Expression of a calmodulin-binding KCNQ2 potassium channel fragment modulates neuronal M-current and membrane excitability

Mohammad Shahidullah, Lindsey Ciali Santarelli, Hua Wen*, and Irwin B. Levitan†

Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Edited by Richard L. Huganir, Johns Hopkins University School of Medicine, Baltimore, MD, and approved September 22, 2005 (received for review May 12, 2005)

KCNQ2 and KCNQ3 ion channel pore-forming subunits coassemble to form a heteromeric voltage-gated potassium channel that underlies the neuronal M-current. We and others showed that calmodulin (CaM) binds to specific sequence motifs in the C-terminal domain of KCNQ2 and KCNQ3. We also found that a fusion protein containing a KCNQ2 CaM-binding motif, coexpressed with KCNQ2 and KCNQ3, competes with the full-length KCNQ2 channel for CaM binding and thereby decreases KCNQ2/3 current density in heterologous cells. We have explored the importance of CaM binding for the generation of the native M-current and regulation of membrane excitability in rat hippocampal neurons in primary cell culture. M-current properties were studied in cultured neurons by using whole-cell patch clamp recording. The M-current density is lower in neurons expressing the CaM-binding motif fusion protein, as compared to control neurons transfected with vector alone. In contrast, no change in M-current density is observed in cells transfected with a mutant fusion protein that is unable to bind CaM. The CaM-binding fusion protein does not influence the rapidly inactivating A-current or the large conductance calcium-activated potassium channel-mediated fast spike afterhyperpolarization in neurons in which the M-current is suppressed. Furthermore, the CaM-binding fusion protein, but not the nonbinding mutant, increases both the number of action potentials evoked by membrane depolarization and the size of the spike afterdepolarization. These results suggest that CaM binding regulates M-channel function and membrane excitability in the native neuronal environment.

A-current | afterdepolarization | afterhyperpolarization

The neuronal M-current is a voltage-dependent potassium current with distinct biophysical characteristics: activation in the subthreshold range of membrane voltage, slow activation and deactivation kinetics, and no inactivation. This current originally was named the M-current because it could be suppressed by muscarine and other agonists that activate muscarinic acetylcholine receptors (1). Its unique combination of biophysical properties, most notably its slow gating, activation at negative voltages, and lack of inactivation, results in sustained activity of the M-current near the action potential threshold. Because it is the major sustained outward current in this voltage range, it plays a dominant role in regulating neuronal excitability (2).

The M-current is ubiquitous in vertebrate central and peripheral neurons (reviewed in ref. 3). It is modulated by the activation of multiple receptor types, including those for classical neurotransmitters such as acetylcholine, serotonin, noradrenaline, and glutamate. In addition, a number of peptide modulators, among which are opioid peptides, somatostatin, angiotensin-II, substance P, bradykinin, and luteinizing hormone releasing hormone can modulate the M-current (e.g., see refs. 3–5). The fact that the M-current is a target for the actions of so many neuromodulators and synaptic neurotransmitters emphasizes its central role in the control of neuronal excitability. Although the molecular mechanisms involved in M-current modulation have been elusive, recent evidence implicates the breakdown of phosphatidylinositol-*bis*-phosphate in the muscarinic suppression of the current (6, 7).

The five recently cloned mammalian KCNQ genes establish a voltage-dependent potassium channel family that is evolutionarily distinct from other voltage-dependent potassium channels. The expression of two KCNQ family members, KCNQ2 and KCNQ3, appears to be nervous system-specific (8). Although they both produce a small but detectable potassium current when expressed alone in heterologous cells, when they are expressed together, they produce a robust current with kinetic and pharmacological properties that closely resemble those of the neuronal M-current (9). Other KCNQ (most notably KCNQ5) and nonrelated potassium channel subunits may also contribute to the M-current (10, 11), but KCNQ2 and KCNQ3 appear to play a prominent role in most neurons (9, 12).

KCNQ channels possess a C-terminal domain after the end of the sixth transmembrane segment that is much longer than those of most other voltage-dependent potassium channels. The ubiquitous calcium sensor calmodulin (CaM) has been shown to associate tightly with the extended C-terminal tail domains of both KCNQ2 and KCNQ3 (13, 14) and to regulate the M-like current produced by these subunits when they are coexpressed in heterologous cells (13, 15). In the present study, we extend this analysis to the M-current in cultured hippocampal neurons. The results are consistent with a central role for CaM in the regulation of the native neuronal M-current and neuronal membrane excitability.

Materials and Methods

Neuronal Cell Culture. Cultures of primary dissociated neurons were prepared from embryonic rat hippocampus as described (16). Pregnant Sprague–Dawley rats were narcotized with CO_2 for 90 s and then killed by cervical dislocation. The embryos (gestational days 18–19) were removed; the brains were dissected and placed on ice, and the hippocampi were dissected under microscopic visualization. The hippocampi were incubated for 20 min in DMEM (BioWhittaker) containing 0.03% trypsin at 37°C and 5% CO_2. They were then resuspended in growth medium containing DMEM, supplemented with 10% bovine calf serum (HyClone), 10% Ham's F12 containing glutamine (BioWhittaker), and 50 units/ml penicillin–streptomycin (Sigma). The cells were triturated with a sterile Pasteur pipette and plated onto poly(L-lysine)-coated glass coverslips (Peptides International) in 35-mm Petri dishes (Nunc), at 150,000 neurons per dish. The coverslips were preincubated for 72 h with growth medium before the neurons were plated onto them. The cultures were maintained in Petri dishes in a

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: ADP, afterdepolarization; AHP, afterhyperpolarization; CaM, calmodulin.

