Potassium channels act as chemosensors for solute transporters

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Potassium channels form physical complexes with solute transporters in vivo, yet little is known about their range of possible signaling modalities and the underlying mechanisms. The KCNQ2/3 potassium channel, which generates neuronal M-current, is voltage-gated and its activity is also stimulated by binding of various small molecules. KCNQ2/3 forms reciprocally regulating complexes with sodium-coupled myo-inositol transporters (SMITs) in mammalian neurons. Here, we report that the neurotransmitter γ-aminobutyric acid (GABA) and other small molecules directly regulate myo-inositol transport in rat dorsal root ganglia, and by human SMIT1-KCNQ2/3 complexes in vitro, by inducing a distinct KCNQ2/3 pore conformation. Reciprocally, SMIT1 tunes KCNQ2/3 sensing of GABA and related metabolites. Ion permeation and mutagenesis studies suggest that SMIT1 and GABA similarly alter KCNQ2/3 pore conformation but via different KCNQ subunits and molecular mechanisms. KCNQ channels therefore act as chemosensors to enable co-assembled myo-inositol transporters to respond to diverse stimuli including neurotransmitters, metabolites and drugs.

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**Results**

**GABA and related metabolites regulate SMIT1 via KCNQ2/3.**

KCNQ channels, like all Kv channels, form as tetramers of α subunits, each subunit of which contains six transmembrane (S) segments, split into the voltage-sensing domain (VSD, S1–4) and the pore module (S5–6). SMIT1 and SMIT2 transporters have a predicted 14 transmembrane segment topology (Fig. 1a). As we previously found that KCNQ2/3 channels co-localize with SMIT1 in rodent neurons and co-assemble with SMIT1 in vitro, we first quantified the effects of GABA on myo-inositol uptake of whole dorsal root ganglia (DRG) isolated from postnatal day 8 rats. GABA (100 µM) reduced [3H]myo-inositol uptake >twofold (Fig. 1b). In Xenopus oocyte expression studies, heterologously expressed SMIT1 activity was insensitive to GABA (100 µM) when expressed alone (Fig. 1c). In contrast, when co-expressed with KCNQ2/3, SMIT1 activity was inhibited >twofold, replicating our observations from rat DRG. Glutamate, which has no effect on KCNQ2/3 channels, had no effect on SMIT1 activity in the presence of KCNQ2/3. However, β-hydroxybutyrate (BHB), γ-aminohydroxybutyrate (GABOB), and the anticonvulsant retigabine, each of which, like GABA, activates KCNQ2/3 by negative shifting its voltage dependence of activation, each inhibited SMIT1 activity in the presence of KCNQ2/3 (Fig. 1d). We previously found that an S5 tryptophan conserved in neuronal KCNQs (e.g., W265 in KCNQ3) is required for binding to and activation of KCNQs by GABA. Here, mutation to leucine of KCNQ2–W236 and KCNQ3–W265 in KCNQ2/3 channels prevented GABA inhibition of co-expressed SMIT1 activity (Fig. 1e). Thus, GABA binding to the S5 tryptophan of KCNQ2/3 channels inhibits myo-inositol uptake by co-expressed SMIT1.

**SMIT1 alters small molecule effects on KCNQ2/3 gating.**

Heterologous co-expression in Xenopus laevis oocytes of human KCNQ2/3 channels and SMIT1 generated slow-activating, slow deactivating, non-inactivating voltage-dependent K+ currents, which we measured using two-electrode voltage clamp (TEVC) (Fig. 2a). Bath application of GABA (10 nM–10 mM) increased the peak tail currents, negative shifted the voltage dependence of activation of KCNQ2/3 channels (Fig. 2a, b) and increased activation rate (Fig. 2c), as we previously found for GABA effects on KCNQ2/3 in the absence of SMIT1. SMIT1 did not alter the maximal effects of GABA on KCNQ2/3 currents; however, SMIT1 co-expression reduced the potency of GABA with respect to KCNQ2/3 activation threefold, as measured by current fold increase at ~60 mV, where changes are higher than at more depolarized voltages but accurately quantifiable (KCNQ2/3, EC50 = 1.1 ± 0.39 µM GABA; KCNQ2/3–SMIT1, EC50 = 3.0 ± 0.67 µM GABA) (Fig. 1d). We observed an approximately similar (fourfold) reduction in KCNQ2/3 GABA sensitivity upon SMIT1 co-expression when we quantified the shift in voltage dependence of activation (ΔV0.5Activation) (KCNQ2/3, EC50 = 137 ± 23 nM GABA; KCNQ2/3–SMIT1, EC50 = 593 ± 125 nM GABA) (Fig. 1e).

