Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

Gabapentin is a potent activator of KCNQ3 and KCNQ5 potassium channels

Rían W. Manville and Geoffrey W. Abbott

Bioelectricity Laboratory, Dept. of Physiology and Biophysics, School of Medicine, University of California, Irvine, CA, USA (RWM and GWA).

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Gabapentin activates KCNQ channels

Corresponding author: Dr. Geoffrey W. Abbott, Bioelectricity Laboratory, Dept. of Physiology and Biophysics, Medical Sciences D, ZOT 4560, School of Medicine, University of California, Irvine, CA 92697, USA.

email: abbottg@uci.edu

phone: 949 924 3269

fax: n/a

Number of text pages: 22

Number of tables: 0

Number of figures: 6

Number of references: 54

Words in Abstract: 169

Words in Introduction: 444

Words in Discussion: 1337

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

Synthetic gabapentinoids, exemplified by gapapentin and pregabalin, are in extensive clinical use for indications including epilepsy, neuropathic pain, anxiety and alcohol withdrawal. Their mechanisms of action are incompletely understood, but are thought to involve inhibition of $\alpha_2\delta$ subunit-containing voltage-gated calcium channels. Here, we report that gabapentin is a potent activator of the heteromeric KCNQ2/3 voltage-gated potassium channel, the primary molecular correlate of the neuronal M-current, and also homomeric KCNQ3 and KCNQ5 channels. In contrast, the structurally related gabapentinoid, pregabalin, does not activate KCNQ2/3, and at higher concentrations (≥10 μM) is inhibitory. Gabapentin activation of KCNQ2/3 (EC_{50} = 4.2 nM) or homomeric KCNQ3* (EC_{50} = 5.3 nM) channels requires KCNQ3-W265, a conserved tryptophan in KCNQ3 transmembrane segment 5. Homomeric KCNQ2 or KCNQ4 channels are insensitive to gabapentin, whereas KCNQ5 is highly sensitive (EC_{50} = 1.9 nM). Given the potent effects and the known anticonvulsant, anti-nociceptive and anxiolytic effects of M-channel activation, our findings suggest the possibility of an unexpected role for M-channel activation in the mechanism of action of gabapentin.

Introduction

Gabapentin (Neurontin) and pregabalin (Lyrica) are synthetic antiepileptic and antinociceptive gabapentinoid compounds originally designed as analogues of the neurotransmitter γ-aminobutyric acid (GABA), and both are in widespread clinical use (Calandre et al., 2016). However, the mechanisms of action of gabapentinoids, exemplified by gabapentin and pregabalin, are incompletely understood. [^{3}H]-gabapentin binding was first described in membrane fractions from rat brain homogenates, and the target protein identified as the α_2-δ subunit of voltage-gated calcium (Ca_V) channels. The findings were later recapitulated using porcine brain tissue, heterologously expressed α_2-δ, and also with pregabalin; binding was found to be exclusive to α_2-δ1 and 2 isoforms (Brown and Gee, 1998; Field et al., 2006; Fuller-Bicer et al., 2009; Gee et al.,

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

1996). Gabapentin and pregabalin are generally considered inactive against canonical $GABA_A$ and $GABA_B$ receptors, despite their structural similarity to GABA (Ben-Menachem, 2004; Jensen et al., 2002; Lanneau et al., 2001; Stringer and Lorenzo, 1999; Taylor, 1997), although some investigators contend that there are some subtype-specific effects on $GABA_B$ receptors (Bertrand et al., 2003a; Ng et al., 2001; Parker et al., 2004). Binding of gabapentin and pregabalin to α_2-δ is suggested to act therapeutically via impairment of Cav channel activity, thus reducing neuronal calcium currents (Stefani et al., 1998; Stefani et al., 2001), although others observed no evidence for gabapentin-induced changes in neuronal Cav activity (Rock et al., 1993; Schumacher et al., 1998).

We recently made the unexpected discovery that GABA can activate voltage-gated potassium (Kv) channels composed of heteromeric assemblies of KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3) pore-forming α subunits (Manville et al., 2018). KCNQ (Kv7) channels comprise tetramers of α subunits, each containing six transmembrane (S) segments, organized into the voltage-sensing domain (VSD, S1-4) and the pore module (S5-6) (**Fig. 1 A, B**). In vertebrate nervous systems, KCNQ2/3 (Kv7.2/3) heteromers are the primary molecular correlate of the M-current, a muscarinic-inhibited Kv current essential for regulating excitability of a wide range of neurons throughout the nervous system (Brown and Adams, 1980; Marrion et al., 1989; Wang et al., 1998). We found that, like the anticonvulsant retigabine (Kim et al., 2015; Schenzer et al., 2005), GABA binds to a conserved tryptophan (W265) on KCNQ3 to activate KCNQ3 homomers and KCNQ2/3 heteromers (Manville et al., 2018) (**Fig. 1B-D**).

Because of the structural similarities between gabapentinoids and GABA, and the known influence of the M-current in many of the disease states responsive to gabapentinoids (epilepsy, pain, anxiety, alcohol withdrawal) (Blackburn-Munro et al., 2005; Kang et al., 2017; Mason et al., 2018), we hypothesized that gabapentinoids might modulate KCNQ2/3 channels. Here, using

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

electrostatic surface mapping, *in silico* docking studies, cellular electrophysiology and site-directed mutagenesis, we examined whether the two gabapentinoids in widespread clinical use (gabapentin and pregabalin) can modulate KCNQ2/3 channel function.

Materials and Methods

Channel subunit cRNA preparation and Xenopus laevis oocyte injection

cRNA transcripts encoding human KCNQ2, KCNQ3, KCNQ4, KCNQ5 (Kv7.2 – Kv7.5) were generated by *in vitro* transcription using the T7 polymerase mMessage mMachine kit (Thermo Fisher Scientific), after vector linearization, from cDNA sub-cloned into plasmids incorporating *Xenopus laevis* β-globin 5' and 3' UTRs flanking the coding region to enhance translation and cRNA stability. cRNA was quantified by spectrophotometry. Mutant KCNQ2 and KCNQ3 cDNAs were generated with site-directed mutagenesis using a QuikChange kit according to the manufacturer's protocol (Stratagene, San Diego, CA) and corresponding cRNAs prepared as above. Defolliculated stage V and VI *Xenopus laevis* oocytes (Ecocyte Bioscience, Austin, TX) were injected with KCNQ channel α subunit cRNAs (5-10 ng). The oocytes were incubated at 16 ^{o}C in Barth's saline solution (Ecocyte) containing penicillin and streptomycin, with daily washing, for 2-5 days prior to two-electrode voltage-clamp (TEVC) recording.

Two-electrode voltage clamp (TEVC)

TEVC recording was performed at room temperature using a OC-725C amplifier (Warner Instruments, Hamden, CT) and pClamp8 software (Molecular Devices, Sunnyvale, CA) 2-5 days after cRNA injection as described in the section above. The oocytes were placed in a small-volume oocyte bath (Warner) and viewed with a dissection microscope. Unless otherwise stated, chemicals were sourced from Sigma. Bath solution was (in mM): 96 NaCl, 4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES (pH 7.6). Gabapentin and pregabalin were stored at -80 ^{o}C as 1 M stocks in

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

molecular grade H_2O and diluted to working concentrations on each experimental day. The drugs were introduced into the recording bath by gravity perfusion at a constant flow of 1 ml per minute for 3 minutes 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. Deactivation was recorded at -80 mV after a single pulse to +40 mV, from a holding potential of -80 mV. Electrophysiology data analysis was performed with Clampfit (Molecular Devices) and Graphpad Prism software (GraphPad, San Diego, CA, USA); values are stated as mean ± SD. Raw or normalized tail currents were plotted versus prepulse voltage and fitted with a single Boltzmann function:

Eq. 1

$$g = \frac{(A_1 - A_2)}{\left\{1 + exp\left[V_{\frac{1}{2}} - V\right]\right\} y + A_2}$$

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_s the slope factor. Activation and deactivation kinetics were fitted with single exponential functions.

Chemical structures and *silico* docking

Chemical structures and electrostatic surface potentials were plotted and viewed using Jmol, an open-source Java viewer for chemical structures in 3D: http://jmol.org/. For *in silico* ligand docking predictions, the *Xenopus laevis* KCNQ1 cryoEM structure (Sun and MacKinnon, 2017) was first altered to incorporate KCNQ3/KCNQ5 residues known to be important for retigabine and ML-213 binding, and their immediate neighbors, followed by energy minimization as we previously described (Manville et al., 2018) using the GROMOS 43B1 force field (van Gunsteren, 1996) in DeepView (Johansson et al., 2012). Thus, *Xenopus laevis* KCNQ1 amino acid sequence LIT*TL*YIGF was converted to LIT*AW*YIGF, the underlined W being W265 in human KCNQ3 and

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

the italicized residues being the immediate neighbors in KCNQ3/KCNQ5. In addition, *Xenopus laevis* KCNQ1 sequence WWG*VV*T*VT*TIGYGD was converted to WWG*LI*T*L**A*TIGYGD, the underlined L being Leu314 in human KCNQ3 and the italicized residues being the immediate neighbors in KCNQ5 and/or KCNQ3. Surrounding non-mutated sequences are shown to illustrate the otherwise high sequence identity in these stretches. No other KCNQ1 residues were changed in the model. Unguided docking of gabapentin and pregabalin, to predict native binding sites, was performed using SwissDock with CHARMM forcefields (Grosdidier et al., 2011a; b).

Statistical analysis

All values are expressed as mean ± standard deviation (SD). 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 $P < 0.05$.

Results

Synthetic anticonvulsants such as retigabine and ML-213 exhibit negative electrostatic surface potential near their carbonyl oxygen moieties, a chemical property thought to be important for activation of KCNQ2/3 channels (Kim et al., 2015). We previously found that GABA also possesses this chemical property, whereas the excitatory neurotransmitter, glutamate (which cannot open KCNQ2/3 channels) does not (Manville et al., 2018). Here, we found that gabapentin exhibits a similar negative electrostatic surface potential pattern to that of GABA, whereas pregabalin does not (**Fig. 2 A**). Using SwissDock, we performed unbiased docking prediction analysis for gabapentin and pregabalin, to a model of KCNQ3 (Manville et al., 2018) based on the recent cryo-EM derived KCNQ1 structure (Sun and MacKinnon, 2017). Strikingly, gabapentin was predicted to bind to KCNQ3-W265 (**Fig. 2 B, C**) whereas pregabalin failed to dock to KCNQ3-W265.

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

We next tested the predictions using the *Xenopus laevis* oocyte expression and two-electrode voltage-clamp (TEVC) electrophysiology. Gabapentin potently activated heteromeric KCNQ2/3 potassium channels, even at low nanomolar concentrations (**Fig. 3 A, B**). In contrast, pregabalin had no augmenting effect on KCNQ2/3 activity, even at 1 µM (**Fig. 3 C, D**). Thus, the experimental data matched the docking predictions. Gabapentin efficacy was highest at -60 mV to -40 mV, leading to a -9 mV shift in the voltage dependence of KCNQ2/3 activation (1 µM gabapentin), but gabapentin also augmented currents at positive membrane potentials (**Fig. 3 B, E**). Dose response studies showed that at -60 mV, gabapentin exhibited an EC_{50} for KCNQ2/3 activation of 4.2 ± 0.13 nM (*n* = 5-7); at 10 nM, gabapentin increased KCNQ2/3 current 3.5-fold at -60 mV (**Fig. 3 F; Supplementary Fig. 1; Supplementary Table 1**). The ability to activate KCNQ2/3 at subthreshold potentials enabled gabapentin to shift the membrane potential (E_M) of KCNQ2/3-expressing oocytes by >-10 mV (EC_{50}, 4.2 nM) (**Fig. 3 G**). Parallel studies showed that pregabalin failed to activate KCNQ2/3 even at 1 µM, and began to inhibit KCNQ2/3 at 10 µM and above (**Fig. 3 E,F; Supplementary Fig. 2; Supplementary Table 2**). Pregabalin likewise failed to shift the oocyte E_M (**Fig. 3 G**). Compared to the established KCNQ2/3 opener and anticonvulsant retigabine, gabapentin acted as a potent partial agonist. Thus, retigabine (30 µM) shifted the voltage dependence of KCNQ2/3 activation by –30 mV (**Fig. 3H**) and increased current at -60 mV sixfold (**Fig. 6I**) but the EC_{50} for retigabine was in the micromolar, not nanomolar range (**Fig. 3J**), ~1000-fold less potent than gabapentin (see **Supplementary Fig. 3; Supplementary Table 3**). In comparison, we recently found that GABA, which also acts at KCNQ3-W265, activates KCNQ2/3 with an EC_{50} of 0.85 µM at -60 mV, increasing current fourfold (Manville et al., 2018). Thus, gabapentin and GABA exhibit similar efficacy but gabapentin is 200-fold more potent.

