MinK, MiRP1, and MiRP2 Diversify Kv3.1 and Kv3.2 Potassium Channel Gating*

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High frequency firing in mammalian neurons requires ultra-rapid delayed rectifier potassium currents generated by homomeric or heteromeric assemblies of Kv3.1 and Kv3.2 potassium channel α subunits. Kv3.1 α subunits can also form slower activating channels by co-assembling with MinK-related peptide 2 (MiRP2), a single transmembrane domain potassium channel ancillary subunit. Here, using channel subunits cloned from rat and expressed in Chinese hamster ovary cells, we show that modulation by MinK, MiRP1, and MiRP2 is a general mechanism for slowing of Kv3.1 and Kv3.2 channel activation and deactivation and acceleration of inactivation, creating a functionally diverse range of channel complexes. MiRP1 also negatively shifts the voltage dependence of Kv3.1 and Kv3.2 channel activation and deactivation and acceleration of inactivation. Furthermore, MinK, MiRP1, and MiRP2 each form channels with Kv3.1-Kv3.2 heteromers that are kinetically distinct from one another and from MiRP/homo-meric Kv3 channels. The findings illustrate a mechanism for dynamic expansion of the functional repertoire of Kv3.1 and Kv3.2 potassium currents and suggest roles for these α subunits outside the scope of sustained rapid neuronal firing.

The distinct electrical properties of different subtypes of neurons are determined by the biophysical characteristics of the intrinsic voltage-dependent conductances that they express. Although neuronal voltage-gated sodium currents are comparatively uniform, a highly varied array of voltage-gated potassium (Kv) currents exist in mammalian brain, generated by a family of Kv channel α subunits with diverse properties and augmented by alternative splicing, extensive regulation, and co-assembly with ancillary subunits. Thus, the fine balance of Kv channel subunits expressed in each neuron is highly influential in setting neuronal resting membrane potential, regulating firing patterns and action potential phenotype, and modulating neurotransmitter release (1, 2).

One Kv channel subfamily, Kv3, is of particular interest in neuronal physiology; Kv3 α subunits form channels with ultra-rapid activation and deactivation kinetics and an especially depolarized activation voltage, activating only when the membrane potential is more positive than ~10 mV (3). These biophysical properties, combined with the expression patterns of Kv3 α subunits in mammalian brain, support a central role for Kv3 channels in determining the ability of neurons to follow high frequency input and sustain rapid firing (3–9). Four mammalian Kv3 genes have been identified, each of which generates by alternative splicing multiple protein products (3). Kv3.1 and Kv3.2 genes express very similar delayed rectifier K⁺ currents in heterologous expression systems, whereas Kv3.4, and at least one splice variant of Kv3.3, can generate A-type (fast inactivating) currents (10, 11). Kv3.1 and Kv3.2 are highly enriched in neurons that fire at high frequencies, such as fast spiking interneurons of the cortex and hippocampus (9, 12), and neurons in the globus pallidus (7, 13). Their unusually rapid activation and deactivation rates allow channels containing Kv3.1 and Kv3.2 α subunits to repolarize action potentials quickly without compromising the threshold for action potential generation by promoting a rapid afterhyperpolarization period, thus minimizing the rate of recovery of sodium channel inactivation. Pharmacological or genetic disruption of Kv3 currents leads to impaired fast spiking in inhibitory neurons and increased seizure susceptibility (6). Furthermore, computational modeling suggests that the unique activation and deactivation rates of Kv3.1 and Kv3.2 channels are kinetically optimized for high frequency action potential generation (9, 14, 15).

Kv3 α subunits are also expressed in non-fast spiking neurons and other cell types (16, 17), suggesting they perform functions other than to sustain rapid firing, despite their perfect adaptation for this task. One mechanism by which Kv α subunits can exhibit varied behavior, thus expanding their potential physiological roles, is by associating with ancillary subunits that alter the functional properties of the resultant mixed channel complex. A family of ancillary subunits, the MinK-related peptides (MiRPs, encoded by KCNE genes), are thought to play a particularly broad role in shaping Kv currents in mammalian heart (18, 19). Recently MinK-related peptide 2 (MiRP2) was demonstrated to form complexes in mammalian brain with Kv3.1 resulting in channels with slowed activation and a reduced ability to support fast spiking in computer simulations (20). Here, we demonstrate that co-assembly with MinK, MiRP1, or MiRP2 is a general mechanism for diversifying the gating of currents generated by Kv3.1 and Kv3.2 α subunits. MiRP-Kv3 channels are, to various degrees, slower activating and deactivating but faster inactivating than MiRP-free Kv3 channels, supporting a role beyond rapid spiking for MiRP-Kv3 channels. The data raise the possibility that MiRP-Kv3 channels may underlie a variety of voltage-gated currents in non-rapid firing cells in brain and other tissues.
MiRP1s Diversify Kv3 Channel Gating