KCNQ2/3–SMIT1 complexes were also sensitive to BHB, as we previously observed for KCNQ2/3; in the presence of SMIT1, BHB increased the peak tail current, negative shifted the voltage dependence of KCNQ2/3 activation (Fig. 2g) and speeded

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KCNQ2/3 activation (Fig. 2h). SMIT1 modulated the effects of BHB on KCNQ2/3, but differently to effects for GABA. Thus, SMIT1 increased the maximal efficacy of BHB 3-fold with respect to KCNQ2/3, yielding an maximal 8-fold increase in current at −60 mV (Fig. 2i) but reduced the BHB potency 60-fold as quantified by fold increase at −60 mV (KCNQ2/3–SMIT1, BHB EC50 = 16.5 ± 0.46 µM, versus 0.26 ± 0.07 µM for KCNQ2/3 without SMIT1) (Fig. 2i) or 13-fold as quantified by ΔV0.5Activation (KCNQ2/3–SMIT1, BHB EC50 = 10.2 ± 0.31 µM, versus 0.79 ± 0.30 µM for KCNQ2/3 without SMIT1) (Fig. 2j).

GABOB is a high-affinity partial agonist of KCNQ2/3 channels8. GABOB effects on KCNQ2/3–SMIT1 voltage dependence and activation rate were minimal (Fig. 2k–n). SMIT1 appears to reduce KCNQ2/3 sensitivity, by threefold as quantified by ΔV0.5Activation (KCNQ2/3–SMIT1) GABOB EC50 = 304 ± 100 nM, versus 89.1 ± 10 nM for KCNQ2/3 alone) (Fig. 2o). The capacity of SMIT1 to tune KCNQ2/3 sensitivity to GABA and related metabolites suggests that SMIT1 impinges on the KCNQ2/3 binding site for these molecules.

GABA and SMIT1 similarly alter KCNQ2/3 ion selectivity. We previously found that SMIT1 binds to the KCNQ2 pore module and decreases KCNQ2/3K+ selectivity, producing a relative increase in Na+ and Cs+ permeability—evidence of an effect of SMIT1 binding on KCNQ2/3 pore conformation13. Here, we found that GABA, which binds to a conserved tryptophan in S5–W265, GABA activates homomeric KCNQ3, but not homomeric KCNQ2. Thus, KCNQ4, which also binds GABA but previously found to give robust KCNQ3 currents without altering other fundamental channel properties19 (Fig. 3g–i). The results suggest that there are some common features to GABA and SMIT1 effects on KCNQ2/3 pore conformation. We next tested the effects of GABA on homomeric KCNQ4s (Fig. 3d–i). We previously found that while GABA binds to the S5 tryptophan of both KCNQ2 (W236) and KCNQ3 (W265), GABA activates homomeric KCNQ3, but not homomeric KCNQ2. Here, we found that GABA has no effect on the ion selectivity of homomeric KCNQ2 (Fig. 3d–f). In contrast, GABA increased the Na+ and Cs+ permeability of homomeric KCNQ3—a consistent observation of KCNQ3 mutant (A315T), previously found to give robust KCNQ3 currents without altering other fundamental channel properties19 (Fig. 3g–i). As an additional control we examined KCNQ4, which also binds GABA but is not activated by it19. GABA also had no effect on KCNQ4 ion selectivity (Fig. 3j–l). Thus, the current-potentiating effects of GABA occur concomitant to a shift in ion selectivity (as we
observe for KCNQ3 homomers and KCNQ2/3 heteromers); the selectivity shift does not occur in KCNQs that are not activated by GABA, even when GABA can bind to them (homomeric KCNQ2 and KCNQ4). These data suggest that the ion selectivity shift signals a conformational shift in the pore that occurs upon activation by GABA.

KCNQ3 is structurally primed to be the GABA sensor for SMIT1. The effects of SMIT1 on homomeric KCNQ3 channels were not previously reported. Here, we found that SMIT1 positively shifts the voltage dependence of KCNQ3+ (Fig. 4a–d), slows its activation (Fig. 4e) and depolarizes KCNQ3+ -expressing oocytes (Fig. 4f), i.e., SMIT1 impedes voltage-dependent KCNQ3+ activation. This is the opposite of SMIT1 effects on KCNQ2 and KCNQ2/3, as we previously reported13. Comparing next the effects of SMIT1 on homomeric KCNQ2 and KCNQ3+ relative ion permeability, we found that SMIT1 greatly increases the relative Na+ and Cs+ permeation compared to K+ of KCNQ2 (Fig. 4g–i), but not...
KCNQ3+ (Fig. 4j–l). This is again consistent with the increased Na+ and Cs+ permeability being the signature of an alternate pore conformation that arises when the voltage dependence of KCNQ channel activation is negative shifted, either by GABA or by SMIT1 (Fig. 3 and ref. 13). This suggests that while GABA activates and alters pore properties of KCNQ2/3 channels via KCNQ3, SMIT1 activates and alters pore properties of KCNQ2/3 via KCNQ2. Furthermore, the results suggest that by maintaining its baseline pore conformation despite interaction with SMIT1, KCNQ3 (but not KCNQ2) is structurally primed to respond to GABA and communicate the effects of GABA binding to SMIT1 to regulate SMIT1 function. Consistent with this hypothesis, KCNQ3–SMIT1 but not KCNQ2–SMIT1 was responsive to GABA, with the functional output being an approximately twofold reduction in SMIT1 myo-inositol uptake (Fig. 4m, n), similar to effects of GABA on KCNQ2/3–SMIT1 complexes (Fig. 1d).

