Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents

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Benign familial neonatal convulsion (BNFC) is a neurological disorder caused by mutations in the potassium channel genes KCNQ2 and KCNQ3, which are thought to contribute to the medium afterhyperpolarization (mAHP). Despite their importance in normal brain function, it is unknown whether they invariably function as heteromeric complexes. Here, we examined the contribution of KCNQ3 and KCNQ2 in mediating the apamin-insensitive mAHP current (ImAHP) in hippocampus. The ImAHP was not impaired in CA1 pyramidal neurons from mice genetically deficient for either KCNQ3 or KCNQ2 but was reduced ≈50% in dentate granule cells. While recording from KCNQ-deficient mice, we observed that the calcium-activated slow afterhyperpolarization current (IsAHP) was also reduced in dentate granule cells, suggesting that KCNQ channels might also contribute to this potassium current whose molecular identity is unknown. Further pharmacological and molecular experiments manipulating KCNQ channels provided evidence in support of this possibility. Together our data suggest that multiple KCNQ subunit compositions can mediate the ImAHP, and that the very same subunits may also contribute to the IsAHP. We also present data suggesting that the neuronal calcium sensor protein hippocalcin may allow for these dual signaling processes.

Epilepsy | KCNQ | M-current | Retigabine | sAHP

In the brain, calcium regulates neuronal responses by activating potassium channels that in turn shape neuronal output (1, 2). A hallmark of pyramidal neuron function is a physiological process known as spike frequency adaptation, which dictates the duration and frequency of action potential firing (1, 2). A train of action potentials leads to the activation of voltage-gated calcium channels and rapid calcium entry into neurons. Calcium then activates a series of potassium channels leading to a pronounced hyperpolarization and subsequent spike frequency adaptation. Classically, this afterhyperpolarization is subdivided into three phases: fast (fAHP), medium (mAHP), and slow afterhyperpolarization (sAHP) (2, 3).

Although the channels underlying the sAHP are unknown, molecular studies over the past decade have identified the channels that mediate the fAHP and the mAHP. The BK family of potassium channels is responsible for the fAHP (4). The mAHP is primarily composed of an apamin-sensitive conductance mediated by SK2 channels and the M-current presumed to be mediated by KCNQ family members (5, 6). However, it is unclear whether KCNQ2, KCNQ3, or KCNQ2/3 heteromers (KCNQ2/3) contribute to the ImAHP in the hippocampus. Therefore, we set out to determine the role of KCNQ subunits to the ImAHP in the hippocampus. We found that both KCNQ2 and KCNQ3 contribute to the ImAHP in a cell-type specific manner, although neither subunit is strictly required. Unexpectedly, the IsAHP is also reduced in KCNQ knockout mice.

Results

Role of KCNQ3 in the ImAHP in Hippocampus. Previous work has suggested that KCNQ2/3 heteromers are responsible for the M-current, a component of the ImAHP (6, 7). To directly determine which KCNQ channels contribute to the ImAHP in hippocampus, we used KCNQ knockout mice. We first focused on KCNQ3 knockout mice ($KCNQ3^{-/-}$). These mice were viable, unlike KCNQ2 knockouts (8), and their breeding resulted in progeny that followed Mendelian inheritance (35 $KCNQ3^{+/+}$, 72 $KCNQ3^{+/-}$, 34 $KCNQ3^{-/-}$, $n = 21$ litters, 141 mice, χ^2 $P = 0.96$). As this was not the focus of our study, we did not characterize the mice for any neurological phenotypes.

To confirm the functional loss of KCNQ3, we used retigabine, an antiepileptic drug that acts as a channel opener by stabilizing the open conformation of KCNQ channels. In heterologous expression systems, it primarily targets KCNQ3-containing channels and, to a lesser extent, KCNQ2 homomers (9). Bath application of retigabine to hippocampal CA1 pyramidal cells from wild-type mice led to the rapid development of an outward current reflecting the opening of KCNQ channels (Fig. 1) (10). However, bath application of retigabine to slices from $KCNQ3^{-/-}$ mice generated little outward current, verifying the absence of KCNQ3 from these cells.