*Present address: Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794.

†To whom correspondence should be addressed. E-mail: levitani@mail.med.upenn.edu.

humidified incubator at 37°C with 5% CO_2 and were fed once or twice per week with preheated growth medium. Electrophysiological recordings were obtained from cells that had been in culture for 8–15 days.

cDNA Constructs and Neuronal Transfection. The pEGFP-C2 vector (Clontech) was used to transfect cultured hippocampal neurons. A cDNA encoding amino acid residues 321–567 of mouse KCNQ2, and another encoding the same region with a point mutation at arginine 345 (R345E), were subcloned separately into this vector to allow expression of fusion proteins containing both GFP and either the CaM-binding WT (GFP-WT) or the nonbinding mutant (GFP-R345E) KCNQ2 sequence. The pEGFP-C2 vector with no inserted channel sequence was used to express GFP alone. Previously, we used GST fusion protein constructs to introduce these WT and mutant channel sequences into heterologous cells (13).

Immediately before transfection, the neuronal culture medium was removed and set aside, and the neurons were washed with DMEM. A mixture of 2 μg of cDNA and 3 μl of Lipofectamine 2000 (Invitrogen) in 0.5 ml of warm Opti-MEM (Invitrogen) was added to the Petri dish, and the dishes were incubated at 37°C for 30 min. The transfection medium was then removed, and 1.5 ml of fresh preheated growth medium supplemented with 0.5 ml of the original growth medium (that had been removed at the start of transfection) was added. Dishes of neurons were incubated at 37°C with 5% CO_2 for 18–24 h after transfection before electrophysiological recording. Only neurons exhibiting bright green fluorescence were used for recordings.

Patch Recording and Data Analysis. The whole-cell patch mode was used to obtain voltage clamp current recordings or current clamp voltage recordings from the cultured transfected hippocampal neurons at room temperature. The extracellular solution contained 120 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11 mM glucose, 5 mM Hepes, 23 mM $NaHCO_3$, and 0.001 mM tetrodotoxin (pH 7.4). The tetrodotoxin was omitted from the extracellular solution for current clamp recordings of action potentials. Glass pipettes (Jencons, Bridgeville, PA) were fire-polished and had resistances of 4–6 MΩ when filled with the internal solution containing 160 mM K-acetate, 3 mM $MgCl_2$, and 20 mM Hepes (pH 7.4). Under these conditions, voltage clamp outward currents remain stable for at least 15–20 min after achieving the whole-cell recording configuration, and all measurements were completed within this time period. Experiments were carried out by using an Axopatch 200A amplifier (Axon Instruments, Union City, CA). Data were digitized with a Digidata 1200 interface (Axon Instruments) and stored on a PC hard drive for further analysis. Fitting of deactivation time constants and other data analysis and plotting were done by using PCLAMP8 (Axon Instruments) and ORIGIN 7 (Origin Lab, Northampton, MA) software. The size of the spike afterdepolarization (ADP) was measured as the integrated area under the voltage trace, during a 450-msec period beginning at the peak of the afterhyperpolarization (AHP) that follows the spike. Statistical significance was assessed by ANOVA by using ORIGIN software.

Results

Expression of a CaM-Binding Fusion Protein Decreases the Neuronal M-Current. All measurements presented here were obtained from transfected hippocampal neurons dissociated in primary cell culture. Transfection rates are low in these cultures (<5%), but because all of the transfected neurons express GFP, they were identified unequivocally by their bright green fluorescence. The M-current was measured by using the voltage clamp protocol introduced by Brown and Adams (1). Neurons were held at −70 mV and stepped to −20 mV for 300 ms to permit the inactivation of most voltage-dependent potassium currents, and then the voltage was stepped to more negative voltages (voltage traces at top of Fig. 1). The slowly deactivating current during the hyperpolarizing voltage step (Fig. 1*A*) was taken as a measure of the M-current. As shown in Fig. 1*D*, essentially all of the slowly deactivating current that we measured by this hyperpolarization protocol was inhibited by the specific M-current blocker linopirdine, confirming that it was