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Chinese Hamster Ovary (CHO) cells were cultured in α minimum essential medium (containing α-glutamine, HEPES, and deoxyribonucleosides; Invitrogen, San Diego, CA) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin and streptomycin at 37 °C in a humidified incubator, gassed with 5% CO2/95% air. CHO cells were cultured in 75-cm2 flasks. On reaching full confluency, the monolayer was washed once with Ca2+- and Mg2+-free phosphate-buffered saline and incubated with 0.05% Trypsin-EDTA (Fisher Scientific, Suwanee, GA) for ≤1 min. The cells were then resuspended in supplemented α minimum essential medium and plated either at a 1:25 dilution into a new 75-cm2 flask, or at a 1/5 dilution in sterile 60-mm culture dishes for transfection. Cell culture medium was changed every 2–3 days and the cells passaged every 4 days.

To study Kv3 channels in the presence and absence of MiRPs, wild-type CHO cells were transiently transfected with cDNA for rat Kv3.1b and/or rat Kv3.2α (a kind gift from B. Rudy) alone or in combination with cDNA for rat MinK, rat or human MiRP1, or rat or human MiRP2, all in cytomegalovirus-based expression vectors. When investigating the effects of MiRPs on heteromeric Kv3 channels, quantities of each transfected α subunit cDNA were adjusted in order to give a sum current density (MiRP-free) similar to that observed when expressed individually (MiRP-free). After reaching 70–80% confluency in 60-mm dishes, CHO cells were rinsed once with phosphate-buffered saline and transfected using SuperFect™ transfection reagent (Qiagen, Hilden, Germany) following the manufacturer’s protocol. Cells were co-transfected with a plasmid containing the cDNA encoding the reporter protein (Qiagen, Hilden, Germany) to determine transfection efficiency. For immunoprecipitation studies, haemagglutinin (HA)-tagged constructs of MinK and MiRP1 were used to facilitate detection with a monoclonal anti-HA antibody. Experiments were carried out 24–48 h post-transfection.

Co-immunoprecipitation—CHO cells transfected with the appropriate channel subunits were lysed 24 h later with immunoprecipitation (IP) buffer: 150 mM NaCl, 50 mM Tris-Cl (pH 7.4), 20 mM NaF, 10 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride (Fisher Scientific), 1% Nonidet P-40 (Pierce, Rockford, IL), 1% CHAPS (Sigma, St. Louis, MO), 1% Triton X-100 (Fisher Scientific), and 0.5% SDS (Sigma). Supernatants were pre-cleared with protein A-Sepharose 4B beads (Amersham Biosciences, Arlington Heights, IL) for 20 min at 4 °C; these beads were collected by centrifugation at 5,000 × g for 3 min and discarded.Pre-cleared supernatants were incubated with polyclonal rabbit antibodies for immunoprecipitation: either anti-Kv3.1 (Sigma) or anti-Kv3.2 (Alomone Laboratories, Jerusalem, Israel). After 5–12 h at 4 °C, protein A-Sepharose 4B beads were added and the mixture incubated for a further 2 h at 4 °C. The complexed beads were collected by centrifugation, washed for 4 × 15 min then immunoprecipitated complexes were eluted by incubating the beads for 20 min at 37 °C in SDS-PAGE loading buffer: 10% dithiothreitol, 0.2% bromophenol blue, 4% SDS, and 20% glycerol in 100 mM Tris-Cl buffer, pH 6.8. After centrifugation, the resulting bead eluates were separated by SDS-PAGE on 15% gels. After transfer onto polyvinylidene difluoride membranes, blots were probed with either monoclonal mouse anti-HA antibodies or polyclonal rabbit anti-Kv3.1 antibodies (Sigma). Secondary antibodies were horse-radish peroxidase-coupled goat anti-mouse or anti-rabbit IgG as appropriate (Bio-Rad, Hercules, CA) to facilitate subsequent detection by fluorography.

