

Cytoplasmic BK_{Ca} channel intron-containing mRNAs contribute to the intrinsic excitability of hippocampal neurons

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High single-channel conductance K⁺ channels, which respond jointly to membrane depolarization and micromolar concentrations of intracellular Ca²⁺ ions, arise from extensive cell-specific alternative splicing of pore-forming α -subunit mRNAs. Here, we report the discovery of an endogenous BK_{Ca} channel α -subunit intron-containing mRNA in the cytoplasm of hippocampal neurons. This partially processed mRNA, which comprises \approx 10% of the total BK_{Ca} channel α -subunit mRNAs, is distributed in a gradient throughout the somatodendritic space. We selectively reduced endogenous cytoplasmic levels of this intron-containing transcript by RNA interference without altering levels of the mature splice forms of the BK_{Ca} channel mRNAs. In doing so, we could demonstrate that changes in a unique BK_{Ca} channel α -subunit intron-containing splice variant mRNA can greatly impact the distribution of the BK_{Ca} channel protein to dendritic spines and intrinsic firing properties of hippocampal neurons. These data suggest a new regulatory mechanism for modulating the membrane properties and ion channel gradients of hippocampal neurons.

dendrite | epilepsy | intron-retention | KCNMA1 | local splicing

In mammalian neurons, the firing of an action potential requires the coordinated gating of at least a dozen different classes of voltage-gated ion channels. The integration of these currents manifests itself as characteristic input–output properties intrinsic to each neuron. One such firing property of some neurons, such as those in the hippocampus (1, 2) or cerebellum (3), is the ability to initiate repetitive burst firing in response to depolarizing current. Voltage-clamp analysis suggests that the small net inward current that drives the depolarizing momentum is a result of a subtle balance of the sum of inward and outward postspike currents (4). Although short-term and long-term feedback mechanisms exist to preserve burst firing (5), it is known that relatively small changes in the size of an individual current may have a dramatic impact on firing activity (6).

One transient current activated during the falling phase of the action potential is the BK_{Ca} channel. In the central nervous system, BK_{Ca} channels are localized to the cell soma as well as the pre- and postsynaptic terminals of neurons, where they regulate fundamental neuronal functions such as burst firing, neurotransmitter release, shaping action potential waveforms, and frequency tuning (7). Native channels are assembled as tetramers of the pore-forming α -subunits encoded by a single gene, *KCNMA1* (8), which is subject to vast tissue-specific (9) and cell-specific alternative splicing (10). The resulting functional heterogeneity in BK_{Ca} channel currents is due in part to these splicing events generating BK_{Ca} channels with altered Ca²⁺ sensitivity and gating kinetics (7) as well as altered channel trafficking (11). Channel differences also arise from modulation via a family of tissue-specific auxiliary β -subunits (10). Currently, the functional diversity among BK_{Ca} channels in neurons

is not fully resolved, but the expression and subcellular distribution patterns of splice variants are expected to be one functionally significant factor in determining their characteristic input–output properties. Indeed, there is some evidence for members of the K⁺ channel family, for example BK_{Ca} (12) or A-type (13), establishing functional gradients within the dendritic processes of mature neurons, where they transform the shape of local synaptic potentials or the size of somatic action potentials (14).

Here we report a novel form of posttranscriptional gene expression (cytoplasmic splicing of a RNA with a retained intron) that plays a role in generating local populations of BK_{Ca} channel mRNAs and proteins in hippocampal neurons. These results show that the regulation of a cytoplasmic BK_{Ca} channel intron-containing mRNAs partially underlie the characteristic input–output properties of hippocampal neurons.

Results

BK_{Ca} Channel Intron-Containing mRNAs Are Present in Hippocampal Dendrites. To determine the repertoire of mRNA splice variants in the postsynaptic compartment, we prepared a cDNA template derived from antisense RNA amplification of polyA mRNA isolated from rat hippocampal dendrites, which were harvested from 14- to 21-day-old cultures *in vitro* (15). Dendrites were carefully harvested individually to ensure that no cell bodies, and thus nuclei, of any neuron or nonneuronal cell were harvested. From this starting material, we detected two separate PCR products using primers specific to intron 16 of *KCNMA1* [supporting information (SI) Fig. 5a]. Intron 16 (i16) is \approx 6,000 nt long and lies upstream of a previously described “hot spot” for alternative exon usage (16). Exons surrounding i16 encode the 3'-end of the first of two highly conserved Ca²⁺-binding RCK (regulators of conductance K⁺ channel) domains and the non-conserved linker region spanning the first and second RCK module (17) (see Fig. 1a). The nucleotide sequence of the PCR products (330 and 390 nt) proved to be identical to the rat genomic BK_{Ca} channel genomic DNA sequence deposited in GenBank. We also observed the presence and absence of introns in other mRNAs in amplified polyA mRNA dendrite samples