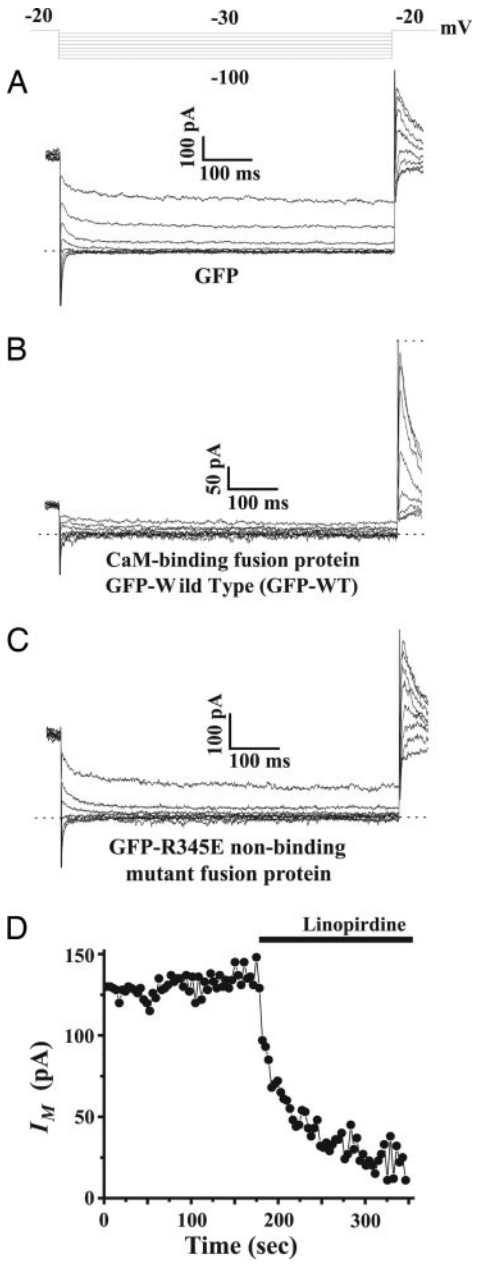

Fig. 1. M-current is suppressed in rat hippocampal neurons by disruption of CaM binding. Neurons were transfected with either GFP alone (*A*), GFP-WT fusion protein (*B*), or the mutant GFP-R345E fusion protein that does not bind CaM (*C*). Whole-cell currents were recorded from voltage-clamped neurons, and a standard hyperpolarizing protocol (top traces) was used to measure the M-current. Cells were held at −70 mV for 5 s and then depolarized to −20 mV for 300 ms before they were stepped back to hyperpolarized voltages to measure the deactivating M-current (see also Fig. 3*A*). Dotted lines represent the zero current level. Note that at the end of the hyperpolarizing pulse, depolarization back to −20 mV activates A-type currents. The examples shown here are typical of results from a large number of neurons (see Fig. 3*D* for quantitation). (*D*) M-current amplitude measured at −40 mV every 3 s in a GFP-transfected neuron. The current is stable in the whole cell recording configuration and is blocked by 30 μM linopirdine.

