Variants of the KCNMB3 regulatory subunit of maxi BK channels affect channel inactivation

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Variants of the KCNMB3 regulatory subunit of maxi BK channels affect channel inactivation. Physiol Genomics 15: 191–198, 2003; 10.1152/physiolgenomics.00110.2003.—The steady-state and kinetic properties of the KCNMB3 regulatory subunits associated with calcium-activated potassium channels (BK channels) are presented. BK channels containing four sequence variants (V1–V4) in the four different isoforms of the β-subunit (β3α, β3b, β3c, and β3d) were expressed in Xenopus oocytes. Reconstituted BK channel inactivation ranged from none to around 90% inactivation. In particular, channels expressing the β3b-V4 variant displayed a right shift in the potassium current voltage-dependence of activation and inactivated to about 30% of the maximum conductance, compared with wild-type β3b channels that showed no inactivation. When the membrane potential was depolarized, BK channels inactivated with a very rapid time course (~2–6 ms). This same variant was previously demonstrated to show subtly higher incidence in patients with idiopathic epilepsy (IE) compared with controls, we have recently found four sequence variants which affect the amino acid sequence (M. Labuda, personal communication). Taken together, these data suggest that neurons expressing BK channels composed of the β3b-V4 variant subunit may experience reduced levels of inhibition and may therefore play permissive roles in high levels of neuronal activity that is characteristic of epilepsy.

POTASSIUM CHANNELS PLAY IMPORTANT roles in controlling the electrical excitability of nerve cells. There are several different classes of potassium channels including voltage-gated (Kv), inward rectifying (Kir), and calcium-activated potassium channels such as the large-conductance (BK) channels. BK channels are especially interesting in that they are activated by both cytoplasmic Ca2+ and membrane depolarization (10, 16, 17) and are therefore uniquely suited to repolarize the membrane during neuronal excitation and Ca2+ entry into the cell. Indeed, the large-conductance (BK) channels are highly concentrated at synaptic terminals where they play crucial roles in membrane repolarization and neurotransmitter release (11). In addition, they have also been suggested to affect the firing properties of neurons (1, 18). BK channels consist of pore-forming α-subunits (24), which are often associated with accessory β-subunits. The function of the β-subunits is to modulate the physiological, pharmacological, and kinetic properties of the channels (7, 13, 24–26). The recent cloning, sequencing, and characterization of several families of β-subunits underscores the diversity of BK channels and the possibility that these channels may play many different roles in the nervous system.

Beta subunits (β1–β4) are expressed in both peripheral tissues (3, 7, 19, 21, 22) and in the central nervous system (CNS) (3, 7, 19, 22), although their CNS expression appears to be less robust (21). The β3 family, also known as KCNMB3, has been reported to be present in human brain (2, 22). The family of KCNMB3 β-subunits consists of four members, termed β3a–d, which arise by alternative splicing of a single gene producing different amino termini. KCNMB3 maps to human chromosome 3q26.3-q27 (19), a region which is duplicated in a syndrome characterized by neurological anomalies and/or seizures. Furthermore, a susceptibility locus for common idiopathic generalized epilepsy (IGE) has been mapped to 3q26 (20). In a mutation search of patients with ideopathic epilepsy (IE; including both generalized and partial subtypes) and matched normal controls, we have recently found four KCNMB3 sequence variants which affect the amino acid sequence (M. Labuda, personal communication). The physiological consequences of these variants remain unknown. However, since ion channels have been implicated in numerous rare monogenic forms of epilepsy (reviewed in Ref. 12), it is possible that these β-subunit variant forms of KCNMB3 contribute to IEG in some patients.

We set out to determine the properties of BK channels expressing the variant forms of the β3a–d subunits. We found that the steady-state and kinetic properties of BK channels expressing some of the variant forms of the β-subunit were altered. In particular, a single base pair deletion resulting in a frame shift and truncation of the protein by 18 amino acids caused a
rightward shift in the activation curve and a rapid inactivation of the BK channel. These results suggest that variants of KCNMB3 may be involved in increased levels of neuronal excitability that is characteristic of epilepsy.

