Contents

20.1 Introduction 276
20.2 Gene identification 276
20.3 Biophysical, pharmacological, and structural features 277
   20.3.1 Overview 277
   20.3.2 Determinants of KCNQ channel assembly 277
   20.3.3 Pharmacology 278
   20.3.4 Receptors and signal transduction 279
   20.3.5 Regulation of KCNQ channels byPIP2 279
   20.3.6 Lipid specificity and location of the PIP2-binding site 281
   20.3.7 Receptor specificity 281

20.4 Auxiliary subunits and regulatory proteins of KCNQ channels 282
   20.4.1 Calmodulin in KCNQ channel function 282
   20.4.2 A-kinase anchoring protein (AKAP) 79/150 and PKC action 283
   20.4.3 Regulation of KCNQ channels by KCNE subunits 283
      20.4.3.1 KCNQ1/KCNE1 283
      20.4.3.2 Other KCNE subunits 285
      20.4.3.3 Effects of KCNEs on KCNQ channel trafficking 286
   20.4.4 Other molecular partners of KCNQ channels 286

20.5 M-channel expression and function in mammalian tissues 286
   20.5.1 Function of KCNQ channels in the peripheral nervous system 286
      20.5.1.1 Sympathetic system 286
      20.5.1.2 Peripheral somatosensory system 286
   20.5.2 Functions of KCNQ channels in the central nervous system 287
      20.5.2.1 KCNQ2/3 287
      20.5.2.2 KCNQ4 289
      20.5.2.3 KCNQ5 289
      20.5.2.4 KCNQ1 289
   20.5.3 Heart 289
   20.5.4 Other tissues 289
      20.5.4.1 Epithelia 289
      20.5.4.2 Smooth and skeletal muscles 290
      20.5.4.3 Other tissues/structures 290

20.6 M-channelopathies and therapeutic potential of KCNQ channels 290
   20.6.1 Arrhythmias 290
      20.6.1.1 Long QT syndrome 290
      20.6.1.2 Short QT syndrome 291
   20.6.2 Deafness 291
   20.6.3 Epilepsy/seizures 291
   20.6.4 Pain 292
   20.6.5 M-channels as drug targets 292
20.1 INTRODUCTION

As for most of the ion channels discussed in this volume, the currents carried by KCNQ K+-channels were characterized by pharmacological, kinetic, or functional features long before their gene identification, and so have established names reflecting those features. In the central nervous system (CNS) and the peripheral nervous system (PNS) neurons, this corresponds to the M-current, first described by David Brown and colleagues in sympathetic ganglia as a voltage-gated, noninactivating K+ current depressed by the stimulation of muscarinic acetylcholine receptors (mACHRs) (1,2). These investigators were searching for the molecular basis of the slow postsynaptic potential (EPSP), the prolonged depolarization occurring with a delay after a synaptic EPSP seen after trains of action potentials (3). The slow EPSP proved to be due to the closure of the M-type K+ current via mAChR stimulation and actions of Gq/11 G proteins, an effect also mediated by a variety of peptide neurotransmitters, such as Gonadotropin-releasing hormone (GnRH) (Luteinizing-hormone-releasing hormone (LHRH) in frog), substance P, angiotensin II, and others (4). The inhibition of M-current (IM) generally increases excitability as the standing K+ conductance at resting potentials is reduced (Figure 20.1). IM is well poised to serve this role due to its lack of inactivation, threshold for activation near neuronal resting potentials, and slow kinetics (5). In the heart, a similar K+ current with even slower kinetics, dubbed IKr, underlies much of the initial repolarization after the cardiac action potential and is sensitive to protein kinase A (PKA), making it partly responsible for the speeding of the heart rate upon adrenergic stimulation (6). Neither IM nor IKr is particularly sensitive to the well-known blocker of most delayed rectifiers, tetraethylammonium (TEA) ions or various scorpion toxins. Much study has been spent answering two fundamental questions for these K+ currents: the molecular correlates underlying them and the signal transduction mechanisms linking muscarinic or β-adrenergic stimulation to modulation of M-current, or IKr, respectively. As we see in the following, the major clues for both questions came from inherited diseases in people linked to specific gene loci.

20.2 GENE IDENTIFICATION

In 1996, an inherited form of human cardiac arrhythmia called long QT syndrome (LQTS) was localized to a novel K+ channel gene, dubbed KvLQT1 (7–9). Similar to the tetrameric arrangement of four subunits of the prototypical Shaker K+ channel, the KvLQT1 gene predicted each subunit to have six membrane-spanning domains (S1–S6), with the voltage sensor residing in S4, and a long carboxyl terminus. Careful study revealed native IKr to be composed of KvLQT1, as the pore-forming subunit, in conjunction with an auxiliary β-subunit, called minK/I,K (this β-subunit was initially thought capable of itself being a channel; see the following text) (10–12). Concurrently, analysis of the genome of the worm, C. elegans, identified novel genes orthologous to KvLQT1, KQT1-2 (13). From this analysis, two other human KQT/KvLQT1-like channels were identified as the gene loci in which mutations underlie inherited epileptic syndromes in human newborns (14,15), mapped to chromosomes 20q1.3 and 8q24 (16,17). KvLQT1 was then renamed KCNQ1, and the two seizure-associated genes, KCNQ2 and KCNQ3. From expression studies in oocytes, KCNQ2/3 heteromers were shown to display all the characteristics of native M-current (18), and expression in mammalian cell lines confirmed suppression of KCNQ2/3 current by M_i mAChRs (19,20).

Figure 20.1 Hallmark features of neuronal M-current. (a) Whole-cell voltage clamp recording from a rat superior cervical ganglion (SCG) neuron, using the pulse protocol shown above. IM is seen as the standing outward current at V_rest = −30 mV that slowly deactivated during the voltage step to −60 mV, and then slowly reactivates when the neuron is stepped back to −30 mV. Application of the muscarinic agonist, oxotremorine methiodide (oxo-M), causes a strong suppression of IM within ~30 s. Im is typically quantified in neurons as the amplitude of the relaxation at −60 mV. (b) A similar rat SCG neuron is studied under whole-cell current clamp, and depolarizing or hyperpolarizing currents applied, as indicated. Injection of the 50 pA current elicits only two action potentials (APs), although the current is maintained, typical of these phasic neurons. When the M-channel opener, retigabine (RTG) is applied, there is a hyperpolarization of V_rest by ~15 mV, an absence of any APs elicited by the 50 pA current, and a profound decrease in input resistance, as manifested by the greatly reduced hyperpolarization induced by the −50 or −100 pA current injections. All of these effects can be explained by the left-shift in the voltage dependence of M-channel activation, and increased open probability at all voltages, induced by retigabine (RTG). When the M-channel blocker, linopirdine (Lino), is applied, the phasic feature is abolished, as now the 50 pA injection causes an uninterrupted train of APs during the current pulse, indicating that the pronounced spike-frequency adaptation of these neurons is heavily M-current dependent. Similar phenomena due to M-currents are observed throughout the PNS and CNS (Unpublished data from the authors).
KCNQ2 is expressed as six to nine alternatively spliced forms in rodent (21) and human (22) brain, with the alternative splicing involving exons 7–15. With one or two exceptions, the isoforms seem functionally similar (23), although systematic investigation of this issue has not been performed.

Another subtype associated with inherited syndromes of deafness, KCNQ4, localizes to the inner ear (24) and to auditory nuclei in the brain (25–28). Finally, KCNQ5 contributes to neuronal M-channel composition in the brain, and sympathetic and sensory ganglia (29–31) and KCNQ5-associated epileptic syndromes have been suggested (32,33). KCNQ1, KCNQ4, and KCNQ5 have also been identified in various smooth muscle types, where they help control excitability and contractility (34–37). KCNQ1-containing channels are also expressed in various epithelia, where their function is almost certainly in K+ transport (38).

20.3 BIOPHYSICAL, PHARMACOLOGICAL, AND STRUCTURAL FEATURES

20.3.1 OVERVIEW

As mentioned earlier, native M-current possesses ideal features for control over neuronal excitability with a threshold for activation around ~60 mV (in some neurons, as negative as ~80 mV (39)), no inactivation and kinetics of activation and deactivation of ~100–200 ms at 0 and ~60 mV, respectively (Table 20.1). These characteristics endow M-channels with the ability to shape the somatic response to synaptic inputs and to be critical to spike-frequency adaptation and input/output relations. During bursts of action potentials, gradual M-channel activation increases the threshold for each subsequent action potential during the burst and, thus, underlies accommodation. As detailed in Table 20.1, the various KCNQ channels differ somewhat in their activation ranges, although all are well described by Boltzmann relations typical of voltage-gated channels (38). Structurally, their architecture is conserved with the other Kv families, possessing six transmembrane domains (S1–S6), a voltage sensor within S4, and an extended carboxy-terminus that is the locus of multiple modulatory signals. The single-channel conductance (γ) varies but is generally small (Table 20.1). The single-channel conductance of KCNQ1 homomers is too low to detect electrophysiologically (1–4 pS), but assembly with KCNE1 increases it considerably (40–42). Whereas KCNQ2-5 channels do not appreciably inactivate, KCNQ1 homomers do so modestly. Recovery from inactivation is faster than deactivation, resulting in a characteristic hook in the tail currents. Coexpression with KCNE1 removes inactivation (43), and boosts current amplitudes (44). The γ of other KCNQ channels is also generally low, with KCNQ3 having the largest (45,46) and KCNQ4 and KCNQ5 the smallest (summarized in Table 20.1; (45–48)). At saturating voltages, the open probability (P_o) of KCNQ2-5 channels is strikingly divergent (46,48,49), related to their differential affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2). The open probability of KCNQ3 is the highest, near unity; the maximal P_o of KCNQ2 is ~0.2; KCNQ4 has the lowest (<0.1), the open probability of KCNQ2/3 heteromers is intermediate between KCNQ2 and KCNQ3 (~0.3), and that of KCNQ1-containing channels remains indeterminate. M-channels display complex gating kinetics, including at least two open states and multiple closed states, and an unusually large number of transitions between states (47,48,50–52). Despite intense work, mostly by the Brown lab, their precise kinetic characteristics and number of state transitions, even for KCNQ2/3, remain unclear.

20.3.2 DETERMINANTS OF KCNQ CHANNEL ASSEMBLY

The extended carboxy-terminus of all KCNQ channels are thought to contain four helices, A–D, of which A and B, in the proximal part, are involved in channel regulation, whereas C and D, in the distal part, are involved in subunit-specific assembly and tetramerization (Figure 20.2; reviewed in (53)). As opposed to Kv1-Kv4 channels, in which homomeric vs. heteromeric assembly is controlled by the N-terminal T1 domain (54), this feature of KCNQ channels resides within the C and D helices. The Schwake and Lerche labs, using the *Xenopus* oocyte expression system, first defined a subunit-interaction domain (sid), encompassing the C and D helices (55–57), a finding later confirmed in hippocampal neurons (58). These domains dictate that KCNQ3 will coassemble with KCNQ2 (18, 20), KCNQ4 (59), and KCNQ5 (60), whereas KCNQ1 does not form heteromers with any of the neuronal KCNQ2-5 subunits (55,57,61). KCNQ2 is also discriminate, not forming heteromers with KCNQ4 (28) or KCNQ5 (29). Both C and D helices are thought to be coiled–coiled in nature, making the C-termini of KCNQ channels hard to purify biochemically. Nevertheless, the D helix alone has been purified (the B helix has been recently purified with calmodulin [CaM]; see the following text) and suggested to make critical intersubunit interactions that may control functional assembly (62).