SMIT1 permits KCNQs to distinguish BHB from GABA and GABOB. We previously showed that, as for GABA, BHB activates homomeric KCNQ3+ but not KCNQ28. Strikingly, here we found that while KCNQ3+ but not KCNQ2 can communicate the GABA binding event to co-expressed SMIT1 to alter its activity, for BHB the isoform selectivity is reversed. Thus, BHB inhibits myo-inositol transport activity of SMIT1 when co-expressed with KCNQ2, but not KCNQ3+. In contrast, for GABOB, as for GABA, KCNQ3+ but not KCNQ2 conferred sensitivity to SMIT1 (Fig. 4m, n). Thus, SMIT1 enables KCNQs to distinguish BHB from the structurally highly similar (Fig. 2) GABA and GABOB.

An S4–5 arginine is required for SMIT1–KCNQ2/3 communication. We previously found that GABA binds to the S5-located KCNQ2–W236 and KCNQ3–W265 (Fig. 5a, b), and that activation of KCNQ2/3 channels requires KCNQ3–W265 (KCNQ2 binds GABA but is not activated by it)8. We showed earlier that complexes formed by SMIT1 and heteromeric mutant W236L–KCNQ2/W265L–KCNQ3 channels do not respond to GABA (Fig. 1c) which is consistent with the role of the S5 W residues in GABA binding. However, W236L–KCNQ2/W265L–KCNQ3 channels remain sensitive to SMIT1, which negative shifts their midpoint voltage dependence of activation (Fig. 5c–f) and hyperpolarizes W236L–KCNQ2/W265L–KCNQ3-expressing oocytes (Fig. 5g) as we previously found for wild-type KCNQ2/3 channels13.

We also recently found that some plant metabolites can hyperpolarize KCNQ channel activation voltage dependence by binding to an arginine (e.g., R242 in KCNQ3) at the foot of S4, abutting the S4–5 linker (Fig. 5b)20–23. More recently, we found that this arginine also influences GABA binding23. Here, the activity of KCNQ2/3 channels with the S4–5 arginine mutated to alanine (KCNQ2–R213A/KCNQ3–R242A) was strongly inhibited by SMIT1 (Fig. 5h, i), in contrast to effects for wild-type13 or WL/WL (Fig. 5c–g) KCNQ2/3. While the low current magnitude of the resulting channels precluded accurate quantification of voltage dependence, SMIT1 co-expression positively shifted the resting membrane potential of KCNQ2–R213A/KCNQ3–R242A channels (Fig. 5j), the opposite of effects on wild-type13 or WL/WL (Fig. 5c–g) KCNQ2/3. Thus, we conclude that GABA binding to the S5 W causes a conformational shift in KCNQ2/3 channels that is communicated to co-assembled SMIT1 via residues other than the S5 W, likely including R213/R242. In accord with this hypothesis, KCNQ2–R213A/KCNQ3–R242A channels were unable to modulate either SMIT1 myo-inositol transport, or the regulation of this process by GABA (Fig. 5k).

Discussion

An increasing number of potassium channel-transporter (“chansporter”) complexes are being uncovered. Thus far, the KCNQ potassium channel family appears particularly influential in this area, but whether this stems from an inherent proclivity of KCNQs to participate in chansporter complexes, or an investigational bias arising from the early KCNQ studies in this area is not yet known. KCNQ1 physically interacts with SMIT1 in the choroid plexus, in tripartite complexes with KCNE2, and in vitro is thought to also form complexes with the structurally related myo-inositol transporter, SMIT213,24. KCNQ2/3 complexes are now known to interact with SMIT1 and SMIT213,14, and DAT and GLT1 (sodium-coupled dopamine and glutamate transporters, respectively)25,26 in the brain and/or peripheral nervous system.

The single Drosophila KCNQ gene product, dKCNQ, interacts with cupcake (dSLC5A11), a non-transporting orthologue of SMIT2 that probably acts as a glucose sensor to help control feeding behavior via its effect on dKCNQ activity27. Similar to what we found for human SMIT2 with KCNQ124, dSLC5A11 inhibits dKCNQ activity; increased cupcake expression in the vinegar fly brain inhibits neuronal dKCNQ activity. This promotes feeding and other hunger-driven activities27.

