake venom-derived toxins that block other voltage-sensitive channels[a,b]. However, Kv3.4 channels have been reported to be specifically blocked by peptides obtained from the venom of the sea anemone *Anemonia sulcata*, known as blood depressing substance I and II (BDS-I and BDS-II)[c].

Kv3.1 and Kv3.2 channels are negatively modulated by intracellular Mg^{2+} in a voltage-dependent manner, producing a negative slope conductance in the conductance–voltage curve[d,e]. Variations in the conductance–voltage relationship between different studies on mammalian cells might be associated with differences in intracellular Mg^{2+} concentrations: lower Mg^{2+} concentrations causing an apparent rightward shift in the voltage dependence.

Recently, Wigmore and Lacey recorded a highly TEA-sensitive Kv3-like current in rat subthalamic nucleus neurons (which are known to express Kv3.1 and Kv3.2 proteins) and found that it was highly permeable to Cs^+ (Ref. f). It has been confirmed that Kv3.2 channels expressed in CHO cells have comparable permeabilities to Cs^+ and K^+ (Y. Amarillo and B. Rudy, unpublished observations). This property could be an additional useful feature for distinguishing Kv3 currents. Other salient features of Kv3 channels include their single channel conductances, which tend to be somewhat larger than those of other voltage-gated K^+ channels (12–30 pS at physiological K^+ concentrations)[a,b].

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the refractory period and allowing high-frequency firing[5,6,23,38].

Such a role for Kv3 channels has been confirmed in experiments that investigated the effects of Kv3 channel-blockers (see Box 2) on the repetitive firing properties of cortical FS GABAergic interneurons and on high frequency firing neurons in the auditory brainstem, together with computer modeling. More recently, analysis of the firing properties of these neurons in Kv3 knockout mice has confirmed and extended these results.

Erisir *et al.* have observed that low tetraethylammonium (TEA) concentrations (≤1 mM) broaden the action potential, nearly abolish the fAHP and disrupt fast spiking of parvalbumin (PV)-containing interneurons in mouse neocortical slices (Fig. 4a,b)[23] (see also Ref. 40). At these concentrations, TEA blocks only a few know K^+ channels, including Kv3 channels (see Box 2). The effects of suppressing the TEA-sensitive current appear specific, as blockers of other K^+ channels increase the firing frequency[23]. Under voltage clamp, the TEA-sensitive current accounts for more than 75% of the total outward current and has properties very similar to those of heterologously expressed Kv3.1–Kv3.2 currents. In hippocampus, application of 0.2 mM 4-aminopyridine to FS basket cells also eliminates the AHP and converts the high-frequency firing phenotype into one with substantially slower firing rate[24]. Similar experiments in the medial nucleus of the trapezoid body (MNTB) suggest that channels containing Kv3.1 subunits (perhaps in channel complexes with Kv3.3 proteins) mediate the ability of these cells to follow high-frequency inputs[5] (Fig. 4c). More recently, similar effects on high frequency firing following blockade of a Kv3-like current have been described in the subthalamic nucleus[25].

Investigation of the firing properties of neocortical GABAergic interneurons[43] and MNTB principal cells[56] in mice deficient in Kv3 proteins suggests that the Kv3-like, TEA-sensitive current recorded from these cells is indeed mediated by Kv3 channels, and provides more direct support for the hypothesis that these neurons require Kv3 channels to fire at high frequencies. For example, Lau *et al.* have found that PV-containing GABAergic interneurons in deep cortical layers from Kv3.2 knockout mice have broader spikes and sustain significantly lower firing frequencies (117 Hz compared with 152 Hz at room temperature), with more firing frequency adaptation than wild type[43]. Such differences are not observed in PV-containing interneurons in superficial layers, where Kv3.2 proteins are only weakly expressed.

Analysis of the repetitive firing properties of FS-interneurons has revealed that although the steady state firing frequency of spike trains is substantially slower after Kv3 channel block, the firing frequency of the initial spikes in the train is unaffected. In fact, the effects of TEA accumulate during subsequent spikes and reach a maximum by about the tenth spike[23]. These results support a model in which Kv3 channels facilitate high-frequency firing in part by reducing the amount of Na^+ channel inactivation that accumulates during the spike train, as hypothesized earlier (see also Refs 23,38). Consistent with this idea, application of 1 mM TEA to cortical FS interneurons significantly reduces the maximum rate of rise of the second, but not the first, spike in a train, suggesting that in the presence of TEA there is significantly greater Na^+ channel inactivation at the onset of the second spike[23]. This interpretation is supported by computer simulations, which show that a decrease in both the amount and speed of recovery from Na^+ channel inactivation accompanies the slowdown in firing rate produced by blocking 95% of the Kv3-like channels[23]. In addition, experiments with knockout mice suggest that the fast repolarization produced by Kv3 channels also prevents the activation of slower K^+ conductances, which, after Kv3 channel