Although KCNQ2/3 heteromers are thought to constitute the M-current and contribute to the apamin-insensitive ImAHP, the apamin-insensitive ImAHP in CA1 pyramidal neurons was unaffected in $KCNQ3^{-/-}$ (Fig. 2*A*). This indicated that KCNQ3 and consequently KCNQ2/3 heteromers are not strictly required for the apamin-insensitive ImAHP. To test whether KCNQ channels mediate the remaining apamin-insensitive ImAHP in $KCNQ3^{-/-}$ mice, we bath applied 10 μmol/l XE991, a blocker specific to KCNQ channels at this concentration. Application of XE991 (Fig. 2*A*) blocked the peak amplitude of the apamin-insensitive ImAHP in $KCNQ3^{-/-}$ mice ($49 \pm 4\%$, $n = 5$) to the same extent as in wild-type mice ($51 \pm 4\%$, $n = 5$; $P = 0.73$) (Fig. 2*A*). The apamin-insensitive ImAHP has also been traditionally recorded using an M-current voltage protocol, holding neurons at −30 mV and stepping down to −50 mV. The M-current is defined as the time-dependent, slowly deactivating portion of this current. Like the ImAHP, there was no significant difference between the M-current recorded in $KCNQ3^{+/+}$ (16 ± 2 pA, $n = 7$) and $KCNQ3^{-/-}$ mice (19 ± 3 pA, $n = 11$, $P = 0.42$) (Fig. 2*B*). We also measured the current blocked by 10 μmol/l XE991 using the classical M-current protocol. We did not find any difference between the current blocked by XE991 in $KCNQ3^{-/-}$ mice (57 ± 10 pA, $n = 11$) and that of $KCNQ3^{+/+}$ mice (64 ± 12 pA, $n = 7$, $P = 0.66$) (Fig. 2*B*).

The maintenance of the ImAHP in $KCNQ3^{-/-}$ mice suggests compensatory mechanisms could be occurring in these neurons. To test whether other afterhyperpolarization currents may have changed, we measured the sAHP and the SK currents. Neither the IsAHP peak amplitude ($KCNQ3^{+/+}$ 37 ± 6 pA, $n = 14$; $KCNQ3^{-/-}$ 36 ± 5 pA, $n = 22$, $p = 0.88$) (Fig. 2*C*) nor the I-SK ($KCNQ3^{+/+}$ 126 ± 14 pA, $n = 10$; $KCNQ3^{+/+}$ 134 ± 11 pA, $n =$

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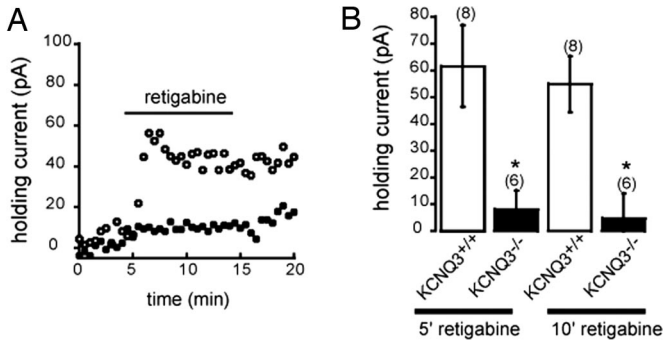

Fig. 1. Neurons from KCNQ3$^{-/-}$ mice are less sensitive to retigabine. (*A*) Time course of outward current induced by bath application of retigabine (40 μmol/l) in brain slices from either KCNQ3$^{+/+}$ ($n = 8$, open circles) or KCNQ3$^{-/-}$ mice ($n = 5$, filled circles). (*B*) Summary graphs showing the amplitude of the outward current after 5-min and 10-min bath application of retigabine in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice. Asterisk indicates $P < 0.05$ (5 min) and $P < 0.01$ (10 min).

18; $p = 0.66$) was reduced in KCNQ3$^{-/-}$ mice. Together, our data suggest that KCNQ3 channels are present in CA1 neurons but are not required for the generation of the apamin-insensitive ImAHP in hippocampal CA1 pyramidal cells.

A complicating factor in using knockout mice is possible compensation by related family members. Inspection of the Allen brain atlas and previously published work (11, 12) indicated that both KCNQ2 and KCNQ5 channels are expressed in CA1 pyramidal neurons in addition to KCNQ3. Although KCNQ2 and KCNQ3 have been implicated in the ImAHP, a role for KCNQ5 has not been excluded. To decrease the likelihood of compensation by KCNQ5, we investigated hippocampal dentate granule cells that express KCNQ2 and KCNQ3 mRNA but little KCNQ5 mRNA (11). In contrast to CA1 pyramidal cells, the absence of KCNQ3 in granule cells led to a significant decrease in the apamin-insensitive ImAHP (Fig. 3*A*) (KCNQ3$^{+/+}$: 178 ± 23 pA, $n = 8$; KCNQ3$^{-/-}$: 86 ± 15 pA, $n = 15$; $P < 0.005$). This suggests that KCNQ3 does contribute to the ImAHP, although its precise contribution is cell type dependent.