The family of KCNQ channels are notable in their highly divergent current amplitudes when heterologously expressed (for a review, see (53)). Thus, KCNQ2/3 heteromers display >10-fold greater currents than KCNQ2 or KCNQ3 expressed alone, whereas KCNQ1, KCNQ4, and KCNQ5 express large homomeric currents. KCNQ4 homomers express so robustly that they are hard to isolate individually in patches (46,63). The situation for KCNQ3 homomers is especially noteworthy, since one is hard-pressed to record significant KCNQ3 homomers in oocytes or mammalian expression systems and most cell-attached patches are empty. Although there is evidence that the sid region plays a role in this (56,62), it is stunning that the replacement of a single alanine residue at the 315 position near the intracellular mouth of the pore (uniquely possessed by KCNQ3) with a single serine residue at 315 has not been performed.

As mentioned earlier, native M-current possesses ideal features for control over neuronal excitability with a threshold for activation around ~60 mV (in some neurons, as negative as ~80 mV (39)), no inactivation and kinetics of activation and deactivation of ~100–200 ms at 0 and ~60 mV, respectively (Table 20.1). These characteristics endow M-channels with the ability to shape the somatic response to synaptic inputs and to be critical to spike-frequency adaptation and input/output relations. During bursts of action potentials, gradual M-channel activation increases the threshold for each subsequent action potential during the burst and, thus, underlies accommodation. As detailed in Table 20.1, the various KCNQ channels differ somewhat in their activation ranges, although all are well described by Boltzmann relations typical of voltage-gated channels (38). Structurally, their architecture is conserved with the other Kv families, possessing six transmembrane domains (S1–S6), a voltage sensor within S4, and an extended carboxy-terminus that is the locus of multiple modulatory signals. The single-channel conductance (γ) varies but is generally small (Table 20.1). The single-channel conductance of KCNQ1 homomers is too low to detect electrophysiologically (1–4 pS), but assembly with KCNE1 increases it considerably (40–42). Whereas KCNQ2-5 channels do not appreciably inactivate, KCNQ1 homomers do so modestly. Recovery from inactivation is faster than deactivation, resulting in a characteristic hook in the tail currents. Coexpression with KCNE1 removes inactivation (43), and boosts current amplitudes (44). The γ of other KCNQ channels is also generally low, with KCNQ3 having the largest (45,46) and KCNQ4 and KCNQ5 the smallest (summarized in Table 20.1; (45–48)). At saturating voltages, the open probability (P_o) of KCNQ2-5 channels is strikingly divergent (46,48,49), related to their differential affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2). The open probability of KCNQ3 is the highest, near unity; the maximal P_o of KCNQ2 is ~0.2; KCNQ4 has the lowest (<0.1), the open probability of KCNQ2/3 heteromers is intermediate between KCNQ2 and KCNQ3 (~0.3), and that of KCNQ1-containing channels remains indeterminate. M-channels display complex gating kinetics, including at least two open states and multiple closed states, and an unusually large number of transitions between states (47,48,50–52). Despite intense work, mostly by the Brown lab, their precise kinetic characteristics and number of state transitions, even for KCNQ2/3, remain unclear.

20.3.2 DETERMINANTS OF KCNQ CHANNEL ASSEMBLY

The extended carboxy-terminus of all KCNQ channels are thought to contain four helices, A–D, of which A and B, in the proximal part, are involved in channel regulation, whereas C and D, in the distal part, are involved in subunit-specific assembly and tetramerization (Figure 20.2; reviewed in (53)). As opposed to Kv1-Kv4 channels, in which homomeric vs. heteromeric assembly is controlled by the N-terminal T1 domain (54), this feature of KCNQ channels resides within the C and D helices. The Schwake and Lerche labs, using the *Xenopus* oocyte expression system, first defined a subunit-interaction domain (sid), encompassing the C and D helices (55–57), a finding later confirmed in hippocampal neurons (58). These domains dictate that KCNQ3 will coassemble with KCNQ2 (18, 20), KCNQ4 (59), and KCNQ5 (60), whereas KCNQ1 does not form heteromers with any of the neuronal KCNQ2-5 subunits (55,57,61). KCNQ2 is also discriminate, not forming heteromers with KCNQ4 (28) or KCNQ5 (29). Both C and D helices are thought to be coiled–coiled in nature, making the C-termini of KCNQ channels hard to purify biochemically. Nevertheless, the D helix alone has been purified (the B helix has been recently purified with calmodulin [CaM]; see the following text) and suggested to make critical intersubunit interactions that may control functional assembly (62).

The family of KCNQ channels are notable in their highly divergent current amplitudes when heterologously expressed (for a review, see (53)). Thus, KCNQ2/3 heteromers display >10-fold greater currents than KCNQ2 or KCNQ3 expressed alone, whereas KCNQ1, KCNQ4, and KCNQ5 express large homomeric currents. KCNQ4 homomers express so robustly that they are hard to isolate individually in patches (46,63). The situation for KCNQ3 homomers is especially noteworthy, since one is hard-pressed to record significant KCNQ3 homomers in oocytes or mammalian expression systems and most cell-attached patches are empty. Although there is evidence that the sid region plays a role in this (56,62), it is stunning that the replacement of a single alanine residue at the 315 position near the intracellular mouth of the pore (uniquely possessed by KCNQ3) with a single serine residue at 315 has not been performed.
in these effects (64). Added to the mix is a postulated requirement of CaM for channel expression and functional density. Study continues on this topic.

### 20.3.3 PHARMACOLOGY

KCNQ channels have a unique, yet subtle, pharmacological profile (see Table 20.1), both presenting opportunities and challenges to study them experimentally, and to generate clinically relevant KCNQ-targeting drugs. With regard to TEA⁺, the tyrosine at turret-position 284 in KCNQ2, analogous to position 449 in Shaker (67,68), uniquely confers high (<1 mM) sensitivity, whereas KCNQ3 and KCNQ1 are largely TEA⁺ insensitive, and that of KCNQ2/3, as expected, displays moderate sensitivity (20,69,70). Given the importance of M-current to neuronal firing, a large effort has been made to develop KCNQ channel-specific drugs. Linopirdine, and its potent analogue, XE991, blocks all M-type channels at low µM concentrations (71–73), and XE991 has emerged as the gold standard for KCNQ channel identification. However, at the higher concentrations typically used by investigators,

### Table 20.1 Properties of KCNQ1-5 channels

<table>
<thead>
<tr>
<th>KCNQ SUBTYPE</th>
<th>V₁/₂ (mV)</th>
<th>τ&lt;sub&gt;ACT&lt;/sub&gt; (0 mV, ms)</th>
<th>τ&lt;sub&gt;DEACT&lt;/sub&gt; (−60 mV, ms)</th>
<th>γ (pS)</th>
<th>DRUG SENSITIVITY</th>
<th>β-SUBUNITS</th>
<th>TISSUE LOCALIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>−15</td>
<td>150–200</td>
<td>200–450</td>
<td>&lt;2</td>
<td>Blocked by lino, XE, chrom; RTG, NEM-insensitive</td>
<td>KCNE1-5</td>
<td>Heart, ear, epithelia, smooth muscle</td>
</tr>
<tr>
<td>KCNQ1/ KCNE1</td>
<td>2–8</td>
<td>&gt;500</td>
<td>&gt;200</td>
<td>3–6, varied</td>
<td>Blocked by lino, XE, chrom; RTG, NEM-insensitive</td>
<td>None</td>
<td>Heart, epithelia</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>−15 to −30</td>
<td>~200</td>
<td>150–280</td>
<td>−6</td>
<td>Blocked by lino, XE; high TEA sensitivity; opened by RTG, FLU, NH29, ZnPy, NEM, H₂O₂</td>
<td>None</td>
<td>Brain, autonomic and sensory ganglia</td>
</tr>
<tr>
<td>KCNQ3</td>
<td>−35 to −50</td>
<td>100–150</td>
<td>100–200</td>
<td>−9</td>
<td>Blocked by lino, XE, TEA insensitive; opened by RTG, FLU, ZnPy, H₂O₂</td>
<td>None</td>
<td>Brain, autonomic and sensory ganglia, gut</td>
</tr>
<tr>
<td>KCNQ2/3</td>
<td>−20 to −27</td>
<td>130–170</td>
<td>75–100</td>
<td>9.0</td>
<td>Blocked by lino, XE, modest TEA sensitivity; opened by RTG, FLU, NH29, PPOs, NSAIDs, NEM, H₂O₂</td>
<td>None</td>
<td>Brain, autonomic and sensory ganglia</td>
</tr>
<tr>
<td>KCNQ4</td>
<td>−20 to −30</td>
<td>125–200</td>
<td>~100</td>
<td>~2</td>
<td>Blocked by lino, XE, slightly TEA sensitive, opened by RTG, FLU, NEM, BMS, ZnPy, NSAIDs, NEM, H₂O₂</td>
<td>KCNE1-4?</td>
<td>Cochlea, auditory nuclei, mechano-sensitive neurons, smooth muscle</td>
</tr>
<tr>
<td>KCNQ5</td>
<td>−25 to −44</td>
<td>~150</td>
<td>~125</td>
<td>~2</td>
<td>Blocked by lino, XE, slightly TEA sensitive, opened by RTG, FLU, NSAIDs, NEM, BMS, ZnPy, H₂O₂</td>
<td>ND</td>
<td>Brain, autonomic and sensory ganglia, smooth muscle</td>
</tr>
<tr>
<td>KCNQ4/5</td>
<td>−38</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Blocked by lino, XE, diclofenac, slightly TEA sensitive, opened by RTG, FLU, ZnPy, NSAIDs</td>
<td>ND</td>
<td>Smooth muscle</td>
</tr>
</tbody>
</table>

**Note:** Table 20.1 Shown are various properties of the KCNQ channels described in the text. For references, see the citations in the text in the corresponding sections.