Gabapentin began to activate KCNQ2/3 immediately upon wash-in, with the current augmentation taking ~2 minutes to plateau. Gabapentin effects washed out relatively slowly (<50% washout after 2 minutes) but the gabapentin-augmented current was rapidly inhibited by washing in the

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

KCNQ channel inhibitor, XE991 (50 µM) (**Fig. 4 A**). Gabapentin effects on KCNQ2/3 gating kinetics were suggestive of it stabilizing the open state; at 10 nM, gabapentin speeded KCNQ2/3 activation and slowed deactivation (**Fig. 4 B, C; Supplementary Fig. 1; Supplementary Table 1**). Again, pregabalin had no effects (**Fig. 4 B, C; Supplementary Fig. 2; Supplementary Table 2**).

We next examined the effects of gabapentin on homomeric channels formed by neuronal KCNQ isoforms. At 1 µM, gabapentin activated KCNQ3* (an expression-optimized KCNQ3-A315T mutant that ensures robust currents (Zaika et al., 2008)) and KCNQ5, especially at subthreshold potentials. In contrast, KCNQ2 and KCNQ4 were insensitive to 1 µM gabapentin (**Fig. 5A-C; Supplementary Figs. 4-7; Supplementary Tables 4-7**). Dose response studies revealed that KCNQ3 and KCNQ5 were, like KCNQ2/3 channels, activated at -60 mV even by 10 nM gabapentin, and that KCNQ3 exhibited similar gabapentin sensitivity and efficacy to that of KCNQ2/3 channels (EC_{50} = 5.3 nM; maximal 4-fold increase in current at -60 mV). In contrast, KCNQ5 channels exhibited higher sensitivity but lower efficacy (EC_{50} = 1.9 nM; maximal 3-fold increase in current at -60 mV) (**Fig. 5D; Supplementary Figs. 4-7; Supplementary Tables 4-7**).

Canonical $GABA_A$ and $GABA_B$ receptors are generally considered to be gabapentin-insensitive (Jensen et al., 2002; Taylor, 1997); in addition, previous studies have concluded that *Xenopus laevis* oocytes do not express endogenous $GABA_A$ or $GABA_B$ receptors (Guyon et al., 2013). Furthermore, the gabapentin-activated currents in KCNQ2/3 expressing oocytes were completely inhibited by the KCNQ-specific inhibitor, XE991 (**Fig. 4A**). These data, combined with docking prediction studies, rapid onset of activation, the lack of effects of pregabalin and the KCNQ isoform-specificity of gabapentin (**Fig. 5**) are consistent with direct activation of KCNQ2/3 channels by gabapentin. This conclusion was further supported by two additional sets of experiments. First, gabapentin (10 nM) had no effect on endogenous currents in non-injected oocytes, discounting the possibility that gabapentin was activating endogenous currents (**Fig. 6A**). Second, substitution to

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

leucine of KCNQ3-W265, the GABA binding site (Manville et al., 2018) and the *in silico* predicted docking site for gabapentin (**Fig. 2**), essentially eliminated the effects of gabapentin on KCNQ2/3 currents (**Fig. 6D, E**); the double mutation of KCNQ2-W236L and KCNQ3-W265L in KCNQ2/3 channels had similar effects (**Fig. 6F, G**). KCNQ2/KCNQ3-W265L channels were insensitive to gabapentin across the voltage range (**Fig. 6H**) and up to 100 µM gabapentin (**Fig. 6I; Supplementary Fig. 8; Supplementary Table 8**). Double-mutant (WL/WL) KCNQ2/3 channels showed slight (≤ 50%) augmentation by gabapentin at -60 mV only at 1 µM and higher gabapentin (**Fig. 6H, I; Supplementary Fig. 9; Supplementary Table 9**).

Discussion

A gabapentin binding site on KCNQ channels

We recently discovered that KCNQ3 and KCNQ5 are directly activated by the inhibitory neurotransmitter GABA, which binds close to the highly conserved S5 tryptophan, KCNQ3-W265 (Manville et al., 2018). In the current study, we show that gabapentin likewise activates KCNQ3 and KCNQ5, whereas the related gabapentinoid, pregabalin, does not. Substitution of KCNQ3-W265 with a leucine prevents activation by GABA and gabapentin, and impairs GABA binding (Manville et al., 2018). KCNQ3-W265 (and its equivalent in KCNQ2, 4 and 5) is also very important for binding of retigabine and structurally related anticonvulsants (Schenzer et al., 2005). This is thought to be because small molecules with a strong negative electrostatic surface potential close to a carbonyl/carbamate oxygen can hydrogen-bond with the W265 (Kim et al., 2015). Indeed, here we found that pregabalin lacks this exposed negative surface potential and neither *in silico* docks, nor activates KCNQ3. Our *in silico* docking studies for gabapentin position it near to W265 and close to where retigabine (Kim et al., 2015) and GABA (Manville et al., 2018) are predicted to bind, but not necessarily overlapping – although no conclusions should be drawn from the small differences in poses, and resolution of the exact pose would require structural analysis

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

and/or further mutagenesis to map the entire binding site. We conclude that the W265-based binding site evolved to accommodate GABA and other endogenous metabolites and analogs of GABA, leading to sensitivity to modern synthetic anticonvulsants including retigabine and gabapentin. Interestingly, KCNQ2-5 all bind GABA but only KCNQ3 and KCNQ5 are activated by GABA or gabapentin (Manville et al., 2018); retigabine activates all four (but not KCNQ1, which lacks the equivalent W) but KCNQ3 is the most sensitive (Tatulian et al., 2001).

Mechanisms of therapeutic action

Gabapentin and pregabalin are in wide clinical use to treat a variety of disorders of the nervous system, including neuropathic pain and epilepsy. There is considerable overlap between the clinical indications for each drug (Alles and Smith, 2018; Calandre et al., 2016; Sills, 2006). This, together with the contrasting ability of gabapentin and pregabalin to activate neuronal KCNQ isoforms found herein, suggests that KCNQ activation cannot be the dominant mechanism of action for the majority of the therapeutic effects of gabapentin. Gabapentinoid binding to the $\alpha_2\delta\text{-}_1$ subunit reportedly inhibits $\alpha_2\delta\text{-}_1$-containing Cav channels (Stefani et al., 1998; Stefani et al., 2001), (Stefani et al., 1998; Stefani et al., 2001) although others found that gabapentinoids have little effect on Cav channel activity or Cav channel-dependent neurotransmitter release at presynaptic nerve terminals (Brown and Randall, 2005; Hoppa et al., 2012; Rock et al., 1993; Schumacher et al., 1998). $\alpha_2\delta\text{-}_1$-NMDA receptor complexes were recently discovered in human and rodent spinal cord; gabapentin inhibited $\alpha_2\delta\text{-}_1$-dependent potentiation of NMDA receptor activity and associated pain hypersensitivity, presenting a plausible mechanism for antinociceptive effects of gabapentin (Chen et al., 2018).

Multiple gabapentinoid targets in neurons – a role for KCNQs?

In a study comparing pregabalin and gabapentin effects on cultured dorsal root ganglion (DRG) neurons from neonatal rats, pregabalin and gabapentin produced biphasic effects (acute inhibition,

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

but longer-term augmentation) on endogenous K^+ currents. The enhancing effect was attenuated by pertussis toxin or by intracellular application of a synthetic cAMP analogue, suggesting an indirect mechanism involving G protein activation (McClelland et al., 2004). Another group also found that effects of gabapentin on inward rectifier K^+ channels and N-type Ca^{2+} channels were pertussis toxin-sensitive (Bertrand et al., 2003b). Pertussis toxin is commonly used to inhibit the downstream effects of $GABA_B$ receptor activation, as it inhibits some (but not all) of the G proteins involved in this process (Asano et al., 1985). Yet, others have shown that $GABA_B$ receptors are insensitive to gabapentinoids (Lanneau et al., 2001), and $GABA_B$ receptor inhibitors did not alter the pregabalin-induced inhibition of Cav currents in neonatal rat DRG neurons (Martin et al., 2002; McClelland et al., 2004). The most likely explanation for this apparent discrepancy is that gabapentinoids can activate pertussis-sensitive G-proteins, but independent of $GABA_B$ receptors (Martin et al., 2002).

With respect to the DRG neuron K^+ channel inhibition by pregabalin, it was apamin-sensitive implying it involved small-conductance Ca^{2+}-activated K^+ channels (McClelland et al., 2004). The K^+ current enhancement did not begin until 10 minutes after initiation of administration of pregabalin, was apamin-insensitive, and was faster when pregabalin was applied intracellularly, suggesting an intracellular signaling mechanism. The gating kinetics and voltage dependence of the DRG Kv current described in the gabapentinoid study do not necessarily suggest against it containing an M-current component. Interestingly, KCNQ2 (which is gabapentin-insensitive) expression precedes that of KCNQ3 (gabapentin-sensitive) during human brain development (Tinel et al., 1998), and the effects of KCNQ channel inhibition upon depolarization-induced GABA release and action potential propagation also alter dramatically from P0-P7 in rat (Okada et al., 2003). Thus, in some neurons, M-current might be insensitive to gabapentin early in development (e.g., the first week), unless KCNQ5 was appreciably expressed. Furthermore, in the study of gabapentinoid action on DRG neurons, K^+ channel activity was quantified at +40 mV, a voltage at

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

which the activating effects of gabapentin (and most Kv channel activators) are minimal. In addition, we find that pregabalin *inhibits* KCNQ2/3 channel activity at concentrations of 10 μM and above, suggesting that at the concentrations used in the prior study (250 μM) (McClelland et al., 2004) pregabalin would inhibit KCNQ2/3 channels and may have similar effects on other KCNQ isoforms that could be expressed in neonatal rat DRG neurons.

It is highly possible, given the somewhat pleiotropic actions of gabapentinoids, that the potent effects of gabapentin on KCNQ3 and KCNQ5 channels might be masked by other effects observed at higher doses, both experimentally and with respect to clinical mechanisms of action. Serum gabapentinoid concentrations may reach 100 μM in patients (although in the brain and spinal cord this concentration is likely to be lower) (Ben-Menachem et al., 1992; Ben-Menachem et al., 1995; Berry et al., 2003), several orders of magnitude higher than the EC_{50} for gabapentin activation of KCNQ2/3, KCNQ3 and KCNQ5 channels, but as noted above, within the range for pregabalin inhibition of KCNQ2/3.

Gabapentin has also been found to augment K_{ATP} currents in rat hippocampal and human neocortical slices (but not incidentally, in rat DRG neurons) (Freiman et al., 2001), and to inhibit the hyperpolarization-activated, cyclic nucleotide-gated channel, HCN4, albeit not at clinically relevant drug concentrations (Tae et al., 2017). Conversely, gabapentin augmented in hippocampal and inhibitory interneurons, cells that highly express HCN1 and HCN2 (Peng et al., 2011; Surges et al., 2003). Thus, indirect modes of action of gabapentin may occur *in vivo*, as reported for Kv currents in rat DRG neurons (McClelland et al., 2004).

Conclusions

Perhaps the two most important take-home points from this study are, first, that we have discovered a new chemical space for KCNQ2/3 activation by synthetic compounds. Future

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

structure-activity relationship studies guided by what we now know regarding the difference between gabapentin versus pregabalin with respect to KCNQ opening, and our previous work identifying endogenous activators for KCNQ3 and KCNQ5, including GABA, GABOB and β-hydroxybutyrate (Manville et al., 2018), can start to inform synthesis of a new class of KCNQ activators for potential therapeutic use. Second, the high potency but relatively low efficacy of gabapentin compared to, e.g., retigabine, suggests the possibility that gabapentin could act as a partial agonist and disrupt therapeutic actions of retigabine and related anticonvulsants. Furthermore, it is possible that gabapentin competes with the binding of endogenous GABA and its metabolites to neuronal KCNQ channels but shares similar or lower efficacy to them with respect to KCNQ activation, possibly explaining why KCNQ activation may not be an important determinant of gabapentin's beneficial effects. Thus, further exploration of gabapentinoids and related compounds with respect to KCNQ activation might uncover superior compounds, which either avoid KCNQ activation and thus potentially disruptive partial agonism, or alternatively are more effective than gabapentin in activating neuronal KCNQs and thus clinically superior because of an additional, beneficial target site.

Acknowledgements

We are grateful to Angele De Silva (University of California, Irvine) for generating mutant channel constructs.

Author Contributions

Participated in research design: Manville and Abbott.

Conducted experiments: Manville.

Contributed new reagents or analytic tools: not applicable.