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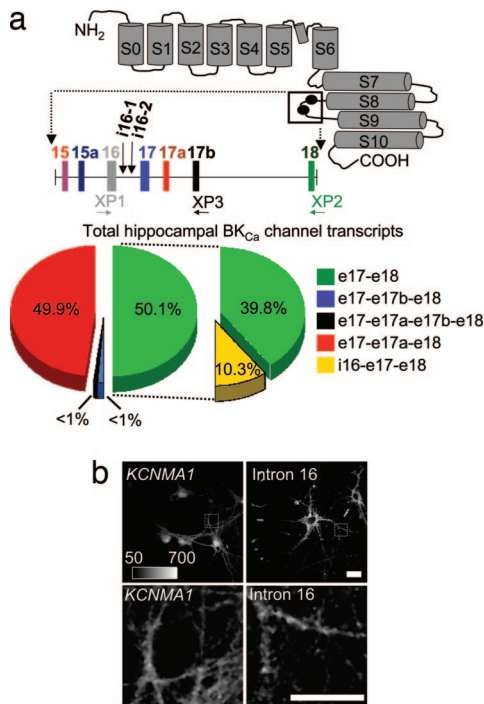


Fig. 1. BK_{Ca} channel intron-containing mRNAs are present in the cytoplasm of hippocampal neurons. (a) Putative membrane topology of the BK_{Ca} channel α -subunit and the location of two sites of alternative splicing (black circles) are shown. Exons are denoted with vertical solid bars and introns with horizontal lines. Exon lengths are 69, 12, 87, 77, 9, 174, and 94 nt for e15, e15a, e16, e17, e17a, e17b, and e18, respectively. Intron lengths are 2,340, 6,222, 6,654, 4,528, 6,421, and 30,920 nt for i15, i15a, i16-containing, i17, i17a, and i17b, respectively. *KCNMA1* splice variant analysis was performed in hippocampal tissue. These data show the abundance of four *KCNMA1* splice variants and the exonic linkage analysis of each splice variant to i16-containing region. For the alternative splicing analysis, the “all transcript” products were analyzed with XP1, XP2, and XP3 on two separate hippocampus samples in replicate ($n = 16$). For i16 transcripts, two samples of both hippocampus and hippocampal dendrites were analyzed in replicate ($n = 2$) with XP2 and XP3. (b) ISH was used to verify the subcellular localization of *KCNMA1* mRNA (e22–e25) and i16-containing BK_{Ca} channel mRNA ($n = 3$). Anti-digoxigenin F_{ab} fragments conjugated to Qdot-565 were used to enhance the detection and visualization. (Upper) Photomicrographs showing ISH of cultured hippocampal neurons with *KCNMA1* e22–e25 probe and i16-containing BK_{Ca} channel mRNA probe. (Lower) Photomicrographs showing enlarged marked regions from Upper.

subjected to microarray analysis (SI Fig. 5b). The presence of select introns in multiple mRNAs, as evidenced by hybridization, shows that intron retention is a generalized phenomenon in hippocampal neurons and not simply restricted to the BK_{Ca} channel gene. The absence of hybridization for multiple select introns further demonstrates that there was no genomic contamination of the dendrite samples. For this study, we will restrict our focus to the analysis of the BK_{Ca} channel mRNA.

i16-Containing Transcripts Are Associated with a Single BK_{Ca} Channel Splice Variant. The levels and endogenous populations of i16-containing transcripts were resolved by MALDI-TOF MS base extension (18). This PCR method uses an identical probe to analyze multiple transcripts (see SI Experimental Procedures). Therefore, it was chosen over real-time PCR, which requires different exon-spanning probes to identify alternative spliced transcripts. Using this approach, we first determined the levels of i16-containing transcripts. Hippocampal tissue cDNA was amplified with PCR primers in exon 16 (e16) forward and e18 reverse to capture the “all BK_{Ca} channel transcripts” population. A single extension primer (XP1) was used to assay the PCR

products. The i16-containing transcript represented $10.3\% \pm 2.3\%$ of the total BK_{Ca} channel transcript population (Fig. 1a). The levels of i16-containing transcripts reported here are in agreement with a previous study showing other *KCNMA1* splice variants typically represent a small subset ($\approx 10\%$) of the total *KCNMA1* transcript population (19).