In complexes with the DAT and GLT1 transporters, KCNQ2/3 channels are thought to counteract the depolarizing force of sodium co-transport into neurons, facilitating optimal neurotransmitter transport through DAT and GLT1 while also preventing excessive membrane depolarization. As previously found for KCNQ–SMIT chansporter complexes24, inhibition of the co-assembled KCNQ2/3 channels using the small molecule XE991 removed the augmenting effects of KCNQ2/3 on DAT and GLT1 transport activity. Complexes formed from DAT and GLT1 with KCNQ2/3 may arise in...
**Fig. 3** GABA increases relative sodium and cesium permeability of KCNQ channels that it activates. All error bars indicate SEM; n values indicate biologically independent experiments. **a** Representative traces from recordings of *Xenopus laevis* oocytes injected with cRNA encoding KCNQ2/3 in 100 mM K\(^+\) (voltage protocol inset). **b** Mean current-voltage relationship for KCNQ2/3 in 100 mM K\(^+\) (black), Cs\(^+\) (blue), Rb\(^+\) (red), and Na\(^+\) (magenta; n = 5), in the absence or presence of GABA (100 µM; n = 6) as indicated, values quantified from arrow in panel (a). **c** Estimated mean ion permeability relative to that of K\(^+\) versus ionic radius (Pauling) through KCNQ2/3 in the absence (black; n = 5) and presence (purple; n = 6) of GABA (100 µM). \(p < 0.05\). **d** Representative traces from recordings of oocytes injected with cRNA encoding KCNQ2 in 100 mM K\(^+\). **e** Mean current-voltage relationship for KCNQ2 in 100 mM K\(^+\) (black), Cs\(^+\) (blue), Rb\(^+\) (red), and Na\(^+\) (magenta), in the absence or presence of GABA (100 µM) as indicated; n = 5. **f** Estimated mean ion permeability relative to that of K\(^+\) versus ionic radius (Pauling) through KCNQ2 in the absence (black) and presence (purple) of GABA (100 µM); n = 5. **g** Representative traces from recordings of oocytes injected with cRNA encoding KCNQ3* in 100 mM K\(^+\). **h** Mean current-voltage relationship for KCNQ3* in 100 mM K\(^+\) (black), Cs\(^+\) (blue), Rb\(^+\) (red), and Na\(^+\) (magenta), in the absence or presence of GABA (100 µM) as indicated; n = 4. **i** Estimated mean ion permeability relative to that of K\(^+\) versus ionic radius (Pauling) through KCNQ3* in the absence (black) and presence (purple) of GABA (100 µM); n = 4, \(p < 0.05\). **j** Representative traces from recordings of oocytes injected with cRNA encoding KCNQ4 in 100 mM K\(^+\). **k** Mean current-voltage relationship for KCNQ4 in 100 mM K\(^+\) (black), Cs\(^+\) (blue), Rb\(^+\) (red), and Na\(^+\) (magenta), in the absence or presence of GABA (100 µM) as indicated; n = 4. **l** Estimated mean ion permeability relative to that of K\(^+\) versus ionic radius (Pauling) through KCNQ4 in the absence (black) and presence (purple) of GABA (100 µM); n = 4.

axons where the restrictive confines could necessitate complex formation to facilitate efficient channel-transporter cross-talk\(^4\) as we also proposed for KCNQ2/3–SMIT1/2 complexes\(^3\).

Outside the KCNQ family, the Ca\(^{2+}\)-activated K\(^+\) channel BK was found to form complexes with the GABA transporter GAT3—another sodium-coupled transporter (encoded by SLC6A11)—in mouse brain lysates\(^28\). Similarly, KCNA2 (Kv1.2) interacts with the LAT1 (SLC7A5) neutral amino acid transporter and the two co-localize in mouse neurons. LAT1 regulates KCNA2 gating and voltage dependence, and the proteins each
alter the effects in one another of gene variants linked to developmental delay and epilepsy.

In the present study we provide evidence that KCNQ channels in transporter complexes can act as a chemosensor for the transporter, enabling the latter to respond to stimuli it does not typically respond to, e.g., GABA, BHB, GABOB, retigabine. Reciprocally, the transporter has the capability to tune the response of the channel to those same stimuli, at least in the case of SMIT1 altering how KCNQ channels sense GABA and related metabolites.

We demonstrate that KCNQ2/3 mediates GABA regulation of SMIT1 activity both in vitro and in ex vivo DRG. We do not yet know the physiological role of this regulation, but it could potentially represent a form of negative feedback regulation.
In this model, increased local GABA concentration subdues neuronal firing by activating KCNQ2/3, but this in turn inhibits co-assembled SMIT1, reducing local [P2P2] and eventually leading to KCNQ2/3 inhibition, increasing excitability once again (see Fig. 6 for schematic).

It is important to mention that GABA binding to KCNQ2/3 is able to overcome inhibition of KCNQ2/3 channels by P2P2 depletion and/or muscarinic receptor activation, therefore the potential excitatory effects of P2P2 depletion would be predicted to come into effect only after GABA concentrations had sufficiently subsided. This combination of effects might be required for timed waves of excitation and inhibition, or conversely even to dampen such oscillations, depending on the magnitude and timing of GABA and P2P2 concentration changes. Overlaid upon this, it was previously discovered that KCNQ2/3 channels themselves regulate GABA release, pointing to further possible layers of feedback regulation.