NEUROSCIENCE

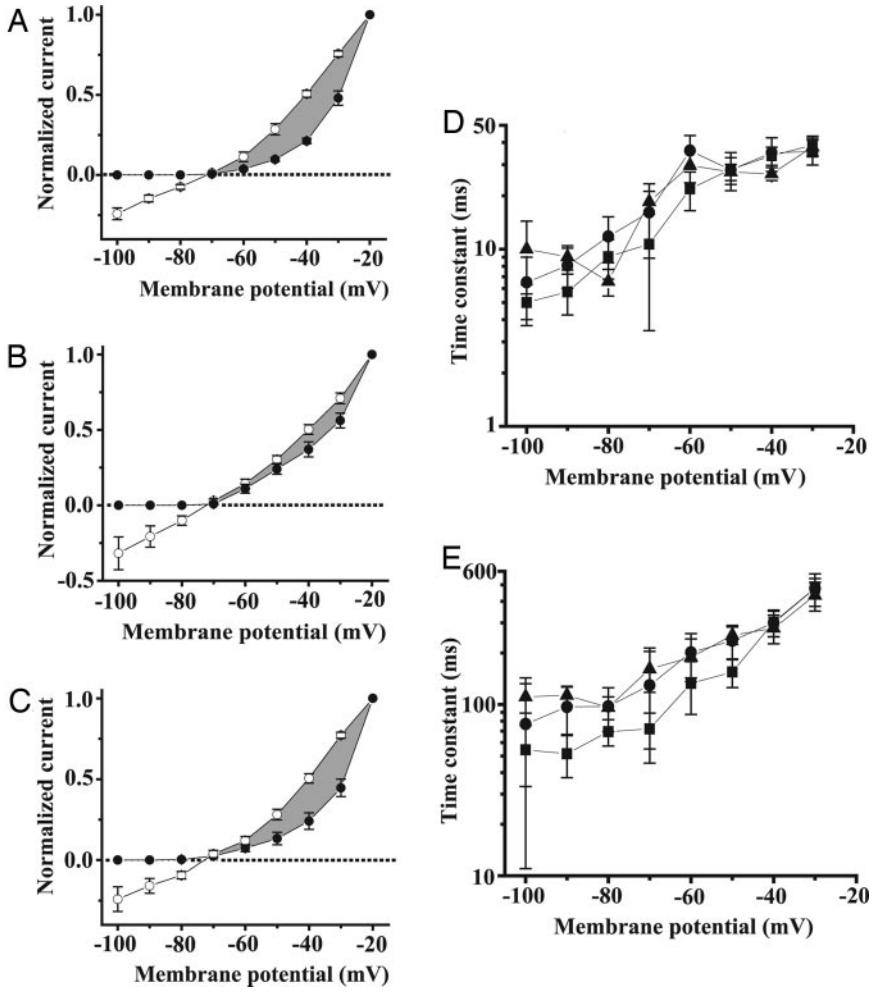

Fig. 2. Voltage-dependence and kinetics of the M-current. Mean instantaneous (○) and leak-subtracted steady-state (●) currents, recorded from hippocampal neurons as shown in Fig. 1, plotted as a function of membrane potential. The instantaneous current is the amplitude of the fast transient, and the steady-state current is the current measured after the slow relaxation is complete at the end of the hyperpolarizing pulse. In all cases, the curves intersect near −73 mV, the reversal potential of the current. The shaded region between these two lines determines the amount of M-current. The amount of M-current is smaller in neurons transfected with WT fusion protein (*B*) as compared to neurons transfected with GFP alone (*A*) or GFP-R345E (*C*). Shown are means ± SEM ($n = 6$–9). (*D* and *E*) Fusion protein expression does not influence M-current deactivation kinetics. Deactivation of the M-current was fitted best by two exponential functions. Shown are semilogarithmic plots of the fast (*D*) and slow (*E*) deactivation time constants, as a function of membrane potential, in neurons transfected with GFP alone (●), GFP-WT (■), or GFP-R345E (▲). Means ± SEM ($n = 6$–9).

indeed M-current. The amplitude and kinetics of the M-current were similar in neurons transfected with the pEGFP-C2 vector (Fig. 1*A*) and in untransfected neurons (data not shown).

The M-current amplitude was much smaller in neurons expressing the CaM-binding GFP-WT fusion protein; in fact, in many such neurons, it was difficult to detect any slowly deactivating current during the hyperpolarizing voltage step (e.g., Fig. 1*B*). The WT sequence comprises the CaM-binding domain (amino acid residues 321–567) of mouse KCNQ2. We have shown in heterologous cells that this sequence competes with full-length KCNQ2 and KCNQ3 for CaM binding, and thereby causes a decrease in the M-like current produced by expression of these cloned subunits (13). Although the transfection efficiency of the cultured hippocampal neurons was too low for us to use biochemical techniques to measure displacement of CaM bound to native KCNQ2 and KCNQ3, we assumed that the CaM-binding WT fusion protein was operating by a similar mechanism to reduce the native M-current. When the neurons were transfected with a mutant fusion protein (GFP-R345E) that is incapable of binding CaM (13), the amplitude of the M-current (Fig. 1*C*) was comparable to that in neurons transfected with GFP alone (Fig. 1*A*).

The current–voltage (*I–V*) relationship for the M-current was derived from the steady-state amplitude of deactivating tail currents during the hyperpolarizing voltage steps to different voltages (2). The point at which this steady-state *I–V* curve crosses the instantaneous *I–V* curve defines the reversal potential of the M-current. The reversal potential under control conditions is approximately −73 mV, and the threshold for activation is approximately −60 mV (Fig. 2*A*); neither of these parameters changed in the presence of either the CaM-binding WT (Fig. 2*B*) or mutant (Fig. 2*C*) fusion protein. The shaded region bounded by the instantaneous and steady-state *I–V* curves, representing the amplitude of the M-current, was smaller only in the presence of the CaM-binding fusion protein (Fig. 2*B*), consistent with the data illustrated in Fig. 1. Two voltage-dependent exponential time constants were required to fit the deactivation of the M-current, in agreement with previous measurements (12, 17), and neither their amplitudes nor their voltage-dependences are affected by expression of either fusion protein (Fig. 2 *D* and *E*). Thus, the basic voltage dependence and kinetic properties of the neuronal M-current are similar under all transfection conditions.