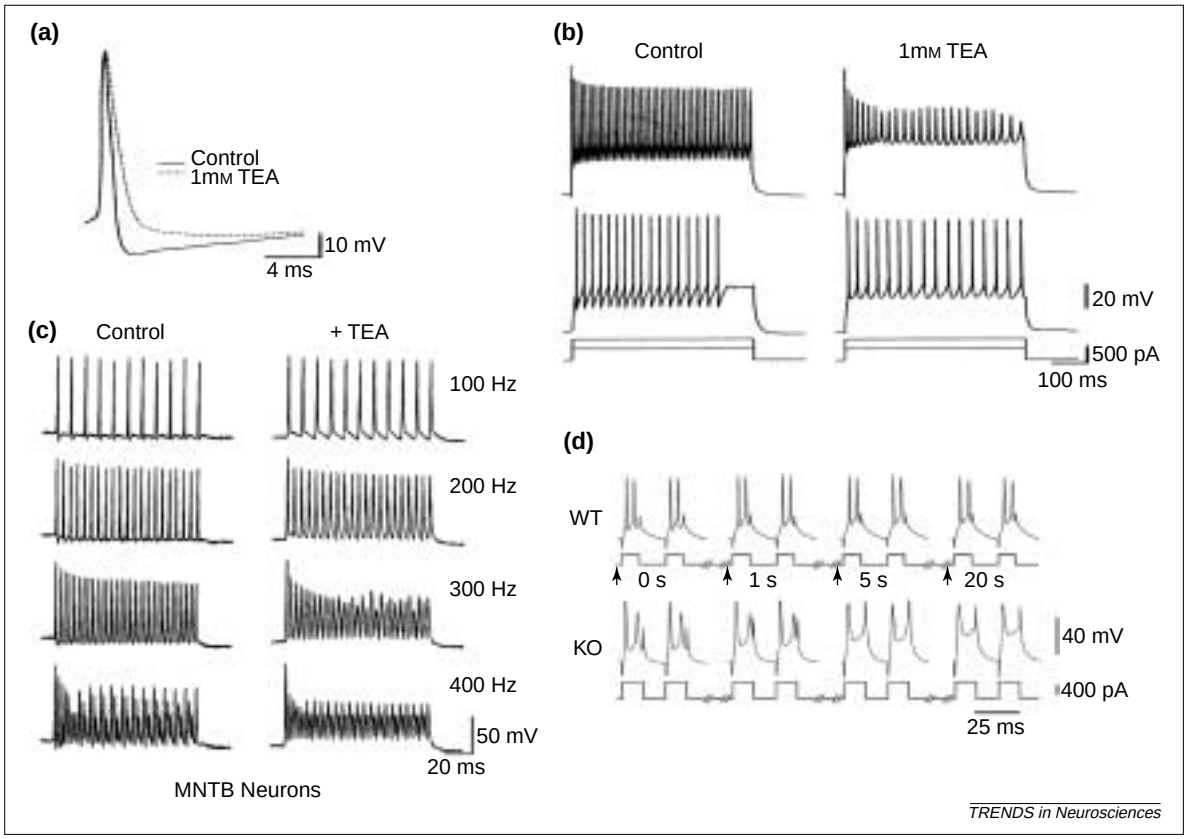

Fig. 4. (a),(b) Low concentrations of tetraethyl ammonium (TEA) impaired spike repolarization and high frequency firing of 'fast spiking' (FS) neocortical interneurons. (a) Cortical FS interneurons are characterized by having brief action potentials (0.60–0.70 ms at half amplitude at room temperature and ~0.3 ms near 37°C) with large fast afterhyperpolarizations (fAHPs). TEA (1 mM) caused spike broadening by reducing the maximum rate of spike repolarization and nearly abolished the fAHP. (b) In response to sustained current injection, FS neurons fire high-frequency spike trains, which appear abruptly as the injected current is increased and show little firing frequency adaptation. Steady firing frequency increases with depolarization and reaches values of 150–200 Hz at room temperature and ~400 Hz near body temperature. The left-hand panels (control) show repetitive firing of the same FS neuron as in (a) in response to 350 pA (bottom) and 650 pA (top) currents under control conditions. The right-hand panels show the responses to identical currents in the presence of 1 mM TEA. TEA reduced the steady firing rate, produced an increase in firing frequency adaptation and a large reduction in spike amplitude during the larger current pulse. These effects of TEA were manifest over the whole range of injected currents and reduced the maximum steady firing rate that could be achieved by direct current injection into FS neurons[23]. (c) TEA impaired the ability of neurons in the medial nucleus of the trapezoid body (MNTB) to respond to high-frequency stimulation. MNTB neurons do not respond with high frequency trains of action potentials when depolarized by steady current injection, because of the presence of a low voltage-activated K^+ current that shunts the membrane following one or a few spikes[4,5]. However, these cells are capable of following high-frequency inputs (up to ~600 Hz). Recordings from an MNTB neuron are shown in response to short current pulses delivered at the indicated frequencies. Application of low concentrations of TEA (1 mM) did not alter the ability of the cells to follow brief stimuli repeated up to 200 Hz (although the action potentials were broader), but reduced their ability to respond faithfully to stimuli of higher frequency. (d) Deep-layer FS neurons from wild-type, but not from Kv3.2-deficient mice can repetitively fire action potential doublets for long periods. FS neurons from wild-type (WT) and Kv3.2$^{-/-}$ (KO) mice were stimulated repetitively at 40 Hz with brief depolarizing current pulses that generated a spike doublet (for a total duration of 1 min). The pulses and voltage responses are shown for the first two stimuli and the first two after 1, 5 and 20 s of continuous stimulation. The wild-type neuron always responded with a spike doublet. By contrast, neurons from knockout mice failed to generate a spike doublet rapidly after the onset of the stimulation. In the knockout, the second spike is already smaller than the first spike during the first current pulse, and it becomes smaller, slower and more delayed with subsequent depolarizations, until the cell fails to generate a second spike. (a),(b) reproduced, with permission from Ref. 6. (c) reproduced, with permission from Ref. 5. (d) reproduced, with permission, from Ref. 43.