Performed data analysis: Manville and Abbott.

Wrote or contributed to the writing of the manuscript: Manville and Abbott.

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

References

Alles SRA and Smith PA (2018) Etiology and Pharmacology of Neuropathic Pain. *Pharmacological reviews* **70**:315-347.

Asano T, Ui M and Ogasawara N (1985) Prevention of the agonist binding to gamma-aminobutyric acid B receptors by guanine nucleotides and islet-activating protein, pertussis toxin, in bovine cerebral cortex. Possible coupling of the toxin-sensitive GTP-binding proteins to receptors. *The Journal of biological chemistry* **260**:12653-12658.

Ben-Menachem E (2004) Pregabalin pharmacology and its relevance to clinical practice. *Epilepsia* **45 Suppl 6**:13-18.

Ben-Menachem E, Persson LI and Hedner T (1992) Selected CSF biochemistry and gabapentin concentrations in the CSF and plasma in patients with partial seizures after a single oral dose of gabapentin. *Epilepsy research* **11**:45-49.

Ben-Menachem E, Soderfelt B, Hamberger A, Hedner T and Persson LI (1995) Seizure frequency and CSF parameters in a double-blind placebo controlled trial of gabapentin in patients with intractable complex partial seizures. *Epilepsy research* **21**:231-236.

Berry DJ, Beran RG, Plunkeft MJ, Clarke LA and Hung WT (2003) The absorption of gabapentin following high dose escalation. *Seizure : the journal of the British Epilepsy Association* **12**:28-36.

Bertrand S, Morin F and Lacaille JC (2003a) Different actions of gabapentin and baclofen in hippocampus from weaver mice. *Hippocampus* **13**:525-528.

Bertrand S, Nouel D, Morin F, Nagy F and Lacaille JC (2003b) Gabapentin actions on Kir3 currents and N-type Ca2+ channels via GABAB receptors in hippocampal pyramidal cells. *Synapse* **50**:95-109.

Blackburn-Munro G, Dalby-Brown W, Mirza NR, Mikkelsen JD and Blackburn-Munro RE (2005) Retigabine: chemical synthesis to clinical application. *CNS drug reviews* **11**:1-20.

Brown DA and Adams PR (1980) Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate

neurone. *Nature* **283**:673-676.

Brown JP and Gee NS (1998) Cloning and deletion mutagenesis of the alpha2 delta calcium channel subunit from porcine cerebral cortex. Expression of a soluble form of the protein that retains [3H]gabapentin binding activity. *The Journal of biological chemistry* **273**:25458-25465.

Brown JT and Randall A (2005) Gabapentin fails to alter P/Q-type Ca2+ channel-mediated synaptic transmission in the hippocampus in vitro. *Synapse* **55**:262-269.

Calandre EP, Rico-Villademoros F and Slim M (2016) Alpha2delta ligands, gabapentin, pregabalin and mirogabalin: a review of their clinical pharmacology and therapeutic use. *Expert Rev Neurother* **16**:1263-1277.

Chen J, Li L, Chen SR, Chen H, Xie JD, Sirrieh RE, MacLean DM, Zhang Y, Zhou MH, Jayaraman V and Pan HL (2018) The alpha2delta-1-NMDA Receptor Complex Is Critically Involved in Neuropathic Pain Development and Gabapentin Therapeutic Actions. *Cell reports* **22**:2307-2321.

Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, Bramwell S, Corradini L, England S, Winks J, Kinloch RA, Hendrich J, Dolphin AC, Webb T and Williams D (2006) Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proceedings of the National Academy of Sciences of the United States of America* **103**:17537-17542.

Freiman TM, Kukolja J, Heinemeyer J, Eckhardt K, Aranda H, Rominger A, Dooley DJ, Zentner J and Feuerstein TJ (2001) Modulation of K+-evoked [3H]-noradrenaline release from rat and human brain slices by gabapentin: involvement of KATP channels. *Naunyn-Schmiedeberg's archives of pharmacology* **363**:537-542.

Fuller-Bicer GA, Varadi G, Koch SE, Ishii M, Bodi I, Kadeer N, Muth JN, Mikala G, Petrashevskaya NN, Jordan MA, Zhang SP, Qin N, Flores CM, Isaacsohn I, Varadi M, Mori Y, Jones WK and Schwartz A (2009) Targeted disruption of the voltage-dependent calcium channel alpha2/delta-1-subunit. *American journal of physiology Heart and circulatory physiology* **297**:H117-124.

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R and Woodruff GN (1996) The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *The Journal of biological chemistry* **271**:5768-5776.

Grosdidier A, Zoete V and Michielin O (2011a) Fast docking using the CHARMM force field with EADock DSS. *Journal of computational chemistry* **32**:2149-2159.

Grosdidier A, Zoete V and Michielin O (2011b) SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic acids research* **39**:W270-277.

Guyon A, Kussrow A, Olmsted IR, Sandoz G, Bornhop DJ and Nahon JL (2013) Baclofen and other GABAB receptor agents are allosteric modulators of the CXCL12 chemokine receptor CXCR4. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**:11643-11654.

Hoppa MB, Lana B, Margas W, Dolphin AC and Ryan TA (2012) alpha2delta expression sets presynaptic calcium channel abundance and release probability. *Nature* **486**:122-125.

Jensen AA, Mosbacher J, Elg S, Lingenhoehl K, Lohmann T, Johansen TN, Abrahamsen B, Mattsson JP, Lehmann A, Bettler B and Brauner-Osborne H (2002) The anticonvulsant gabapentin (neurontin) does not act through gamma-aminobutyric acid-B receptors. *Molecular pharmacology* **61**:1377-1384.

Johansson MU, Zoete V, Michielin O and Guex N (2012) Defining and searching for structural motifs using DeepView/Swiss-PdbViewer. *BMC bioinformatics* **13**:173.

Kang S, Li J, Zuo W, Fu R, Gregor D, Krnjevic K, Bekker A and Ye JH (2017) Ethanol Withdrawal Drives Anxiety-Related Behaviors by Reducing M-type Potassium Channel Activity in the Lateral Habenula. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **42**:1813-1824.

Kim RY, Yau MC, Galpin JD, Seebohm G, Ahern CA, Pless SA and Kurata HT (2015) Atomic basis for therapeutic activation of neuronal potassium channels. *Nature communications* **6**:8116.

Lanneau C, Green A, Hirst WD, Wise A, Brown JT, Donnier E, Charles KJ, Wood M, Davies CH and Pangalos MN (2001) Gabapentin is not a GABAB receptor agonist. *Neuropharmacology* **41**:965-975.

Manville RW, Papanikolaou M and Abbott GW (2018) Direct neurotransmitter activation of voltage-gated potassium channels. *Nature communications* **9**:1847.

Marrion NV, Smart TG, Marsh SJ and Brown DA (1989) Muscarinic suppression of the M-current in the rat sympathetic ganglion is mediated by receptors of the M1-subtype. *British journal of pharmacology* **98**:557-573.

Martin DJ, McClelland D, Herd MB, Sutton KG, Hall MD, Lee K, Pinnock RD and Scott RH (2002) Gabapentin-mediated inhibition of voltage-activated Ca2+ channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. *Neuropharmacology* **42**:353-366.

Mason BJ, Quello S and Shadan F (2018) Gabapentin for the treatment of alcohol use disorder. *Expert opinion on investigational drugs* **27**:113-124.

McClelland D, Evans RM, Barkworth L, Martin DJ and Scott RH (2004) A study comparing the actions of gabapentin and pregabalin on the electrophysiological properties of cultured DRG neurones from neonatal rats. *BMC pharmacology* **4**:14.

Ng GY, Bertrand S, Sullivan R, Ethier N, Wang J, Yergey J, Belley M, Trimble L, Bateman K, Alder L, Smith A, McKernan R, Metters K, O'Neill GP, Lacaille JC and Hebert TE (2001) Gamma-aminobutyric acid type B receptors with specific heterodimer composition and postsynaptic actions in hippocampal neurons are targets of anticonvulsant gabapentin action. *Molecular pharmacology* **59**:144-152.

Okada M, Zhu G, Hirose S, Ito KI, Murakami T, Wakui M and Kaneko S (2003) Age-dependent modulation of hippocampal excitability by KCNQ-channels. *Epilepsy research* **53**:81-94.

Parker DA, Ong J, Marino V and Kerr DI (2004) Gabapentin activates presynaptic GABAB heteroreceptors in rat cortical slices. *European journal of pharmacology* **495**:137-143.

Peng BW, Justice JA, Zhang K, Li JX, He XH and Sanchez RM (2011) Gabapentin promotes inhibition by enhancing hyperpolarization-activated cation currents and spontaneous firing in hippocampal CA1 interneurons. *Neuroscience letters* **494**:19-23.

Rock DM, Kelly KM and Macdonald RL (1993) Gabapentin actions on ligand- and voltage-gated responses in

cultured rodent neurons. *Epilepsy research* **16**:89-98.

Schenzer A, Friedrich T, Pusch M, Saftig P, Jentsch TJ, Grotzinger J and Schwake M (2005) Molecular determinants of KCNQ (Kv7) K+ channel sensitivity to the anticonvulsant retigabine. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**:5051-5060.

Schumacher TB, Beck H, Steinhauser C, Schramm J and Elger CE (1998) Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. *Epilepsia* **39**:355-363.

Sills GJ (2006) The mechanisms of action of gabapentin and pregabalin. *Current opinion in pharmacology* **6**:108-113.

Stefani A, Spadoni F and Bernardi G (1998) Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology* **37**:83-91.

Stefani A, Spadoni F, Giacomini P, Lavaroni F and Bernardi G (2001) The effects of gabapentin on different ligand- and voltage-gated currents in isolated cortical neurons. *Epilepsy research* **43**:239-248.

Stringer JL and Lorenzo N (1999) The reduction in paired-pulse inhibition in the rat hippocampus by gabapentin is independent of GABA(B) receptor receptor activation. *Epilepsy research* **33**:169-176.

Sun J and MacKinnon R (2017) Cryo-EM Structure of a KCNQ1/CaM Complex Reveals Insights into Congenital Long QT Syndrome. *Cell* **169**:1042-1050 e1049.

Surges R, Freiman TM and Feuerstein TJ (2003) Gabapentin increases the hyperpolarization-activated cation current Ih in rat CA1 pyramidal cells. *Epilepsia* **44**:150-156.

Tae HS, Smith KM, Phillips AM, Boyle KA, Li M, Forster IC, Hatch RJ, Richardson R, Hughes DI, Graham BA, Petrou S and Reid CA (2017) Gabapentin Modulates HCN4 Channel Voltage-Dependence. *Frontiers in pharmacology* **8**:554.

Tatulian L, Delmas P, Abogadie FC and Brown DA (2001) Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**:5535-5545.

Taylor CP (1997) Mechanisms of action of gabapentin. *Revue neurologique* **153 Suppl 1**:S39-45.

Tinel N, Lauritzen I, Chouabe C, Lazdunski M and Borsotto M (1998) The KCNQ2 potassium channel: splice variants, functional and developmental expression. Brain localization and comparison with KCNQ3. *FEBS letters* **438**:171-176.

van Gunsteren WF (1996) *Biomolecular simulation: the GROMOS96 manual and user guide.*, Vdf Hochschulverlag ETHZ.

Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE and McKinnon D (1998) KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* **282**:1890-1893.

Zaika O, Hernandez CC, Bal M, Tolstykh GP and Shapiro MS (2008) Determinants within the turret and pore-loop domains of KCNQ3 K+ channels governing functional activity. *Biophysical journal* **95**:5121-5137.

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

This study was supported by a National Institutes of Health, National Institute of General Medical Sciences R01 award [GM115189] to GWA.

Please address correspondence and reprint requests to Dr. Geoffrey W. Abbott, Bioelectricity Laboratory, Dept. of Physiology and Biophysics, Medical Sciences D, ZOT 4560, School of Medicine, University of California, Irvine, CA 92697, USA; email: abbottg@uci.edu

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legends

Figure 1. KCNQ3 contains a conserved neurotransmitter binding pocket.

A. Topological representation of KCNQ3 showing two of the four subunits, without domain swapping for clarity. *Pentagon*, approximate position of KCNQ3-W265; VSD, voltage sensing domain.

B. Chimeric KCNQ1/KCNQ3 structural model (*red*, KCNQ3-W265). Domain coloring as in A.

C,D. Close-up side views of KCNQ structure as in panel B, showing results of SwissDock

Figure 2. Gabapentin is predicted to bind to KCNQ3-W265.

A. Electrostatic surface potentials (red, electron-dense; blue, electron-poor; green, neutral) and structures calculated and plotted using Jmol.