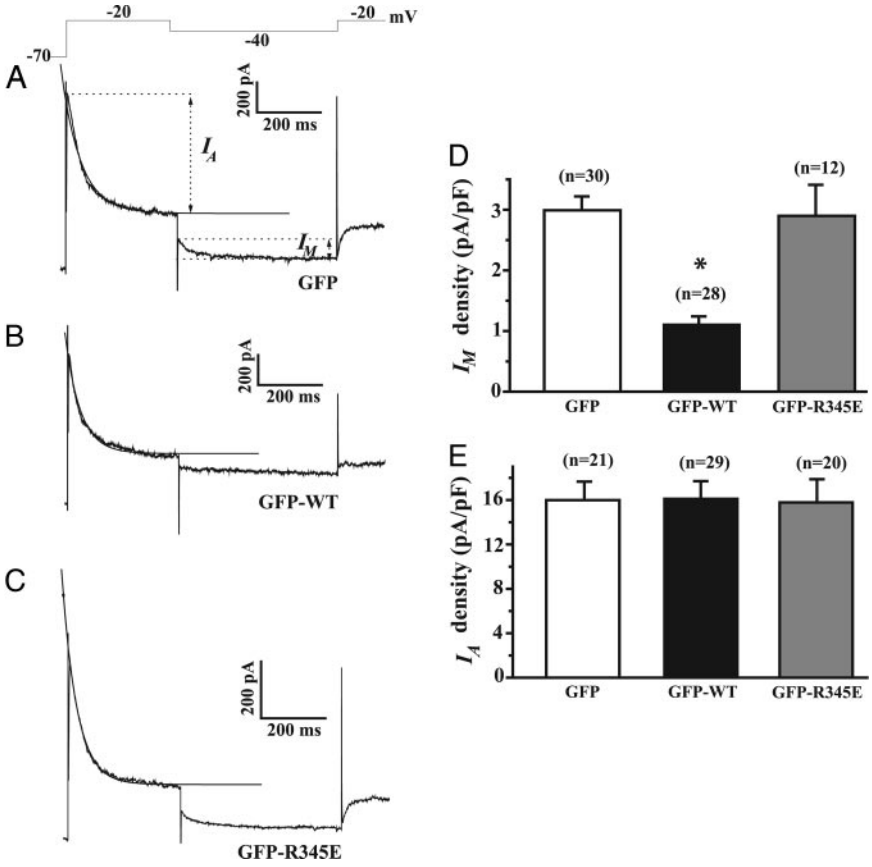

Fig. 3. Expression of the CaM-binding WT fusion protein selectively influences the amplitude of the M-current. The voltage protocol for measurement of both the A-current and the M-current is shown at the top left. Cells were held at −70 mV for 5 s. Outward current was generated by depolarizing the cell to −20 mV, and current was then deactivated by a hyperpolarizing pulse to −40 mV. The inactivating A-current (I_A) was fitted with a single exponential function (solid line), and its amplitude was measured from the extrapolated fitted line. The deactivating M-current (I_M) was measured at −40 mV. Neurons were transfected with GFP alone (*A*), GFP-WT (*B*), or GFP-R345E (*C*). (*D*) Mean (±SEM) M-current density, measured at −40 mV by using the voltage protocols illustrated here and in Fig. 1 (all data combined), in neurons transfected with either GFP alone or the WT or R345E fusion protein. The current density is ≈3-fold lower in neurons transfected with GFP-WT than in those transfected with either GFP alone or GFP-R345E. *, significantly different from others, $P < 0.0001$. (*E*) Mean (±SEM) A-current density, measured at −20 mV as in *A–C*, is the same in the presence of all three fusion proteins.

The CaM-Binding Fusion Protein Selectively Decreases the M-Current. To determine whether another neuronal potassium current might be influenced by transfection of the CaM-binding fusion protein, we devised a voltage clamp protocol to simultaneously measure the rapidly inactivating A-type current and the M-current (Fig. 3*A*). Neurons were held at −70 mV and then were stepped to −20 mV to activate the M-current and also allow activation and subsequent inactivation of the A-current. The neurons were then hyperpolarized to −40 mV for 500 ms to deactivate the M-current. The inactivation of the A-current was fitted by a single exponential function, and the amplitude of the current (I_A) was determined from extrapolation of the fitted line. The amplitude of the M-current (I_M) was measured as the difference between the instantaneous and steady-state currents at −40 mV. Expression of the CaM-binding WT fusion protein decreased the M-current substantially but did not affect the A-current (Fig. 3*B*), whereas the mutant fusion protein was without effect on either current (Fig. 3*C*). The current density for each current, averaged from a large number of neurons, is shown in Fig. 3 *D* and *E*. The M-current density was decreased by a factor of three, after expression of the CaM-binding WT but not the mutant fusion protein (Fig. 3*D*), whereas the A-current density was the same under all transfection conditions (Fig. 3*E*).

The CaM-Binding Fusion Protein Modulates Neuronal Membrane Excitability. We measured the membrane excitability of the hippocampal neurons by examining the generation of action potentials in the whole-cell current clamp recording mode. The membrane potential of the neurons was adjusted to −80 mV by the injection of a constant current, and then action potentials were evoked by the injection of additional depolarizing current for 500 ms. As shown in Fig. 4*A*, the injection of a 300-pA depolarizing current was sufficient to evoke a single action potential in most neurons transfected with GFP alone, whereas smaller depolarizing currents generated an electrotonic potential but no spike. This accommodation to a sustained depolarizing stimulus resulted from the slow activation of the M-current during the depolarization (18). In contrast, multiple action potentials were evoked by this same depolarization in neurons transfected with the CaM-binding fusion protein (Fig. 4*B*), consistent with the finding that there is less M-current in these neurons. Neurons that express the mutant fusion protein (Fig. 4*C*) accommodate to the same extent as the GFP-transfected controls. The number of action potentials evoked during the depolarization was plotted as a function of depolarizing stimulus strength in Fig. 4*D*. It is evident that there was less accommodation in neurons that express the CaM-binding WT fusion protein, as compared to GFP or mutant fusion protein controls.

As another measure of membrane excitability, we determined the size of the spike ADP and AHP during depolarizations that evoked a single action potential (Fig. 5). As shown by several laboratories (e.g., refs. 19 and 20), the AHP consists of multiple kinetic components and was mediated by several different potassium currents. In our hands, the rapid component of the hippocam-

