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Pharmacogenetics of KCNQ channel activation in two potassium channelopathy mouse models of epilepsy

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SUMMARY

Objectives—Anti-seizure drugs are the leading therapeutic choice for treatment of epilepsy, but their efficacy is limited by pharmacoresistance and the occurrence of unwanted side effects. Here, we examined the therapeutic efficacy of KCNQ channel activation by retigabine in preventing seizures and neurocardiac dysfunction in two potassium channelopathy mouse models of epilepsy with differing severity that have been associated with increased risk of sudden unexpected death in epilepsy (SUDEP): the *Kcna1*$^{-/-}$ model of severe epilepsy and the *Kcnq1*$^{A340E/A340E}$ model of mild epilepsy.

Methods—A combination of behavioral, seizure threshold, electrophysiological, and gene expression analyses was used to determine the effects of KCNQ activation in mice.

Results—Behaviorally, *Kcna1*$^{-/-}$ mice exhibited unexpected hyperexcitability instead of the expected sedative-like response. In flurothyl-induced seizure tests, KCNQ activation decreased seizure latency by ≥50% in *Kcnq1* strain mice but had no effect in the *Kcna1* strain, suggesting the influence of genetic background. However, in simultaneous electroencephalography and electrocardiography recordings, KCNQ activation significantly reduced spontaneous seizure frequency in *Kcna1*$^{-/-}$ mice by ~60%. In *Kcnq1*$^{A340E/A340E}$ mice, KCNQ activation produced adverse cardiac effects including profound bradycardia and abnormal increases in heart rate variability and atrioventricular conduction blocks. Analyses of *Kcnq2* and *Kcnq3* mRNA levels revealed significantly elevated *Kcnq2* expression in *Kcna1*$^{-/-}$ brains, suggesting drug target alterations may contribute to the altered drug responses.

Significance—This study shows that treatment strategies in channelopathy may have unexpected outcomes and that effective rebalancing of channel defects requires improved understanding of channel interactions at the circuit and tissue levels. The efficacy of KCNQ channel activation and manifestation of adverse effects were greatly affected by genetic background, potentially limiting KCNQ modulation as a way to prevent neurocardiac dysfunction in epilepsy and thereby SUDEP

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DISCLOSURE OF CONFLICTS OF INTEREST

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ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

risk. Our data also uncovers a potential role for KCNQ2-5 channels in autonomic control of chronotropy.

Keywords

retigabine; *Kcna1*; *Kcnq1*; Kv1.1; Kv7.1

INTRODUCTION

Studies in mouse models suggest that activation of voltage-gated KCNQ (Kv7) potassium channels could be an effective pharmacological approach to prevent aberrant neurocardiac activity and thereby decrease risk for sudden unexpected death in epilepsy (SUDEP) risk. In *ex vivo* experiments using *Kcna1* knockout *(Kcna1*$^{-/-}$) mice, a well-characterized model of SUDEP involving brain-heart dysregulation via the parasympathetic nervous system, *Kcna1*$^{-/-}$ vagal axons exhibit abnormal spontaneous firing that is almost completely abolished by augmenting Kv7 currents with the KCNQ channel opener flupirtine.[1] In Sentrin/SUMO-specific protease 2 deficient mice, which exhibit seizures and sudden death as a result of hyper-SUMOylation of multiple potassium channels, *in vivo* treatment with retigabine (RTG), a more potent analog of flupirtine, prevents acoustically-induced seizures and vagally-mediated, seizure-associated atrioventricular (AV) blocks.[2] Thus, KCNQ openers show promise for preventing SUDEP in cases involving neurocardiac or parasympathetic mechanisms since they can not only suppress seizures, but also potentially help maintain normal vagal activity.

Here, we explored the potential of KCNQ channel activation as a pharmacological strategy to prevent seizures and neurocardiac dysfunction in two potassium channelopathy mouse models of epilepsy of differing severity that have been associated with sudden death: the *Kcna1*$^{-/-}$ model of severe epilepsy and the *Kcnq1*$^{A340E/A340E}$ model of mild epilepsy. *Kcna1*$^{-/-}$ mice lack Kv1.1 voltage-gated potassium channel α-subunits and exhibit several key features of human SUDEP including severe early-onset epilepsy characterized by frequent generalized tonic-clonic seizures (up to ~24/d); premature sudden death beginning at a young age (~P16); and seizure-evoked bradyarrhythmias that progress to cardiac arrest.[3–6] Kv1.1 channel mutations have been identified as a molecular cause of epilepsy in patients and a *de novo* copy number variant in *KCNA1* was identified in a human case of SUDEP.[7,8]