SMIT1, by apparently impacting the KCNQ-binding site for GABA and related small molecules, can tune KCNQ responses to these important neuronal signaling moieties. Perhaps most surprisingly, SMIT1 switched the KCNQ isoform selectivity of BHB such that its binding was communicated to SMIT1 not by GABA and GABOB, but by in vitro transcription using mMessage mMachine kits (Thermo Fisher Scientific), after CDNA linearization. cRNA was quantified by spectrophotometry. Mutant cDNAs were generated by site-directed mutagenesis using a QuikChange kit according to manufacturer’s protocol (Stratagene, San Diego, CA) and the corresponding cRNAs were prepared as above. We injected defolliculated stage V oocyte injection. cRNA transcripts encoding human KCNQ2, KCNQ3, KCNQ4, or SMIT1 were generated as before by in vitro transcription using mMessage mMachine kits (Thermo Fisher Scientific), after CDNA linearization. cRNA was quantified by spectrophotometry. Mutant cDNAs were generated by site-directed mutagenesis using a QuikChange kit according to manufacturer’s protocol (Stratagene, San Diego, CA) and the corresponding cRNAs were prepared as above. We injected defolliculated stage V oocytes (Eccyotoc Biosciences, Austin, TX) with Kv channel α subunit cRNAs (3–20 ng) and/or SMIT1 cRNA (10–20 ng). Oocytes were incubated at 16°C in Barth’s saline solution (Eccyotoc) containing penicillin and streptomycin, with daily washing, for 3–5 days prior to TEVC recording and/or radioligand uptake studies.

**Methods**

cRNA preparation and and X. laevis oocyte injection. cRNA transcripts encoding human KCNQ2, KCNQ3, KCNQ4, or SMIT1 were generated as before by in vitro transcription using mMessage mMachine kits (Thermo Fisher Scientific), after CDNA linearization. cRNA was quantified by spectrophotometry. Mutant cDNAs were generated by site-directed mutagenesis using a QuikChange kit according to manufacturer’s protocol (Stratagene, San Diego, CA) and the corresponding cRNAs were prepared as above. We injected defolliculated stage V oocytes (Eccyotoc Biosciences, Austin, TX) with Kv channel α subunit cRNAs (3–20 ng) and/or SMIT1 cRNA (10–20 ng). Oocytes were incubated at 16°C in Barth’s saline solution (Eccyotoc) containing penicillin and streptomycin, with daily washing, for 3–5 days prior to TEVC recording and/or radioligand uptake studies.

Radioligand uptake studies. For DRG studies, we conducted radiolabeled uptake assays using myo-[3H(N)]inositol (American Radiolabeled Chemicals Inc.) on whole, freshly isolated postnatal day 8 rat DRG purchased from BrainBits (Springfield, Illinois). DRG were removed from the Hibernate AB storage solution (BrainBits) and placed into NaBactivsivum (BrainBits) containing 25 ng/ml of nerve growth factor and incubated overnight at 37°C. Isolated DRG were cut into 3 mm pieces and placed in 1 ml Eppendorf tubes containing NaBactivsivum (BrainBits) containing 25 ng/ml of nerve growth factor and incubated overnight at 37°C. Isolated DRG were cut into 3 mm pieces and placed in 1 ml Eppendorf tubes containing NaBactivsivum (brainBits) containing 25 ng/ml of nerve growth factor and incubated overnight at 37°C. Isolated DRG were cut into 3 mm pieces and placed in 1 ml Eppendorf tubes containing NaBactivsivum (brainBits) containing 25 ng/ml of nerve growth factor and incubated overnight at 37°C. Isolated DRG were cut into 3 mm pieces and placed in 1 ml Eppendorf tubes containing NaBactivsivum (brainBits) containing 25 ng/ml of nerve growth factor and incubated overnight at 37°C.
Fig. 5 KCNQ2/3 channels require an arginine at the foot of the voltage sensor for communicating GABA binding to SMIT1. All error bars indicate SEM.

a Topology of KCNQ3 (two of four subunits shown) showing positions of W265 (red) and R242 (yellow) and segment numbering. VSD voltage-sensing domain.

b Structural model of KCNQ3 showing positions of W265 and R242. Color coding as in (a).

c Mean traces showing currents generated by KCNQ2–W236L/KCNQ3–W265L (Q2WL/Q3WL) (n = 8) alone or with SMIT1 (n = 7) in Xenopus oocytes.

d Mean peak currents for KCNQ2–W236L/KCNQ3–W265L (Q2WL/Q3WL) alone (black; n = 8) or with SMIT1 (red; n = 7) quantified from traces as in (c).

e Mean peak tail currents for KCNQ2–W236L/KCNQ3–W265L (Q2WL/Q3WL) alone (black; n = 8) or with SMIT1 (red; n = 7) quantified from traces as in (c).

f Mean G/Gmax relationship for KCNQ2–W236L/KCNQ3–W265L (Q2WL/Q3WL) alone (black; n = 8) or with SMIT1 (red; n = 7) quantified from traces as in (c).

g Mean E_M of unclamped Xenopus oocytes expressing KCNQ2–W236L/KCNQ3–W265L (Q2WL/Q3WL) alone (black) (n = 8) or with SMIT1 (red) (n = 7). **p < 0.01.