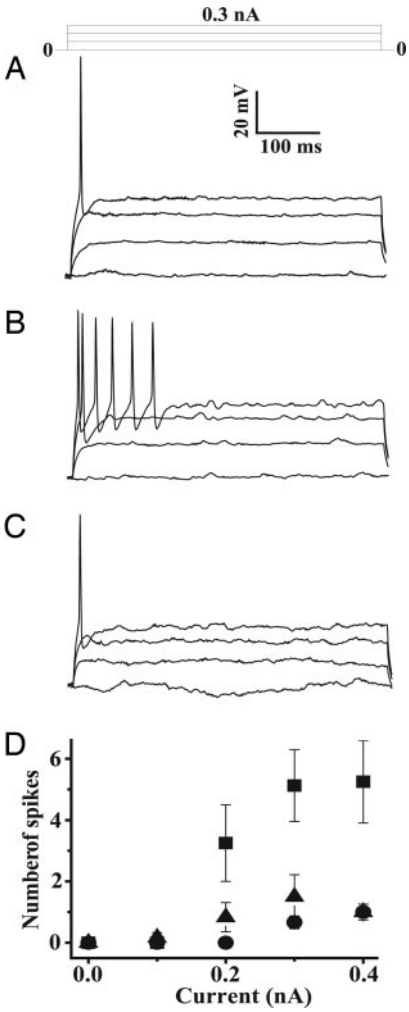

Fig. 4. The CaM-binding fusion protein modulates spike accommodation. Whole-cell current clamp recordings from hippocampal neurons transfected with GFP alone (*A*), GFP-WT (*B*), or GFP-R345E (*C*). In all cases, the starting voltage was set to −80 mV by the injection of an appropriate amount of current, and action potentials were evoked by the injection of depolarizing currents ranging from 0 to 0.3 nA (top traces). (*D*) The number of spikes evoked is plotted as a function of the injected current for neurons transfected with GFP alone (●), GFP-WT (■), or GFP-R345E (▲). Means ± SEM ($n = 7–10$). GFP-WT causes a significant increase in the number of action potentials evoked by 0.2-, 0.3-, and 0.4-nA current injections ($P < 0.0004$).

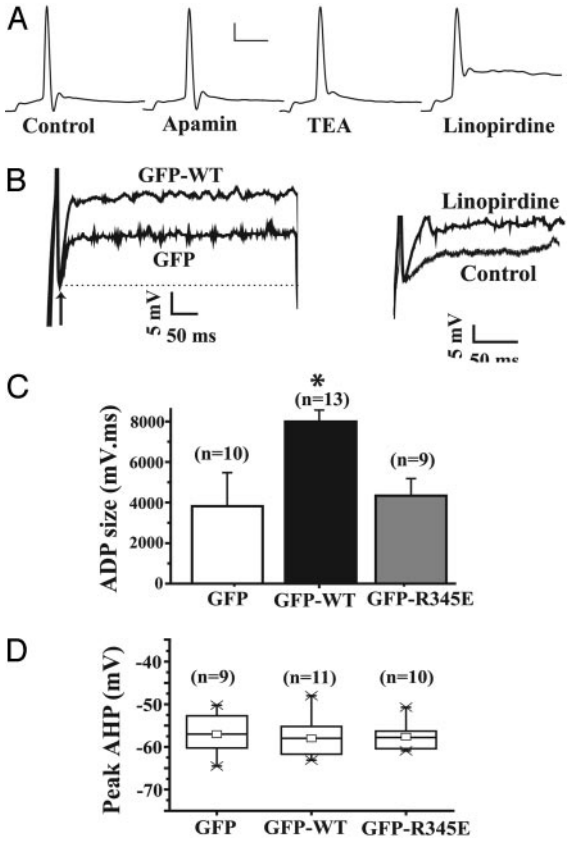

Fig. 5. The CaM-binding fusion protein modulates spike ADP, but not AHP. (*A*) Single action potentials were evoked by a 300-pA depolarizing current step in a GFP-transfected neuron with the resting membrane voltage set to −80 mV (*A*). Potassium channel blockers were applied sequentially to the same neuron. Tetraethylammonium (1 mM) blocks the fast AHP whereas linopirdine (30 μM) increases the amplitude of the ADP. Note that 10 μM apamin does not affect the AHP in these neurons. (Scale bars, 20 mV, 20 ms). (*B*) Examples of whole-cell current clamp recordings from neurons that fired a single action potential in response to injection of 300 pA of depolarizing current. The traces for neurons transfected with GFP alone and GFP-R345E (data not shown) overlay each other, whereas the ADP is larger (more depolarized trace) in neurons transfected with GFP-WT (*Left*). The effect of 10 μM linopirdine on the ADP is shown for comparison (*Right*). The tops of the spikes are clipped to better illustrate the ADP and AHP. The arrow indicates the peak of the AHP. (*C*) ADP amplitude, measured as described in *Materials and Methods*, in neurons expressing GFP alone or the CaM-binding GFP-WT fusion protein. All neurons used for this analysis were comparable in size, as determined by the membrane capacitance. *, significantly different from others, $P < 0.0001$. (*D*) Voltage reached at the peak of the AHP in neurons expressing the three fusion proteins. Box plot (mean ± range) for the number of neurons shown in parentheses.

pal neuron AHP was blocked by a low concentration (1 mM) of tetraethylammonium (Fig. 5*A*), suggesting that a large conductance calcium-activated potassium channel contributes prominently to the rapid AHP as it does in other neuronal types (21, 22). Surprisingly, apamin, a blocker of small conductance calcium-activated potassium channels, has little or no effect on the AHP in these neurons (Fig. 5*A*). Linopirdine does not alter the peak of the rapid AHP, but markedly enhanced the ADP (Fig. 5*A*), consistent with the idea that the slowly activating M-current participated only minimally in the generation of the rapid AHP but was important during the ADP.