**MATERIALS AND METHODS**

**Sequence variants of KCNMB3.** KCNMB3 codes for four isoforms, each with a different 5′ end (β3a, accession no. NP_741979; β3b, NP_741980; β3c, NP_741981; and β3d, NP_055222). The location of the four sequence variants found in a previous study (M. Labuda, personal communication), are shown in Fig. 1. Variant 1 L71V (V1), located in exon 2, is a recognized single nucleotide polymorphism (SNP) (dbSNP:2276802) with a frequency of 0.973 for leucine. V2 (N161S) in exon 4 was reported previously by Uebele et al. (22). The remaining two variants, V3 (M226T) and V4 (delA750), both in exon 4, are novel. V4 is a single A deletion which causes a frame shift that changes 3 amino acids and then truncates the protein by 18 amino acids. Numbering of variants listed above is based on the sequence of isoform β3c.

**Expression of BK channels.** The four KCNMB3 subunits differ in their first exon and share exons 2 through 4 (22). Each isoform, with about 150 bp upstream from each translation start codon, was inserted into the KpnI-XhoI site of Xenopus expression plasmid pX77. This plasmid is a modified pGEM-42 plasmid with the Xenopus globin 3′-untranslated region (3′-UTR) inserted 3′ to the cloning site (courtesy of S. Sokol, Beth Israel Deaconess Medical Center and Harvard Medical School) and confirmed to be correct by sequencing. The four sequence variants of each of the four KCNMB3 subunits were produced through site-specific mutagenesis (4).

Plasmids containing the KCNMA1 α-subunit (a gift from Martin Wallner) and each of the KCNMB3 β-subunits were linearized by NotI and BamHI digestion, respectively, then transcribed by using the MessageMachine Kit (Ambion). The quality and quantity of the synthesized cRNAs were checked by electrophoresis.

**Mature Xenopus laevis** were obtained from a breeding colony at the University of Alberta. Ovary tissue was surgically harvested, and stage V and VI oocytes were isolated using partial collagenase digestion (collagenase A, 0.002 g/ml in Ca2+- and Mg2+-free ND96) followed by manual defolliculation. Oocytes were maintained in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1.1 mM MgCl2, 5 mM HEPES, 2.5 mM sodium pyruvate, 0.1 U/ml gentamicin, pH 7.4, 210 mosmol/kgH2O). After 24 h, ~5 ng of α-subunit cRNA with fivefold molar β-subunit (~5 ng) cRNA was injected into each Xenopus oocyte using a Drummond pipette (50 nl volume). Water injection (50 nl) served as a control. Oocytes were maintained at 18°C in ND96, and the K+ channel function was measured 3–6 days after injection.

**Electrophysiology.** All currents were recorded in the inside-out patch clamp configuration according to established procedures (8), and all voltages refer to the inside face of the membrane patch (intracellular surface) with reference to the pipette. Oocytes were devitellinized prior to recording, then transferred to the recording chamber on the stage of an upright microscope (Leica, model DM LFSA). Patches were obtained under visual observation, and seals usually resulted after 30–60 s of either light suction or a combination of light suction and mild hyperpolarization of the patch. Pipettes were pulled to a final resistance of 0.7–1.5 MΩ after filling with the pipette solution, and currents were recorded on an Axopatch 200B amplifier (Axon Instruments). Data was acquired at a rate of either 50 or 100 kHz (for KCNMB3b-containing channels) using a Digidata 1322A Interface (Axon Instruments) and low-pass filtered at 5–10 kHz (~3 dB, 8-pole Bessel filter). Recordings were performed in solutions containing symmetrical potassium. Extracellular (pipette) solution consisted of the following: 105 mM potassium gluconate, 10 mM KCl, 2 mM MgCl2, 10 mM HEPES, and 10 mM d-glucose. The intracellular (bath) solution consisted of 105 mM potassium gluconate, 10 mM KCl, 2 mM MgCl2, 10 mM HEPES, 10 mM EGTA, and 9.9 mM CaCl2. All solutions were adjusted to a pH of 7.2 with KOH. The combined EGTA and CaCl2 concentrations resulted in a free calcium concentration of 10 μM, as calculated using the free-calcium concentration computation of WEBMAXCLITE v1.00 (Stanford, CA).

**Data analysis and statistics.** Data was stored and analyzed offline using pCLAMP software 8.0. Graphs were plotted using SigmaPlot 7.0 (SPSS). Data are expressed as means ± SE. Statistical significance was tested with Student’s t-test.