In contrast, *Kcnq1*$^{A340E/A340E}$ mice carry a dominant negative missense mutation (A340E) leading to loss-of-function of the *Kcnq1* gene, which encodes KCNQ1 (Kv7.1) voltage-gated potassium channel α-subunits. The A340E mutation in mice causes infrequent spontaneous seizures (~0.5/d) and multiple cardiac abnormalities, including AV blocks that occur concomitantly with cortical discharges suggesting they are brain-driven.[9] Although SUDEP has not been documented in *Kcnq1*$^{A340E/A340E}$ mice, the homologous A341E mutation in humans causes long QT syndrome type 1 (LQT1), which is strongly associated with sudden death due to ventricular tachyarrhythmias.[9,10] *KCNQ1* mutations have been identified in patients with epilepsy or a history of seizures suggesting *KCNQ1* is also a human epilepsy gene.[11,12] In this study, we tested the hypothesis that KCNQ channel

activation with RTG normalizes neurocardiac function in *Kcna1*$^{-/-}$ and *Kcnq1*$^{A340E/A340E}$ mice leading to a reduction in seizure susceptibility and cardiac dysfunction. RTG is a potent activator of KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3) voltage-gated postassium channel α-subunits but has no effect on KCNQ1.[13] Our results reveal unexpected effects of genotype on the efficacy and tolerability of KCNQ activation, as well as potentially new cardiovascular roles for KCNQ2-5 channels in the autonomic control of chrontropy.

MATERIALS AND METHODS

Animals and Genotyping

Kcna1$^{-/-}$ knockout (KO) mice (Tac:N:NIHS-BC genetic background) carry null alleles of the *Kcna1* gene resulting from targeted deletion of the open reading frame, as previously described.[6] *Kcnq1*$^{A340E/A340E}$ mutant mice (C57BL/6 genetic background) carry a point mutation of the *Kcnq1* gene, as previously described.[14] Both strains were maintained by breeding heterozygous mice with non-sibling heterozygous mice of the same strain. Animals were housed at 22°C, fed irradiated rodent chow *ad libitum* (PicoLab Diet 20; LabDiet, St. Louis, MO), and submitted to a 12 h light/dark cycle. Mice of both sexes were used in experiments. All procedures were performed in accordance with the guidelines of the National Institutes of Health (NIH), as approved by the Institutional Animal Care and Use Committee of the Louisiana State University Health Sciences Center-Shreveport. Genotyping was performed as described previously (see Supporting Information for details).[3,9]

Drug Administration

Retigabine dihydrochloride (RTG; Alomone Labs, Jerusalem, Israel) was administered at a concentration of 5, 10, or 20 mg·kg^{-1}, as specified in the text. RTG was dissolved in 0.9% NaCl vehicle (VEH) and injected intraperitoneally (ip) at a volume of 10 mL·kg^{-1}.

Behavioral Studies

Individually housed *Kcna1* and *Kcnq1* homozygous mutants, heterozygotes, and WT littermate controls (4–6 weeks old; n = 6 per genotype) were injected once daily for four days with vehicle or RTG according to the following injection schedule: VEH was given the first day (day 1), followed by progressively increasing RTG doses of 5 mg·kg^{-1} (day 2), 10 mg·kg^{-1} (day 3), and 20 mg·kg^{-1} (day 4). Injections were always performed at a similar time of day between 9:30 am to 2:00 pm. Behavioral responses to VEH or drug were video-monitored for 1 hour post-injection using digital network cameras (DCS-942L, D-Link, Fountain Valley, CA). During the first 30 min post-injection, the following behaviors were manually scored offline and quantified by an observer blinded to genotype: immobility, myoclonus, hopping, and vocalizing.

Flurothyl-Induced Seizure Susceptibility

Flurothyl-induced seizure latencies were measured using the same procedure as described previously (see Supporting Information for details).[4] Forty minutes prior to seizure induction, mice (age P29–31) were administered VEH or RTG (10 mg·kg^{-1}, ip). Mice were then placed in an air-tight Plexiglas chamber (18.4 × 15.0 × 34.5 cm) and allowed to

acclimate for five minutes before exposure to the liquid convulsant flurothyl (2,2,2-trifluoroethyl ether; Sigma-Aldrich, St. Louis, MO).