Contribution of KCNQ2 to ImAHP in Hippocampus. Given that KCNQ2 is also present in dentate granule cells, we wondered whether KCNQ2 also contribute to the ImAHP. To test this hypothesis, we obtained mice genetically deficient for KCNQ2. KCNQ2$^{-/-}$ mice do not survive because of pulmonary atelectasis; therefore, we investigated KCNQ2$^{+/-}$ mice, which have a decreased threshold for chemically induced seizures (8). We found that the peak amplitude of the ImAHP was significantly decreased in KCNQ2$^{+/-}$ dentate granule cells compared to KCNQ2$^{+/+}$ littermates (KCNQ2$^{+/+}$: 199 ± 19 pA, $n = 7$; KCNQ2$^{+/-}$: 81 ± 16 pA, $n = 10$; $P < 0.0005$) (Fig. 3*B*). Unlike dentate granule cells in CA1 pyramidal neurons, KCNQ2 deficiency did not significantly impair the ImAHP (KCNQ2$^{+/+}$:

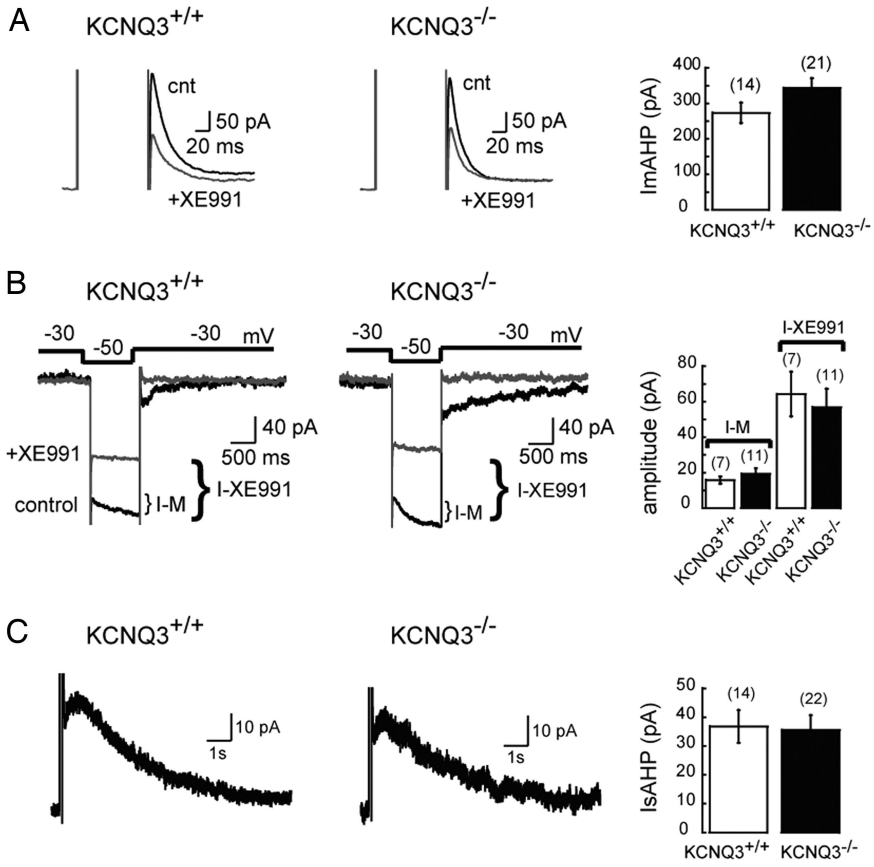

Fig. 2. KCNQ3 is not required for the ImAHP/M-current in CA1 pyramidal neurons. (*A*) Left and middle panels show ImAHP in either KCNQ3$^{+/+}$ or KCNQ3$^{-/-}$ mice before and after application of 10 μm XE991. Right panel is a summary graph of the ImAHP amplitude in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice. (*B*) Left and middle panels show the M-current induced by a step hyperpolarization (1s) to −50 mV from a holding potential of −30 mV from either KCNQ3$^{+/+}$ or KCNQ3$^{-/-}$ before and after application of 10 μmol/l XE991. Brackets indicate the traditional M-current (I-M) or the current blocked by 10 μmol/l XE991 (I-XE991). Right panel is a summary graph of the M-current amplitude (I-M and I-XE991) in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice. (*C*) Left and middle panels show IsAHP in either KCNQ3$^{+/+}$ or KCNQ3$^{-/-}$ mice; right panel is a summary graph of the IsAHP amplitude in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice.