We next determined the endogenous *KCNMA1* exonic combination between i16 to e18 in both hippocampal tissue and isolated dendrites. This region consists of four downstream exons: two constitutive exons (e17 and e18) and two alternatively spliced exons (e17a and e17b). Here we amplified cDNA with two sets of PCR primers: (i) e16 forward and e18 reverse to capture “all BK_{Ca} channel transcripts” or (ii) i16–1 forward and e18 reverse to capture “only i16-containing transcripts” (Fig. 1a). The PCR products were simultaneously assayed with the two different extension primers (XP2 and XP3) to capture all four exonic combinations (Fig. 1a). In the “all BK_{Ca} channel transcripts” population, every exonic combination was detected, but two highly abundant variants, e17–e18 and e17–e17a–e18, made up the bulk of the BK_{Ca} channel transcript population (Fig. 1a). A remarkably different pattern of splicing was detected in the “only i16-containing transcript” population. Unexpectedly, only one splice variant was detected; all of the i16-containing transcripts skip e17a and e17b and splice e17 directly to e18 (Fig. 1a). These data show that i16-containing mRNAs are restricted to a subset of the *KCNMA1* transcript population containing correctly spliced downstream exons, e17 and e18. Furthermore, these results highlight two key properties of i16-containing transcripts. First, they are present at biologically significant levels in the hippocampus, and, more surprisingly, the overall complexity of the i16-containing transcripts transcript population is less diverse than would be possible if all combinations of the downstream exons were associated with the retained intron.

The Subcellular Localization of BK_{Ca} Channel and the i16-Containing Splice Variant mRNAs in Hippocampal Neurons. *In situ* hybridization (ISH) is one method for assessing the dendritic localization of mRNA transcripts in neurons, but the methodology as standardly practiced is not particularly robust. For our studies, we developed a novel, highly sensitive Quantum Dot (Qdot)-based ISH protocol with i16-containing BK_{Ca} channel mRNA-specific probes to visualize its endogenous subcellular localization throughout the somatodendritic compartment (Fig. 1b and SI Fig. 6). This procedure allows detection of low abundance signals because of a prephotobleaching step eliminates any endogenous background cellular autofluorescence (SI Fig. 6). For the i16-containing BK_{Ca} channel mRNA, a series of puncta are detectable in the cell soma that extends into the proximal and distal dendrite. Signal intensity is strongest in the first 50- μ m proximal segment of the dendrite and diminishes as a function of distance from the cell soma (Fig. 1b and SI Fig. 7). A comparison with the ISH signal of the *KCNMA1* e22–e25 exon probe of the mature BK_{Ca} channel mRNA shows a similar pattern of distribution (Fig. 1b and SI Fig. 7). This pattern is coincident with where dendritic BK_{Ca} channel activity is most predominant in the most proximal portions of the dendrite while decaying with distance from the cell body (12). The importance of these data are twofold. First, they provide independent corroboration of the somatodendritic presence of the i16-containing BK_{Ca} channel mRNA. Second, they represent, to our knowledge, the first report of an endogenous intron-containing mRNA that is exported from the nucleus and transported to the somatodendritic cytoplasm. Furthermore, these phenomena are not restricted to cultured hippocampal neurons. Similar levels of i16-containing BK_{Ca} channel mRNA were detected by our quantitative PCR approach in fetal and adult brain tissues (data not shown). We followed this observation by using ISH to show the presence of i16-containing BK_{Ca} channel mRNA in the somatodendritic