**Abbreviations:** Lino, linopirdine; XE, XE991; TEA, tetraethylammonium; RTG, retigabine; FLU, flupirtine; NEM, N-ethylmaleimide; ZnPy, zinc pyrithione; PPOs, pyrazolo[1,5-a]pyrimidin-7(4H)-ones; NH29, N-phenylanthranilic acid-29; NSAIDs, nonsteroidal anti-inflammatory drugs; H₂O₂, hydrogen peroxide, BMS, BMS-204352, ND, not determined.
both compounds will block many other $K^+$ currents (74); unpublished observations by the authors); thus, care must be used in interpreting results from such doses. With the hope of developing novel anticonvulsants, a number of M-channel enhancers (openers) have appeared. The prototype, retigabine (RTG), potently augments opening of KCNQ2-5 and KCNQ2/3 channels (75–78), both by shifting the voltage dependence of activation toward more negative potentials, and by increasing their $P_o$ at saturating voltages (49,79). KCNQ1-containing channels are RTG insensitive, due to its site of action on KCNQ2-5 at a tryptophan residue in S5 that KCNQ1 lacks (61,80). In line with expectations, animal models show RTG to be a potent anticonvulsant (81,82) and analgesic agent (31,83–85). RTG also induces a profound depression of neurotransmitter release (86). Novel lines of investigation have also showed RTG, and its close analogue, flupirtine (FLU), to be neuroprotective in models of stroke (87,88), and to regulate neuronal activity in various regions of the brain involved in the control of blood pressure (89), movement disorders, drug-seeking behavior, and emotional responses (90) (see the following text for a more subtle role for PKC). The answer to the mystery came with the identification of the KCNQ genes underlying M-current and the discovery that M-channels require PIP2 to activate ((Figure 20.3 (109–111), reviewed in (112)). This theme is elaborated next.

20.3.4 RECEPTORS AND SIGNAL TRANSDUCTION

As alluded to in Section 20.1, M-current was discovered as a $K^+$ conductance depressed by muscarinic stimulation, prompting a long-lasting and intense search for the signaling mechanism linking mAChRs to M-channels. The mechanism was found to be via $M_1$ receptors (100–102), $G_{q/11}$ G proteins (103) acting via phospholipase C (PLC) (104,105) and requiring some intermediate second messenger (106). Although IP3-mediated rises of intracellular $Ca^{2+}$ and production of diacylglycerol (DAG) by activation of protein kinase C (PKC) are canonical second messengers in this pathway, neither was shown to be needed for muscarinic M-current depression (104,107,108) (see the following text for a more subtle role for PKC). The answer to the mystery came with the identification of the KCNQ genes underlying M-current and the discovery that M-channels require PIP2 to activate (Figure 20.3 (109–111), reviewed in (112)). This theme is elaborated next.

20.3.5 REGULATION OF KCNQ CHANNELS BY PIP2

Although the pool of phosphoinositides, such as PIP2, in mammalian membranes were thought to be nearly limitless, Hilgemann and colleagues were the first to show that significant depletions could occur, which would be sensed by membrane transport proteins that need the presence of PIP2 to be activated (113,114). The field as a whole has a very robust literature, for which many inclusive reviews are available (115–120). The early work focused on the PIP2 sensitivity of inward-rectifier (Kir) channels, soon followed by Transient Receptor Potential M7 (TRPM7) as a channel whose currents are depressed by depletion of membrane PIP2 subsequent to stimulation of $G_{q/11}$-coupled
Figure 20.3 M-channels are regulated by plasma membrane PIP₂. (a) Recovery of KCNQ2/3 channels from M, receptor inhibition is blocked by the inhibitor of phosphatidylinositol kinases (PI-K), wortmannin (Wort). Plotted are the amplitudes of KCNQ2/3 currents at −20 mV from a tsA-201 cell that was heterologously transfected for KCNQ2 + KCNQ3 subunits, and M, receptors and studied under perforated-patch voltage clamp. With the cell exposed to Wort at 1 μM, a concentration sufficient to block PI 3-K, but not PI 4-K, application of the muscarinic agonist oxotremorine methiodide (oxo-M, 10 μM) nearly abolishes the KCNQ2/3 current within 30 s, and recovery is almost complete within ~7 min. However, at concentrations that block PI 4-K, I M depression by o xo-M is unaltered, but recovery is increasingly abolished due to the inability of the cell to resynthesize PIP₂. (From Suh, B. and Hille, B., Neuron, 35, 507, 2002.) (b) Run-down of cloned KCNQ2/3 channels is rescued by PIP₂. Plotted is the current at 0 mV in a macropatch on an oocyte heterologously expressing KCNQ2 + KCNQ3 subunits. Upon excision into inside-out mode, the current rapidly runs down, is fully restored by bath application of PIP₂ (10 μM), and then abolished by bath application of a PIP₂ antibody or the PIP₂ scavenger, poly-L-lysine. (From Zhang, H. et al., Neuron, 37, 963, 2003.) (c) Translocation of the PIP₂/IP₃ probe, EGFP-PLCδ-PH, correlates with depression of I M by stimulation of muscarinic receptors. Shown are I M amplitudes and the cytosolic EGFP fluorescence from an SCG neuron transfected with EGFP-PLCδ-PH, and representative fluorescent micrographs of the neuron in the inset. Upon application of oxo-M, I M is strongly depressed, and the EGFP-PLCδ-PH probe robustly translocates to the cytoplasm, with very similar time courses. (From Winks, J.S. et al., J. Neurosci., 25, 3400, 2005.) (d) Dephosphorylation of PIP₂ suppresses M-channels. Shown are whole-cell current traces from a tsA-201 cell transfected with KCNQ2 + KCNQ3, alone (left) or together with a voltage-sensitive phosphatase from zebrafish (Dr-VSP) (right). In the latter, after a brief depolarization to 100 mV (voltage at which Dr-VSP is fully activated), the KCNQ2/3 current is strongly depressed, recovering within 60 s, reflecting the rate of recovery of PIP₂ levels by PI(4)P 5-kinase, and indicating that PI(4)P cannot maintain KCNQ2/3-channel activity. (From Falkenburger, B.H. et al., J. Gen. Physiol., 135, 99, 2010.) (e) Single-channel recordings reveal KCNQ2 and KCNQ3 to have highly divergent apparent affinities for PIP₂. CHO cells were transfected with KCNQ2 or KCNQ3, alone and the channels studied at the single-channel level in patches. After excision to the inside-out mode, all the channels ran down, but could be reactivated by bath application of the water-soluble PIP₂ analogue, diC₈-PIP₂, at divergent concentrations. Left: shown are current traces at 0 mV from an example of such an experiment for KCNQ2 and KCNQ3. (f) shown are pooled dose–response curves of P₀ at 0 mV vs. [diC₈-PIP₂] from experiments shown in (e), showing the highly divergent apparent affinities of KCNQ2, KCNQ3, and KCNQ2/3 heteromers. (From Li, Y. et al., J. Neurosci., 25, 9825, 2005.)
M_{2} mAChRs (121). Using blockade of PIP_{2} synthesis (Figure 20.3a; (109)) and analysis of inside-out macropatches (Figure 20.3b; (110)), M-channels were shown to be sensitive to PIP_{2} abundance, and the muscarinic depression of I_{M} in rat sympathetic neurones (which continue to be a prime model neurone to study this topic) to be likely due mostly to PIP_{2} depletion by PLC hydrolysis (Figure 20.3c). Similar results were found for KCNQ1-containing channels (122). Further support for this mechanism was found in frog ganglia (111,123), in mammalian cell lines heterologously expressing KCNQ2-4 channels (63), and by using membrane-permeant PIP_{2} sequestering peptides (124). Li et al. (63), studying KCNQ channel activity in inside-out patches at the single-channel level, revealed the channels to have sharply divergent apparent affinities for PIP_{2}, with that of KCNQ3 being ~30 fold higher than for KCNQ2 or KCNQ4 (Figure 20.3e and f). As was predicted, the single-channel P_{o} of KCNQ isoforms is related to their apparent PIP_{2} affinity, with the maximal P_{o} of KCNQ3 (at saturating voltages) nearing unity at tonic PIP_{2} levels, whereas the maximal P_{o} of KCNQ2 and KCNQ4 is 5–10 times lower. Increasing tonic PIP_{2} abundance by overexpression of a PI(4)P 5-kinase dramatically increases the KCNQ2 or KCNQ4 current amplitudes and their maximal P_{o} (63,125,126).

A very useful tool in the study of channel regulation by lipids has been the development of optical probes, allowing single-cell monitoring of PIP_{2} metabolism in real time (127). The trick is to fuse short peptide domains that bind specific phosphoinositides to fluorescent proteins and then to monitor their translocation between plasma membrane and cytoplasm. For the case of PIP_{2}, the pleckstrin homology (PH) domain of PLCδ is most often used (128–130). At rest, the PLCδ-PH probe is localized to the membrane, bound to PIP_{2}. Upon stimulation of PLC-linked receptors (110,131), or depletion of PIP_{2} by 5-phosphatases (125,132), the probe translocates to the IP_{3} in the cytoplasm. Although the 10-fold higher affinity of this probe for IP_{3} than for PIP_{2} (133) yields uncertainty in whether depletion of PIP_{2} or production of IP_{3} is being reported by the probe (134,135), the ability to correlate PIP_{2} hydrolysis with KCNQ current regulation has proved invaluable. Indeed, Winks and colleagues exploited these properties of PLCδ-PH binding to strengthen the PIP_{2} depletion hypothesis of muscarinic modulation of M-current using kinetic argument (Figure 20.3c) and careful titration of reactants (126). The Hille lab has embarked on a series of papers that dissect out the various steps in this signaling cascade, using fluorescently tagged components as Förster resonance energy transfer (FRET) probes, coupled with cellular modeling (134,136) of enzymes, reactants, and probes involved in the M_{2}/G_{α_{11}}/PLC/PIP_{2} cascade (137–141). All the modeling in these labs is consistent with M-channel modulation by loss of bound PIP_{2}, at least in small mammalian cells.

### 20.3.6 LIPID SPECIFICITY AND LOCATION OF THE PIP_{2}-BINDING SITE

Many phosphoinositide-sensitive proteins discriminate between poly-phosphorylated phosphoinositides on the basis of their phosphorylation at the 3′ position of the inositol ring (142). The 3′-phosphorylated species, such as PIP(3,4)P_{2} and PIP(3,4,5)P_{3}, have very high affinity for Akt, Grb1, and other enzymes involved in the Ras/mitogen-activated protein kinase (MAPK) pathway of cell growth and proliferation. Indeed, the affinity of Akt or Grb1 for PIP(3,4)P_{2} over PIP(3,4,5)P_{3} (i.e., PIP_{3}) is several orders of magnitude, as is necessary since the plasma membrane abundance of the latter is nearly 100-fold greater than the former, even after stimulation of PI(3)-kinase. In contrast, the PLC isoforms activated by G_{α_{11}}-coupled receptors specifically hydrolyze PIP_{2}, which is most relevant for modulation of KCNQ channels. However, inside-out patch recordings have shown most KCNQ channels to be nearly as well activated by PI(3,4)P_{2} or PI(3,4,5)P_{3} as by PIP_{2} (63,143), similar to certain Kir channels (144). The physiological relevance of these minor phosphoinositides species for KCNQ modulation is likely to be low, given the profound difference in their abundance. The use of invertebrate voltage-sensitive PIP_{2} 5′-phosphatases (VSPs) has shown the singly phosphorylated PI(4)P to minimally activate KCNQ2/3 channels (Figure 20.3d; (140) but cf. (143)), and the synthesis of PI(4)P by PI 4-kinase to be the rate-limiting step in recovery of M-current after its muscarinic depression. A similar conclusion was reached using the chemically induced dimerization method (132) of rapidly dephosphorylating PIP_{2} into PI(4)P (125).