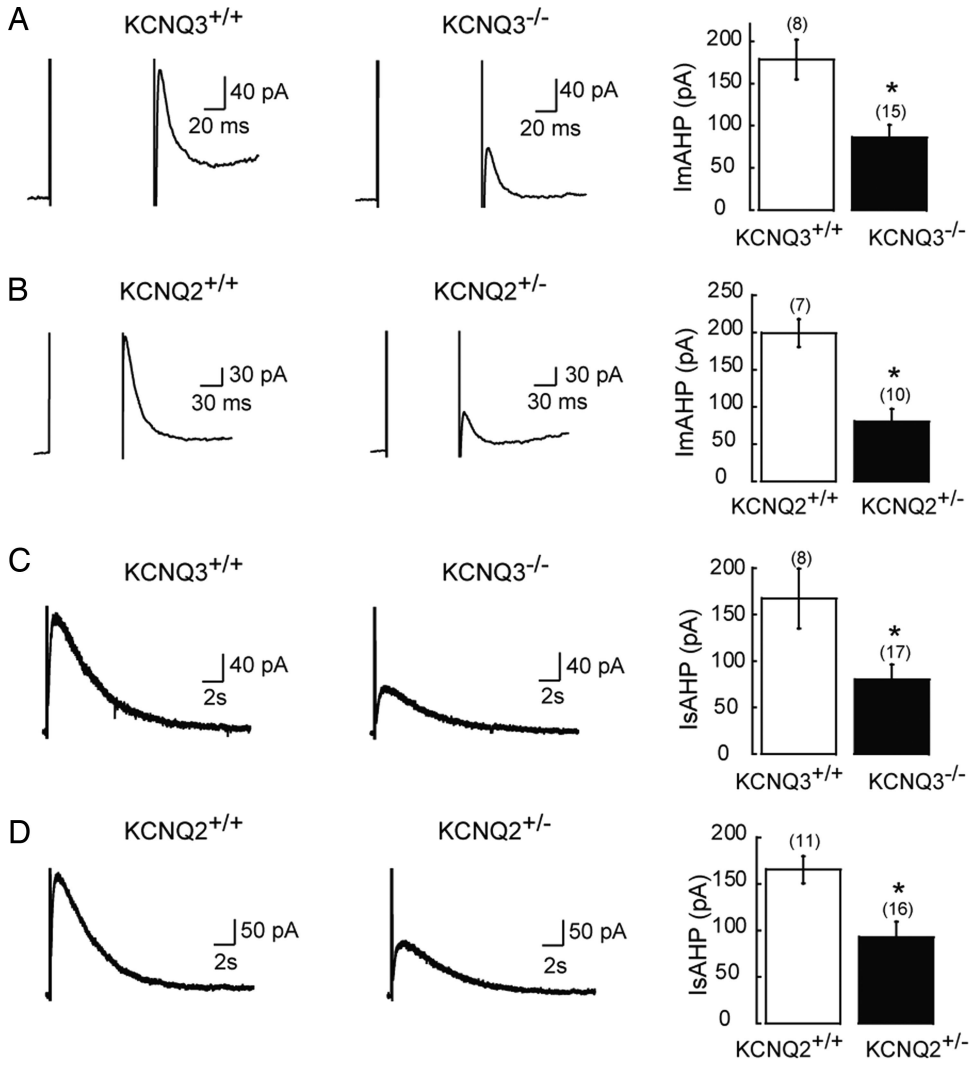

Fig. 3. Loss of KCNQ3 or KCNQ2 impairs the ImAHP and IsAHP in dentate gyrus granule cells. (*A*) Left and middle panels show an ImAHP in either KCNQ3$^{+/+}$ or KCNQ3$^{-/-}$ mice. Right panel is a summary graph of the ImAHP amplitude in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice. Asterisk indicates a $P < 0.005$. (*B*) Left and middle panels show an ImAHP in either KCNQ2$^{+/+}$ or KCNQ2$^{+/-}$ mice. Right panel is a summary graph of the ImAHP amplitude in KCNQ2$^{+/+}$ and KCNQ3$^{+/-}$ mice. Asterisk indicates $P < 0.005$. (*C*) Left and middle panels show IsAHP in either KCNQ3$^{+/+}$ or KCNQ3$^{-/-}$ mice. Right panel is a summary graph of the IsAHP amplitude in KCNQ3$^{+/+}$ and KCNQ3$^{-/-}$ mice. Asterisk indicates $P < 0.05$. (*D*) Left and middle panels show an IsAHP in either KCNQ2$^{+/+}$ or KCNQ2$^{+/-}$. Right panel is a summary graph of the IsAHP amplitude in KCNQ2$^{+/+}$ and KCNQ2$^{+/-}$ mice. Asterisk indicates a $P < 0.005$.