Electrophysiological Analysis—Electrophysiological recordings from CHO cells were performed by resistive-feedback voltage-clamp under cell voltage-clamp experiments. For immunoprecipitation studies, he

RESULTS

Rat MiRP and MiRP1 Form Stable Complexes with Kv3.1 and Kv3.2 α Subunits—Previously, MiRP2 was shown to form stable complexes with Kv3.1 α subunits. Here, co-immunoprecipitations were performed to assess whether Kv3.1 or Kv3.2 α subunits form stable complexes with MinK and MiRP1 when expressed in CHO cells. Kv3 α subunits were immunoprecipitated with appropriate anti-α subunit antibodies, and co-immunoprecipitated HA-tagged rat MinK or MiRP1 (Fig. 1A) were probed for with anti-HA antibodies by Western blotting. First, Western blots of lysates expressing HA-tagged rat MinK or MiRP1 alone or with Kv3 α subunits showed bands at ~25 kDa (as expected for fully glycosylated MinK and MiRP1) that were absent in lysate from non-transfected CHO cells, indicating expression and detection of either KCNE subunit when expressed alone or with Kv3 α subunits (Fig. 1B). Immunoprecipitations with antibodies against Kv3.1 or Kv3.2 gave a band of ~25 kDa in Western blots probed with anti-HA antibody when either α subunit was co-expressed with rat MinK or rat MiRP1 (Fig. 1, C and D). Rat MinK or MiRP1 did not immunoprecipitate with any of the anti-Kv3.3 α subunit antibodies when expressed in the absence of the α subunit, neither did immunoprecipitation in cells expressing α subunits alone, but no KCNE subunits give an anti-HA band at 25 kDa, indicating specificity of the previous positive immunoprecipitations (Fig. 1, C and D). The additional band sizes exhibited by MiRP1 in panel D probably represent immature forms of MiRP1 (MiRP1 contains two glycosylation sites). The results suggest that rat variants of MinK and MiRP1 form stable complexes with rat Kv3.1 and Kv3.2 when co-expressed in CHO cells. Finally, as previously reported (7), Kv3.1 and Kv3.2 α subunits formed heteromeric complexes when co-expressed, as assessed by co-immunoprecipitation using anti-Kv3.2 antibodies followed by Western blotting and detection with anti-Kv3.1 antibodies (Fig. 1E). The two upper bands observed in this blot are probably due to alternative post-translationally modified forms of Kv3.1, which can be glycosylated and also phosphorylated (5). The lower band of the three is the immunoprecipitating rabbit anti-Kv3.2 antibody, recognized by the goat anti-rabbit secondary antibody.

Rat MinK, MiRP1, and MiRP2 alter Kv3.1 Gating—Transfection of CHO cells with Kv3.1 gave ultra-rapid activating and deactivating currents as previously described (22) (Fig. 2A). Co-transfection of either rat MinK, rat MiRP1, or rat MiRP2 produced a slowing of both activation and deactivation (Fig. 2, A and B). Interestingly, co-transfection with the human variant
of MiRP1 had no effect on any of the attributes of rat Kv3.1 channels, despite efficient human MiRP1 expression, assessed both by Western blot and by ability to modulate human ether-a-go-go related gene currents (Protocol 3, data not shown). Furthermore, effects of rat MiRP2 on Kv3.1 gating kinetics were quantitatively different from those previously observed for human MiRP2 on rat Kv3.1 under similar conditions, and rat MiRP2 significantly increased Kv3.1 current density, opposite to the effect observed for human MiRP2 (20). Rat MiRP1 also shifted the voltage dependence of Kv3.1 activation 9 mV more negative, whereas rat MinK and MiRP2 had no effect on this parameter (Fig. 2C). Normalization of the first 100 ms of exemplar traces at 0 mV emphasizes the slowing of Kv3.1 activation by MinK, MiRP1, and MiRP2 (Fig. 2D). This slowing of activation rate was quantified (Fig. 2E), revealing a significant increase (~2- to 4-fold) in the time constant of activation (\( \tau_{act} \)) at all activating voltages. Deactivation rates at ~30 mV were fitted with double exponential functions (Fig. 2F), revealing an 80–100% increase in the \( \tau \) of both slow and fast components of Kv3.1 deactivation and a 50% increase in the amplitude of the slow component when co-expressed with rat MinK or MiRP1; rat MiRP2 similarly slowed the fast component but had less effect on the slow component and no effect on the relative amplitudes (Fig. 2G). Effects on Kv3.1 gating are summarized in Table I. The data indicate that rat MinK, MiRP1, and MiRP2 modulate Kv3.1 channel function to create distinct channel complexes with unique activation and deactivation properties.