**RESULTS**

**Xenopus** oocytes expressing the mRNA coding for either the α-subunit alone, or the α-subunit plus one of the wild-type or variant forms of the β-subunits, were examined to determine the steady-state and kinetic properties of the various BK channels. Inside-out patches were pulled from Xenopus oocytes 3–6 days post mRNA injection, since this gave a compromise

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Fig. 1. Protein sequence of the four KCNMB3 isoforms (β3a–β3d) showing rare variants (labeled V1 to V4). The four isoforms differ only at the amino terminus, as shown. Transmembrane domains are under-scored; substituted amino acids are in boldface; the deletion of adenine 750 (delA750, numbered using the β3c sequence) is marked with an arrowhead; the truncated part of the protein resulting from delA750 is in italics.
between high channel expression and good health of the oocyte. Inside-out patches were exposed to 10 μM free Ca\(^{2+}\), a value that had previously been determined to be sufficient for reasonable and adequate activation of the BK channels (3, 5, 21). Patches were then depolarized according to several different voltage-clamp protocols to determine steady-state channel properties. Negative controls (inside-out patches pulled from both H\(_2\)O-injected and uninjected oocytes) showed no evidence of channel activity under our recording conditions (data not shown).

**Potassium currents.** Oocyte inside-out patches were held at a potential of -100 mV before hyperpolarizing to -150 mV to remove inactivation. Membrane patches were then depolarized stepwise from -120 mV to +120 mV for a duration of 600 ms (Fig. 2). Currents obtained from channels composed of only the \(\alpha\)-subunit displayed no inactivation over the course of the experiment (\(n = 10\)). Similarly, wild-type channels composed of either \(\alpha + \beta3b\) or \(\alpha + \beta3d\) subunits did not inactivate when depolarized in the presence of 10 μM Ca\(^{2+}\) (\(n = 9–11\)). However, currents obtained from wild-type channels composed of \(\alpha + \beta3a\) and \(\alpha + \beta3c\) (\(n = 9–10\)) subunits inactivated to varying degrees throughout the depolarization protocols (Fig. 2). These results were comparable to previously published data (22). When currents were obtained from channels expressing the \(\alpha\)-subunit and one of the four variant forms (V1–V4) in the four families of \(\beta3\) subunits (\(\beta3a–d\)), only one variant (\(\alpha + \beta3b-V4\), Fig. 2, Table 1, \(n = 9\)) altered the general shape of the potassium currents compared with the wild-type currents. These channels inactivated, whereas the wild-type \(\alpha + \beta3b\) channels showed virtually no inactivation (Fig. 2 and 4). In a few patches (4 of 10) expressing the wild-type \(\beta3b\) subunit, the currents showed a small peak and then a barely detectable but rapid inactivation, similar to data recently published (22). No inactivation whatsoever was detected in the remaining wild-type patches (6 of 10), even when sampling at a very high acquisition fre-

![Fig. 2. Current traces from inside-out patches taken from Xenopus oocytes expressing the calcium-activated \(\alpha\)-subunit alone, or in combination with the \(\beta\)-subunit. Current recordings were obtained in symmetrical K-glucurate containing 10 μM free Ca\(^{2+}\). Patches were held at -100 mV before undergoing a hyperpolarization step to -150 mV to remove inactivation, immediately followed by 600-ms depolarizing steps from a range of potentials from -120 mV to +120 mV. **Inset:** current traces (from \(\alpha + \beta3b\) and \(\alpha + \beta3b-V4\)) on an expanded time scale to see the peak current and subsequent inactivation of the channels containing the mutant \(\beta3b-V4\) subunit. Vertical scale bars are 1 nA, except \(\alpha + \beta3a\) and \(\alpha + \beta3d\), which are 0.5 nA.](image-url)
Table 1. Activation and inactivation kinetics