415 ± 28 pA, $n = 11$; KCNQ2$^{+/-}$: 355 ± 18 pA, $n = 27$; $P = 0.08$). Together our data indicate that both KCNQ2 and KCNQ3 contribute to the apamin-insensitive ImAHP but that neither is strictly required. However, our data do not exclude the possibility that native channels are predominantly heteromers.

Possible Contribution of the KCNQ Channels in IsAHP. During the course of experiments, we found that the IsAHP was reduced ≈50% in dentate granule cells in both KCNQ3$^{-/-}$ mice (KCNQ3$^{+/+}$: 167 ± 32 pA, $n = 8$; KCNQ3$^{-/-}$ 80 ± 16 pA, $n = 15$; $P < 0.05$) and KCNQ2$^{+/-}$ mice (KCNQ2$^{+/+}$: 165 ± 15 pA, $n = 11$; KCNQ2$^{+/-}$ 93 ± 17 pA, $n = 16$; $P < 0.005$) (Figs. 3*C*, 3*D*). Our data might indicate that KCNQ channels contribute to the IsAHP or that compensatory mechanisms indirectly impair the IsAHP.

We decided to test the possibility that KCNQ channels underlie a component of the IsAHP. KCNQ-dependent processes are typically sensitive to XE991 and retigabine. Application of 10 μmol/l XE991 to CA1 pyramidal neurons of wild-type mice led to a small but reliable reduction of the IsAHP (XE991 block 33 ± 4%, $n = 13$) (Fig. 4*A*). Application of 40 μmol/l retigabine altered the IsAHP waveform, making it difficult to measure a peak response (Fig. 4*B*). Consequently, we measured the IsAHP charge transfer by integrating the IsAHP response over 19 seconds and found that retigabine significantly decreased the IsAHP charge (control: 324 ± 37 pC, $n = 10$; retigabine 98 ± 24 pC, $n = 10$; $P < 0.001$ paired Student's *t* test) (Fig. 4*B*). This reduction probably occurs through occlusion given that retigabine acts as a KCNQ channel facilitator (9). It is unlikely that the retigabine-induced changes are caused by voltage escape or shunting because of poor space clamp, as previous work has shown that KCNQ-dependent processes are primarily found in the soma and axon hillock, regions that are sufficiently voltage clamped.

As a further test to determine whether KCNQ channels contribute to the IsAHP in hippocampus, we co-transfected human KCNQ2- and KCNQ3-dominant negative constructs (KCNQ2GS, KCNQ3GS) in cultured hippocampal slices. These constructs have a point mutation in their selectivity filter, rendering them nonconductive, and were previously shown to inhibit the ImAHP in neurons (6, 13). We co-transfected