Rat MinK, MiRP1, and MiRP2 Slow Kv3.2 Activation and Deactivation—Kv3.2 \( \alpha \) subunits form delayed rectifier potassium channels characterized by ultra-rapid activation and deactivation kinetics making them ideally suited to promoting fast spiking in neurons (3). Functionally, homomeric Kv3.2 channels are almost identical to homomeric Kv3.1 channels (7). In CHO cell co-expression experiments rat MiRP1 caused a slight (~6 mV) negative shift in the voltage dependence of Kv3.2 activation and rat MiRP2 significantly increased Kv3.2 current density (Fig. 3, A–C). All three MiRPs significantly slowed Kv3.2 activation, illustrated in Fig. 3D, in which the time base has been expanded to show the initial 100 ms of activation at 0 mV. Fitting of the Kv3.2 activation rate over a range of voltages revealed a 4- to 6-fold increase in the mean time constant of activation (\( \tau_{act} \)) with rat MinK or MiRP1 and a 2- to 3-fold increase with rat MiRP2 (Fig. 3E). Co-expression with rat MinK, MiRP1, or MiRP2 also slowed the deactivation rate of Kv3.2 channels (Fig. 3F). Deactivation kinetics were quantified by fitting the deactivating tail at ~30 mV with a double exponential function, indicating a doubling of both components of deactivation by MinK, a 3-fold slowing with MiRP1, and lesser effects with MiRP2. MinK and MiRP1 also both significantly increased the amplitude of the slow component (Fig. 3G). Thus rat MinK, MiRP1, and MiRP2 form functionally distinct channel complexes with Kv3.2. Effects on Kv3.2 gating are summarized in Table I. Human MiRP1 had no effects on Kv3.2 function, and effects of human MiRP2 were quantitatively different from those of rat MiRP2, again indicating species specificity of MiRP action (data not shown).

Rat MinK, MiRP1, and MiRP2 Form Functionally Distinct Channels with Heteromeric Kv3.1–Kv3.2 Channels—Like other Kv \( \alpha \) subunits, Kv3 \( \alpha \) subunits can form intra-subfamily heteromeric \( \alpha \) subunit complexes. Consistent with previous observations (7), Kv3.1 and Kv3.2 formed heteromeric Kv3.1-Kv3.2 channels when co-expressed in CHO cells in the present study, generating a current essentially indistinguishable from the current produced by the corresponding parental homomers. Co-expression with rat MinK, MiRP1, or MiRP2 altered this
phenotype, slowing both current activation and deactivation (Fig. 4, A and B) and generating a modest degree of current inactivation, discussed in more detail below (Fig. 5). MiRP1 also reduced Kv3.1-Kv3.2 heteromer current density 2-fold, and negative-shifted the voltage dependence of activation by 12 mV, whereas MiRP2 significantly increased Kv3.1-Kv3.2 current density (Fig. 4, A–C). Normalization of the first 100 ms of exemplar traces showing activation to peak at 0 mV demonstrated that rat MinK, MiRP1, and MiRP2 all slowed the activation rate of heteromeric Kv3.1-Kv3.2 currents (Fig. 4D). This slowing of activation rate was quantified (Fig. 4E), revealing a significant increase (~5- to 12-fold with rat MinK; ~10- to 17-fold with rat MiRP1; ~2- to 3-fold with rat MiRP2) in the mean time constant of activation ($\tau_{\text{act}}$). Association with rat MinK, MiRP1, or MiRP2 also slowed Kv3.1-Kv3.2 channel deactivation (Fig. 4F). Deactivation rates at ~30 mV were fitted with double exponential functions (Fig. 4G), revealing for rat MinK an approximate doubling in both the $\tau$ of slow and fast components of deactivation; for rat MiRP1 a 2- to 3-fold increase, and for both a doubling of the relative amplitude of the slow component of deactivation. MiRP2 produced a subtler slowing of deactivation.

Human MiRP1 had no effects on the functional attributes of Kv3.1-Kv3.2 channels (data not shown).