h Mean traces showing currents generated by KCNQ2–R213A/KCNQ3–R242A (Q2RA/Q3RA) alone (n = 14) or with SMIT1 (red; n = 16) in Xenopus oocytes.

i Mean peak prepulse and peak tail currents for KCNQ2–R213A/KCNQ3–R242A (Q2RA/Q3RA) alone (black; n = 14) or with SMIT1 (red; n = 16) quantified from traces as in panel (h).

j Mean E_M of unclamped Xenopus oocytes expressing KCNQ2–R213A/KCNQ3–R242A (Q2RA/Q3RA) alone (black) (n = 14) or with SMIT1 (red) (n = 16).

k [3H]Myo-inositol uptake (30 min) for Xenopus oocytes expressing subunits indicated in the absence or presence of GABA (100 µM) as indicated (SMIT1 alone, n = 19; Q2RA/Q3RA-S1, n = 29; Q2RA/Q3RA-S1 + GABA, n = 32).
NbActiv4 medium containing 1 mM cold myo-inositol (Sigma), followed by 1–2 washes in 3 ml NbActiv4 medium. DRG were homogenized over a period of 6 h in 100 μl of 2% sodium dodecyl sulfate (SDS) in NbActiv4; each homogenized DRG section was then transferred to a scintillation vial (1 DRG section per vial) containing Ready Protein Plus scintillation fluid (Beckmann Coulter) (6 ml). Vials were capped, shaken vigorously, and then allowed to sit at room temperature for at least 30 min before scintillation counting in a Beckmann Coulter 6500. Each experiment was performed on two batches of DRG from different deliveries; each experiment always contained intra-batch controls and results presented are pooled data from both batches.

For oocyte studies, we conducted radiolabeled uptake assays using myo-[2-3H](N)inositol (American Radiolabeled Chemicals Inc.) 5 days after oocyte cRNA injection. For each condition/expression group, oocytes were placed in a round-bottomed, 10-ml culture tube, washed, and resuspended in ND96 (200 μl per tube) containing myo-[2-3H](N)inositol (3 μCi/ml) with or without GABA, glutamate, β-Hydroxybutyric acid (BHB), GABOB (Sigma), or retigabine (Tocris) at pH 7.5. After 30 min at room temperature, oocytes were washed 2–3 times in 3 ml of ND96 containing 1 mM cold myo-inositol (Sigma), followed by 1–2 washes in 3 ml of ND96. Oocytes were next individually placed in wells of a 96-well plate and lysed in 100 μl of 2% SDS in ND96; each lysed oocyte was then transferred to a scintillation vial (1 oocyte per vial) containing Ready Protein Plus scintillation fluid (Beckmann Coulter) (6 ml). Vials were capped, shaken vigorously, and then allowed to sit at room temperature for at least 30 min before scintillation counting in a Beckmann Coulter 6500. Each experiment was performed on two batches of oocytes from different deliveries; each experiment always contained intra-batch controls and results presented are pooled data from two or more batches.

**Two-electrode voltage clamp (TEVC).** We conducted TEVC recording at room temperature as before with an OC-725C amplifier (Warner Instruments, Hamden, CT) and pClamp8 software (Molecular Devices, Sunnyvale, CA) 3 days after cRNA injection as described in section above. Oocytes were placed in a small-volume oocyte bath (Warner) and viewed with a dissection microscope. Chemicals were sourced from Sigma. Bath solution was in mM: 96 NaCl, 4 KCl, 1 MgCl2, 1 CaCl2, 10 HEPES (pH 7.6). GABA and GABOB were stored at −80 °C as 1 M stock in 100% ethanol and diluted to working concentrations on each experimental day. BHB was stored at 4 °C as a 480 mM stock in 100% ethanol and diluted to working concentrations each experimental day. All compounds were introduced to the recording bath via gravity perfusion at a constant flow of 1 ml per minute for 3 min prior to recording. Pipettes were of 1–2 MΩ resistance when filled with 3 M KCl.

Currents were recorded in response to pulses between −80 mV and +40 mV at 20 mV intervals, or a single pulse to +40 mV, from a holding potential of −80 mV, to yield current–voltage relationships, current magnitude, and for quantifying activation rate. TEVC data analysis was performed with Clampfit (Molecular Devices) and Graphpad Prism software (GraphPad, San Diego, CA, USA); values are stated as mean ± SEM. Normalized tail currents were plotted versus prepulse voltage and fitted with a single Boltzmann function

\[
g = \frac{(A_1 - A_2)}{\left(1 + \exp \left(\frac{V_{1/2} - V}{\gamma} \right)\right)} + A_2, \tag{1}
\]

where \(g\) is the normalized tail conductance, \(A_1\) is the initial value at \(-∞\), \(A_2\) is the final value at \(+∞\), \(V_{1/2}\) is the half-maximal voltage of activation and \(V\) the slope factor. Activation and deactivation kinetics were fitted with single exponential functions.