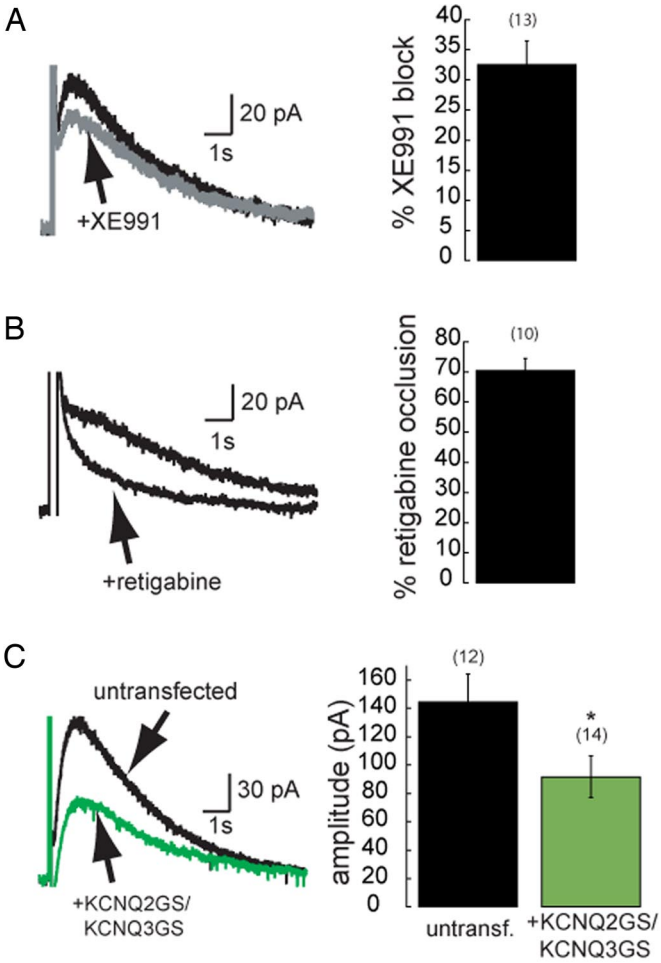

Fig. 4. KCNQ channels might contribute to the IsAHP. (*A*) Left panel shows representative traces of the IsAHP before and after bath application of 10 μmol/l XE991. Right panel is a summary graph of the block induced by XE991. (*B*) Left panel shows representative traces of the IsAHP before and after bath application of 40 μmol/l retigabine. Right panel is a summary graph of the occlusion induced by retigabine. (*C*) Left panel shows overlaid traces of the evoked IsAHP recorded from transfected (green) and untransfected (black) neurons. Right panel is a bar graph summarizing the effect of cotransfecting KCNQ2GS and KCNQ3GS-IRES-EGFP on IsAHPs. We recorded from CA3 neurons because of the larger IsAHP amplitude. Asterisk indicates statistical significance of $P < 0.05$ (IsAHP). A 100 nmol/l quantity of apamin was present during the course of these recordings.

KCNQ2GS and KCNQ3GS because previous work demonstrated that both are required for robust surface expression in pyramidal neurons (14). As shown in Fig. 4*C*, the IsAHP was significantly decreased in neurons transfected with the dominant negative constructs (untransfected: 145 ± 19 pA, $n = 12$; transfected: 92 ± 15 pA, $n = 14$; $P < 0.05$). As a control for the effectiveness of the dominant negative constructs, we also measured the ImAHP in the same neurons and found that the ImAHP was severely reduced (untransfected: 109 ± 25 pA, $n = 12$; transfected: 28 ± 11 pA, $n = 14$; $P < 0.005$).

Together these data provide the first evidence that KCNQ channels might contribute to the IsAHP. However, the absence of complete block by XE991 could suggest that additional potassium channels also contribute to the IsAHP.

Hippocalcin Unmasks a sAHP-Like Current in Superior Cervical Ganglion Neurons. If KCNQ channels contribute to two distinct physiological processes, the mAHP and the sAHP, why do some neurons exhibit only an apamin-insensitive ImAHP/M-current, but not an IsAHP? Based on our previous work (15), one possibility is that these neurons express KCNQ subunits but do not express hippocalcin, the calcium sensor required for the IsAHP.

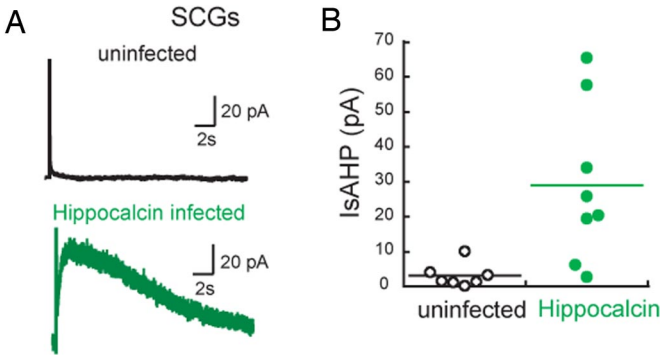

Fig. 5. Hippocalcin generates a sAHP-like current in SCG neurons. (*A*) Traces showing the dependence of IsAHP on hippocalcin. Top trace is from an uninfected neuron on a coverslip infected with hippocalcin-EGFP. Bottom panel shows a trace from an infected neuron (green) expressing hippocalcin-EGFP. (*B*) Scatter plot of the IsAHP peak amplitudes from either uninfected or infected (green) SCG neurons.

To test this idea, we recorded from dissociated superior cervical ganglion (SCG) neurons, which are the model system for studying the M-current and are known to express KCNQ channels (16, 17). Importantly, sympathetic neurons do not have an IsAHP (18). We infected SCG neurons with a hippocalcin-expressing virus (15) to determine whether this might "rescue" the IsAHP in these KCNQ expressing neurons. One day postinfection, we found that SCGs generated a sAHP-like current based on its waveform that was similar to the IsAHP in hippocampal neurons (Fig. 5). The simplest interpretation of our data are that the addition of hippocalcin allows SGCs to generate a sAHP-like current, and this may involve KCNQ channels. However, it is possible that the IsAHP generated in SCGs might not be identical to the IsAHP in hippocampus.