head. Importantly, there was no difference in the intensity of phalloidin staining between untreated and si16-treated neurons (Fig. 3*b*, control; 419.09 ± 43.01 , $n = 60$ and si16; 415.20 ± 32.46 , $n = 60$). There was, in contrast, a quantifiable difference in BK_{Ca} channel protein distribution (Fig. 3*b*, control; 274.47 ± 14.97 and si16; 179.44 ± 4.74 , $n = 60$, $P < 0.001$). To further refine this difference, we transformed the BK_{Ca} channel fluorescence intensity differences relative to phalloidin signal in Fig. 3*b* to reflect a measure of their incidence of colocalization (Fig. 3*c*). The observed BK channel fluorescence ranged from $\approx 50\%$ to 75% of the overall phalloidin signal in the spine heads of untreated neurons. Therefore, to be more rigorous we selected a lower value (50%) as our minimum parameter to establish the incidence of BK_{Ca} channel and phalloidin colocalization. The spine heads showing $\geq 50\%$ BK_{Ca} channel fluorescence intensity relative to phalloidin intensity were annotated as normal. By using these parameters of colocalization within a region of interest in a spine head, untreated cells are about two times more likely to have BK_{Ca} channel protein in their spine heads above our 50% threshold as compared with si16-treated cells (Fig. 3*c*). In untreated neurons, the BK_{Ca} channel puncta predominately showed normal colocalization with phalloidin ($\approx 88\%$; Fig. 3*c*). We see a dramatic change in si16-treated neurons in the colocalization pattern of BK_{Ca} channel puncta in dendritic spines. Here, the colocalization incidence was significantly reduced ($\approx 42\%$; Fig. 3*c*). Collectively, these experiments highlight the local contribution i16-containing mRNAs make to the BK_{Ca} channel protein distribution in hippocampal dendritic spines.

In parallel control experiments, we examined the differential distribution of another member of the K⁺ channel family, Kv2.1. As a prevalent component of the somatodendritic delayed-rectifier potassium currents in mammalian neurons, Kv2.1 plays a prominent role in regulating Ca²⁺ influx and suppressing neuronal excitability (20). We assessed the colocalization of Kv2.1 channels with the phalloidin signature of individual dendritic spines. In contrast to the changes observed in BK_{Ca} channel differential distribution, the localization of Kv2.1 was unaltered between control and si16-treated cultures ($n = 15$; control, 85.0 ± 7.2 ; si16, 83.3 ± 8.0 ; Student's *t* test, $P = 0.88$; image shown in SI Fig. 8). As an additional control, we assessed the spine colocalization of the NR1 subunit of the NMDA receptor (21) with and without i16 siRNA treatment. Again, no discernible difference was found ($n = 13$; control, 80.8 ± 6.4 , si16-treated, 75.0 ± 9.4 ; Student's *t* test, $P = 0.66$). These controls show that the siRNA-induced difference in BK_{Ca} channel spine localization is selective. Given this striking change in the pattern of BK_{Ca} channel protein distribution in dendritic spines, we next screened for associated functional consequences.

BK_{Ca} Channel Intron-Containing mRNAs Contribute to the Excitability of Hippocampal Neurons. Hippocampal cells typically fire a burst of action potentials characterized by spike accommodation in which subsequent action potentials broaden often leading to spike failure (22). In hippocampal neurons, BK_{Ca} channels play a role in both action potential repolarization (12) as well as spike broadening during repetitive firing (23). Complex spike bursts of this sort are thought to underlie some adaptive processes during the acquisition of learning and memory (24). Abnormally large BK_{Ca} channel currents are the primary cause for changes in the patterns of complex spikes in some forms of epilepsy and dyskinesia (25). Having established an effective siRNA treatment protocol to selectively reduce i16-containing BK_{Ca} channel splice variant mRNA and protein levels, we next recorded from hippocampal neurons and analyzed their firing patterns and action potential profiles. Mock-treated, non-*KCNMA1* siRNA-treated (Ambion negative controls), or si16-treated neurons were not detectably different from the control sample in either the shape of the evoked actions potentials, action potential