**Rat MinK, MiRP1, and MiRP2 Modulate Kv3.1 and Kv3.2 Channel Inactivation**—Kv3.1 and Kv3.2 channels exhibit little slow (C-type or U-type) inactivation over short-duration voltage pulses (hundreds of milliseconds). Over longer duration voltage pulses (seconds), slow inactivation becomes evident (3). Furthermore, Kv3.1 and Kv3.2 channels are not significantly inactivated by depolarizing prepulses (7) and display little cumulative inactivation (23). However, recordings of Kv3.1 and Kv3.2 currents in mammalian cells often demonstrate a significant initial fast transient component (see Figs. 2–4), which resembles rapid inactivation of a subset of the channel population. This phenomenon has been well documented and probably arises from local extracellular potassium accumulation due to the rapidity of Kv3.1 and Kv3.2 activation (see "Discussion") (3, 5, 24–26). In all cases, co-expression with rat MinK, MiRP1, or MiRP2 substantially reduced or eliminated this transient component, but in many cases an enhanced degree (compared with MiRP-free currents) of slow inactivation was...
observed throughout the duration of the voltage pulse. These observations were extended both by recording and quantifying slow inactivation during longer duration voltage pulses, and quantifying changes in the transient current component, that occur when Kv3.1 and Kv3.2 α subunits are co-expressed with MiRPs (Fig. 5). Exemplar traces illustrate a significant increase in both the rate and extent of slow inactivation of Kv3.1–Kv3.2 currents upon co-expression with MinK (Fig. 5A). The rates of slow inactivation of a variety of Kv3-MiRP complexes were quantified by assessing the time taken to inactivate by 25% at +40 mV (because the rate and limited extent of slow inactivation in MiRP-free Kv3 channels preclude accurate fitting of parameters such as $\tau_{\text{inactivation}}$) (Fig. 5B). Although no significant effects on the slow inactivation of Kv3.1 currents were observed, rat MinK, MiRP1, and MiRP2 each roughly halved the time taken for Kv3.2 currents to inactivate by 25% (Fig. 5, A and B; n = 5–14 cells per group). Rat MinK and MiRP1 also doubled the rate of slow inactivation of heteromeric Kv3.1-Kv3.2 channels, whereas rat MiRP2 had no effect (Fig. 5, A and B). Cumulative inactivation was measured by the decrease in peak current after 20 successive 1-s pulses to +40 mV; the only significant effects were an increase in cumulative inactivation of Kv3.1-Kv3.2 heteromers by rat MinK and MiRP1 (n = 7–19, summarized in Table I).

The reduction or loss of the characteristic Kv3.1 and Kv3.2 transient current component upon co-expression with rat MinK, MiRP1, or MiRP2 was quantified separately as it occurred over a much shorter timescale than classic slow inactivation (Fig. 5, C–E). Currents recorded 50 ms after the start of the voltage pulse were compared with corresponding peak currents at positive voltages. The results demonstrate that the transient current component increased with voltage for Kv3.1 (Fig. 5C), Kv3.2 (Fig. 5D), and heteromeric Kv3.1-Kv3.2 (Fig. 5E) channels, and that co-expression of rat MinK, MiRP1, or MiRP2 eliminated or greatly reduced current decay over the first 50 ms in Kv3.1, Kv3.2, or Kv3.1–Kv3.2 channels (Fig. 5, C–E). Human MiRP1 had no effects on either slow inactivation or the initial transient component of Kv3.1, Kv3.2, or Kv3.1–Kv3.2 currents (data not shown). Effects on slow inactivation and the transient current component are summarized in Table I.

### DISCUSSION

The functional diversity of potassium channels underlies the complex nature of repolarization in excitable tissues, and unique cellular electrophysiological characteristics derive from expression of specific ion channel subunits. Kv3 channels are highly expressed in mammalian brain, often in neuronal cell types with fast spiking action potential phenotypes (see Ref. 4 for review). Kv3.1 and Kv3.2 channels are characterized by their ultra-rapid activation and deactivation, and activation at membrane potentials more positive than −10 mV. Biophysically, Kv3.1 and Kv3.2 channels currents are virtually indistinguishable and can be separated only by their different sensitivities to phosphorylation by protein kinase A (27). Kv3.1 and Kv3.2 have distinct expression patterns throughout the brain, but their expression overlaps in certain neuronal populations, particularly in the anterior part of the brain that includes the cortex, hippocampus, and globus pallidus. These data together with biochemical and electrophysiological data from native and heterologous systems suggest that Kv3.1 and Kv3.2 can form functional heteromeric complexes in certain cells (3). Thus, channels formed by homeric or heteromeric complexes of Kv3.1 and Kv3.2 α subunits are considered central determinants of rapid firing in neurons of the globus pallidus and interneurons of the hippocampus (6, 7, 9, 12, 13, 28).