Simultaneous Electroencephalography-Electrocardiography (EEG-ECG) Recordings

Mice (~4 weeks old) were anesthetized with avertin (0.02 $mL \cdot g^{-1}$, ip) and surgically implanted with bilateral silver wire EEG (temporal and frontal) and ECG (thoracic) electrodes (0.005-inch diameter) attached to a microminiature connector for recording in a tethered configuration as done previously (see Supporting Information for details).[4] Avertin was prepared by mixing 250 mg 2,2,2-tribromoethanol (Sigma-Aldrich, St. Louis, MO) with 155 μL 2-methyl-2-butanol (Sigma-Aldrich, St. Louis, MO) dissolved in 19.75 mL double-distilled water heated to 40°C, followed by filter-sterilization prior to use. Simultaneous video and cortical EEG-ECG activity were recorded in freely moving mice using a digital video EEG-ECG monitoring system (Data Sciences International; St. Paul, MN). Mice were monitored by video EEG-ECG over an 8-h daytime recording period for 3 consecutive days. Vehicle was administered at the beginning of each 8-h recording session, followed by RTG 4 h later. To determine drug effects, mice were used as their own controls by comparing their responses to VEH and drug. EEG-ECG recordings were analyzed offline using Ponemah software (Data Sciences International; St. Paul, MN) to manually identify spontaneous seizures and cardiac events by an observer blinded to genotype as described in detail in the Supporting Information.

Heart Rate (HR) and Heart Rate Variability (HRV) Analyses

Ponemah software (Data Sciences International; St. Paul, MN) was used to automatically generate RR interval data for the first day of EEG-ECG recording for each animal. The RR intervals were used to calculate the average HR for each minute of recording for the 2 h immediately following VEH and RTG administration. For HR and HRV analyses, values for each animal were derived by averaging three stable 3-min ECG segments when the mouse was mostly stationary, beginning at 30-min after administration of either VEH or RTG. HRV was measured in the time domain using the standard deviation of the RR intervals (SDNN) and the root mean square of successive differences (RMSSD) as done previously.[4] All cardiac parameters were measured during the light phase of the day between 10:30 a.m. and 4:30 p.m.

Quantitative PCR (qPCR) Analyses

Following isoflurane euthanasia, brains of mice (age P29–31) were quickly harvested, dissected, and collected. Total RNA was isolated using TRI-Reagent® (Zymo Research, Irvine, CA), purified using PureLink® RNA Mini Kit (Thermo Fisher, Waltham, MA), and processed for relative qPCR analyses of mRNA transcript levels using TaqMan probes (Thermo Fisher, Waltham, MA), as described in detail in the Supporting Information. Reactions were normalized to the housekeeping gene hypoxanthine phosphoribosyl-transferase 1 (*Hprt1*). Relative mRNA expression was calculated as normalized values by use of the $2^{-\Delta Ct}$ formula.

Statistical Analyses

All data are expressed as means ± standard deviation. Prism 6 for Windows (GraphPad Software Inc, La Jolla, CA) was used for statistical analyses. For comparisons involving only two groups, either paired or unpaired two-tailed Student's *t* tests were employed as appropriate. For comparisons involving three or more groups, one-way analysis of variance (ANOVA) or two-way repeated measures ANOVA were performed with either Tukey's or Sidak's multiple comparison post hoc tests.

RESULTS

RTG increases behavioral excitability in *Kcna1*$^{-/-}$ mice

Age-matched *Kcna1* and *Kcnq1* mutants (homozygotes and heterozygotes) and WT littermate controls were injected with either VEH or RTG (5, 10, or 20 mg·kg^{-1}, ip) followed by video monitoring to observe behavior. KCNQ channel openers have muscle-relaxant properties in rodents,[15,16] so we hypothesized that RTG would induce this type of behavior, especially at higher doses. At the highest dose tested (20 mg·kg^{-1}), WT and heterozygous mice of both strains exhibited varying degrees of the expected muscle-relaxant behavior, which we termed immobility, which was characterized by a motionless flattened posture with thoracic and abdominal areas resting against the floor of the cage (Fig. 1A, B). However, RTG-induced immobility was much more pronounced and long lasting in mice from the *Kcnq1* strain (C57BL/6) compared to the *Kcna1* strain (Tac:N:NIHS-BC), even at lower doses (Fig. 1A, B).

In contrast to the normal sedative-like drug response in WT animals, RTG (5–20 mg·kg^{-1}) never induced immobility in *Kcna1*$^{-/-}$ mice (Fig. 1A, B). Instead, *Kcna1*$^{-/-}$ mice exhibited unexpected behavioral responses to RTG, including full-body jerking and twitching (termed myoclonus) beginning at low doses (5 mg·kg^{-1}), which evolved to include hopping and vocalization at higher doses (10–20 mg·kg^{-1}; Fig. 1C, E, F). Unlike *Kcna1*$^{-/-}$ animals, *Kcnq1*$^{A340E/A340E}$ mice rarely exhibited myoclonic behavior in response to RTG (Fig. 1D), and hopping and vocalizing was never observed at any dose (data not shown).