The study of the location of PIP_{2} interactions with K_{c} channels first showed their electrostatic interaction with negatively charged basic residues of channels (114,145). Zhang and colleagues identified a histidine just after S6 of KCNQ2 as being partly responsible (Figure 20.2; (110)), and a cluster of basic residues in the same region was identified for KCNQ1 (122,146). However, exploiting the dramatic difference in apparent affinity for PIP_{2} among KCNQ2-4 channels, chimera, and homology modeling within the proximal half of the C-terminus suggested the linker between the A and B helices of KCNQ2 and KCNQ3 (Figure 20.2) to be the primary interaction domain for PIP_{2}, and site-directed mutagenesis coupled with single-channel recording localized a cationic cluster of basic residues in this linker domain as being critical (45,147). Further support for this site comes from PIP strip immunoblots (143), although a more proximal site of interaction with PIP_{2} has recently been suggested by this group (148). The recently solved crystal structure of a Kir channel bound by PIP_{2} that cross-links the end of M2 (analogous to S6 in voltage-gated channels) to the beginning of the C-terminus (149) adds impetus to the clarification and further study of this issue.

### 20.3.7 RECEPTOR SPECIFICITY

Although numerous other G_{α_{11}}-coupled receptors also activate PLC and hydrolyze PIP_{2}, to produce IP_{3} and DAG, and would thus be thought to act similarly toward M-channels, their mechanisms surprisingly diverge. Again, autonomic ganglia have served as best understood model neuron for these studies. Surprisingly, among the four G_{α_{11}}-coupled receptors most intensely studied for their modulation of M-current in sympathetic neurons: muscarinic M_{1}, bradykinin B_{2}, angiotensin AT_{1}, and purinergic P2Y receptors, stimulation of neither the M_{1} nor AT_{1} receptors provokes IP_{3}-mediated [Ca^{2+}]_{i} rises, whereas the B_{2} and P2Y receptors do. Nevertheless, stimulation of all four receptors induces robust PIP_{2} hydrolysis, as reported by the PLCδ-PH probe (for a review, see (150)). This divergence has been ascribed to microdomains containing B_{2} (and presumably P2Y) receptors together with IP_{3} receptors (151), such that the IP_{3} produced is sufficient to open reluctant IP_{3} receptors (152).
Furthermore, whereas blockade of IP$_3$ signals has no effect on muscarinic or angiotensin II depression of I$_M$, it severely blocks I$_M$ depression by bradykinin or purinergic agonists (Figure 20.4a). The mechanism of the latter was shown to be via $[Ca^{2+}]_i$, signals and CaM (153–156), which binds directly to M-type channels (Figure 20.4b; (59, 157–160)). More specific discussion on the possible mechanisms of receptor-specific modulation of KCNQ channels can be found in recent reviews (119, 150). Alternative roles for CaM binding and regulation of M-channel function and expression are discussed in the next section.

Although several signaling intermediates of PLC cascade have been shown to contribute to receptor-mediated KCNQ channel inhibition, it is thought that many of these signals converge on PIP$_2$. Thus, PIP$_2$ unbinding from the channels can occur via cellular depletion of PIP$_2$, or by alteration of the affinity of a channel for PIP$_2$ by other signaling molecules (120). These modalities thus require the PIP$_2$ affinity of the channels to be only moderate for G protein-coupled receptor (GPCR) signaling to be effective, such that modest changes in PIP$_2$ abundance or PIP$_2$, affinity are physiologically sensed by the channels; for KCNQ subtypes with very high PIP$_2$, affinity (i.e., KCNQ3), their control by these pathways is probably not significant (119). This paradigm is similar to that postulated for control over the diverse family of Kir channels (161). In other peripheral ganglia, and in the CNS, the repertoire of G$_{q/11}$-coupled receptors differs from that of sympathetic ganglia, but the signal transduction mechanisms are very likely conserved. Thus, stimulation of B$_2$ (85, 162), protease–activated receptor 2 (163) and MrgD (164) receptors inhibits M-channels in sensory neurons (elaborated in the following). G$_{q/11}$-coupled 5HT$_3$ (165, 166) and ghrelin (167) receptor action depress I$_M$ in dopaminergic or serotonergic neurons; stimulation of P2Y (168), metabotropic glutamate (168), or muscarinic receptors (169, 170) suppresses M-currents in the hippocampus and M$_1$ receptors do so in cholinergic striatum (171). All of these receptors depress I$_M$ via some combination of $Ca^{2+}$ signals, PKC and PIP$_2$ depletion, but their precise differences in intracellular signaling mechanism have yet to be elucidated.

### 20.4 Auxiliary Subunits and Regulatory Proteins of KCNQ Channels

#### 20.4.1 Calmodulin in KCNQ Channel Function

There are CaM-binding sites on both the A and B helices in the carboxy-terminus of all KCNQ subtypes (Figure 20.2; [153, 157–159, 172, 173]), which have been suggested to have multiple functions. As introduced earlier, CaM is proposed to serve as the sensor in $Ca^{2+}$-dependent modulation of I$_M$, without the need of any CaM-binding kinase or phosphatase, similar to the role of CaM in activation of SK-type K$^+$ channels (174). As for SK channels (175), $Ca^{2+}$ binding to the N-terminal, but not the C-terminal, lobe of CaM is required for the $Ca^{2+}$-dependent action (154). Given the obligate requirement for PIP$_2$ binding to M-channels, an obvious
20.4 Auxiliary subunits and regulatory proteins of KCNQ channels

20.4.2 A-KINASE ANCHORING PROTEIN (AKAP) 79/150 AND PKC ACTION

During the search for the mystery second messenger linking muscarinic receptors to M-channels, PKC was ruled out early on using pharmacological blockers (107), and indeed unbinding of PIP$_2$ is central to the muscarinic action. However, the Scott lab demonstrated PKC to phosphorylate KCNQ2, and AKAP79/150 was shown to have an important role in muscarinic, but not bradykinin, suppression of I$_{M}$ in sympathetic neurons (184–186). As in its coordination of PKA to target proteins (187), AKAP79/150 (human/rodent) recruits PKC to KCNQ subunits, shown for KCNQ2 to phosphorylate S534 and S541 in the rat ortholog (184) (corresponding to S551 and S558 in the canonical human sequence), located in the distal B-helix. AKAP79/150 interacts with rat KCNQ2 at a site within residues 321–499 (184), which spans the A and B helices, including the linker between them. The action is to sensitize KCNQ2 channels (as well as endogenous M-channels in sympathetic neurons) to muscarinic suppression, such that the dose–response relationship of muscarinic agonist vs. current inhibition is shifted to lower concentrations (184,185,188).

FRET experiments revealed AKAP79 to intimately associate also with KCNQ3-5 (likely within the corresponding domain), but not KCNQ1 (although a different AKAP, yotiao, interacts with KCNQ1, see the following text), subunits, and to sensitize KCNQ3-5 and KCNQ2/3 channels to muscarinic depression as well (188). It is presumed that AKAP79/150-recruited PKC also phosphorylates KCNQ3-5, at their threonine residues corresponding to S558 in KCNQ2 (KCNQ3-5 do not have a corresponding S/T analogous to S551), consistent with the T553A KCNQ4 mutant being insensitive to the presence of AKAP79 (188). In rodent sympathetic neurons, the suppression of I$_{M}$ by AKAP150 RNAi (185) or expression of a dominant-negative (DN) AKAP79 (189) blunts I$_{M}$ suppression at half-maximal agonist concentrations, similar to the responses in AKAP150 KO mice (190,191).

As mentioned earlier, the influence of AKAP79/150 is strikingly specific among the four G$_{q/11}$-coupled receptors in sympathetic neurons, sensitizing I$_{M}$ to depression by M$_{1}$ and AT$_{1}$ receptors, but not by B$_{2}$ or P2Y receptors (Figure 20.5a; 185,189,190). We have suggested this specificity to arise from the involvement of Ca$^{2+}$/CaM in B$_{2}$ and P2Y, but not M$_{1}$ or AT$_{1}$, receptor modulation of I$_{M}$, and the overlapping site of AKAP79/150 interaction with M-channels, and of the PKC phosphorylation site, within the same B-helix that is critical to interactions with CaM (see earlier references). Thus, Ca$^{2+}$/CaM binding to the channels is suggested to block the interaction with AKAP79/150, preventing PKC B helix phosphorylation that sensitizes the channels to PLC-mediated inhibition. Consistent with that hypothesis, cotransfection of WT, but not DN, CaM, with KCNQ2/3 channels in CHO cells abrogated FRET with AKAP79 and the channels, and in cells coexpressing M$_{1}$ receptors, including WT CaM but not DN CaM, wholly blocked the sensitization of the KCNQ2/3 current by muscarinic stimulation (188). Furthermore, the expression of a DN AKAP79, or gene deletion of AKAP150 in rodent sympathetic neurons, reduced the depression of I$_{M}$ by the stimulation of M$_{1}$ or AT$_{1}$ receptors but had no effect on I$_{M}$ depression by B$_{2}$ or P2Y receptors (189). Interestingly, the earlier negative conclusions on PKC involvement in muscarinic depression of M-current, which were based on pharmacological tests (20,107), were shown due to the association of AKAP79/150 with PKC at its catalytic core (192), precisely the site where several PKC inhibitors act. Thus, those inhibitors have no effect on muscarinic depression of I$_{M}$, whereas those acting at the DAG binding site reduce the modulation at subsaturating doses of agonist (184). This AKAP79/150-dependent pharmacological profile was confirmed using a FRET-based reporter of PKC activity, and molecular modeling of the PKC/AKAP79 complex (179).

The AKAP, yotiao, is required for modulation of KCNQ1/ KCNE1 ($I_{KS}$) channels by PKA (Figure 20.5c; also see the following text). In addition, all KCNQ channels, except KCNQ1 and KCNQ2 homomers, have also been found to be regulated by Srk tyrosine kinases (Figure 20.5d); (193,194) and epidermal growth factor receptor tyrosine kinase (195). The physiological relevance of this pathway is yet to be determined.

20.4.3 REGULATION OF KCNQ CHANNELS BY KCNE SUBUNITS

One of the most studied examples of KCNQ channel auxiliary proteins is the family of small $\beta$ subunits, KCNE1-5, also known as minK (KCNEL) and minK-related peptides (MiRP1-4) (196). KCNEs are small transmembrane proteins featuring a single transmembrane domain, an extracellular N-terminus, and cytosolic C-terminus (Figure 20.6a). Of the five KCNE isoforms, the most well studied is KCNE1 as, together with KCNQ1, this subunit forms the cardiac I$_{KS}$ channel. In addition to KCNQ, KCNEs interact with and regulate many other ion channels; however, these interactions are outside the scope of this review (the interested reader is directed to the recent review by Pongs et al. (197)).