**Relative permeability calculations.** According to the Goldman–Hodgkin–Katz (GHK) voltage equation

\[
E_{rev} = \frac{RT}{2F} \ln \left(\frac{P_K}{\beta_o P_C} \frac{C_0}{C_1} \right) \quad \tag{2}
\]

where \(E_{rev}\) is the absolute reversal potential and \(P\) is permeability. This permits calculation of the relative permeability of each ion if concentrations on either side of the membrane are known. A modified version of this equation was used here to determine relative permeability of two ions in a system in which only the extracellular ion concentration was known. Thus, relative permeability of Rb⁺, Ca⁺, and Na⁺ compared to K⁺ ions was calculated for all channels by plotting the I/V relationships for each channel with each extracellular ion (100 mM) and comparing them to that with 100 mM extracellular K⁺ ion to yield a change in reversal potential (ΔE<sub>rev</sub>) for each ion compared to that of K⁺. Permeability ratios for each ion (X) compared to K⁺ were then calculated as

\[
\Delta E_{rev} = E_{rev,X} - E_{rev,K} = \frac{RT}{2F} \ln \frac{P_X}{P_K} \quad \tag{3}
\]

Values were compared between channel types and statistical significance assessed using ANOVA.

**Chemical structures, in silico docking, and sequence analyses.** Chemical structures and electrostatic surface potentials (range: −0.1 to 0.1) were plotted using Jmol, an open-source Java viewer for chemical structures in 3D: http://jmol.org/. Illustrations of KCNQ structure were based on the X. laevis KCNQ1 cryoEM structure, which we altered to incorporate KCNQ3 residues known to be important for GABA and mallowtoxin binding, followed by energy minimization using the GROMOS 43B1 force field, in DeepView. Thus, X. laevis KCNQ1 amino acid sequence LITTLYIGF was converted to LITAAYYGIF, the underlined W being W265 in human KCNQ3. In addition, X. laevis KCNQ1 sequence WWGVVTVTTGGYG was converted to WWGLTLATTGGYG, the underlined L being Leu314 in human KCNQ3. Surrounding non-mutated sequences are shown to illustrate the otherwise high sequence identity in these stretches. Structures were visualized in UCSF Chimera.

**Statistics and reproducibility.** All values are expressed as mean ± SEM. One-way ANOVA was applied for all other tests; if multiple comparisons were performed, a post hoc Tukey’s HSD test was performed following ANOVA. All p values were two-sided. Statistical significance was defined as \(p < 0.05\).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Data availability
The authors declare that all data supporting the findings of this study are available within the article.