Kv3 α subunits are also expressed in neuronal subtypes that...
do not exhibit rapid firing (16, 17). We previously demonstrated that Kv3.1 forms native complexes with MiRP2 in rat brain and that the resultant slowing of activation was sufficient to limit the frequency of action potential trains in computer simulations of neuronal firing (20). Here, we report that Kv3 channel properties are highly regulated by rat MinK, MiRP1, and MiRP2. Each subunit combination produced a functionally unique channel complex, creating a far wider array of gating properties than is generated by the Kv3.1 and Kv3.2 subunits alone (Fig. 6). This diversification expands the potential role of Kv3.1 and Kv3.2 channels and may be representative of the general role of MiRPs in physiology. Rather than forming a small group of distinct, rigid partnerships, MiRPs appear to provide a combinatorial increase in the already diverse array of potassium currents that Kv α subunits can generate when acting without MiRPs. A notable aspect of delayed rectifier Kv3 current modulation was the loss of the transient current component, previously attributed to local extracellular potassium ion accumulation because of the rapid activation of Kv3 channels, partly because it is abolished in the presence of 100 mM external K\(^{+}\) (26). Our data are consistent with this hypothesis, because the slowing of Kv3 activation by MinK, MiRP1, and MiRP2 would be predicted to alleviate the K\(^{+}\) ion accumulation and thus the transient current component. Aside from effects on gating kinetics, rat MiRP1 negative-shifted the voltage dependence of Kv3.1, Kv3.2, and Kv3.1-Kv3.2 activation, and

![Figure 3](image-url)

**Fig. 3. Effects of MinK, MiRP1, and MiRP2 on Kv3.2 activation and deactivation.** Voltage-clamp studies of CHO cells transfected with rat Kv3.2 alone or with rat MinK, MiRP1, or MiRP2. Data are represented as mean ± S.E. Asterisks denote statistical significance: single asterisk, \(p < 0.05\); double asterisk, \(p < 0.01\). All values are stated in Table I. A, exemplar traces showing currents recorded using Protocol 1 (inset) in CHO cells transfected with Kv3.2 alone or co-transfected with MinK, MiRP1, or MiRP2 as indicated. B, mean peak current density from CHO cells transfected with Kv3.2 alone (filled squares, \(n = 24\)) or with MinK (open circles, \(n = 17\)), MiRP1 (open triangles, \(n = 16\)), or MiRP2 (open squares, \(n = 15\)) as in panel A. Significant difference was observed at voltages between +20 and +60 mV. C, mean normalized conductance-voltage relationship for currents as in panel B; data were fit with a Boltzmann function. D, normalized exemplar traces showing expanded view of activation at 0 mV. E, mean activation rates at voltages between −10 mV and +60 mV, fitted with a single exponential function, expressed as \(\tau_{\text{act}}\). F, normalized exemplar traces showing expanded view of deactivation at −30 mV. G, mean deactivation rates of Kv3.2, Kv3.2-MinK (+ MinK\(^{o}\)), Kv3.2-MiRP1 (+ M1), and Kv3.2-MiRP2 (+ M2) currents at −30 mV as in panel B, fitted with a double exponential function, showing slow and fast \(\tau_{\text{deact}}\) components.
reduced Kv3.1-Kv3.2 current density 2-fold. In a previous study of A-type Kv3.4 subunits, human MiRP2 caused a dramatic negative shift in the voltage dependence of activation (29). This heterogeneity of effects on closely related subunits is consistent with previous studies of, for example, KCNQ modulation: MinK greatly slows KCNQ1 activation, whereas MiRP1 and MiRP2 uncouple the voltage dependence of the majority of KCNQ1 current; MiRP2 reportedly suppresses KCNQ4 currents in oocytes; MiRP1 alters the gating kinetics of KCNQ2-KCNQ3 heteromers (30–36).