20.4.3.1 KCNQ1/KCNE1

KCNEL is abundantly expressed in tissues also known to express KCNQ1, that is, in the heart and epithelia. Several KCNE...
KCNQ channels

![Normalized I_M amplitudes](image)

Figure 20.5 KCNQ channels are modulated by kinases, often recruited by AKAPs (a) AKAP79/150 sensitizes I_M to depression by muscarinic stimulation. Plotted are normalized I_M amplitudes under whole-cell voltage clamp, of a control sympathetic neuron, one injected with RNAi against AKAP150, or one injected with RNAi against AKAP150 and also heterologously expressing AKAP79 as rescue. The depression of I_M in response to the muscarinic agonist, oxotremorine methiodide (oxo-M), near its EC_50 value for inhibition is much greater with functional AKAP79/150, than without. (From Hoshi, N. et al., Nat. Cell. Biol., 7(11), 1066, 2005.) (b) AKAP79/150 sensitization of G_{s,i}-mediated suppression of I_M is receptor specific. Plotted are I_M amplitudes from sympathetic neurons studied under perforated-patch voltage clamp, from a WT AKAP150+/+, or AKAP150−/− (KO), mouse. Inhibition of I_M was assayed in response to the P2Y, AT_r, or M1 receptors agonists, uridine 5'-triphosphate (UTP), angiotensin II (AngII), or oxo-M, at concentrations near their EC_50 values for I_M suppression. In the WT mouse, all three agonists elicit robust suppressions of I_M, but in the AKAP150 KO, that by AngII or oxo-M was markedly attenuated, whereas that by UTP was unaltered. (From Zaika, O. et al., J. Neurosci., 27, 8914, 2007.) (c) The AKAP, yotiao, orchestrates PKA action on KCNQ1/KCNE1 (I_M) channels. CHO cells were transfected with KCNQ1 + KCNE1, either alone (Left) or together with yotiao (Right), and studied under whole-cell voltage clamp. Shown are the current traces and current/voltage relations with a control pipette solution, one with added cAMP (200 μM), or one with added cAMP (200 μM) and the phosphatase inhibitor, okadaic acid (OA). Open symbols, no yotiao; solid symbols, with yotiao. Squares, control; triangles, cAMP; circles, cAMP/OA. The increase in I_M is nearly absent in the absence of yotiao. (From Marx, S.O. et al., Science, 295, 496, 2002.) (d) Inhibition of KCNQ2/3 channels by Src tyrosine kinase. Shown are families of currents from CHO cells transfected with KCNQ2 + KCNQ3, either alone or together with c-Src. Besides suppression of current amplitudes (Left), Src slows the kinetics of activation (Right). (From Gamper, N. et al., J. Neurosci., 23, 84, 2003.)

Mutations are associated with LQTS. When coexpressed with KCNQ1 in expression systems, KCNE1 dramatically slows KCNQ1 activation without a marked effect on deactivation, removes inactivation, shifts the voltage dependence of activation by approximately 20 mV toward more positive potentials, and increases γ by threefold to sevenfold (Figure 20.6c; (10,40,41,43,198,199). In addition, KCNE1 dramatically reduces the sensitivity of KCNQ1 to XE991 (200) and increases its sensitivity to the I_K channels, chromanol 293B and azimilide (201).

The stoichiometry of KCNQ1/KCNE1 assembly has been a matter of some debate as both fixed and variable stoichiometry models have been suggested (reviewed in (202)). According to the most common view, two KCNE molecules bind to KCNQ1 tetramers (203–206). Solution NMR, mutagenesis, and various simulations have resulted in several models of KCNQ1-KCNE1 interactions that share the idea of KCNE1 somehow aligning along the I_K channels, possibly by interacting with pore-forming S5-P-S6 residues of KCNQ1 (Figure 20.6b; (205,207,208)). It was also suggested that the single transmembrane domain of the KCNE1 protein is located in a cleft between voltage sensor domain of one KCNQ1 subunit and the pore helix of a neighboring subunit (205).

Various structural and kinetic models have been put forward to explain slowing of KCNQ1 gating by KCNE1 (some of these discussed in the recent review by Wrobel et al. (202)). Thus, it was suggested that KCNE1 might stabilize resting (closed)
states of KCNQ1 (209), retard pore opening (210), decelerate the movement of the S4 segment of the voltage-sensing domain of KCNQ1 (209,211), or disturb coupling between the voltage sensor movement and the pore opening (212). A recent study demonstrated that KCNE1 dramatically slows KCNQ1 gating currents (211), suggesting that slowing of S4 movement is indeed at the core of the KCNE1 action. At the single-channel level, the KCNE1 effect is manifested as an introduction of very long latencies to first opening to the first subconductance state and by increased occupancy of partially open subconductance states before channels reach the fully open state (42).

Importantly, although the association of KCNQ1 with KCNE1 reproduces kinetic properties of $I_{Ks}$ fairly well, apparently for a full recapitulation of properties of native $I_{Ks}$ the KCNQ1/KCNE1 complex needs to also include yotiao (213–217). Yotiao binds to a coiled-coil domain within the KCNQ1 C-terminus and acts as a scaffolding protein to recruit and position PKA and the phosphatase 1 (PP1) at the cytosolic domains of KCNQ1, thus controlling the phosphorylation/dephosphorylation of KCNQ1 channel at N-terminal Ser-27 (197,213,215,216). This control over KCNQ1 phosphorylation has an important physiological outcome as β-adrenergic stimulation results in PKA-dependent phosphorylation of KCNQ1 within the $I_{Ks}$ complex which, in turn, leads to an increase in $I_{Ks}$ amplitude and acceleration of action potential repolarization (Figure 20.5c; (197)). An LQTS-associated KCNQ1 mutation (G589D), which disrupts assembly of the KCNQ1/KCNE1/yotiao complex, renders the KCNQ1 channel insensitive to PKA phosphorylation, thus emphasizing the importance of yotiao for normal $I_{Ks}$ function and regulation by β-adrenergic signaling (197,213,215).

20.4.3.2 Other KCNE subunits

The association of KCNQ1 with KCNE2 or KCNE3 results in constitutively open, voltage-independent channels (Figure 20.6c), but with divergent effects on current densities; KCNE2 decreases it by over threefold (218,219), whereas KCNE3 increases it over 10-fold (218,220). The constitutively open gating of KCNQ1/KCNE3 or KCNQ1/KCNE2 channels resembles those of the

---

**Figure 20.6 Regulation of KCNQ1 by KCNE subunits.** (a), simplified diagram of KCNE protein family (KCNE1-5, labeled as E1–E5). Cytosolic domains are shown as gray boxes. (b) Structural model for KCNQ1-KCNE1 interaction (open and closed states, as labeled) obtained by a high-resolution docking simulation. The transmembrane domain of KCN1 (residues 45–71) occupies a central position in the closed state model on the right and aligns to the left of the pore in the open state model on the left. (From Kang, C. et al., Biochem., 47, 7999, 2008.) (c) Effects of KCNE subunits on the KCNQ1 currents studied by the whole-cell voltage clamp recordings from COS-7 cells transfected with KCNQ1 alone or with KCNQ1 and each of the 5 KCNE subunits (as indicated). Cells were held at −80 mV and currents elicited by train of voltage steps from −100 to + 40 mV, in 10 mV increment, for KCNE1 to KCNE3, and from −100 to + 90 or +110 mV for KCNE4 and KCNE5 respectively. (From Bendahhou, S. et al., Cardiovasc. Res., 67, 529, 2005.)
Ion channel families

KCNQ channels

Mammalian tissues, particularly (1) CNS and PNS neurons; (2) heart, smooth, and skeletal muscles; and (3) epithelia. In excitable cells, M-channels are mainly involved in control of excitability and synaptic transmission, whereas in epithelia, they recycle K+ ions and provide the driving force for epithelial transport.

20.5.1 FUNCTION OF KCNQ CHANNELS IN THE PERIPHERAL NERVOUS SYSTEM

20.5.1.1 Sympathetic system

M-current has been originally discovered in the peripheral sympathetic neurons and ever since, cultured sympathetic neurons (i.e., SCG) remain one of the most popular native cell preparation for studying M-channel properties and regulation (reviewed in (236,237)). Since much of the work discussed in this chapter is indeed obtained from such neurons, we will not specifically discuss the role of M-channels in these cells. Of note, however, is the fact that sympathetic neurons can be subdivided into tonic (firing continuously during depolarization) and phasic neurons (firing a single action potential or a short burst) and the relative abundance of M-current largely defines the pattern: tonic neurons display small tonic M-current amplitudes, whereas M-currents in phasic neurons are much larger (238,239). This exemplifies how tonic M-current density can define the firing properties of a neuron.

20.5.1.2 Peripheral somatosensory system

Another peripheral neuron type in which M-current is a major regulator of excitability is the peripheral somatosensory neuron and, in particular, painful stimuli-sensing (nociceptive) neuron. The recent literature suggests M-channels to be a major player in regulation of peripheral nociceptive transmission. Nociceptive and nonnociceptive somatosensory neurons express KCNQ2, KCNQ3, and KCNQ5 in various combinations (89,240–242); in addition, a subset of rapidly adapting mechano-sensitive neurons of both spinal and trigeminal systems abundantly express KCNQ4 (27,243). M-channels are expressed in cell bodies of dorsal root ganglion (DRG) (Figure 20.7a; (31,240,241)) and trigeminal ganglion (TG) (96,244) where they contribute to the slow delayed rectifier K+ current. Functional expression of M-channels is also confirmed in afferent fibers (240–242,245,246), as well as in the nociceptive nerve endings in the skin (Figure 20.7b; (242)). M-channel activity strongly contributes to the control of somatic (Figure 20.7c and d) and axonal (Figure 20.7e) firing in vitro (83,163,247–249). Moreover, growing evidence suggests that M-channels control peripheral fiber excitability in vivo. Thus, hind paw injection of the M-channel blocker XE991 induces moderate pain in rats (162,163), while similar injections of pharmacological M-channel enhancers such as RTG and FLU produce analgesic effect (162,240). Functional M-channels are also found in the central terminals of nociceptive fibers (250).

Among the voltage-gated K+ channels expressed in nociceptors, M-channels arguably have the most negative threshold for activation (negative to −60 mV). Given the resting membrane potential of these neurons in the range of −60 mV (85,96,251), M-current is probably the most abundant voltage-gated K+ current fraction present at rest. Indeed, in small DRG neurons, voltage clamped at −60 mV, there is a small outward current, which can be almost completely blocked by XE991; this current is also strongly augmented by M-channel openers (Figure 20.7c; (85)). Therefore, inhibition or downregulation of M-channel
activity/expression in nociceptive fibers is expected to produce hyperexcitability. The role of M-channels in pain will be discussed in more detail in Section 20.6.4.