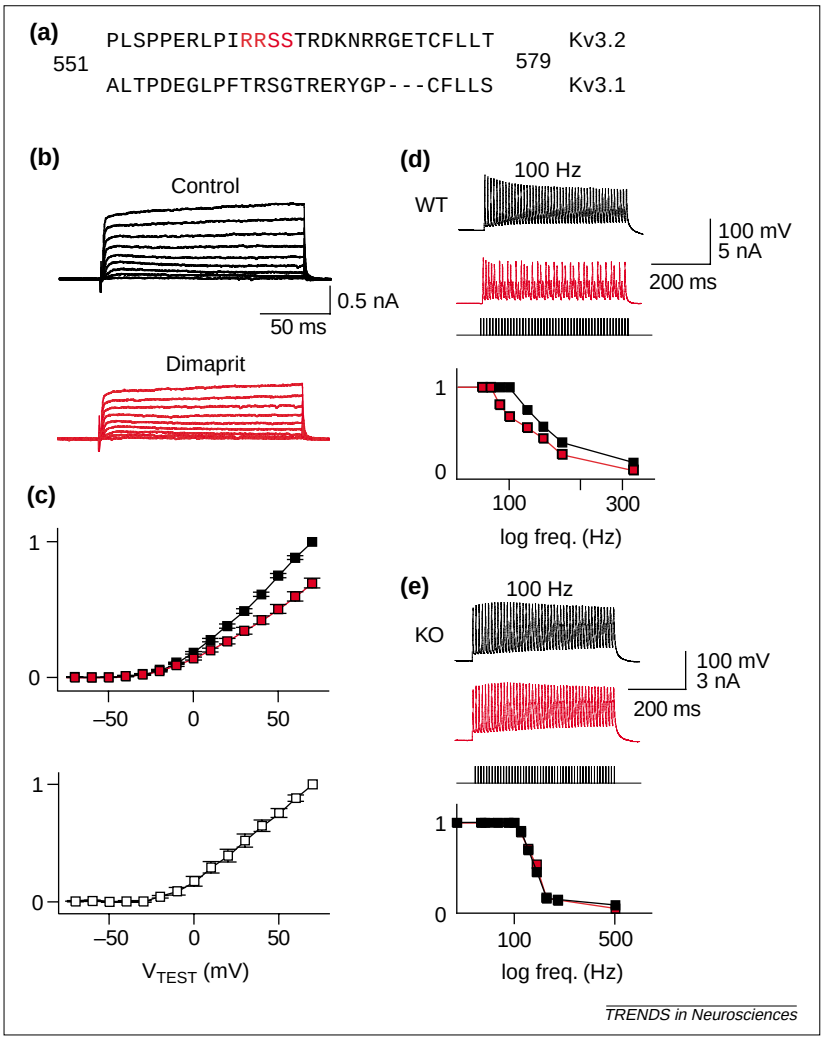

Fig. 5. Protein kinase A (PKA)-mediated modulation of Kv3.2 K^+ currents by H_2 receptor activation in hippocampal interneurons. (a) The amino acid sequence of residues 551–579 in the C-terminal domain of Kv3.2 proteins containing the putative PKA phosphorylation site (RRSS, indicated in red) is compared with the equivalent region of Kv3.1b. Although the two sequences are very similar, Kv3.1b proteins lack the PKA phosphorylation site. (b) Whole-cell sustained outward K^+ currents in hilar interneurons (activated by a 300 ms test pulse between –50 mV and +70 mV, 10 mV increments, V_{hold} = –70 mV) were reduced by the selective H_2 receptor agonist dimaprit (10 μM), which phosphorylates channels containing the Kv3.2 subunit via PKA. (c) Averaged normalized current–voltage (I–V) relationships of outward currents measured in control or in the presence of dimaprit (n = 6). The lower panel illustrates the averaged difference current carried by Kv3.2-containing channels, isolated by the digital subtraction of the current in dimaprit from control. (d) In wild-type (WT) interneurons, dimaprit (10 μM) reduces the maximal firing frequency. A representative interneuron showing that under control conditions a train of stimuli delivered at 100 Hz can be followed for the entire 500 ms duration (top trace). After application of dimaprit, action potential firing was observed on ~50% of the suprathreshold stimuli (bottom trace). A plot of the data obtained at all stimulus frequencies demonstrates that dimaprit reduced the cut frequency, f_c (f_c = 77 Hz with dimaprit versus 100 Hz in controls). (e) By contrast, in Kv3.2 knockout animals, H_2 receptor agonists are without effect on the maximum sustainable firing frequency. Modified, with permission, from Ref. 49.

suppression, decay slowly during the interspike interval, shunt depolarizing currents and contribute to delaying the onset of the subsequent spike[43].