Discussion

Molecular Composition of the M-Current in Hippocampus. Our study is a direct demonstration *in vivo* that KCNQ2 and KCNQ3 channels contribute to the apamin-insensitive ImAHP. However, our data do not support the widely held hypothesis that KCNQ2/3 heteromers are the only possible mediators of this process. Previous work in heterologous systems suggested that KCNQ2 and KCNQ3 homomers do not traffic efficiently or produce robust currents (7, 19), leading to the hypothesis that only KCNQ2/KCNQ3 or KCNQ3/KCNQ5 heteromers mediate the M-current. However, our data showing that loss of KCNQ3 in CA1 pyramidal neurons does not impair the ImAHP suggests that KCNQ2 and/or KCNQ5 homomers mediates the remaining ImAHP as KCNQ2 and KCNQ5 do not form functional heteromers. Consistent with this idea, McBain *et al.* have suggested that KCNQ2 channels most likely mediate the M-current recorded in some interneurons of the hippocampus (20).

Although not all biophysical properties of the native M-current are reconstituted by KCNQ2/3 in heterologous cells (7, 21), the proposed model of KCNQ2/3 heteromers in mediating the M-current in SCGs is well accepted. The first description of the M-current was in frog sympathetic ganglia and SCGs, and it was in these preparations that most of the pharmacological and biophysical properties of the M-current were elucidated (22). However, the KCNQ2/3 heteromer model might not be an accurate representation of the M-current in hippocampus. For

instance, the SCG M-current and KCNQ2/3 channels are sensitive to phorbol ester activation (17), but phorbol ester application has no effect on the M-current in hippocampus (23, 24). In addition, the M-current in SCG neurons activates at membrane potentials more positive to −60 mV having a $V_{1/2}$ of approximately −45 to −35 mV (16), whereas in CA1 pyramidal neurons the M-current is active even below resting membrane potentials with a $V_{1/2}$ of approximately −60 mV (figure S7 of ref. 25). Furthermore, in hippocampal pyramidal neurons the M-current is segregated into two populations, a TEA-sensitive and a TEA-insensitive component (12), further supporting the notion that multiple KCNQ channel combinations contribute to the apamin-insensitive ImAHP.

Possible Mediation of the IsAHP by KCNQ Channels. The proposal that KCNQ channels might contribute to the IsAHP is unexpected and provocative, as KCNQ channels were already known to underlie the M-current, a potassium current that has been traditionally thought of as a distinct molecular entity based on their differing voltage dependence, sensitivity to norephinephrine block, and inhibition by XE991. Unlike the M-current and KCNQ channels, the current-to-voltage relationship (I-V) and kinetics of the IsAHP have been described as voltage independent (3). However, the kinetics of the IsAHP might not solely depend on intrinsic channel kinetics but rather on the calcium sensor binding/unbinding kinetics, which are unlikely to be voltage dependent. In addition, norepinephrine-induced PKA activation robustly inhibits the IsAHP, but not the M-current (26). This difference might be explained if the IsAHP norepinephrine sensitivity is not due solely to the underlying channels. Consistent with this possibility, the IsAHP in hippocalcin knockout mice has greatly reduced sensitivity to norepinephrine (15). The ImAHP and KCNQ-dependent processes are both blocked by 10 μmol/l XE991, whereas the IsAHP was not reported to be XE991 sensitive (10). We found that XE991 led to a small but consistent block of the IsAHP in mice. This might suggest that members of the KCNQ channel family are not the sole mediators of the IsAHP or that their contribution is small. Alternatively, the lack of complete XE991 block may be due to the presence of an auxillary subunit (7), holding membrane potential (27), or subunit composition (28).

Overall, our data raise the possibility that KCNQ channels at least in part underlie the IsAHP. If true, this provides an elegant example of modular architecture. That is, the same ion channel family mediates two temporally and functionally distinct processes by simply engaging a diffusible calcium sensor, hippocalcin. Such arrangement provides an enormous flexibility to neurons in controlling and regulating their rate of action potential discharge, as the mAHP and sAHP have distinct temporal profiles and are regulated by different signaling cascades. However, in the absence of complete elimination of the IsAHP by KCNQ knockout mice or reconstitution in a heterologous expression system, we cannot unequivocally conclude that KCNQ channels mediate the IsAHP. Previous work using transgenic mice expressing KCNQ2 dominant-negative subunits showed that these mice had very little spike frequency adaptation and a severely diminished mAHP (6). Given the prominent role that IsAHP plays in spike frequency adaptation in CA1 pyramidal neurons, we predict that IsAHP is diminished in these mice and that this reduction might contribute to their striking behavioral phenotype.