20.5.2 FUNCTIONS OF KCNQ CHANNELS IN THE CENTRAL NERVOUS SYSTEM

20.5.2.1 KCNQ2/3

Neuronal KCNQ channel subunits (KCNQ2-5) are abundantly expressed throughout the mammalian CNS, with M-current identified in the hippocampus (169) shortly after its description in peripheral ganglia. KCNQ2 and KCNQ3 have very widespread CNS distribution, whereas KCNQ4 and KCNQ5 are also expressed in the CNS but in a more restricted fashion (see the following text). KCNQ2 and KCNQ3 subunits have been found in abundance throughout almost all major brain structures with particularly high expression at key sites controlling rhythmic neuronal activity and synchronization (252–255). In particular, Cooper et al. (252), using an immunohistochemical approach, found KCNQ2 to be most abundantly expressed in the following structures of the mouse brain: (1) In the basal ganglia, strong staining was observed in somata of dopaminergic and GABAergic cells of the substantia nigra, cholinergic large aspiny neurons of the striatum, and GABAergic and cholinergic neurons of the globus pallidus. (2) In the septum, somatic KCNQ2 labeling was found in GABAergic neurons of the substantia nigra, cholinergic large aspiny neurons of the striatum, and GABAergic and cholinergic neurons of the globus pallidus. (3) In the thalamus, anti-KCNQ2 antibodies labeled GABAergic nucleus reticularis neurons that regulate thalamocortical oscillations. (4) In the hippocampus, parvalbumin-positive and parvalbumin-negative interneurons were KCNQ2 positive. In another study, it was found that in hippocampus, KCNQ2 and KCNQ3 localize primarily to the somatic layers of dentate gyrus and CA1-3 (253).
In CNS neurons, KCNQ2 and KCNQ3 channels can be found in the both soma and axons (233, 234, 245, 253, 256) with almost no expression in dendrites (253, 257–259). Growing evidence suggests that KCNQ subunit expression within the soma/axon is not uniform and that functional channels are localized in higher abundance at the sites of action potential generation and propagation: AIS and nodes of Ranvier (see the preceding text) (233, 234, 245, 253, 256). Numerous studies, mostly performed in the hippocampus, have investigated the neurophysiological significance of M-channels in the CNS. In general terms, activation of M-channels located presynaptically reduces neurotransmission, whereas the activation of M-channels expressed at postsynaptic sites (i.e., at the AIS) dampens excitability by increasing the threshold for action potential firing, shortening EPSPs, reducing synaptic integration, promoting accommodation within the action potential bursts, etc. (170, 257–264).

The postsynaptic function of M-channels is relatively well understood. Thus, perisomatic M-channels have been shown to reduce somatic EPSP summation in CA1 pyramidal cells (Figure 20.8a; (258, 259)). At the same time, due to the large distance between the soma and distal dendrites, these perisomatic M-channels have little impact on dendritic EPSP integration. Nevertheless, this perisomatic effect is sufficient for inhibition of spiking in response to dendritic EPSPs (258, 259). As suggested by Hu and colleagues, the high density of M-channels at the AIS is probably an effective regulator of action potentials, as the pool of M-channels can effectively attenuate EPSPs before they reach the threshold for spike initiation; therefore, M-channels at the AIS may to some degree electrically isolate the somatodendritic and the axonal compartments of the neuron. M-channels also contribute to the medium component of afterhyperpolarization (mAHP) following an action potential (259, 265–267), as well as possibly the slow AHP as well (268, 269), phenomena that shunt action potential bursting. In addition, it has been suggested that in hippocampal neurons, M-channels, together with the persistent sodium current and I_p, generate subthreshold resonance in the theta frequency range (5–8 Hz) (259, 265) and gamma oscillations (20–80 Hz) underlying synchronized network activity and seizure initiation (270, 271). Theta oscillations in the hippocampal network are thought to be essential for hippocampal coding of navigation, learning, and memory (272) and, therefore, M-channel activity is likely to be involved in regulation of these processes. Axonally localized M-channels apparently do not contribute to EPSP integration but define action potential thresholds and resting membrane potentials of the axonal (nodal) membrane (257). In addition to the hippocampus, a functional role of KCNQ/M-channels in controlling neuronal excitability has also been demonstrated electrophysiologically in murine (273, 274) and human (275) neocortex, cerebellar Golgi cells (276), striatum (171, 277, 278), and several other brain regions (e.g., (165, 279)).

Growing evidence suggests that M-channels also have a presynaptic function (39, 86, 91, 150, 263, 274, 277). Thus, the Taglialatela group has found that KCNQ2 is localized presynaptically in cortical (274) and striatal (277) neurons; accordingly, M-channel activation with RTG reduced glutamate or dopamine release from cortical or striatal synaptosomes, respectively (274, 277). Mechanistically, this effect can be explained by hyperpolarization of presynaptic terminals, which,
in turn, reduces Ca\(^{2+}\) influx into the terminals via voltage-gated Ca\(^{2+}\) channels and, thus, reduces the amount of neurotransmitter-containing vesicles released per arriving action potential. It should be noted that presynaptic localization of M-channels is somewhat inconsistent with the suggested AIS/nodal targeting of KCNQ2 and KCNQ3 and the lack of M-currents reported in dendrites (257–259). This matter will require further investigation; perhaps, M-channels in presynaptic terminals of some neurons are composed of different KCNQ subunit(s) (e.g., KCNQ5; see the following text).

### 20.5.2.2 KCNQ4

In contrast to KCNQ2 and KCNQ3, KCNQ4 has a much more restricted expression profile throughout the mammalian nervous system as it is specifically expressed in the auditory and vestibular pathways and structures (27,280,281). Thus, KCNQ4 is expressed in the cochlea where it is localized at the basal pole of outer hair cells (OHCs) (280). It is also found in postsynaptic calyx terminals innervating vestibular type I hair cells (282,283) and in auditory brainstem nuclei and tracts (27). KCNQ4 is important for hearing and vestibular function, and multiple mutations within KCNQ4 result in a dominant form of deafness, DFNA2 (see the following text) (26,27,280). In some individuals, this disorder is also accompanied with vestibular abnormalities (284,285). In addition to auditory structures, KCNQ4 is also expressed in the peripheral nerve endings of cutaneous rapidly adapting hair follicle and Meissner corpuscle mechanoreceptors (as well as in the cell bodies of these neurons in DRG and TG). In these rapidly adapting mechanoreceptors, KCNQ4 shortens the duration of stimulus-induced bursts (243). Accordingly, human DFNA2 carriers are more sensitive to low-frequency tactile vibrations as compared to age-matched control subjects (243).

### 20.5.2.3 KCNQ5

KCNQ5 subunits are expressed throughout the CNS and PNS alongside KCNQ2 and KCNQ3, although it is believed that its contribution to perisomatic M-currents is less prominent. However, KCNQ5 has been found to express abundantly in postsynaptic calyx terminals within the calyx of Held, a giant glutamatergic terminal in the medial nucleus of the trapezoid body (MNTB), where this subunit is likely to be the main constituent of M-channels (39). It has been demonstrated that at calyx terminals, KCNQ5 determines the presynaptic resting potential and controls synaptic strength (Figure 20.8b; (39)). Along with KCNQ4, KCNQ5 is also abundantly expressed throughout the auditory system where it localizes predominantly to synaptic endings (286), a finding that is compatible with the idea that KCNQ5 may represent a presynaptic KCNQ subunit. KCNQ5 has also been identified as underlying at least part of the slow AHP in the hippocampus (266).

### 20.5.2.4 KCNQ1

Although KCNQ1 is a cardiac KCNQ subunit, which is not considered to be widely expressed in the nervous system, one study reported abundant KCNQ1 expression in many brain regions, including forebrain and brainstem (287). Moreover, it was also shown that mice carrying LQT-associated mutations within KCNQ1 develop not only LQT but also the epilepsy. The coinheritance of LQT and epilepsy (in a form of KCNQ1 mutations) may explain sudden unexplained death in epilepsy (SUDEP) that can follow a seizure. This hypothesis is still under investigation.

### 20.5.3 HEART

Heteromeric association of KCNQ1 and KCNE1 results in a K\(^{+}\) current with very slow kinetics that is abundantly expressed in the heart and underlies the slow component of the cardiac delayed rectifier current, I\(_{Kr}\). The delayed rectifier K\(^{+}\) current in the heart (I\(_{Kr}\)) was first described by Noble and Tsien (288,289), who also showed that the I\(_{Ks}\) is composed of two kinetically distinct components that were later identified as KCNQ1/KCNE1-mediated I\(_{Ks}\) (10,198) and HERG-mediated I\(_{Kr}\) (290–292). Cardiac I\(_{Kr}\) is responsible for the late repolarization phase of the cardiac action potential and regulates action potential duration in many species (see (293) for review). Particularly, the contribution of I\(_{Ks}\) is enhanced at high stimulation rates as its slow kinetics results in the incomplete deactivation at high pacing rates (294). In human heart, I\(_{Ks}\) probably plays little role in normal ventricular muscle action potential repolarization, but when the action potential duration is abnormally long, I\(_{Kr}\) is likely to provide an important safety mechanism that, when removed, increases arrhythmic risk (295). Accordingly, mutations within both KCNQ1 and HERG are associated with cardiac arrhythmias, particularly with a form of inherited LQTS, a cardiac disorder characterized by prolonged ventricular repolarization, resulting in episodic ventricular tachyarrhythmias that can lead to ventricular fibrillation and sudden death (296). Although the I\(_{Ks}\) has been known for decades, the elucidation of its molecular correlate required considerable study. I\(_{Ks}\) was originally attributed to the subunit that we now know as KCNE1 (see the preceding text) (297–299) as this subunit, when expressed in Xenopus oocytes, gives rise to a current with similar characteristics to mammalian I\(_{Kr}\). This subunit was originally named minK (for minimal K\(^{+}\) channel (299)) although it was soon realized that such a small protein with only one transmembrane domain was unlikely to be able to assemble into functional voltage-gated K\(^{+}\) channels on its own. Indeed, it was later discovered that minK, now called KCNE1, is indeed an auxiliary β-subunit of the KCNQ1 channel (10,198). Xenopus oocytes express an endogenous KCNQ1 orthologue (XK,LQT1), which, when coassembled with exogenous KCNE1, recapitulates an I\(_{Ks}\)-like current (10). The biophysics of I\(_{Ks}\) is discussed in Section 20.4.3.1 and the I\(_{Kr}\) pathologies will be considered in Section 20.6.

### 20.5.4 OTHER TISSUES

Beside their roles in neurons and cardiac myocytes, KCNQ channels are expressed in epithelia, smooth and skeletal muscle, and also in some specialized cell types.

#### 20.5.4.1 Epithelia

KCNQ1, in combination with KCNE1, KCNE2, or KCNE3, is expressed in the basolateral membranes of many epithelia including kidney, stomach, colon, and small intestine (220,300–304). Epithelial cells are polarized, and their apical (or luminal) and basolateral membranes serve distinct functions. One of the
KCNQ channels

Basic functions of the epithelia is transepithelial transport. The major driving force for this transport is the electrochemical gradient of Na+ and K+ across the epithelial membranes. KCNQ1 channels (together with other epithelial K+ channels) are essential for both maintaining a negative membrane potential, providing a driving force for transepithelial transport, and for K+ absorption or secretion. Interestingly, in many epithelia, voltage-gated KCNQ1 channels are converted into voltage-independent, constitutively open K+ channels by coassembly with KCNE2 (219) or KCNE3 (220) (see the preceding text). The functional role of KCNQ1 in epithelia has been recently reviewed in references (44,305,306), so here we mention only some examples. Thus, basolateral KCNQ1-KCNE3 channels may be required for providing a driving force for cAMP-stimulated Cl− secretion in the colon (220) and airway (307), whereas KCNQ1-KCNE2 channels mediating apical K+ recycling in gastric parietal cells are necessary for gastric acid (H+) secretion (308,309). KCNQ1/KCNE1 channels are expressed in the stria vascularis of the inner ear where they conduct a potassium current into the scala media to generate the K+-rich endolymph (44,310).