B,C. Long-range (B) and close-up (C) side views of KCNQ1/3 chimera model structure showing results of SwissDock unguided *in silico* docking of gabapentin. Domain colors as in Fig. 1.

Figure 3. Gabapentin is a potent activator of heteromeric KCNQ2/3 potassium channels.

A. *Left*, mean TEVC traces for KCNQ2/3 expressed in *Xenopus* oocytes in the absence (control) or presence of 10 nM gabapentin (n = 7-8). Dashed line here and throughout, zero current level. *Right*, voltage protocol. Arrow indicates time point at which tail currents are measured throughout this study.

B. Mean tail current and normalized tail currents (G/Gmax) versus prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (red) of 10 nM or 1 μM gabapentin as indicated (n = 7-8). Error bars indicate SD. Voltage protocol as in A.

C. Mean TEVC traces for KCNQ2/3 expressed in *Xenopus* oocytes in the absence (control) or presence of 1 μM pregabalin (n = 7-8). *Lower inset*, voltage protocol.

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

D. Mean tail current and normalized tail currents (G/Gmax) versus prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (blue) of 1 μM pregabalin as indicated (*n* = 5). Error bars indicate SD. Voltage protocol as in C.

E. Voltage dependence of KCNQ2/3 current fold-increase by gabapentin versus pregabalin (10 nM), plotted from traces as in panels A and C (*n* = 5-8). Error bars indicate SD. *P<0.05 versus pregabalin current at -60 mV.

F. Gabapentin and pregabalin dose responses at -60 mV for KCNQ2/3 activation, quantified from data as in panels A-E (*n* = 7-8). Error bars indicate SD.

G. Dose response for gabapentin and pregabalin effects on resting membrane potential (E_M) of unclamped oocytes expressing KCNQ2/3 (*n* = 7-8). Error bars indicate SD.

H. Mean tail current versus prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (green) of 30 μM retigabine as indicated (*n* = 4). Error bars indicate SD. Voltage protocol as in A.

I. Voltage dependence of KCNQ2/3 current fold-increase by retigabine (30 μM), *n* = 4). Error bars indicate SD.

J. Retigabine dose responses at -60 mV for KCNQ2/3 activation, quantified from data as in panels A-E (*n* = 4). Error bars indicate SD.

Figure 4. Gabapentin-activated current is XE991-sensitive and exhibits altered gating kinetics.

A. Exemplar -60 mV KCNQ2/3 current before (*left*, black), during wash-in of gabapentin (red), partial washout with bath solution in the absence of drug (black), and then wash-in of XE991 (blue).

B,C. Mean activation at +40 mV (B) and deactivation at -80 mV (C) rates for KCNQ2/3 before (control) and after wash-in of 1 μM Gabapentin (GABAP) (*n* = 7). Activation rate was quantified

Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

using voltage protocol as in Fig. 3A. Deactivation rate was quantified using voltage protocol shown above. Error bars indicate SD.

Figure 5. Gabapentin is a potent activator of homomeric KCNQ3 and KCNQ5 potassium channels.

A. Mean TEVC traces for homomeric KCNQ2, 3*, 4 or 5 channels (as indicated) expressed in *Xenopus* oocytes in the absence (control) or presence of 1 μM gabapentin (*n* = 4-8). Voltage protocol, *upper inset*.

B. Mean tail current (*left*) and normalized tail currents (G/Gmax; *right*) versus prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing homomeric KCNQ2, 3*, 4 or 5 channels (as indicated) in the absence (black) or presence (red) of 1 μM gabapentin as indicated (*n* = 4-8). Error bars indicate SD.

C. Voltage dependence of current fold-increase by gabapentin (1 μM) for homomeric KCNQ2, 3*, 4 or 5 channels, plotted from traces as in panel A (*n* = 4-8). Error bars indicate SD.

D. Gabapentin dose responses at -60 mV for homomeric KCNQ2, 3*, 4 or 5 channel activation, quantified from data as in panel A (*n* = 4-8). Error bars indicate SD.

Figure 6. Gabapentin activation of KCNQ2/3 requires KCNQ3-W265.

A-C. TEVC of water-injected *Xenopus laevis* oocytes showing no effect of gabapentin (10 nM) on endogenous currents or membrane potential (E_M) (*n* = 5). A, mean traces; B, mean peak current; C, mean EM, in the absence (Control) or presence of 10 nM gabapentin. Voltage protocol, panel A upper inset. Error bars indicate SD.

D,E. TEVC of *Xenopus laevis* oocytes showing effects of gabapentin (10 nM) on heteromeric KCNQ2/KCNQ3-W265L channels. D, mean traces; E, mean tail current (*left*) and mean normalized tail current (G/Gmax; *right*). *n* = 5. Error bars indicate SD.

F,G. TEVC of *Xenopus laevis* oocytes showing effects of gabapentin (10 nM) on heteromeric

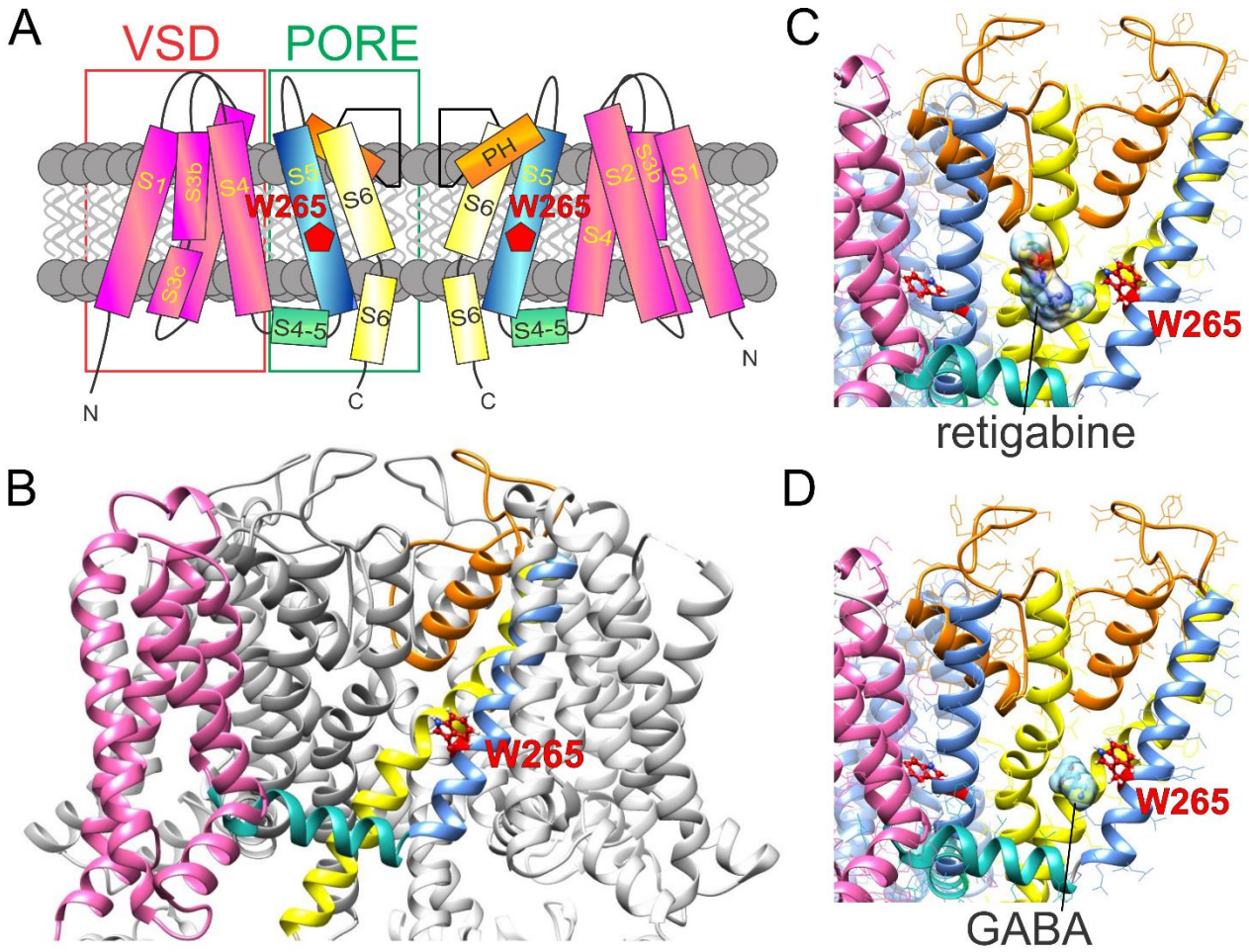
Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

KCNQ2-W236L/KCNQ3-W265L channels. F, mean traces; G, mean tail current (*left*) and mean normalized tail current (G/Gmax; *right*); *n* = 5. Error bars indicate SD.

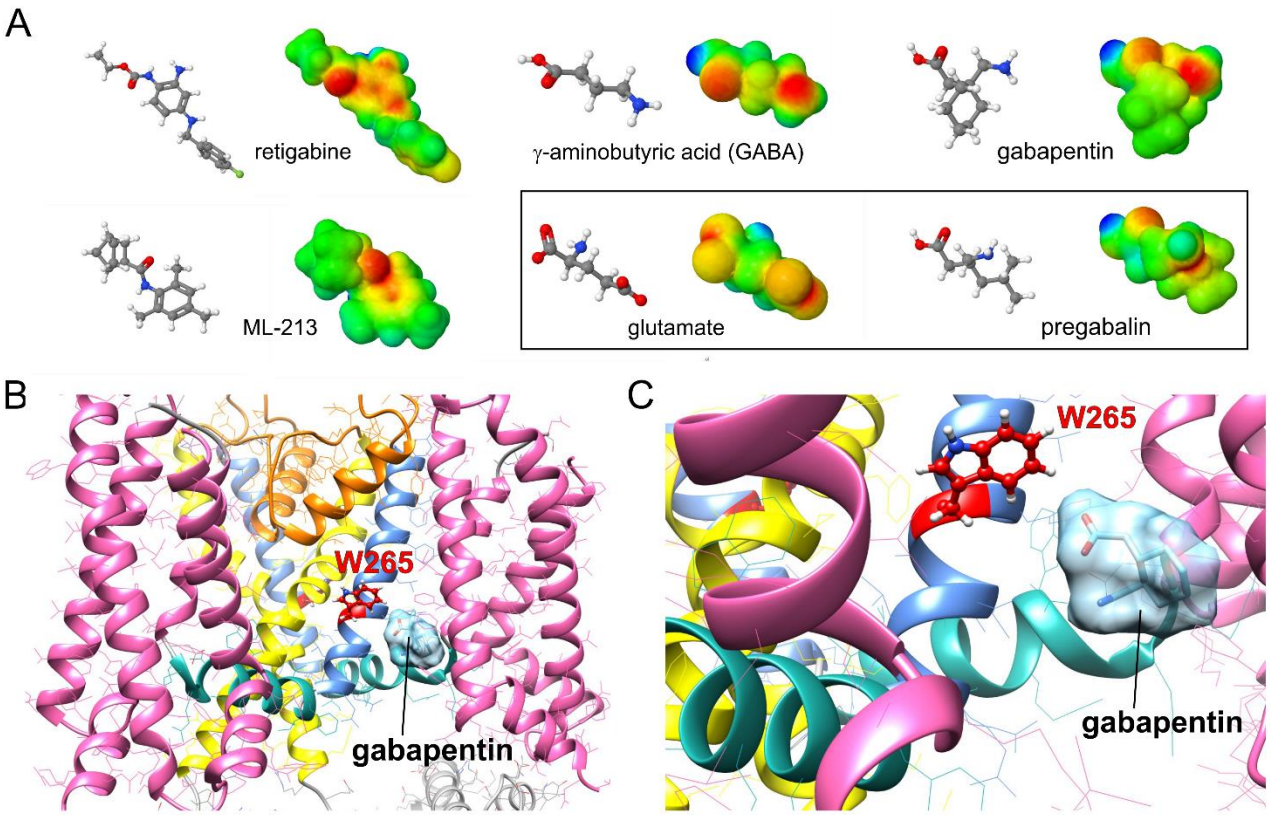
H. Mean tail current fold-changes versus prepulse voltages for channels as indicated; KCNQ2/KCNQ3 results (*black line*) from Fig. 3E shown for comparison; *n* = 5. Error bars indicate SD. *P<0.05 versus other groups at -60 mV.

I. Mean dose responses for channels as indicated; KCNQ2/KCNQ3 results (*black line*) from Fig. 3F shown for comparison; *n* = 5. Error bars indicate SD.