The slowing of Kv3.1, Kv3.2, and Kv3.1-Kv3.2 activation and deactivation by MinK, MiRP1, and MiRP2 has predicted ramifications for the electrical phenotype of neurons in which these subunits are expressed. The slower gating would be predicted to set a much lower firing frequency than that observed in cells expressing Kv3.1 and Kv3.2 subunits alone. This is a possible explanation for the existence of Kv3.1 and Kv3.2 subunits in non fast-spiking neurons such as certain somatostatin- and calbindin-containing neocortical interneurons (16, 17), and further studies are required to examine the presence and role of MiRPs in these neurons. Speeding of Kv3 slow inactivation by MiRPs also has potential physiological implications. In an ele-
Giant study by Lien and Jonas (14), dynamic clamp techniques were applied to CA1 oriens alveus interneurons in hippocampal slices, illustrating that slowing of Kv3.1 deactivation rate or increasing the rate of Kv3.1 inactivation severely impaired the ability of these neurons to fire rapid trains of action potentials. Modulation of Kv3 slow inactivation by MiRPs presents a potential mechanism for controlling long trains or ultra-rapid bursts of action potentials. A wide range of firing frequencies are required even within distinct specialized neuronal populations, and differences in the firing properties of subsets of fast spiking neurons are evident within the medial nucleus of the trapezoid body, medial vestibular nucleus, and interneurons of the hippocampus and neocortex, all of which are characterized by high frequency firing and all of which express Kv3 channel members (6). Although suggested to be a function of variations in expression of other ionic conductances, heterogeneity in the expression of Kv3 ancillary subunits such as MiRPs could equally contribute to this variation. Hence, MiRPs could function as dynamic toggles in neurons expressing Kv3 subunits, providing a simple mechanism for the modulation of current phenotypes in response to defined cellular signals, environmental cues, and perhaps during neuronal development or disease states.

MiRP2 expression in the brain was recently demonstrated at the mRNA and protein levels, with transcripts being detected in such areas as hippocampus, cerebellum, cerebral cortex, and temporal, occipital, and frontal lobes of the brain (20). MiRP1 transcripts are reported in neocortex, cerebellum, and hippocampal formation (33). Studies of MinK expression in neu-
rons have led to inconclusive results, with most but not all studies, relying on mRNA detection, indicating a failure to observe MinK expression (37–39). Although widely expressed in neuronal tissues, Kv3.1 and Kv3.2 show limited expression outside the brain, but both MinK and Kv3.1 are expressed in T-lymphocytes (22), and one group reports expression of Kv3.1 in canine heart (40), raising the possibility of association with MinK, which is highly expressed in cardiac tissue (41).

When considering the physiological implications of Kv3 modulation by MiRPs, the species specificity of the effects of MiRP1 and MiRP2 observed here are potentially of great significance. Studies are currently underway to determine whether the lack of effect of human MiRP1 is also observed when human variants of Kv3.1 and Kv3.2 are used, although in previous studies both rat and human MiRP1 modulated human ether-a-go-go related gene channels to similar degrees (19, 42–45). Studies are also underway to discover which of the sequence differences between human and rat variants of MiRP1 and MiRP2 mediate the observed functional differences. Previously, opposite responses to PKC of guinea pig and rat or mouse MinK were observed, and alleviated by mutation of asparagine 102 to serine in guinea pig MinK (46, 47). Differences such as these highlight the necessity to state which MiRP species variants are being employed in particular experiments.

In summary, MinK, MiRP1, and MiRP2 specifically enhance the functional diversity of Kv3 subfamily delayed rectifiers. It is becoming apparent that promiscuous association with MiRPs provides a versatile mechanism for diversification of voltage-gated potassium currents. The significance of MiRPs in generating normal cardiac rhythm is emphasized by the pathological association of inherited mutations in MinK and MiRP1 (19, 42–45), yet given the promiscuity of these two KCNE-encoded subunits and the ability of some mutations to disrupt currents carried by several α subunit partners (33, 48–51), the etiology of arrhythmia associated with KCNE mutations may actually arise from disruption of a range of cardiac currents upon which MiRPs impinge. The recent findings here and elsewhere that neuronal channels can form functional partnerships with MiRPs in heterologous expression systems and in native brain tissue, both in mammals (20) and in invertebrates (52), illustrate the potential importance of MiRPs to neurophysiology and pathophysiology.

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