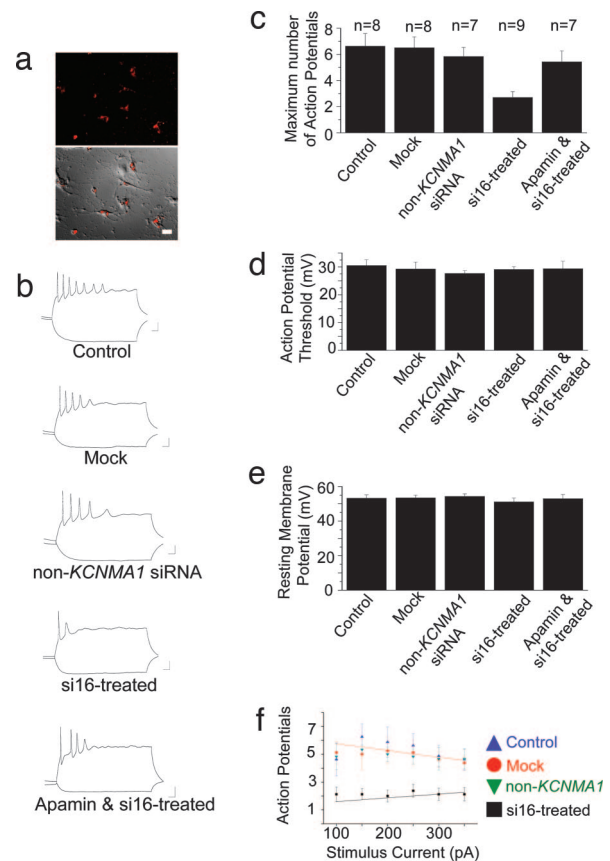


Fig. 4. Firing properties of hippocampal neurons are altered by i16-specific siRNAs. Hippocampal neurons were transfected with pools of siRNAs directed against separate *KCNMA1* i16 sequences or a non-*KCNMA1* target sequence (Ambion negative control nos. 1 and 7) and 20 nM siRNA-Glo Rhodamine marker. The neurons were current-clamped at -80 mV, and brief current injections (500 ms) were applied to evoke a train of action potential. The input impedance of treated neurons was not significantly different compared with control neurons (control, 0.484 ± 0.09 , $n = 8$; mock-treated, 0.479 ± 0.064 , $n = 8$; non-*KCNMA1* siRNA-treated, 0.487 ± 0.080 , $n = 7$; si16-treated, 0.590 ± 0.070 , $n = 9$; apamin and si16-treated, 0.422 ± 0.07 , $n = 7$). (a Upper) A fluorescence image showing the siRNA-Glo Rhodamine signal in the transfected neurons. (Lower) Photomicrograph is a merged image of fluorescence signal over DIC image. (b) Representative action potential traces from -150 and $+150$ pA current injection. (c) Histogram summary of the maximum number of action potentials generated from one current injection for each condition. (d) Histogram summary of the action potential threshold for each condition. (e) Histogram summary of the resting membrane for each condition. (f) Summary plot of the maximum number of action potentials generated at each current injection from si16-treated and mock neurons. Data are means \pm SEM.

generation threshold, resting membrane potential, or input resistance (Fig. 4). However, the maximum number of evoked action potentials was significantly reduced in the si16-treated neurons compared with control, mock-treated, and non-*KCNMA1* siRNA-treated neurons (Fig. 4*c*). These findings show that the i16-containing mRNA is integral to the regulation of functional BK_{Ca} channel expression mediated membrane excitability.

Spike Accommodation in Hippocampal Neurons Requires the Presence of BK_{Ca} Channel Intron-Containing mRNAs. To further characterize the functional significance of the i16-containing BK_{Ca} channel transcript, we compared the input-output function of mock-treated and i16-specific siRNA-treated neurons. With small current injections, the control, mock-treated or non-*KCNMA1*

siRNA-treated neurons showed brisk spiking activity. However, as the size of the current injections increased, spike accommodation became more apparent and reduced the number of spikes fired (Fig. 4*f*). In contrast, the si16-treated cells exhibited marked spike accommodation even for small current injections, essentially leaving the neurons unable to encode different stimulus levels (Fig. 4*f*). These results show that a reduction in BK_{Ca} channel α -subunit intron-containing mRNA levels alters membrane excitability of hippocampal neurons. These data are consistent with previous studies demonstrating that changes in BK_{Ca} channel activity alter the membrane properties (2).