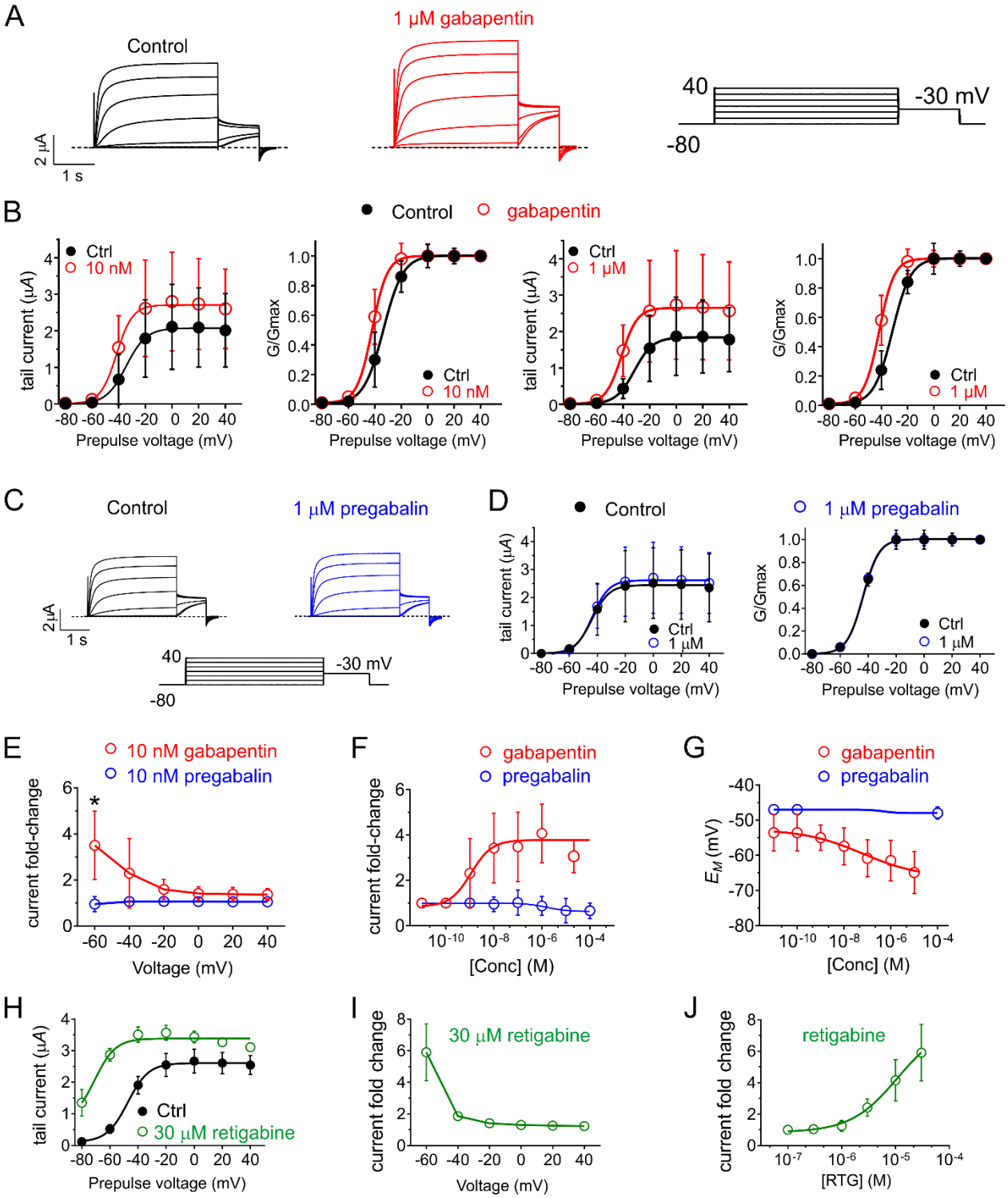
Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.



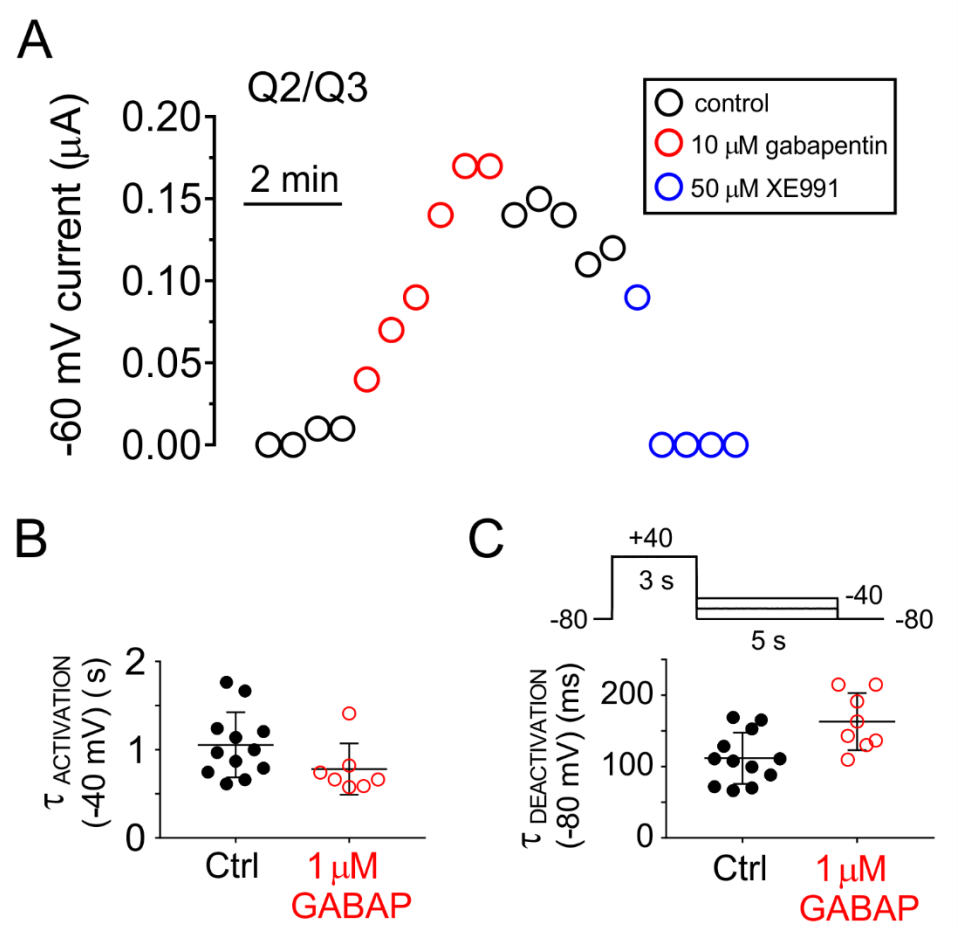
Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.



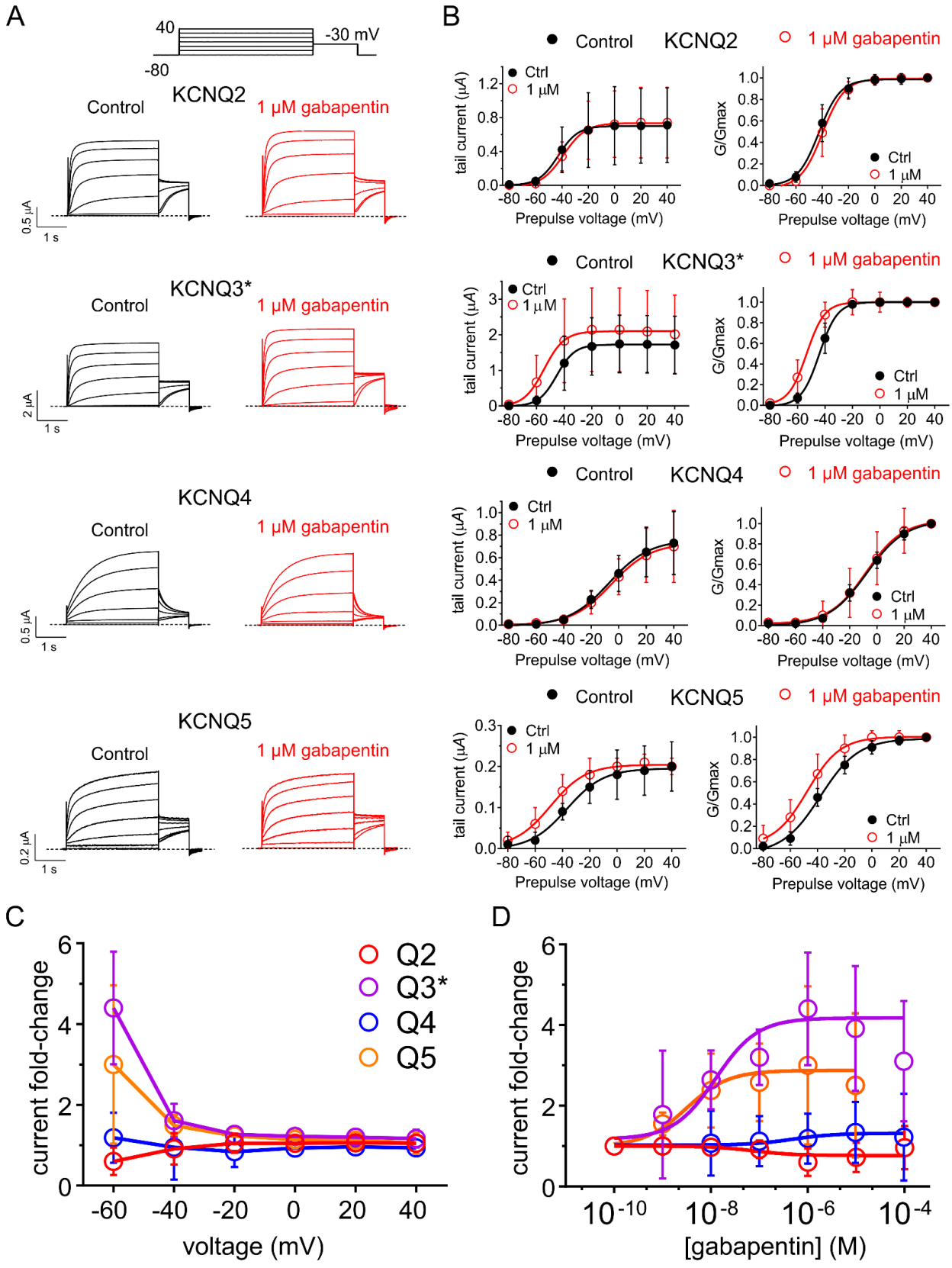
Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.



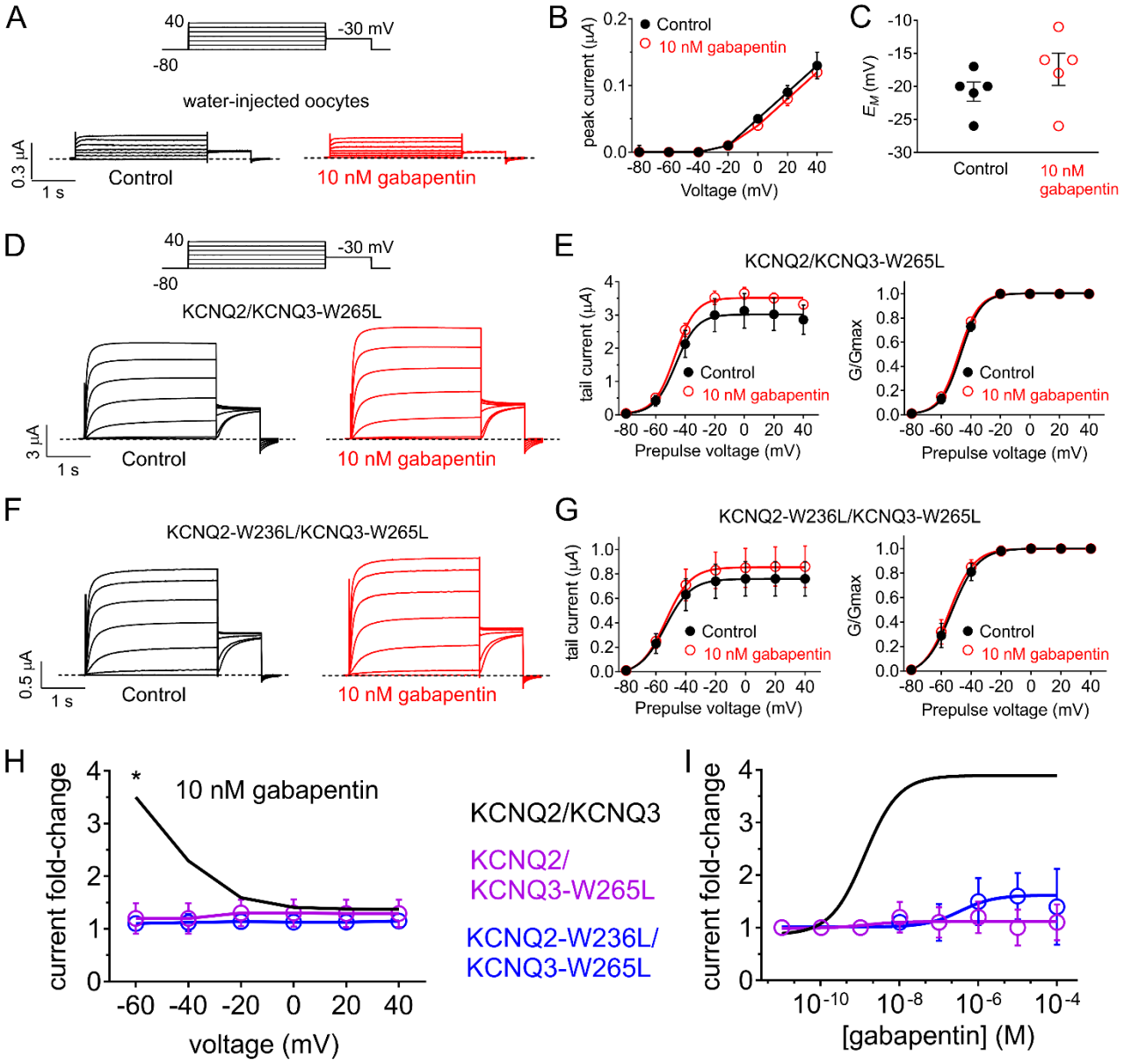
Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.



Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.



Molecular Pharmacology Fast Forward. Published on July 18, 2018 as DOI: 10.1124/mol.118.112953
This article has not been copyedited and formatted. The final version may differ from this version.

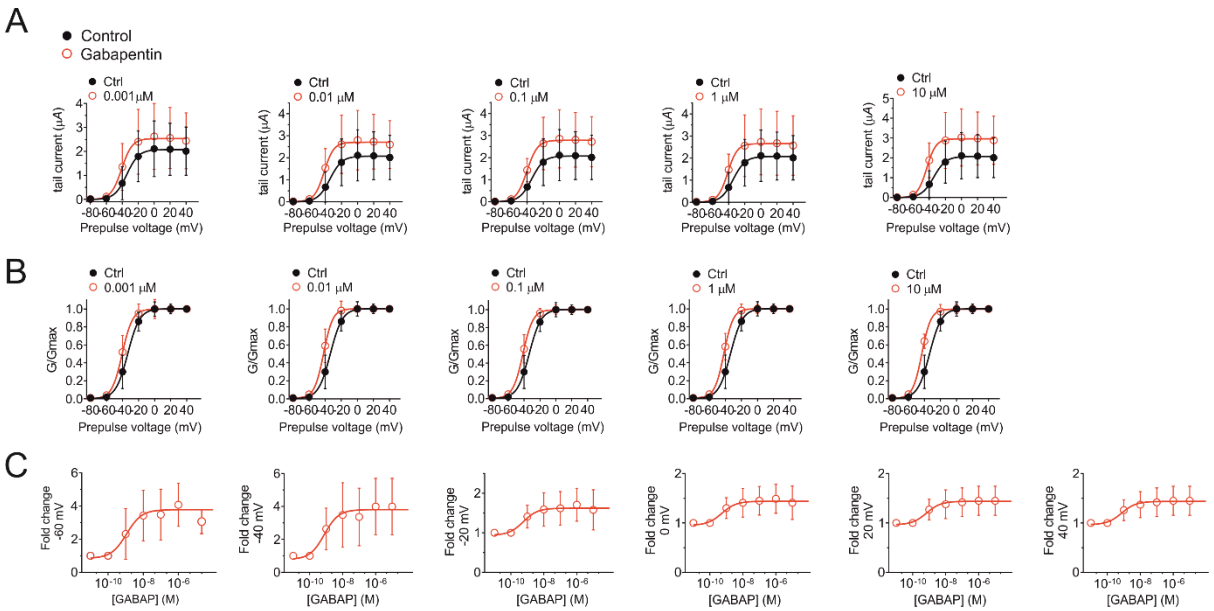


Gabapentin is a potent activator of KCNQ3 and KCNQ5 potassium channels

Rían W. Manville and Geoffrey W. Abbott

MOLECULAR PHARMACOLOGY

SUPPLEMENTAL DATA



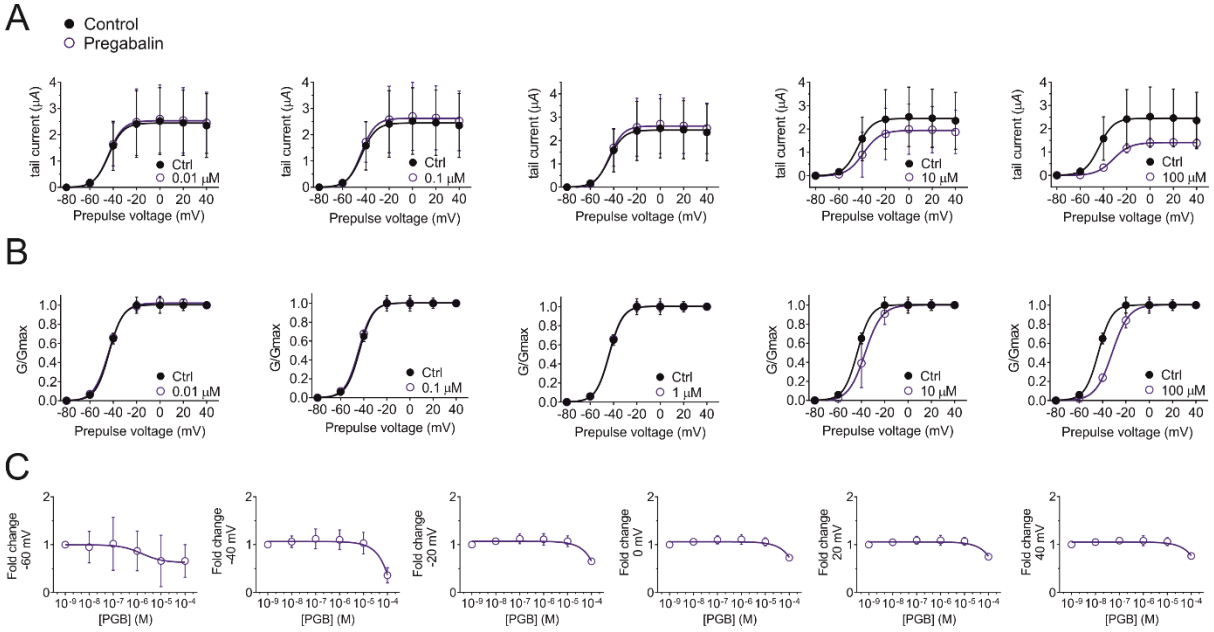
Supplementary Figure 1. Effects of Gabapentin on KCNQ2/3 channels

A. Mean tail current versus prepulse voltage relationship for KCNQ2/3 channels in the absence (black) and presence (red) of Gabapentin, *n* = 7-8.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 7-8.
C. Dose response of KCNQ2/3 channels between -40 and +40 mV, *n* = 7-8. Error bars indicate SD.

KCNQ2/3	τ act, -40 mV (ms)	τ deact, -80 mV (ms)	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	1141 ± 369 (n =8)	115 ± 35 (n =8)	-33.5 ± 3.8 (n =8)	-33.4 ± 15.8 (n =8)	7.4 ± 2.9 (n =8)
0.001 µM Gabapentin	853 ± 379 (n =7)	146 ± 49 (n =7)	-40.5 ± 2.8 (n =7) **	-40.9 ± 12.8 (n =7)	6.4 ± 4.3 (n =7)
0.01 µM Gabapentin	797 ± 379 (n =7)	153 ± 17 (n =7) *	-41.9 ± 2.7 (n =7) ***	-41.6 ± 11.6 (n =7)	5.7 ± 4.2 (n =7)
0.1 µM Gabapentin	835 ± 350 (n =7)	156 ± 26 (n =7) *	-41.4 ± 2.1 (n =7) ***	-41.3 ± 10.2 (n =7)	6.3 ± 3.0 (n =7)
1 µM Gabapentin	800 ± 290 (n =7)	154 ± 36 (n =7)	-42.8 ± 2.3 (n =7) ***	-42.2 ± 12.3 (n =7)	6.0 ± 3.4 (n =7)
10 µM Gabapentin	820 ± 186 (n =8)	160 ± 46 (n =8)	-43.0 ± 1.2 (n =8) ***	-43.2 ± 13.5 (n =8)	5.6 ± 1.8 (n =8)

Supplementary Table 1. Summary of Effects of Gabapentin on KCNQ2/3 channels.

Statistics versus same channel in absence of Gabapentin: ***p=0.0006, **p=0.002. Values indicate mean ± SD.



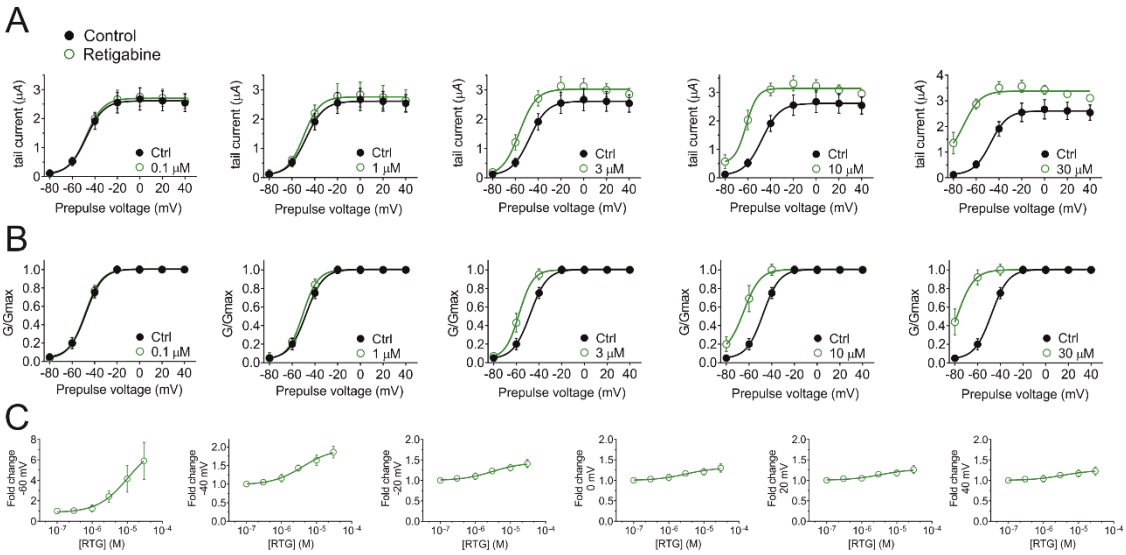
Supplementary Figure 2. Effects of Pregabalin on KCNQ2/3 channels

A. Mean tail current versus prepulse voltage relationship for KCNQ2/3 channels in the absence (black) and presence (red) of Pregabalin, *n* = 4-8.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 4-8.
C. Dose response of KCNQ2/3 channels between -40 and +40 mV, *n* = 4-8. Error bars indicate SD.

KCNQ2/3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-43.5 ± 4.5 (n =8)	-43.7 ± 13.8 (n =8)	5.7 ± 4.9 (n =8)
0.01 μM Pregabalin	-43.8 ± 1.1 (n =4)	-43.8 ± 14.8 (n =4)	6.4 ± 1.2 (n =4)
0.1 μM Pregabalin	-44.4 ± 1.2 (n =4)	-43.9 ± 14.4 (n =4)	5.8 ± 1.1 (n =4)
1 μM Pregabalin	-43.8 ± 1.4 (n =4)	-43.7 ± 13.9 (n =4)	5.7 ± 1.4 (n =4)
10 μM Pregabalin	-36.8 ± 3.7 (n =8) **	-39.0 ± 14.1 (n =8)	7.1 ± 3.9 (n =8)
100 μM Pregabalin	-31.9 ± 0.8 (n =4) ***	-31.5 ± 1.9 (n =4)	7.1 ± 0.5 (n =4)

Supplementary Table 2. Summary of Effects of Pregabalin on KCNQ2/3 channels.

Statistics versus same channel in absence of Pregabalin: ***p=0.0001, **p=0.006. Values indicate mean ± SD.