Methods

Experiments were carried out according to the guidelines of the University of California–San Francisco Institutional Animal Care and Use Committee.

Knockout Mice and PCR Genotyping. (1) We bred KCNQ3$^{+/-}$ mice (Deltagen Inc., San Mateo, CA) to generated KCNQ3$^{-/-}$ mice and wild-type littermates. KCNQ3$^{-/-}$ mice have a deletion from base 1803 to 1824 by insertion of a LacZ-Neo cassette construct. Three sets of primers were used for PCR genotyping: reverse 5′- ATGGCAATAGAAGGGCTTACCTGTC-3′, Neo forward 5′-GGGTGGGATTAGATAAATGCCTGCTCT-3′, and wild-type forward 5′-GTGAGT-GAAAAACCATGGTCCCATG-3′. The primers amplified a 210-bp fragment from the wild-type allele and a 461-bp fragment from the knockout allele. (2) Kcnq2^{tm1Dgen}/Kcnq2^{+} (KCNQ2$^{+/-}$) mice (Jackson laboratory, Bar Harbor, ME) contain a deletion in the KCNQ2 gene from base 418 to 535 by insertion of a Lac0-SA-IRES-lacZ-Neo555G/Kan construct. Phenotypic data regarding these mice are available from Jackson Laboratories. As reported before, Kcnq2$^{tm1Dgen/tm1Dgen}$ was lethal. For KCNQ2 genotyping, three primers were included in each PCR: wild-type forward 5′-ATCGTGACTATCGTGGTATTCG-GTG-3′, Neo forward 5′-GGGCCAGCTCATTCCTCCCACTCAT-3′, and reverse 5′-GGTGATAAG AAGGAACTTCCAGAAG-3′. The primers amplified a 240-bp fragment from the wild-type allele and a 427-bp product from the knockout allele.

Recordings. Transverse hippocampal slices (300 μm) were prepared as described in detail previously (15). Briefly, the hippocampus was cut in ice-cold cutting solution (in mmol/l): 50 NaCl, 25 $NaHCO_3$, 150 sucrose, 10 glucose, 2.5 KCl, 1 NaH_2PO_4, 0.5 $CaCl_2$, and 7 $MgCl_2$, and stored for 30 min at 35 °C in artificial cerebrospinal fluid (ACSF) (in mmol/l): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose. Slices were then maintained at room temperature. Whole-cell recordings were made with glass pipettes (2–4 MΩ) filled with an intracellular solution (in mmol/l): 150 potassium methylsulfate, 10 KCl, 10 HEPES, 4 NaCl, 4 Mg_2ATP, and 0.4 Na_4GTP. Osmolarity was adjusted to 300–305 mOsm and pH to 7.25–7.35 with KOH. The slices were bathed in a modified ACSF containing 500 nmol/l TTX to block voltage-gated sodium channels. Current responses were collected with an Axopatch-1D amplifier, filtered at 2 kHz, digitized at 5 kHz and analyzed using Igor Pro software (Wavemetrics, Lake Oswego, OR).

Organotypic Slice Cultures. Hippocampi were dissected from rat pups at P9-P10 as described previously (29). Slices were transfected with 1.0 μm DNA-coated gold particles using the Helios Gene Gun (Bio-Rad) after 3–4 days in culture. Dominant negative hKCNQ2 and hKCNQ3 constructs were generated by site-directed mutagenesis using Quickchange II (Stratagene, La Jolla, CA) per the manufacturer's instruction. The mutations were confirmed by sequencing. The KCNQ3GS was cloned into pIRES2-EGFP (Clontech) and hKCNQ2GS in pcDNA3 (Invitrogen). Previously, our laboratory has determined that the gene gun method results in >90% co-transfection (29).

Dissociated Superior Cervical Ganglion Neurons. Dissociated SCGs were prepared from P22–30 C57BL/6 mice as described previously (30). For viral infection of dissociated SCG neurons, hippocalcin-EGFP viral particles were added to acutely dissociated SCG neurons (15). We recorded 16–20 h postinfection using the same recording solutions and protocols described for acute slices.

Statistical Analysis. Statistical significance was determined using unpaired Student's *t* tests. All data shown are the mean ± SEM.

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