<table>
<thead>
<tr>
<th>K⁺ Channel Expression</th>
<th>Activation</th>
<th>Inactivation</th>
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<tbody>
<tr>
<td></td>
<td>$V_{50}$, mV</td>
<td>Slope factor</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>-5 ± 2(9)</td>
<td>25.2 ± 2.5</td>
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<tr>
<td>$\alpha + \beta 3a$</td>
<td>6 ± 8(10)</td>
<td>17.9 ± 1.9</td>
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<tr>
<td>$\alpha + \beta 3a V 1$</td>
<td>-3 ± 9(9)</td>
<td>26.8 ± 2*a</td>
</tr>
<tr>
<td>$\alpha + \beta 3a V 2$</td>
<td>13 ± 7(9)</td>
<td>23.3 ± 1.5*a</td>
</tr>
<tr>
<td>$\alpha + \beta 3a V 3$</td>
<td>23 ± 5(13)</td>
<td>20.3 ± 0.8</td>
</tr>
<tr>
<td>$\alpha + \beta 3a V 4$</td>
<td>20 ± 8(10)</td>
<td>24.4 ± 0.9*a</td>
</tr>
<tr>
<td>$\alpha + \beta 3b$</td>
<td>38 ± 5(9)</td>
<td>21.2 ± 1.6</td>
</tr>
<tr>
<td>$\alpha + \beta 3b V 1$</td>
<td>37 ± 7(8)</td>
<td>29.1 ± 1.5*a</td>
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<tr>
<td>$\alpha + \beta 3b V 2$</td>
<td>47 ± 10(10)</td>
<td>23.0 ± 0.5</td>
</tr>
<tr>
<td>$\alpha + \beta 3b V 3$</td>
<td>47 ± 7(8)</td>
<td>33.5 ± 0.9*a</td>
</tr>
<tr>
<td>$\alpha + \beta 3b V 4$</td>
<td>81 ± 7(10)*</td>
<td>32.8 ± 1.1</td>
</tr>
<tr>
<td>$\alpha + \beta 3c$</td>
<td>29 ± 10(9)</td>
<td>26.8 ± 2.8</td>
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<tr>
<td>$\alpha + \beta 3c V 1$</td>
<td>21 ± 8(10)</td>
<td>22.7 ± 1.4</td>
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<td>50 ± 8(8)</td>
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<td>$\alpha + \beta 3c V 3$</td>
<td>32 ± 6(10)</td>
<td>18.6 ± 0.3</td>
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<tr>
<td>$\alpha + \beta 3c V 4$</td>
<td>29 ± 11(8)</td>
<td>23.5 ± 1.7</td>
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<tr>
<td>$\alpha + \beta 3d$</td>
<td>51 ± 12(9)</td>
<td>32.2 ± 3.4</td>
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<tr>
<td>$\alpha + \beta 3d V 1$</td>
<td>32 ± 6(11)</td>
<td>21.4 ± 1.7</td>
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<tr>
<td>$\alpha + \beta 3d V 2$</td>
<td>42 ± 7(9)</td>
<td>18.6 ± 1.6*a</td>
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<tr>
<td>$\alpha + \beta 3d V 3$</td>
<td>35 ± 8(11)</td>
<td>22.2 ± 2.4</td>
</tr>
<tr>
<td>$\alpha + \beta 3d V 4$</td>
<td>32 ± 10(10)</td>
<td>19.8 ± 1.5</td>
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Values are means ± SE; $n$ is in parentheses. $V_{50}$, half-activation potential. *Significance from wild type. NA, not applicable due to no inactivation.

quency of 100 kHz. The only subgroup within the $\beta 3b$ variant to obtain tail currents large enough to be measured accurately. The tail currents occurring at −80 mV are isochronal and isopotential and give an estimate of the extent of channel activation. The amplitude of the tail currents were plotted against the value of the depolarizing voltage steps (Fig. 3), and the resulting activation curves were fitted to a Boltzmann sigmoid (Fig. 3C). The half-activation potentials, at which 50% of the channels are activated ($V_{50}$), were estimated from the fits of each individual curve, from which the mean values were then calculated and shown in Table 1. The only channel that showed a significantly different $V_{50}$ for activation compared with the wild-type channels was the $\beta 3b$-V4 variant, whose $V_{50}$ (81 ± 7 mV; $n = 10$) is right-shifted compared with the wild-type value of 38 ± 5 mV ($n = 9$) (Fig. 3; Table 1; $P < 0.05$). We found the slope factors for several of the variant $\beta$-subunits were significantly different from the wild-type channels (Table 1), suggesting differences in the number of channels affected per unit change of the membrane potential.