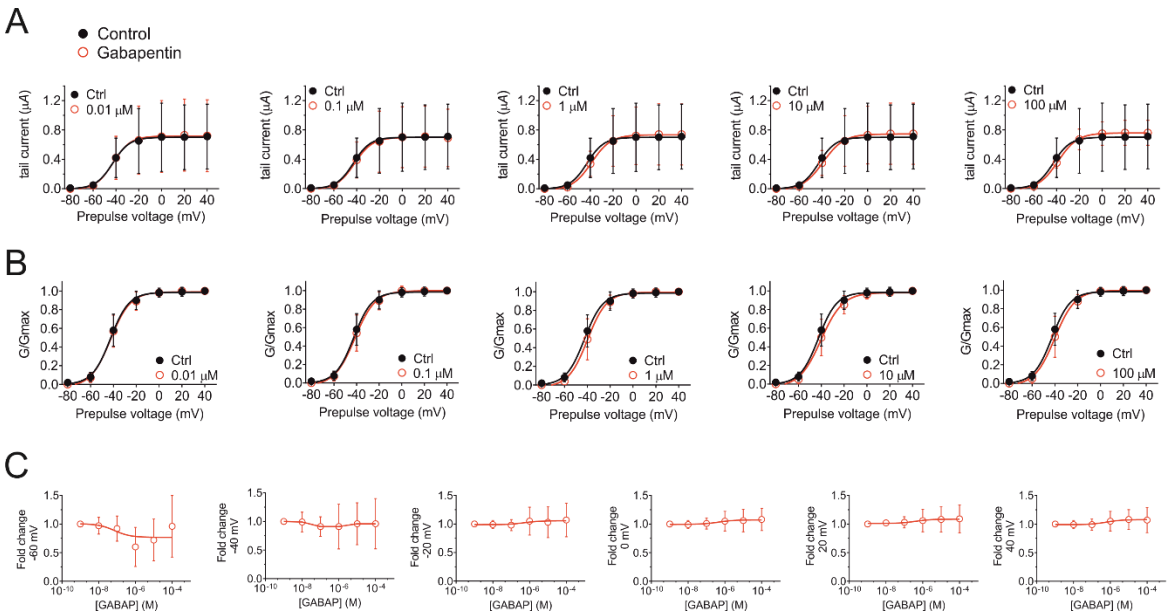
Supplementary Figure 3. Effects of retigabine on KCNQ2/3 channels

A. Mean tail current versus prepulse voltage relationship for Q2/Q3 channels in the absence (black) and presence (green) of retigabine, *n* = 4.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 4.
C. Dose response of Q2/Q3 channels between -40 and +40 mV, *n* = 4. Error bars indicate SD.

KCNQ2/3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-47.8 ± 1.7 (n =4)	-47.6 ± 4.9 (n =4)	7.4 ± 1.2 (n =4)
0.1 μM Retigabine	-48.6 ± 1.6 (n =4)	-47.9 ± 4.1 (n =4)	7.3 ± 1.0 (n =4)
1 μM Retigabine	-50.7 ± 1.6 (n =4) *	-49.7 ± 5.2 (n =4)	6.7 ± 1.0 (n =4)
3 μM Retigabine	-57.6 ± 1.6 (n =4) ***	-56.5 ± 3.2 (n =4) *	6.1 ± 2.2 (n =4)
10 μM Retigabine	-64.6 ± 2.8 (n =4) ***	-61.6 ± 5.0 (n =4) **	7.2 ± 1.4 (n =4)
30 μM Retigabine	-78.1 ± 2.5 (n =4) ****	-70.8 ± 8.1 (n =4) **	7.4 ± 1.6 (n =4)

Supplementary Table 3. Summary of Effects of retigabine on KCNQ2/3 channels.

Statistics versus same channel in absence of retigabine: ****p<0.0001, ***p=0.0002, *p=0.04. Values indicate mean ± SD.



Supplementary Figure 4. Effects of Gabapentin on KCNQ2 channels

A. Mean tail current versus prepulse voltage relationship for KCNQ2 channels in the absence (black) and presence (red) of Gabapentin, *n* = 6.

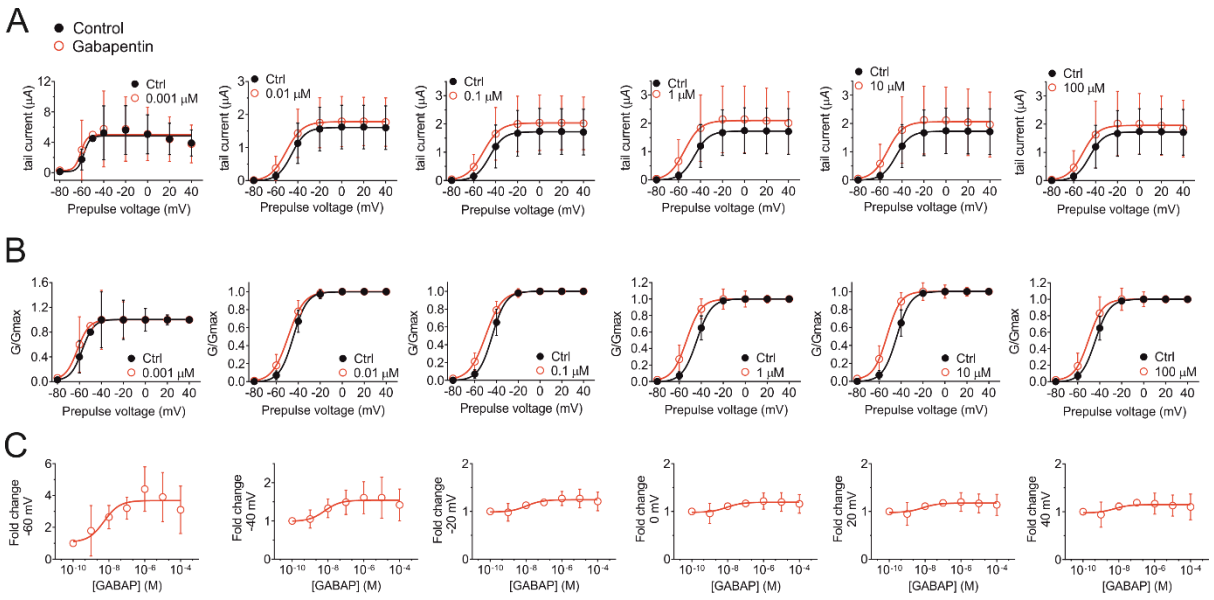
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 6.

C. Dose response of KCNQ2 channels between -40 and +40 mV, *n* = 6. Error bars indicate SD.

KCNQ2	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-42.5 ± 3.0 (n =6)	-42.8 ± 19 (n =6)	8.1 ± 3.4 (n =6)
0.01 μM Gabapentin	-42.5 ± 3.3 (n =6)	-42.6 ± 19.8 (n =6)	8.6 ± 3.3 (n =6)
0.1 μM Gabapentin	-41.4 ± 3.8 (n =6)	-41.8 ± 17.4 (n =6)	8.9 ± 3.8 (n =6)
1 μM Gabapentin	-39.8 ± 3.4 (n =6)	-38.8 ± 15.4 (n =6)	8.4 ± 3.7 (n =6)
10 μM Gabapentin	-40.3 ± 3.9 (n =6)	-38.9 ± 16.4 (n =6)	9.5 ± 3.9 (n =6)
100 μM Gabapentin	-40.1 ± 3.5 (n =6)	-38.8 ± 15.3 (n =6)	8.9 ± 3.6 (n =6)

Supplementary Table 4. Summary of Effects of Gabapentin on KCNQ2 channels.

Values indicate mean ± SD.



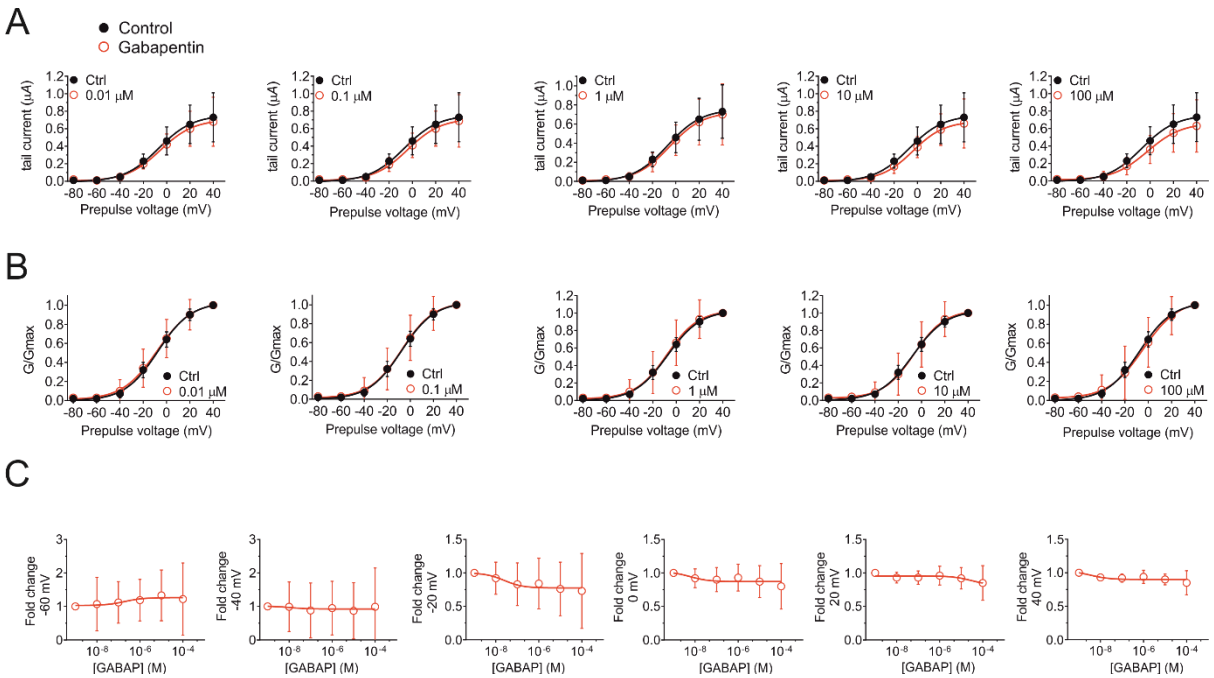
Supplementary Figure 5. Effects of Gabapentin on KCNQ3* channels

A. Mean tail current versus prepulse voltage relationship for KCNQ3* channels in the absence (black) and presence (red) of Gabapentin, *n* = 6.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 6.
C. Dose response of KCNQ3* channels between -40 and +40 mV, *n* = 6. Error bars indicate SD.

KCNQ3*	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-44.5 ± 2.0 (n =6)	-45.5 ± 13.9 (n =6)	6.2 ± 1.8 (n =6)
0.001 µM Gabapentin	-44.9 ± 2.1 (n =6)	-60.1 ± 17.0 (n =7)	n.d.
0.01 µM Gabapentin	-49.2 ± 2.4 (n =6) **	-50.5 ± 17.7 (n =6)	7.2 ± 1.6 (n =6)
0.1 µM Gabapentin	-49.6 ± 3.3 (n =6) *	-51.1 ± 17.7 (n =6)	7.5 ± 2.1 (n =6)
1 µM Gabapentin	-53.3 ± 3.2 (n =6) ***	-54.5 ± 19.2 (n =6)	n.d.
10 µM Gabapentin	-52.7 ± 3.5 (n =6) **	-53.2 ± 19.9 (n =6)	n.d.
100 µM Gabapentin	-50.2 ± 3.7 (n =6) *	51.7 ± 19.7 (n =6)	n.d.

Supplementary Table 5. Summary of Effects of Gabapentin on KCNQ3* channels.

Statistics versus same channel in absence of Gabapentin: ***p=0.0004, **p=0.003, *p=0.01. Values indicate mean ± SD.