These ideas might explain why Kv3 channels are not expressed in several neuronal populations that fire brief high-frequency spike bursts, as in bursting cortical pyramidal cells, principal neurons in the striatum and dopaminergic neurons in the substantia nigra[51,57–59]. Kv3 channels might become necessary for repetitive bursting (as might in fact occur under certain conditions in FS neurons *in vivo*), such that the unique contributions of these channels in preventing the accumulation of Na^+ channel inactivation and shunting currents become necessary. Consistent with this idea, Lau *et al.* have observed that the specific ability of FS cortical interneurons to follow stimuli that generated high frequency spike doublets repeated at 40 Hz was impaired in Kv3.2 knockout mice, as well as in neurons from wild-type animals after application of low TEA concentrations[43] (Fig. 4d).

Modulation of Kv3 K^+ channels

In numerous systems, modulation of voltage-gated channels by kinases and phosphatases provides additional mechanisms to dynamically regulate membrane properties[60–63]. Channels formed by Kv3 subfamily members are targets for such modulation[11,37,49,64,65,69]. Given the dominant functional role of Kv3 channels in some neurons, their phosphorylated state could have profound influences on the physiological properties of the cell and the network to which it is connected (Fig. 5).

Kv3.2 subunits possess a phosphorylation site that can be phosphorylated *in situ* by intrinsic protein kinase A (PKA)[6,37] (Fig. 5). Phosphorylation of heterologously expressed Kv3.2 channels by PKA inhibits currents by a mechanism that possibly involves alteration of the open probability, but not voltage dependence[37]. By contrast, Kv3.1b homotetramers, which lack a consensus site for PKA phosphorylation, are not affected by PKA (Refs 6,37). This distinguishes homomeric Kv3.1 and Kv3.2 channels, which otherwise possess similar (but not identical) biophysical properties.