Inactivation. The $\beta$-subunit confers a wide variety of inactivation properties to BK channels, including variations in the voltage dependence of inactivation, the rate of inactivation, and the proportion of inactivation (7, 24, 25). Voltage dependence and the proportion of inactivation were investigated using a paired-pulse protocol whereby inside-out patches were stepped to a range of potentials between −210 and 110 mV (10-mV steps). The depolarization during the first step was sufficiently long to completely inactivate the channels (Fig. 4, A and B). Patches were then immediately stepped to either +50 mV (for $\beta 3a$, $\beta 3c$, and $\beta 3d$) or +120 mV (for $\beta 3b$), to elicit current responses that were sufficiently large to be accurately measured. The amplitude of currents obtained during the second depolarizing step was plotted against the depolarizing potential during the first step and normalized to the maximum peak current. These inactivation curves were fitted with Boltzmann sigmoid (Fig. 4C). The potential of half-inactivation was measured for each individual curve for every experiment, and the mean value was calculated from the individual values. The proportion of inactivation of the variant channels was also estimated (Table 1).

Most interesting was the inactivation of $\beta 3b$-V4 by 68%, compared with wild-type channels that did not inactivate (Fig. 4C; Table 1). The $V_{50}$ for inactivation of $\beta 3b$-V4 was −22 ± 4 mV ($n = 9$). In addition, other variant channels also exhibited half-inactivation voltages that were significantly shifted with respect to the wild-type channels. In particular the $\beta 3c$-V2 ($V_{50} = −73 ± 6 mV$) exhibited a significant ($P < 0.05$) rightward shift in the voltage dependence of inactivation (Fig. 4 and Table 1) compared with the wild-type $\beta 3c$ channels ($V_{50} = −94 ± 7 mV$). The extent of inactiva-
tion also varied among variant channels, with the β3c-V1 and β3c-V2 channels exhibiting less inactivation compared with the wild type (Table 1). In addition, the β3a-V1 channels were ~90% inactivated within 300 ms of depolarization, compared with the β3a wild-type channels that showed only 62% inactivation under similar conditions (Fig. 4 and Table 1).

**Time course of inactivation.** The time course for inactivation of the currents (τ) was determined from the raw traces acquired during the current/voltage protocol. To measure τ, we fit the currents obtained during depolarizing steps to simple, single exponential fits, from the peak of the current to the baseline. These fits were performed over a range of depolarizations above +10 mV, since equimolar concentrations of K⁺ on either face of the patch resulted in no current at 0 mV, and only very little current at potentials immediately positive to 0 mV, making it either difficult or impossible to measure exponential decays at these potentials. The τ varied over the range of potentials tested for each channel (Fig. 5). Values recorded at two arbitrary levels of depolarization (+60 mV and +100 mV; Fig. 5, B, D, and F) reveal that at +60 mV the wild-type β3a channels inactivate with a time constant of 66.5 ± 10.7 ms (n = 6), which is significantly different from all of the β3a variant channels (Fig. 5B). When patches were held at +100 mV, only the V1 and V4 variants (27.6 ± 2.3 and 34.9 ± 3.4 ms, respectively) inactivated with a τ that was significantly different from wild-type β3a channels (65.2 ± 10.8 ms; n = 6; P < 0.05). Similarly, at +60 mV the wild-type β3c channels inactivate with a time constant of 68.0 ± 6.9 ms, which is significantly different from only the β3c-V2 variant channel (Fig. 5F; n = 8–10; P < 0.05). Whereas, at +100 mV, the β3c variants V1 and V2 inactivated slower (τ = 73.2 ± 8.8 and 78.8 ± 8.0 ms, respectively) than the wild-type channels that inactivated with a time constant of 55.3 ± 5.4 ms (Fig. 5F; n = 8–10; P < 0.05). Interestingly, the β3b-V4 variant inactivates extremely rapidly, with an average τ value of 6.1 ± 2.2 ms at +60 mV (n = 7) and 1.9 ± 0.2 ms at +100 mV (Fig. 5, C and D; n = 8). To accurately detect the τ of these rapidly
inactivating currents, we sampled at a much higher frequency (100 kHz) and filtered the currents at 10 kHz.

DISCUSSION

Idiopathic epilepsy (IE), estimated to affect 0.4% of the population, is heterogeneous in origin. There are numerous rare monogenic forms which have been shown to be caused by mutations in ion channel subunits (reviewed in Ref. 12). However, most cases of this disorder seem to be multifactorial, involving multiple genes as well as environmental factors. It is possible that some of the genes that predispose toward epilepsy are also variants of ion channel subunits.