SK Channel Inhibition Increases the Excitability of i16-Specific siRNA-Treated Neurons. Reduction of BK_{Ca} channel intron-containing mRNA levels mimics the proposed role for the β 4 subunit in the regulation of the *KCNMA1* α -subunit, which has been reported to play the functional role of inhibiting BK_{Ca} channels from contributing to membrane repolarization (26). BK_{Ca} channels are negative feedback regulators of calcium influx in hippocampal and many other neurons; therefore, the down-regulation of BK_{Ca} channel activity by si16-treated or β 4 subunit regulation should result in sustained calcium influx. This indeed could reduce the firing properties of si16-treated neurons by enhancing the activation of calcium-mediated after-hyperpolarization currents via SK channels. One apamin-sensitive isoform, SK2, is localized throughout the postsynaptic compartment in both the shaft and spines of dendrites (27). Thus, to test the notion that the reduction of i16-containing splice variant levels is reducing functional BK_{Ca} channel activity and increasing SK channel activity, we analyzed the firing patterns of si16-treated neurons in the presence of SK channel blocker apamin. Here, we show the maximum number of evoked action potentials is significantly increased in si16-treated neurons in the presence of apamin (Fig. 4*c*). Collectively, these results are consistent with previous studies, which suggest that BK_{Ca} channel activity is an intrinsic determinant of membrane properties. Moreover, they offer the first evidence for a functional role of a cytoplasmically localized, endogenous intron-containing mRNA in altering the membrane excitability of hippocampal neurons.

Discussion

Under normal cellular conditions, unspliced or incompletely spliced intron-containing mRNAs are routinely sequestered within the nucleus (28) and when transported to the cytoplasm they are subject to cellular nonsense-mediated degradation (29). Although intron retention is not an entirely unique phenomenon to neurons, having been described for cyclooxygenase 3 (30) and EAAT2 variants (31), these events are rarely observed in higher eukaryotes (32). In fact, database entries annotating intron-containing mRNAs often represent them as artifacts with little likelihood of influencing cellular physiology. Yet, several reports have begun to pinpoint a mechanism for promoting the nuclear export of incompletely spliced intron-containing mRNAs (33, 34).

At least two posttranscriptional regulatory mechanisms are used to generate BK_{Ca} channel heterogeneity: (i) extensive alternative splicing of BK_{Ca} mRNA and (ii) differential use of auxiliary β -subunits. We have previously speculated that by coupling the extranuclear splicing capability of the somatodendritic compartment with local protein synthesis (15) or simply locally translating (35, 36) intron-containing mRNAs as readthrough products, there may be another novel layer of posttranscriptional regulation supplementing the functional complexity of individual synapses (15). As recent reports suggest, splicing activity residing outside the nucleus exists as both stimulus-dependent and constitutive mechanisms for regulating gene expression (33, 37). With particular reference to the i16-containing BK_{Ca} channel mRNA, the position of the intron is notable. For maximal activation, BK_{Ca} channels require a

source of intracellular Ca²⁺. Often, these spikes of Ca²⁺ are generated by selectively coupling the opening of Cav channels subtypes in close proximity to BK_{Ca} channels (38) and perhaps as part of large macromolecular signaling complexes (39). Intron 16 immediately precedes an exon that codes for the nonconserved region linking the Ca²⁺-sensing RCK1 and RCK2 domains. Within the proline-rich sequence encoded in this exon, a noncanonical Src homology domain 3 (SH3) is expressed which allows BK_{Ca} channels to coordinate their spatial distribution within the actin cytoskeleton through the actin adaptor protein cortactin (40). In the i16-containing mRNA knockdown experiments, we observed a radically different distribution where the BK_{Ca} channel protein was far less likely to colocalize with filamentous actin in dendritic spines. Changes in BK_{Ca} channels firing properties are synchronized with alterations in actin cytoskeletal dynamics (41) in some forms of stroke and epilepsy (40). One obvious question is to what extent neurons use an i16-containing mRNA to generate a functional BK_{Ca} channel that can properly coordinate with the actin cytoskeleton. The anti-BK_{Ca} channel antibody used here recognizes an epitope encoded within final intracellular C-terminal residues (e.g., exons 24 and 25). In the context of our BK_{Ca} channel immunofluorescence data, the most likely scenario is the cytoplasmic pre-mRNA splicing of the endogenous i16-containing BK_{Ca} transcript yielding a protein with C-terminal sequence intact. Such a cytoplasmic pre-mRNA splicing regulatory checkpoint by spliceosome-competent ribonucleoprotein complexes has recently been described in anucleate platelets (33). It is unclear if such a cellular process in the nervous system may create any novel, alternatively spliced mRNAs encoding a protein which generates a gain-of-function characteristic that alters the excitability of hippocampal neurons.