We defined the ADP amplitude as the integrated area under the voltage trace (18), from the peak of the AHP to an arbitrary time point (450 ms) during the 500-ms depolarizing current stimulus (as in Fig. 5*B*). Examples of the sustained ADP are illustrated in Fig. 5*B*. Note that the effect of the CaM-binding WT fusion protein on the ADP (Fig. 5*B Left*) was comparable to that of linopirdine (Fig. 5*B Right*). As shown in Fig. 5*C*, the amplitude of the ADP was substantially larger in neurons expressing the CaM-binding WT but not the mutant fusion protein. This result may be contrasted with the peak of the AHP, which was the same in the presence of all three fusion proteins (Fig. 5 *B* and *D*), consistent with the idea that the large conductance calcium-activated potassium channels that contribute to the rapid AHP were not affected by fusion protein expression.

Discussion

CaM is the calcium sensor and transducer for a large number of calcium-dependent cellular pathways. Among the many molecular targets of CaM are membrane ion channels. CaM has been shown to bind to several different ion channels and participate in their regulation (reviewed in refs. 23 and 24). For example, CaM acts as the calcium sensor for small conductance calcium-activated potas-

sium channels and mediates their calcium-dependent gating (25). The calcium-dependent inactivation and facilitation of voltage-dependent calcium channels have also been shown to be mediated by CaM bound to the channel (26–29). In both of these cases, CaM binds constitutively to the channel in the absence of calcium, and subsequent calcium binding triggers a conformational change in CaM that modulates channel function (30, 31).

A yeast two-hybrid screen identified CaM as a constitutive binding partner of the KCNQ2 and KCNQ3 potassium channel subunits that were responsible for much of the neuronal M-current (13, 14). CaM binding requires the presence of two small binding motifs separated by several hundred amino acids in the extended C-terminal tail of KCNQ2 that follows the sixth membrane-spanning domain; specific point mutations in either of these motifs can disrupt CaM binding (13, 14). We found that these same mutations eliminated the ability of KCNQ2 to form a functional channel when it is coexpressed with KCNQ3 in heterologous cells, consistent with the idea that CaM binding was essential for the generation of the KCNQ2/KCNQ3 M-like current (13). Interestingly, a robust M-like current can be generated in the presence of a CaM mutant that was incapable of binding calcium, suggesting that this functional effect of CaM was calcium-independent (13). On the other hand, CaM also participated in the calcium-dependent modulation of KCNQ2/KCNQ3 currents (15).

To devise another test, independent of channel mutagenesis, for the necessity of CaM binding in the generation of KCNQ2/KCNQ3 current, we constructed a GST-fusion protein comprising the CaM-binding domain of KCNQ2. When this fusion protein is expressed in heterologous cells, it competes with coexpressed KCNQ2 for CaM binding, and thereby decreases the amount of KCNQ2/KCNQ3 current (13). In the present study, we extended this approach to an examination of the native M-current in hippocampal neurons, because CaM also binds to KCNQ channel subunits in native brain tissue (13). GFP was used as the fusion protein construct in place of GST to ensure that we were recording exclusively from transfected neurons. Expression of the CaM-binding fusion protein decreases the amplitude of the neuronal M-current, but the kinetics of the residual current remained unchanged. Importantly, a single point mutation in the fusion protein that renders it unable to bind CaM does not affect the M-current, confirming that the decrease in current amplitude results from competition for available endogenous CaM by the CaM-binding WT fusion protein. The ability of an exogenous protein to compete for binding raises the interesting possibility that the interaction of CaM with the channel was subject to dynamic regulation. We have not observed modulation of the hippocampal neuron M-current by WT fusion protein placed in the patch electrode (data not shown), but it is possible that the dissociation of the CaM-channel complex was slow relative to the time course of a patch recording experiment.

The CaM-binding fusion protein would also be expected to compete with other cellular signaling pathways that depend on CaM, and thus might influence neuronal membrane currents indirectly. However, not all potassium currents are altered by expression of the CaM-binding WT fusion protein. For example, the rapidly inactivating A-type potassium current was not affected by fusion protein expression in the same neurons in which M-current amplitude is markedly decreased. In addition, fusion protein expression does not alter large conductance calcium-activated potassium (or any other) current that participates significantly in the generation of the rapid AHP. Thus, there was at least some selectivity in the membrane currents that are affected by disruption of CaM binding.

The M-current is important for normal neuronal physiology (e.g., ref. 18), and mutations in KCNQ channels result in pathological conditions including epilepsy and deafness (32). The regulation of KCNQ channels and the neuronal M-current involves multiple molecular players, including phosphatidylinositol-bis-phosphate (6, 7), protein kinases and phosphatases (33, 34), and CaM (13, 15). At least some of these molecular participants play critical roles in the control of neuronal excitability, as indicated by the effects of the CaM-binding fusion protein on spike accommodation and ADP amplitude shown here. The findings that CaM can participate in both calcium-dependent (15) and calcium-independent (13) KCNQ channel regulation, and that an important role for CaM was recapitulated in hippocampal neurons, emphasize further the complexity of modulation of the neuronal M-current.

We thank Margaret Maronski and Marc Dichter (University of Pennsylvania School of Medicine) for providing us with cultured hippocampal neurons. This work was supported by a grant from the National Institutes of Health (to I.B.L.).