We tested variants of KCNMB3 isoforms to detect any physiological effects on BK channel properties. In particular, we focused on parameters dealing with channel activation and inactivation, since the presence of the β-subunit is reported to affect these properties (25). We found that the voltage dependence of activation did not change significantly in the vast majority of the variant β-subunits, although the activation curve for the β3b-V4 channel was shifted to the right compared with wild-type channels (Fig. 3C). This particular channel displayed highly significant changes in almost all of the parameters investigated (Table 1). Several of the variants exhibited interesting characteristics that could potentially play roles in predisposing toward epilepsy. For instance, the β3a-V1 variant inactivated to a significantly greater degree than the wild-type channels, suggesting that they almost completely shut off if activated for a sufficient length of time. Interestingly, these channels also inactivate much faster than the wild-type channels with a time constant (τ) of 25 ms, which is ~2.5 times smaller than τ for the wild-type channels (~66 ms). These τ values are relatively long compared with the time course over which an action potential may occur. Nevertheless, it will be important to determine which cell types express these variants to determine whether the channels play a significant role in neuronal function. Other findings of interest include variants of the β3c channels (β3c-
V2) that inactivate slower and to a lesser degree than the wild-type channels, suggesting that this variant may actually hasten cell repolarization rather than hinder it.

We have shown that BK channels containing β3b-V4 subunits show striking differences in channel inactivation from wild-type subunits. Furthermore, these channels need greater levels of depolarization to fully activate. Taken together these three significant changes in channel properties, 1) rightward shift in the activation curve, 2) inactivating channel, and 3) a very rapid inactivation rate, suggest that this variant could lead to a reduced ability to repolarize following stimulation, resulting in neuronal hyperexcitability that is typical of epilepsy. However, this remains to be demonstrated directly in neurons or neuronal-like cells expressing the β3b-V4 variant. Another important caveat is that we used 10 μM Ca²⁺ to adequately activate the channels. This concentration had previously been determined to work sufficiently well when activating channels containing β3a–d subunits (3). However, since different values of intracellular Ca²⁺ will shift the activation and inactivation curves (3, 5, 21), our results should be interpreted within the narrow range of parameters of our experimental design. For instance, intracellular Ca²⁺ concentration will vary and will therefore affect channel activity differentially as intracellular Ca²⁺ concentration rises and falls in the channel’s immediate vicinity (14, 6, 9).

Our functional data supports an earlier finding that demonstrated a subtle difference in the distribution of the V4 variant (M. Labuda, personal communication). Although the incidence of V4 was not significantly different between 226 epileptics and 259 matched normal controls, the number of double heterozygotes (individuals carrying two variants, predominantly) significantly deviated from Hardy-Weinberg equilibrium among individuals with epilepsy. Furthermore, the transmission-disequilibrium test of 41 families from two different data sets showed that V4 was transmitted to affected offspring significantly more often than nonaffected offspring. If KCNMB3 can act as one of multiple susceptibility factors for IE, then one would expect such subtle changes that might take large patient studies to detect.

To have a role in epilepsy, a channel subunit gene must be expressed in the brain. Expression of KCNMB3 in the brain has been controversial (3, 15);
KCNM4 is the predominantly expressed β-subunit in this tissue (3, 15). However, Uebele et al. (22) have shown with RT-PCR that β-b, β-c, and β-d all show some expression in the brain. We have also shown by Northern blot (M. Labuda, personal communication) that KCNM3 is expressed, albeit weakly, in all brain tissues tested (cerebellum, cerebral cortex, medulla, spinal cord, occipital pole, frontal lobe, temporal lobe, and putamen). RT-PCR and Northern blots will not distinguish between low level expression throughout the tissue and high-level expression in a small subset of the cells in a particular region. Since expression in the brain is low, and all the KCNM3 variants are also found in normal individuals, we might expect that any effect on channel function would be subtle and not sufficient to cause epilepsy on its own, again typical of a susceptibility locus for IE. Thus our evidence suggests that KCNM3 variants, in combination with variants in other genes, could predispose individuals to IE. The V4 variant alters/truncates the terminal 21 amino acids of the β3b subunit. Interestingly, this region is not present in the other three β-subunits (β1, β2, or β4), nor is it conserved in rodent β3b subunits, but it was detected in gorilla and chimp (M. Labuda, personal communication). This suggests that this region may have a unique function in neurons, leading to hyperexcitability if it is lost.

GRANTS

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