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References
1. Brown, D. A. & Adams, P. R. Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neuron. *Nature* 283, 673–676 (1980).
2. Biervert, C. et al. A potassium channel mutation in neonatal human epilepsy. *Science* 279, 403–406 (1998).
3. Cooper, E. C., Harrington, E., Jan, Y. N. & Jan, L. Y. M channel.
4. Wang, H. S. et al. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* 282, 1890–1893 (1998).
5. Klinger, F., Gould, G., Boehm, S. & Shapiro, M. S. Distribution of M-channel subunits KCNQ2 and KCNQ3 in rat hippocampus. *Neuroreport* 58, 761–769 (2011).
6. Sigel, E. & Steinmann, M. E. Structure, function, and modulation of GABA(A) receptors. *J. Biol. Chem.* 287, 40224–40231 (2012).
7. Andrade, R., Malenka, R. C. & Nicoll, R. A. A G protein couples serotonin and GABAB receptors to the same channels in hippocampus. *Science* 234, 1261–1265 (1986).
8. Manville, R. W., Papanikolaou, M. & Abbott, G. W. Direct neurotransmitter activation of voltage-gated potassium channels. *Nat. Commun.* 9, 1847 (2018).
9. Cooper, E. C., Harrington, E., Jan, Y. N. & Jan, L. Y. M channel. KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. *J. Neurosci.* 21, 9529–9540 (2001).
10. Martire, M. et al. M channels containing KCNQ2 subunits modulate norepinephrine, aspartate, and GABA release from hippocampal nerve terminals. *J. Neurosci.* 24, 592–597 (2004).
11. Peretz, A. et al. Pre- and postsynaptic activation of M-channels by a novel opener dampens neuronal firing and transmitter release. *J. Neurophysiol.* 97, 283–295 (2007).
12. Uchida, T. et al. Abnormal gamma-aminobutyric acid neurotransmission in a KrFab model of early onset myoclonic epilepsy. *Epilepsia* 58, 1430–1439 (2017).
13. Manville, R. W., Neverisky, D. L. & Abbott, G. W. SMIT1 modifies KCNQ channel function and pharmacology by physical interaction with the pore. *Biophys. J.* 113, 613–626 (2017).
14. Neverisky, D. L. & Abbott, G. W. KCNQ-SMIT complex formation facilitates ion channel-solute transporter cross-talk. *FASEB J.* 31, 2828–2838 (2017).
15. Loussoarou, G. et al. Phosphatidylinositol-4,5-bisphosphate, PI(2), controls KCNQ1/KCNEl voltage-gated potassium channels: a functional homology between voltage-gated and inward rectifier K+ channels. *EMBO J.* 22, 5412–5421 (2003).
16. Suh, B. C. & Hille, B. Electrostatic interaction of internal Mg2+ with membrane PI(2) seen with KCNQ K+ channels. *J. Gen. Physiol.* 130, 241–256 (2007).
17. Suh, B. C. & Hille, B. PI(2) is a necessary cofactor for ion channel function: how and why? *Annu. Rev. Biophys.* 37, 175–195 (2008).
18. Kim, K. S., Duignan, K. M., Hawryluk, J. M., Soh, H. & Tzingounis, A. V. The voltage activation of cortical KCNQ channels depends on global PI(2) levels. *Biophys. J.* 110, 1089–1098 (2016).
19. Etxekabia, A., Santana-Castro, I., Regalado, M. P., Aivar, P. & Villarroya, A. Three mechanisms underlie KCNQ2/3 hetereric potassium M-channel potentiation. *J. Neurosci.* 24, 9146–9152 (2004).
20. De Silva, A. M., Manville, R. W. & Abbott, G. W. Deconstruction of an African folk medicine uncovers a novel molecular strategy for therapeutic potassium channel activation. *Sci. Adv.* 4, eaav0242 (2018).
21. Manville, R. W. & Abbott, G. W. Ancient and modern anticonvulsants act synergistically in a KCNQ potassium channel binding pocket. *Nat. Commun.* 9, 3845 (2018).
22. Manville, R. W. & Abbott, G. W. Clantrol leaf harbers a potent potassium channel-activating anticonvulsant. *FASEB J.* 33, 11349–11363 (2019).
23. Manville, R. W. et al. KCNQ5 activation is a unifying molecular mechanism shared by genetically and culturally diverse botanical hypotensive folk medicines. *Proc. Natl Acad. Sci. USA* 116, 21236–21245 (2019).
24. Abbott, G. W. et al. KCNJ1, KCNE2, and Na+–coupled solute transporters form reciprocally regulating complexes that affect neuronal excitability. *Sci. Signal.* 7, ra22 (2014).
25. Bartolome-Martin, D. et al. Identification of potassium channel proteins Kv7.2/7.3 as common partners of the dopamine and glutamate transporters DAT and GLT-1. *Neuropsycharmacology* https://doi.org/10.1016/j.neuropsychologia.2019.03.011 (2019).
26. Manville, R. W. & Abbott, G. W. Teamwork: Ion channels and transporters join forces in the brain. *Neuropsychopharmacology* 161, 107601 (2019).
27. Park, J. Y. et al. Drosophila SLC5A11 mediates hunger by regulating K(+) channel activity. *Curr. Biol.* 26, 1965–1974 (2016).
28. Singh, H. et al. MaxiK channel interface reveals its interaction with GABA transporter 3 and heat shock protein 60 in the mammalian brain. *Neuroscience* 317, 76–107 (2016).
29. Barons, V. A., Yang, R. Y., Morales, L. C., Sipione, S. & Kurata, H. T. SLC7A5 regulates Kv1.2 channels and modifies functional outcomes of epilepsy-linked channel mutations. *Nat. Commun.* 9, 4417 (2018).
30. Manville, R. W., Papanikolaou, M. & Abbott, G. W. M-channel activation contributes to the anticonvulsant action of the ketone body beta-hydroxybutyrate. *J. Pharmacol. Exp. Ther.* 372, 148–156 (2020).
31. Pan, J. W., Rothman, T. L., Behar, K. L., Stein, D. T. & Hetherington, H. P. Human brain beta-hydroxybutyrate and lactate increase in fasting-induced ketosis. *J. Cereb. Blood Flow Metab.* 20, 1502–1507 (2000).
32. Splinter, M. Y. Efficacy of retigabine in adjunctive treatment of partial onset seizures in adults. *J. Cent. Nerv. Syst. Dis.* 5, 31–41 (2013).
33. Sun, J. & MacKinnon, R. Cryo-EM structure of a KCNQ1/CaM complex reveals insights into congenital long QT syndrome. *Cell* 169, 1042–1050 e1049 (2017).
34. van Gunsteren, W. F. Biomolecular Simulation: The GROMOS96 Manual and User Guide. (Vdf Hochschulverlag ETHZ, 1996).
35. Johansson, M. U., Zoete, V., Michielin, O. & Gues, N. Defining and searching for structural motifs using DeepView/Swiss-ProtViewer. *BMC Bioinform.* 13, 173 (2012).
36. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* 25, 1605–1612 (2004).

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Author contributions
R.W.M. performed all the electrophysiological experiments, transport studies and data analysis, prepared most of the figure panels, and edited the manuscript. G.W.A. conceived the study, generated docking figures and other models, wrote the manuscript and prepared the figures.

Competing interests
The authors declare no competing interests.

Additional information
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