20.5.4.2 Smooth and skeletal muscles

Smooth muscle is nonstriated muscle that mediates involuntary contraction and support organ dimensions. These muscles are found within the walls of blood vessels, as well as in urinary bladder, uterus, and reproductive, gastrointestinal, and respiratory tracts, and in some other more specified structures. In diverse smooth muscles, contractions are generated either mostly by the influx of Ca2+ through voltage-gated Ca2+ channels (bladder, gut), by a mixture of Ca2+ influx and Ca2+ release from intracellular stores (vascular, uterus), or mostly by release from intracellular stores (airway) (see (311,312) for reviews). Interestingly, M-type channels are localized to all of these smooth muscle types. The expression of KCNQ1, KCNQ4, and KCNQ5 has been found in several types of blood vessels, including portal vein, thoracic aorta, carotid artery, cerebral basilar artery, and femoral artery of mice (34,313–316) as well as in rat aorta and mesenteric artery (35,36). Although multiple KCNQ subunits were detected in these studies, the emerging pattern suggests that KCNQ4 homomers or KCNQ4/5 heteromers are probably the main KCNQ channels in smooth muscle. Functional expression of KCNQ4 in human arteries has also been demonstrated (317). A number of studies have described KCNQ expression in nonvascular smooth muscle. Thus, Ohya et al. (318) found KCNQ1, KCNQ3, KCNE1, and KCNE2 transcripts in rat gastric antral smooth muscle. Transcripts for KCNQ4, KCNQ5, and KCNE4, as well as functional M-like currents, were detected in murine gastrointestinal tract (319). Expression of KCNQ1, KCNQ5, and KCNE4 has been reported in murine myometrial smooth muscle throughout the estrus cycle (320). Recently, M-like currents have been recorded from rod photoreceptors (330) and retinal pigment epithelial cells (331). Recently, functional expression of KCNQ2 has been found in keratinocytes (332).

20.6 M-CHANNELOPATHIES AND THERAPEUTIC POTENTIAL OF KCNQ CHANNELS

As discussed earlier, genes encoding KCNQ subunits were discovered through the analysis of human diseases and, indeed, the KCNQ channel family is unique among other ion channel families in the variety of severe disorders that are associated with genetic mutations within KCNQ genes. In this section, we will consider channelopathies that are linked to KCNQ channels.

20.6.1 ARRHYTHMIAS

Mutations within genes encoding both subunits of IKs, KCNQ1 and KCNE1, underlie several arrhythmic heart disorders, including LQTS and short QT (SQT) syndrome, familial atrial fibrillation, and others (see the following text). The up-to-date database of LQTS-associated mutations (including over 200 LQTS1-associated mutations within KCNQ1 alone) can be found at http://www.fsm.it/cardmoc/.

20.6.1.1 Long QT syndrome

LQTS is an arrhythmic heart disorder characterized by the lengthening of the QT interval of the electrocardiogram, either due to the enhancement of inward depolarizing currents or the reduction in outward repolarizing currents (333,334). Most LQTS
patients are asymptomatic until a triggering event (i.e., heavy exercise, stress, etc.) which initiates ventricular tachyarrhythmia (i.e., torsades de pointes) and may result in sudden cardiac death (306,333,334). Loss-of-function mutations within KCNQ1 often result in LQTS form 1 (LQTS1), with two major forms identified: (1) the autosomal dominant Romano–Ward syndrome (RW) and (2) the autosomal recessive Jervell and Lange-Nielsen syndrome (JLN). The latter is associated with bilateral deafness (335). A number of JLN KCNQ1 mutations have been identified (336–343), reviewed in (293,344), many of them resulting in C-terminal A-domain truncations or missense sequences and, as discussed earlier, bring about defects in KCNQ1 subunit tetramerization. An additional reason for Im loss of function due to JLN mutations could be in defective trafficking due to impaired interaction with yotiao (215,345). Generally, JLN mutations do not feature strong dominant-negative effects on impaired interaction with yotiao (215,345). Generally, JLN KCNQ1 mutations associated with RW often affect channel gating and RW KCNQ1 mutants confer strong dominant-negative effects when assembled with WT KCNQ1 (293,346). A number of RW mutations affecting KCNQ1 trafficking have also been identified (347–349). Mutations in KCNE1 are associated with another form of LQTS, LQT5 (350–352).

20.6.1.2 Short QT syndrome

Several gain-of-function KCNQ1 mutations are associated with a different form of arrhythmia that is characterized by a shortened QT interval, due to accelerated action potential repolarization (353–355). For example, an SQT-associated V307L KCNQ1 mutation was shown to shift the KCNQ1 voltage dependence toward more negative potentials and to accelerate activation kinetics, thus increasing Im and shortening the QT interval (353). SQT is also associated with atrial fibrillation and sudden death (333).

20.6.2 DEAFNESS

Mutations within KCNQ4 are often associated with autosomal dominant type 2 deafness (DFNA2), a progressive form of sensory neuronal hearing loss, which starts with a loss of perception of the high sound frequencies but later in life develops into the progressive loss of hearing in the middle and low frequencies (26). KCNQ4 is expressed at high levels in the basolateral membrane of cochlear OHCs where it provides a major contribution to the K+ conductance defined as GK,n, (356,357). GK,n activates negative to −70 mV and functions to hold the OHC membrane potential near the equilibrium potential for K+, thus maintaining a driving force for K+ entry through the apical transducer channels (356). A similar K+ conductance has also been found in the inner hair cells (306). KCNQ4 expression in the auditory hair cells has been verified by immunohistochemistry and RT-PCR (358), and accordingly, GK,n in cochlear hair cells is sensitive to linopirdine and XE991 (359–361). Genetic deletion of KCNQ4 in a mice model results in progressive hearing loss, which is paralleled by the loss of cochlear OHCs (280); therefore, it was hypothesized that loss of KCNQ4 currents may lead to chronic K+ overload in these cells, causing the degeneration responsible for progressive hearing loss (280,357). There are however some noticeable biophysical and pharmacological differences between native K+ currents in OHCs and heterologously expressed KCNQ4 (reviewed in (306,357)), which may result from heteromeric assembly of KCNQ4 with some other subunits (i.e., KCNE4 (223)) in cochlea. Nevertheless, the link between KCNQ4 and DFNA2 is firmly established by genetic analysis. A number of DFNA2-associated missense mutations and deletions of KCNQ4 have been identified (25,26,362–370). Five point mutations in the pore region (L274H, W276S, L281S, G285C, and G296S) as well as the C-terminal mutant G321S result in the endoplasmic reticulum retention of the mutant channels (371). Other mutations result in the pore collapse, defective assembly, and other loss-of-function defects (reviewed in (357)).

20.6.3 EPILEPSY/SEIZURES

As mentioned in Section 20.1, KCNQ2 and KCNQ3 were identified through positional cloning of chromosomal loci associated with an autosomal dominant form of epilepsy, benign familial neonatal seizures (BFNSs) (15–17). In this syndrome, seizures (generalized or focal tonic-clonic seizures) occur in otherwise healthy infants starting around 3 days of age. The seizures usually disappear spontaneously within the first 6 months of life, although 10–15% of infants with BFNC develop other forms of seizures later in life (372).

Sixty to seventy percent of families with BFNC carry mutations within KCNQ2 or KCNQ3; NCBI’s GeneReview (372) http://www.ncbi.nlm.nih.gov/books/NBK325340/ lists over 70 KCNQ2 mutations and 8 KCNQ3 mutations leading to BFNC; thus, KCNQ2 mutations are nearly 10 times more frequent than these in KCNQ3. All BFNC-associated KCNQ3 mutations identified thus far are missense mutations; mutations within KCNQ2 are more diverse and include various truncations, deletions, frame-shift, and missense mutations (306,372). Heterologous expression of these mutants in expression systems revealed a number of loss-of-function mechanisms: defective trafficking (373), CaM or other accessory protein binding (374,375), and gating abnormalities (376–378). Interestingly, it has been noted that heterologous expression of some BFNC-associated KCNQ2 and KCNQ3 mutants resulted in only rather mild (20–30%) reduction in Im amplitude (379,380). This fact is usually interpreted as demonstrating the importance of M-current for control of neuronal excitability so that even a modest reduction in amplitude is sufficient to cause seizures (381). Another explanation has been suggested recently, in which some BFNC mutations may impair trafficking of KCNQ2/3 channels to axons (58,382) (an effect that may not manifest itself in the large reduction of current amplitude when tested in the nonneuronal expression system); this would result in decreased axonal abundance of M-channels and, thus, abnormal excitability. The reasons why BFNC seizures disappear with age are not entirely clear, although it was suggested that it may have to do with the maturation of GABAergic system (382). Immature CNS neurons have high intracellular Cl− concentration, which renders GABAergic transmission excitatory in early life, before becoming inhibitory (383). It was also reported that KCNQ channel expression in neonatal brain is low but increases with age (384). Thus, the developmental absence of one major inhibitory mechanism (GABA inhibition) in combination with low expression and genetic impairment in another (M-current) may result in the epileptic phenotype. With age, the GABAergic system engages fully and M-channel density in neurons increases, which may explain the disappearance of seizures.
20.6.4 PAIN

Pathological pain is a hyperexcitability disorder and, as such, has been referred to as a channelopathy (385). M-channels play a major role in controlling nociceptive neuron excitability (31,85,96,162,163,242). Accordingly, growing evidence suggests that inhibition or downregulation of M-channel activity/abundance in nociceptors may result in pain sensations. Thus, peripheral injection of M-channel blockers induces moderate pain (162,163) and hyperalgesia (242). Similarly, painful are the injections of inflammatory mediator bradykinin (96,162). Bradykinin inhibits M-current in a PLC- and Ca2+-dependent manner (similarly to its action in sympathetic neurons described in detail earlier) and produces excitability of cultured nociceptive neurons, an action which is mimicked by XE991 and antagonized by FLU (85,162). Therefore, M-channel inhibition is likely to contribute significantly to the bradykinin-induced pain (85,162,386,387). Similarly, M-channels in small DRG neurons are inhibited by other PLC-coupled receptors such as PAR-2 (163) and MrgD (164). A recent study demonstrated that M-channels in trigeminal nociceptors can also be inhibited by nitric oxide (NO) via S-nitrosylation at the redox-sensitive module within the cytosolic loop between S2 and S3 (244). NO-mediated M-channel inhibition correlated with increased excitability and CGRP release in nociceptors and was suggested to contribute to excitatory effects of NO in trigeminal disorders such as headache and migraine (244).