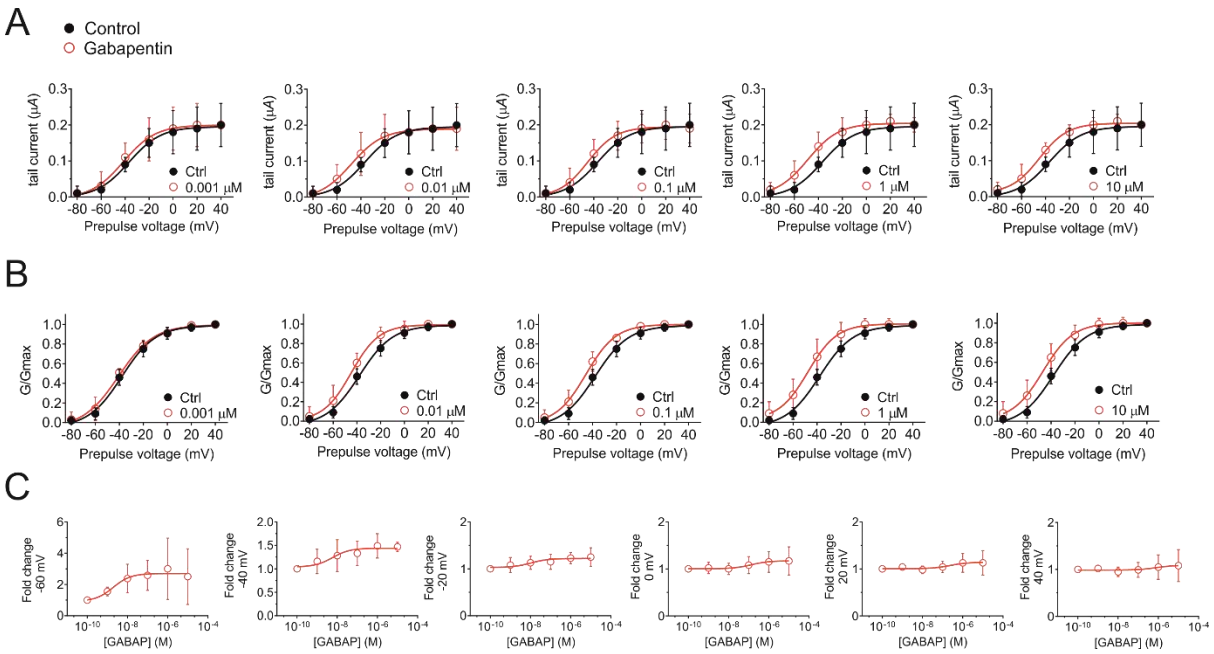
Supplementary Figure 6. Effects of Gabapentin on KCNQ4 channels

A. Mean tail current versus prepulse voltage relationship for KCNQ4 channels in the absence (black) and presence (red) of Gabapentin, *n* = 4.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 4.
C. Dose response of KCNQ4 channels between -40 and +40 mV, *n* = 4. Error bars indicate SD.

KCNQ4	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-42.5 ± 3.3 (n =4)	-42.8 ± 13.2 (n =4)	8.6 ± 3.1 (n =4)
0.01 µM Gabapentin	-42.5 ± 2.1 (n =4)	-42.6 ± 12.9 (n =4)	8.7 ± 1.9 (n =4)
0.1 µM Gabapentin	-41.4 ± 2.0 (n =4)	-41.8 ± 14.6 (n =4)	8.6 ± 1.9 (n =4)
1 µM Gabapentin	-39.8 ± 2.8 (n =4)	-38.8 ± 15.7 (n =4)	8.4 ± 2.6 (n =4)
10 µM Gabapentin	-40.3 ± 1.8 (n =4)	-38.9 ± 13.0 (n =4)	9.5 ±1.6 (n =4)
100 µM Gabapentin	-40.1 ± 2.6 (n =4)	-38.8 ± 18.0 (n =4)	8.9 ± 2.4 (n =4)

Supplementary Table 6. Summary of Effects of Gabapentin on KCNQ4 channels.

Values indicate mean ± SD.



Supplementary Figure 7. Effects of Gabapentin on KCNQ5 channels

A. Mean tail current versus prepulse voltage relationship for KCNQ5 channels in the absence (black) and presence (red) of Gabapentin, *n* = 4.

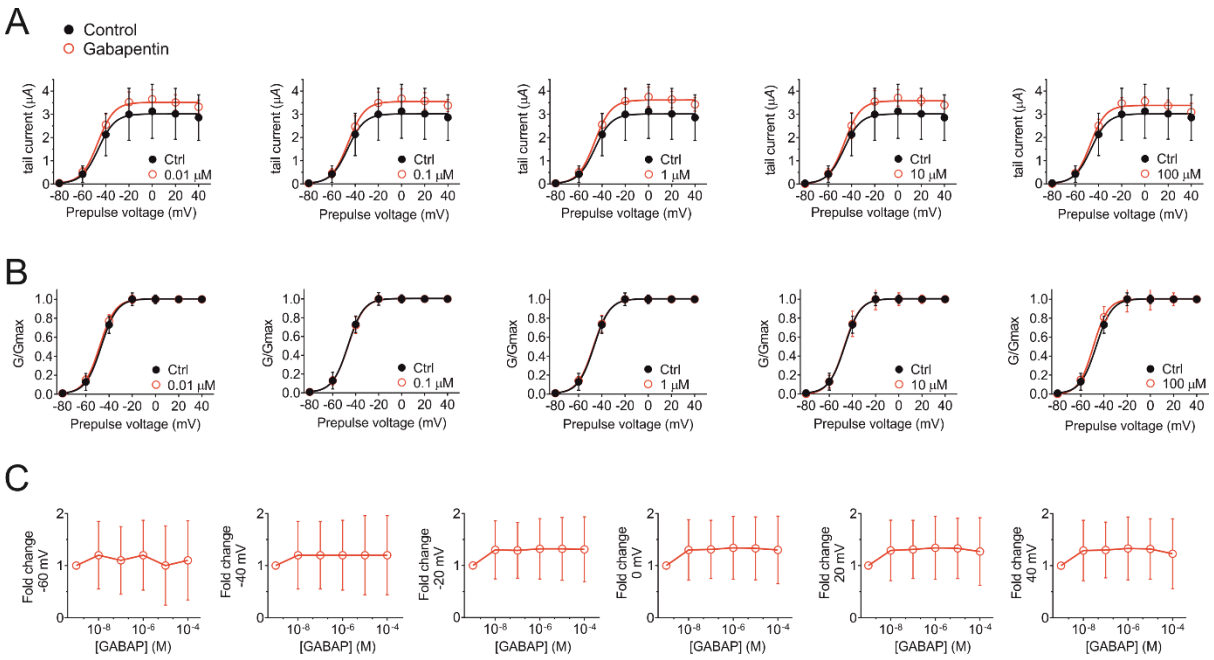
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 4.

C. Dose response of KCNQ5 channels between -40 and +40 mV, *n* = 4. Error bars indicate SD.

KCNQ5	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-37.9 ± 3.8 (n =4)	-36.8 ± 12.8 (n =4)	13.9 ± 4.4 (n =4)
0.001 µM Gabapentin	-40.7 ± 4.9 (n =4)	-41.5 ± 12.9 (n =4)	14.7 ± 4.9 (n =4)
0.01 µM Gabapentin	-44.8 ± 5.7 (n =4)	-44.9 ± 12.3 (n =4)	11.7 ± 5.7 (n =4)
0.1 µM Gabapentin	-45.7 ± 4.8 (n =4) *	-45.0 ± 12.6 (n =4)	12.5 ± 4.8 (n =4)
1 µM Gabapentin	-47.6 ± 4.2 (n =4) *	-48.9 ± 8.2 (n =4)	12.2 ± 8.2 (n =4)
10 µM Gabapentin	-46.8 ± 7.5 (n =4)	-45.0 ± 7.5 (n =4)	12.6 ± 7.5 (n =4)

Supplementary Table 7. Summary of Effects of Gabapentin on KCNQ5 channels.

Statistics versus same channel in absence of Gabapentin: *p=0.02. Values indicate mean ± SD.



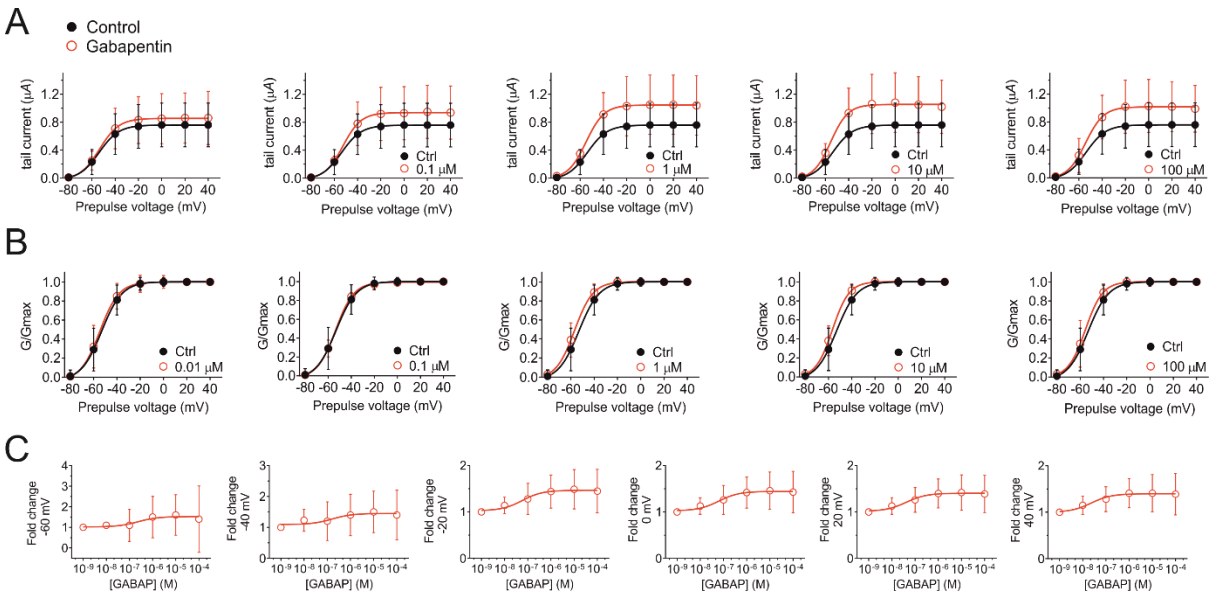
Supplementary Figure 8. Effects of Gabapentin on KCNQ2/KCNQ3-W265L channels

A. Mean tail current versus prepulse voltage relationship for KCNQ2/KCNQ3-W265L channels in the absence (black) and presence (red) of Gabapentin, *n* = 5.
B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 5.
C. Dose response of KCNQ2/KCNQ3-W265L channels between -40 and +40 mV, *n* = 5. Error bars indicate SD.

Q2/Q3-W265L	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-46.7 ± 2.2 (n =5)	-46.4 ± 9.6 (n =5)	6.7 ± 2.2 (n =5)
0.01 μM Gabapentin	-48.1 ± 1.7 (n =5)	-46.9 ± 4.1 (n =5)	6.7 ± 1.1 (n =5)
0.1 μM Gabapentin	-46.4 ± 1.8 (n =5)	-46.0 ± 4.1 (n =5)	6.9 ± 1.8 (n =5)
1 μM Gabapentin	-47.2 ± 2.1 (n =5)	-46.4 ± 4.2 (n =5)	6.9 ± 2.1 (n =5)
10 μM Gabapentin	-47.2 ± 3.3 (n =5)	-46.1 ± 3.7 (n =5)	6.8 ± 3.3 (n =5)
100 μM Gabapentin	-49.2 ± 3.3 (n =5)	-47.2 ± 2.7 (n =5)	6.4 ± 3.3 (n =5)

Supplementary Table 8. Summary of Effects of Gabapentin on KCNQ2/KCNQ3-W265L channels.

Values indicate mean ± SD.



Supplementary Figure 9. Effects of Gabapentin on KCNQ2-W236L/KCNQ3-W265L channels

A. Mean tail current versus prepulse voltage relationship for KCNQ2-W236L/KCNQ3-W265L channels in the absence (black) and presence (red) of Gabapentin, *n* = 5.

B. Normalized tail current versus prepulse voltage relationships as in panel A, *n* = 5.

C. Dose response of KCNQ2-W236L/KCNQ3-W265L channels between -40 and +40 mV, *n* = 5. Error bars indicate SD.

Q2-W236L/ Q3-W265L	**Normalized tail current $V_{0.5}$ (mV)**	**Non-normalized tail current $V_{0.5}$ (mV)**	**Slope (mV)**
Ctrl	-53.1 ± 5.2 (n =5)	-53.8 ± 15.9 (n =5)	8.8 ± 4.0 (n =5)
0.01 μM Gabapentin	-54.7 ± 4.7 (n =5)	-53.5 ± 15.6 (n =5)	8.3 ± 3.8 (n =5)
0.1 μM Gabapentin	-53.6 ± 1.9 (n =5)	-52.6 ± 13.9 (n =5)	7.9 ± 1.4 (n =5)
1 μM Gabapentin	-56.9 ± 3.2 (n =5)	-54.8 ± 13.7 (n =5)	7.9 ± 3.1 (n =5)
10 μM Gabapentin	-56.6 ± 2.7 (n =5)	-55.4 ± 12.7 (n =5)	7.1 ± 2.7 (n =5)
100 μM Gabapentin	-55.5 ± 4.0 (n =5)	-54.3 ± 13.0 (n =5)	7.4 ± 3.5 (n =5)

Supplementary Table 9. Summary of Effects of Gabapentin on KCNQ2-W236L/KCNQ3-W265L channels.

Values indicate mean ± SD.