Histaminergic fibers that originate in the tuberomammillary nucleus of the hypothalamus make a widespread projection throughout the CNS, including the hippocampus[66]. Within the hippocampal formation, histamine H_2 receptors are expressed on cells of the hilar formation, a termination zone for histaminergic fibers[67]. Native channels containing Kv3.2 subunits in FS somatostatin- and parvalbumin-containing interneurons of the dentate gyrus (Fig. 3c) are targets for PKA-mediated phosphorylation by histamine via H_2 receptor activation, with H_2 receptor activation resulting in a reduction in the sustained outward current (Fig. 5)[49]. This broadens the action potential and reduces the maximal firing frequency of these neurons, all via a mechanism involving PKA-mediated phosphorylation of Kv3.2 (Fig. 5c)[49]. PKA-mediated phosphorylation of Kv3.2 impacts not only interneuron firing patterns but negatively modulates high frequency population oscillations recorded in principal cell layers, confirming a role for these interneurons in pacing intrinsic oscillations[49,68]. In animals in which the gene encoding Kv3.2 is disrupted, H_2 receptor modulation of outward currents and high frequency firing is absent (Fig. 5e). The observation that FS is under control of

PKA-mediated phosphorylation of Kv3.2 suggests that any neurotransmitter or neuromodulator that activates adenylyl cyclase cascades could potentially impact interneuron function.

Despite the absence of a site for PKA phosphorylation, Kv3.1b proteins contain about 11 putative protein kinase C (PKC) phosphorylation sites and 10 putative sites for casein kinase 2 (CK2)[7,69]. In both CHO cells and MNTB neurons, dephosphorylation of Kv3.1b using alkaline phosphatase treatment produces an increase in whole cell current accompanied by a 20 mV negative shift in both the voltage dependence of activation and slow inactivation[69]. Of particular interest, application of inhibitors of CK2 (but not protein kinase C) mimics the action of alkaline phosphatase, suggesting basal phosphorylation of Kv3.1b channels in MNTB neurons (and in CHO cells) by constitutively active CK2. Dephosphorylation of Kv3.1, which results from the application of inhibitors of CK2, renders MNTB neurons unable to follow high-frequency stimuli[69]. These data suggest that the phosphorylated state of Kv3.1b (like Kv3.2) can play a major role in determining the high-frequency firing properties of specific neuron populations.

Other modulators have been shown to affect Kv3 channels expressed in heterologous systems. These include PKC activators, which inhibit Kv3.1b currents[11,64], suppress inactivation of Kv3.4 and Kv3.3 channels[12–14], and produce a several fold enhancement of Kv3.3 currents[13,14]. Lastly, intracellular increases in cGMP concentrations, produced by the addition of cGMP analogues or by the activation of guanylyl cyclase by NO, inhibit Kv3.1 and Kv3.2 channels in a voltage-dependent manner. These effects are blocked by cGMP-dependent protein kinase inhibitors, but appear not to result from channel phosphorylation but from dephosphorylation at sites important for voltage-dependent activation to proceed[65]. Whether these modulations are important in neurons remains to be investigated.

Concluding remarks

Together, the experimental data and computer modeling support the idea that the activation range and fast deactivation kinetics of Kv3 channels function specifically to enable neurons to fire repetitively at high frequencies[5,6,23,24,40,43,49,69]. Kv3 channels might have been a common solution in vertebrates to the problems imposed by high-frequency repetitive firing, as they appear to be present in most neurons that can fire repetitively at high frequencies. It remains to be seen whether there are exceptions to this (such as 'chattering' neurons), a finding that would indicate that alternative solutions to achieve high-frequency repetitive firing are used by some neurons.

Kv3 gene products have also been reported in neurons that are not known to fire at high frequencies, suggesting that Kv3 channels might have other physiological roles. Moreover, as described earlier, Kv3 channels are prominently expressed in axons and presynaptic terminals (Fig. 3). The presynaptic function of Kv3 channels remains to be investigated. However, we expect these additional functions of Kv3 channels to be related to their ability to maximize fast action potential repolarization specifically and the consequences of this function: brief action potentials with quick restoration of membrane properties after activity. Consequently, Kv3 channels might be important in regulating the levels of accumulation of intracellular Ca^{2+}, in increasing the fidelity of signal transmission in synapses in which temporal precision is important and in facilitating the faithful transmission of action potentials in particularly active axons by minimizing the accumulation of factors (Na^+ channel inactivation, intracellular Ca^{2+}, extracellular ions, etc.) that can compromise the safety factor for action potential transmission[70–72].

The neuronal Kv3-like channels studied so far probably consist of Kv3.1, Kv3.2 and Kv3.3 proteins. The functional consequences of the fast inactivation produced by Kv3.4 subunits still remains to be studied. In this context, the recent finding that Kv3.4 subunits associate with MiRP2 proteins in skeletal muscle to form subthreshold-operating channels that contribute to setting the resting potential of muscle cells[73] is interesting. Thus, association with accessory subunits, as in this intriguing example, could increase the repertoire of functions of Kv3 channels in neurons.

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