Functionally, most K⁺ channels tend to hyperpolarize the cell and moderate the effects of excitatory input (22). In contrast, BK_{Ca} channels cause rapid spike repolarization and a post-burst fast after hyperpolarization to facilitate repetitive firing in hippocampal neurons (2). The facilitatory effect of BK_{Ca} channels is most conspicuous in the form of gain-of-function phenotypes that greatly enhance neuronal excitability to great detriment. A single point mutation in the first RCK domain underlies an enhanced rate of repolarization in the human syndrome of coexistent generalized epilepsy and paroxysmal dyskinesia (25). A similar gain-of-function phenotype that sharpens action potentials, supports higher firing rates with the loss of SK channel recruitment, and shows distinctive temporal lobe seizures was revealed when the auxiliary BK_{Ca} channel β 4 subunit was deleted in β 4-null mice (26). In the absence of BK_{Ca} channel activity whether genetically (42) or pharmacologically induced (2), the characteristic bursting properties of neurons are attenuated with increasing current injection as spike accommodation becomes more apparent. BK_{Ca} channels are potential drug targets for several clinical disorders such as autism (43) and epilepsy (25). Therefore, the functional significance of cytoplasmic intron-containing BK_{Ca} channel mRNAs suggests that intron-retained channel mRNAs and their locally translated proteins may be a novel class of therapeutic targets for disorders linked to this channel.

Although small changes in an individual ion channel conductance or localization can greatly impact both action potential generation and the intrinsic firing properties of a neuron, this role has never been assigned to an endogenous cytoplasmic intron-containing mRNA. Furthermore, these data highlight the influence BK_{Ca} channel α -subunit intron-containing mRNAs and mRNA splice variant expression will have on heterogeneity of BK_{Ca} currents at the subcellular level in hippocampal neurons. The interrelationship of these distinct subclasses may account, in part, for flexibility in the creation of functional phenotypes for the encoded protein.

Experimental Procedures

Hippocampal Cultures. Primary cultures were plated as previously described (15).

Dendrite RT-PCR Analysis. The pools of dendrites were harvested, subjected to two rounds of antisense RNA amplification procedure as previously described (15), and used as a template for PCR.

MALDI-TOF MS and Quantitative *KCNMA1* Splice Variant Detection. MALDI-TOF MS analysis of PCR amplicons of dendrite and hippocampus *KCNMA1* cDNA was performed by using PCR primers selecting for either all transcripts or intron-containing transcripts using previously described methods (18). See *SI Experimental Procedures*.

ISH Using Cultured Neurons. Antisense digoxigenin-labeled *KCNMA1* RNA probes were generated by *in vitro* transcription. Anti-digoxigenin F_{ab} fragments conjugated to Qdot 565 were used for detection (Invitrogen). The samples were subjected to photobleaching, and the Qdot signal was detected by using the Olympus Fluoview 1000 confocal scan head attached on inverted microscope. See *SI Experimental Procedures*.

ISH Using Adult Rat Brain Sections. Fresh frozen adult rat brains were sectioned at 15- μ m thickness. Anti-digoxigenin F_{ab} Fragments conjugated to alkaline phosphatase (Roche) were used for detection with NBT/BCIP (Nitro blue

tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate; Roche) for visualization under brightfield optics.

Immunocytochemistry. Primary neurons were fixed 10–14 days after plating and as described in *SI Experimental Procedures*. For each cell, five randomly placed line scans were taken from three separate regions of interest for each dendritic segment and analyzed with Metamorph image processing software.

siRNA Treatments. Cultured primary rat hippocampal neurons were transfected in DharmaFect 3 (Dharmacon) 7–9 days after plating (see *SI Experimental Procedures*). The cultures were maintained at 37°C with 5% CO₂ for 72 hr.

Whole-Cell Recordings. Bathing solution consisted of 140 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 1 mM Mg Cl₂, and 10 mM Hepes, adjusted to a pH of 7.4 with NaOH. The internal solution consisted of 120 mM potassium gluconate, 20 mM KCl, 10 mM Hepes, 0.1 mM EGTA, 2 mM MgCl₂, 2 mM ATP, and 0.25 mM GTP, adjusted to a pH of 7.4 with KOH.

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