1. Brown, D. A. & Adams, P. R. (1980) *Nature* **283,** 673–676.
2. Adams, P. R., Brown, D. A. & Constanti, A. (1982) *J. Physiol. (London)* **330,** 537–572.
3. Marrion, N. V. (1997) *Annu. Rev. Physiol.* **59,** 483–504.
4. Jones, S. W. (1985) *J. Physiol. (London)* **366,** 63–87.
5. Jones, S. W. & Adams, P. R. (1987) in *Neuromodulation*, eds. Kaczmarek, L. K. & Levitan, I. B. (Oxford Univ. Press, New York), pp. 159–186.
6. Suh, B.-C. & Hille, B. (2002) *Neuron* **35,** 507–520.
7. Zhang, H., Craciun, L. C., Mirshahi, T., Rohacs, T., Lopes, C. M., Jin, T. & Logothetis, D. E. (2003) *Neuron* **37,** 963–975.
8. Jentsch, T. J. (2000) *Nat. Rev. Neurosci.* **1,** 21–30.
9. Wang, H. S., Pan, Z., Shi, W., Brown, B. S., Wymore, R. S., Cohen, I. S., Dixon, J. E. & McKinnon, D. (1998) *Science* **282,** 1890–1893.
10. Schroeder, B. C., Hechenberger, M., Weinreich, F., Kubisch, C. & Jentsch, T. J. (2000) *J. Biol. Chem.* **275,** 24089–24095.
11. Selyanko, A. A., Hadley, J. K., Wood, I. C., Abogadie, F. C., Delmas, P., Buckley, N. J., London, B. & Brown, D. A. (1999) *J. Neurosci.* **19,** 7742–7756.
12. Shah, M. M., Mistry, M., Marsh, S. J., Brown, D. A. & Delmas, P. (2002) *J. Physiol. (London)* **544,** 29–37.
13. Wen, H. & Levitan, I. B. (2002) *J. Neurosci.* **22,** 7991–8001.
14. Yus-Nájera, E., Santana-Castro, I. & Villarroel, A. (2002) *J. Biol. Chem.* **277,** 28545–28553.
15. Gamper, N. & Shapiro, M. S. (2003) *J. Gen. Physiol* **122,** 17–31.
16. Buchhalter, J. & Dichter, M. A. (1991) *Brain Res. Bull.* **26,** 333–338.
17. Passmore, G. M., Selyanko, A. A., Mistry, M., Al-Qatari, M., Marsh, S. J., Matthews, E. A., Dickenson, A. H., Brown, T. A., Burbidge, S. A., Main, M., *et al.* (2003) *J. Neurosci.* **23,** 7227–7236.
18. Yue, C. & Yaari, Y. (2004) *J. Neurosci.* **24,** 4614–4624.
19. Shah, M. M., Miscony, Z., Javadzadeh-Tabatabaie, M., Ganellin, C. R. & Haylett, D. G. (2001) *Br. J. Pharmacol.* **132,** 889–898.
20. Stocker, M., Hirzel, K., D'hoedt, D. & Pedarzani, P. (2004) *Toxicon* **43,** 933–949.
21. Storm, J. F. (1987) *J. Physiol. (London)* **385,** 733–759.
22. Marrion, N. V. & Tavalin, S. J. (1998) *Nature* **395,** 900–905.
23. Levitan, I. B. (1999) *Neuron* **22,** 645–648.
24. Saimi, Y. & Kung, C. (2002) *Annu. Rev. Physiol.* **64,** 289–311.
25. Xia, X. M., Fakler, B., Rivard, A., Wayman, G., Johnson-Pais, T., Keen, J. E., Ishii, T., Hirschberg, B., Bond, C. T., Lutsenko, S., *et al.* (1998) *Nature* **395,** 503–507.
26. Lee, A., Scheuer, T. & Catterall, W. A. (2000) *J. Neurosci.* **20,** 6830–6838.
27. Peterson, B. Z., DeMaria, C. D., Adelman, J. P. & Yue, D. T. (1999) *Neuron* **22,** 549–558.
28. Qin, N., Olcese, R., Bransby, M., Lin, T. & Birnbaumer, L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2435–2438.
29. Zuhlke, R. D., Pitt, G. S., Deisseroth, K., Tsien, R. W. & Reuter, H. (1999) *Nature* **399,** 159–162.
30. Erickson, M. G., Alseikhan, B. A., Peterson, B. Z. & Yue, D. T. (2001) *Neuron* **31,** 973–985.
31. Schumacher, M. A., Rivard, A. F., Bachinger, H. P. & Adelman, J. P. (2001) *Nature* **410,** 1120–1124.
32. Jentsch, T. J., Schroeder, B. C., Kubisch, C., Friedrich, T. & Stein, V. (2000) *Epilepsia* **41,** 1068–1069.
33. Gamper, N., Stockand, J. D. & Shapiro, M. S. (2003) *J. Neurosci.* **23,** 84–95.
34. Hoshi, N., Zhang, J. S., Omaki, M., Takeuchi, T., Yokoyama, S., Wanaverbecq, N., Langeberg, L. K., Yoneda, Y., Scott, J. D., Brown, D. A., *et al.* (2003) *Nat. Neurosci.* **6,** 564–571.