In addition to inflammatory pain resulting from the acute inhibition of M-channels via GPCR cascades by the inflammatory mediators, longer-lasting downregulation of M-channel expression may contribute to chronic pain. Thus, Rose et al. (240) reported strong downregulation of KCNQ2 expression in rat DRG following neuropathic injury. Similarly, a significant loss of KCNQ5 immunoreactivity was reported in DRG of rats after sciatic nerve transection (241). In accord with previous findings suggesting that augmentation of M-current in sensory fibers is anti-excitatory (83,248,249,388), thermal hyperalgesia produced by neuropathic injury in rats was significantly reduced by injection of FLU directly into the site of nerve injury, an effect completely prevented by XE991 (240). Similarly, a strong downregulation of both KCNQ2 and KCNQ3 in DRG neurons has been reported following the development of bone cancer pain in rats (389). This effect was accompanied by a marked decrease in $I_{Na}$ amplitude in nociceptive neurons. Application of RTG inhibited the bone cancer–induced hyperexcitability of DRG neurons and alleviated mechanical allodynia and thermal hyperalgesia in rats with bone cancer. These studies suggest that despite the decreased abundance of M-channels in nociceptive neurons affected by a chronic pain condition, the remaining M-channels can still be effectively targeted by the pharmacological enhancers in order to reduce nociceptor excitability (see the following text).

20.6.5 M-CHANNELS AS DRUG TARGETS

KCNQ channels are widely highlighted as prospective drug targets for the treatment of diverse hyperexcitability disorders. M-channel openers have been proposed to be used for the treatment of epilepsy, various types of pain, attention-deficit/hyperactivity disorder, stroke, hearing loss, bipolar disease, schizophrenia, mania, addiction to psychostimulants, and other disease states (390–392). Recently, RTG (Ezogabine™) was approved by the U.S. FDA for the adjunctive treatment of partial-onset seizures in adults (393). Since identification of RTG as an M-channel enhancer (76,79), hundreds if not thousands (e.g., screening by (394) alone reported over 600 unique M-channel enhancers) of new M-channel activators have been identified or synthesized in a number of large screens conducted by industrial and academic labs (87,93,394–400). A review of some of these efforts can be found in (390).

Although RTG has been introduced to the market only recently, its very close chemical analogue, FLU (Kataadolontm, Awegal™, etc.), has been used as a nonopioid analgesic since the 1980s in Europe (although it was never certified in the United States) (401,402). Although FLU is not as selective as some of the latest-generation M-channel activators, it is believed that the analgesic efficacy of FLU arises mainly from its M-channel enhancer activity (403). FLU effectively reduces postoperative pain, chronic musculoskeletal pain, migraine, and neuralgia (401,403). As mentioned before, some nonsteroidal anti-inflammatory drugs such as diclofenac (92) and celecoxib (404,405) possess strong M-channel opener activity, which can be responsible for at least some of their analgesic efficacy.

Despite the facts that (1) the chemical structures of FLU and RTG are very similar and (2) RTG consistently reduced pain in a variety of animal models (83,406), a phase IIa proof-of-concept clinical trial of RTG for the treatment of postherpetic neuralgia pain was inconclusive (407). It is likely that this failure reflects the broad expression profile of M-channels within and outside of the nervous system (see the preceding text), and, therefore, a high risk for off-target effects of systemically applied broad-spectrum M-channel modulators. It is therefore hypothesized that modulators that cross the blood–brain barrier poorly and are more selective for KCNQ2 and KCNQ3 over KCNQ1 and KCNQ4 (which are highly expressed in the heart, epithelia and smooth muscle) should have a better safety profile while retaining analgesic activity (390).

20.7 REGULATION OF KCNQ GENE EXPRESSION

20.7.1 REGULATION BY REST

Until recently, little has been known about the transcriptional regulation of KCNQ genes. However, as much as the acute regulation of M-channel activity is important for regulation of neuronal excitability in the short term, long-term mechanisms of regulation of M-channel abundance are expected to contribute to mechanisms of plasticity within neuronal circuits. KCNQ genes have a highly conserved repressor element 1 (RE1, NRSE) binding site situated between the exons 1 and 2 (Figure 20.9a; (408)). Functional interactions of repressor element 1 silencing transcription factor (REST, NRSF) with KCNQ2, KCNQ3, KCNQ5 (408), and KCNQ4 (409) have been demonstrated. REST inhibits expression of genes through the recruitment of multiple chromatin-modifying enzymes (410). Accordingly, overexpression of REST in DRG neurons robustly suppressed native M-current...
20.7 Regulation of KCNQ gene expression

Figure 20.9 Emerging mechanisms of regulation of KCNQ2 gene transcription. (a–c) Regulation of KCNQ gene expression by REST. (a) Schematic representation of the 5′ of the KCNQ2 gene. Black boxes represent exons, and the transcription start site is marked by an arrow. The location of regulatory elements, Sp1 and RE1, are shown as white boxes. (b) REST inhibits M-current and increases excitability of small-diameter DRG neurons. Top panel shows whole-cell voltage clamp recordings from cultured DRG neurons infected with an adenoviral construct expressing GFP only (Vector) or REST and GFP (AdREST). In cultures infected with REST and GFP, both green (AdREST) and nongreen (noninfected) neurons were tested. The XE991 (3 μM)-sensitive component of the whole-cell current elicited by voltage pulses from −30 to −60 mV was virtually absent in the REST-overexpressing neurons. Bottom panel shows whole-cell current-clamp traces in which the voltage was adjusted to −65 mV by current injection and 4 s square current pulses to different test currents applied. Consistent with the decrease in M-current density, REST-overexpressing neurons were overexcitable. (a, b; From Mucha, M. et al., J. Neurosci., 30, 13235, 2010.) (c) Reciprocal changes in KCNQ2 and REST expression in DRG neurons following neuropathic injury in rat. Top panel shows KCNQ2 immunostaining of DRG sections from control animal (sham operated) and from rat with partial sciatic nerve ligation 30 days postsurgery (partial sciatic nerve ligation, PSNL) or control (sham) animals. Bottom left shows western blots of the nuclear-enriched fraction of DRG neurons from sham-operated and neuropathic animals using antiepileptogenic protective role for activity-induced upregulation of M-channels in the brain. (f) AKAP79/150 model. Shown schematically are the signaling-protein complexes recruited to M-channels and L-type CaV1.3 (L-channels) by AKAP79/150 that underlie receptor-specific modulation of M-channels, PKA phosphorylation of L-channels, and upregulation of M-channel transcriptional expression. Left, arranged in a complex around KCNQ2/3 channels are CaM, AKAP79/150, PKC, and the Gαi-coupled M, or AT, receptor types, and PIP2, bound to both the channels, and AKAP. Right, clustered to L-channels is AKAP79/150, which recruits CaN and PKA, the latter mediating upregulation of L-channel currents. In response to activity, Ca2+ influx through L-channels calcifies CaN, dephosphorylating NFATs, revealing nuclear localization signals that direct NFATs into the nucleus, where they act on NFAT-binding regulatory domains of KCNQ2 and KCNQ3, increasing their transcription. (d–f; From Zhang, J. and Shapiro, M.S., Neuron, 76, 1133, 2012.)
density and increased tonic excitability of these neurons (Figure 20.9b; (408)). Tonic expression of REST in peripheral neurons is believed to be low, but it may increase greatly following inflammation (408) or after neuropathic injury (240,411). Interestingly, dramatic reduction in KCNQ2 (240,389) and KCNQ3 (389) transcripts and proteins following experimentally induced chronic pain development has been reported. It has therefore been suggested that M-channel downregulation upon the development of chronic pain can be mediated by the transcriptional suppression by REST. Indeed, increase of REST immunoreactivity and mRNA after nerve injury mirrored the reciprocal reduction in KCNQ2 transcripts and KCNQ2 protein levels (Figure 20.9c; (240)). It is likely that transcriptional suppression of KCNQ2 and KCNQ3 expression in nociceptive neurons contributes to the long-lasting hyperexcitability of peripheral fibers observed in chronic pain states.

20.7.2 Regulation by NFAT

Recently, activity-dependent upregulation of M-channels at the transcriptional level has been reported in rodents, mediated by the Ca\(^{2+}\)-dependent phosphatase, calcineurin (CaN), nuclear-factor of activated T-cells (NFATs) transcription factors, and the same AKAP79/150 scaffold proteins mediating PKC phosphorylation (Figure 20.9d through f; (191)). Those authors found the reporter of neuronal activity to be Ca\(^{2+}\), L-type Ca\(^{2+}\) channels, to which AKAP79/150 recruits CaN as the Ca\(^{2+}\) sensor, similar to the role of Ca,1.2 channels (412). Upon binding Ca\(^{2+}\), CaN dephosphorylates NFAT (413), which then translocates into the nucleus where it interacts with NFAT-binding regulatory elements of KCNQ2 and KCNQ3 genes, resulting in augmented mRNA levels and increased I\(_M\) amplitudes (191). If CaN activity is suppressed, L-type channels blocked, or AKAP150 knocked out in the animal, both effects were completely absent. Simultaneous imaging of GFP-tagged NFATs and \([Ca^{2+}]_i\) revealed selectivity for NFATc1 and NFATc2, and a time course of NFAT translocation to the nucleus much slower than that of [Ca\(^{2+}\)], rises induced by high-[K\(^+\)], or stimulation of nicotinic AChRs in sympathetic neurons, probably reflecting this NFAT pathway acting as a working memory of Ca\(^{2+}\) signals (414). The L-channel/CaN/NFAT pathway seen by Zhang and Shapiro (191) is similar to that first described in hippocampal neurons (415), suggesting a mechanism conserved in the CNS and PNS. Indeed, upon induction of a single chemoconvulsant seizure in mice, mRNA for KCNQ2 and KCNQ3 in the hippocampus was found to be increased by 3- to 30-fold, an effect likewise wholly absent in AKAP150 KO mice (191). Thus, transcriptional upregulation of I\(_M\) may represent an antiepileptogenic mechanism, acting to prevent an isolated seizure from progressing to full-blown epileptic disease.

Finally, increased transcription of KCNQ2 and KCNQ3 genes by the transcription factor Sp1 has also been described (408); however, the physiological relevance of this regulation remains to be determined.

20.8 Concluding Remarks

KCNQ/M-type channels have emerged as pivotal players in diverse excitable and nonexcitable tissues. In the former, they play powerful roles in control over electrical and chemical excitability, with corresponding effects on cognitive, autonomic, somatosensory, rhythmic activity, and visceral function. In the latter, M-type currents are crucial to normal K\(^+\) flux and homeostasis. Not unexpectedly, dysfunction of KCNQ channels leads to severe dysfunction throughout the body and is seen as one of the hottest targets for novel modes of therapeutic intervention against a wide spectrum of human diseases. Equally logical is the employment of many modes of regulation of M-currents in cells in all of these tissues, mediated by G proteins, Ca\(^{2+}\)-binding proteins, kinases/phosphatases, lipid-signaling molecules, gasotransmitters, and regulators of transcription. Thus, not only are changes in KCNQ channels themselves exerting powerful actions on cellular states of excitability or activity, but subtle alterations among the plethora regulatory pathways and auxiliary subunits described in this chapter are expected to manifest critically in KCNQ current activity, with correspondingly powerful control over organismal function.

REFERENCES


KCNQ channels


KCNQ channels


Ion channel families

KCNQ channels


211. Nakajo, K. and Y. Kubo. 2007. KCNE1 and KCNE3 stabilize and/or slow voltage sensing S4 segment of KCNQ1 channel. J Gen Physiol 130:269–281.


KCNQ channels