RTG increases seizure threshold in *Kcnq1* mice, but has no effect in *Kcna1* mice

To determine whether RTG decreases seizure susceptibility in *Kcna1* and *Kcnq1* mice, animals were injected with either VEH or RTG (10 mg·kg^{-1}) followed by exposure to the convulsant flurothyl to induce seizure activity, which manifests as initial myoclonic jerks that progress to generalized tonic-clonic seizure. In this and subsequent experiments, RTG was administered at a dose of 10 mg·kg^{-1} (unless otherwise stated) to avoid excessive confounding effects of behavioral toxicity such as the myoclonus, hopping, and vocalizing that occurs at higher doses in *Kcna1*$^{-/-}$ mice. Upon flurothyl exposure, VEH-injected *Kcna1*$^{-/-}$ mice exhibited latencies to myoclonic jerks and tonic-clonic seizure that were reduced by about 50% compared to their Tac:N:NIHS-BC WT littermates (Fig. 2A, C), similar to previous reports.[4] However, VEH-injected *Kcnq1*$^{A340E/A340E}$ mice showed latencies that were not significantly different from their C57BL/6 WT counterparts (Fig. 2B, D), indicating the *Kcnq1*^{A340E} mutation does not affect seizure threshold despite the occurrence of infrequent spontaneous seizures in this model.[9] When injected with RTG,

Kcna1$^{-/-}$ mice and Tac:N:NIHS-BC WT littermates exhibited latencies that were unchanged compared to VEH, suggesting that a 10 mg·kg^{-1} dose of RTG has no effect on seizure susceptibility in this mouse strain (Fig. 2A, C). Even when administered higher RTG doses of 20 mg·kg^{-1}, Tac:N:NIHS-BC *Kcna1*$^{+/+}$ mice still showed no changes in seizures latencies compared to VEH-treated animals (Fig. S1). In contrast, RTG (10 mg·kg^{-1}) significantly increased the latency to tonic-clonic seizure by >50% in both C57BL/6 *Kcnq1*$^{+/+}$ (t = 2.68, $P \leq 0.05$, unpaired t-test) and *Kcnq1*$^{A340E/A340E}$ mice (t = 3.179, $P \leq 0.01$, unpaired t-test; Fig. 2D), indicating a therapeutic anti-seizure response. However, RTG (10 mg·kg^{-1}) had no significant effect on the latency to myoclonic jerks in C57BL/6 *Kcnq1*$^{+/+}$ animals, and instead slightly shortened the latency to myoclonic jerks in *Kcnq1*$^{A340E/A340E}$ mice (t = 2.194, $P \leq 0.05$, unpaired t-test; Fig. 2B).

RTG decreases spontaneous seizure frequency in *Kcna1*$^{-/-}$ mice

To determine whether short-term administration of RTG decreases the frequency and/or duration of spontaneous seizures in *Kcna1*$^{-/-}$ and *Kcnq1*$^{A340E/A340E}$ mice, video EEG-ECG recordings were performed over three consecutive days. In *Kcna1*$^{-/-}$ mice, RTG (10 mg·kg^{-1}) significantly reduced the number of spontaneous seizures per 4-h recording session from 1.2 ± 1.0 seizures with VEH to 0.5 ± 0.7 seizures following treatment (t = 2.6, $P < 0.05$, paired t-test), without modifying seizure durations (Fig. 2E–G). Although spontaneous seizures have been reported in *Kcnq1*$^{A340E/A340E}$ mice, they are relatively rare, occurring only about once every 1–2 d.[9] Consistent with these previous studies, no EEG seizures were observed during the 3-day recording period in *Kcnq1*$^{A340E/A340E}$ mice; therefore, modification of spontaneous seizure phenotypes could not be readily assessed in this strain. In addition, interictal spikes did not occur at a high enough frequency in our cohort to allow utility as a marker of spontaneous epileptiform activity.

RTG increases HRV in *Kcnq1*$^{A340E/A340E}$ mice

To determine whether KCNQ activation normalizes heart function in *Kcna1* and *Kcnq1* mice, various cardiac measures were analyzed using video EEG-ECG recordings: HR, HRV, and skipped heart beats (i.e., AV conduction blocks). At baseline with VEH, mice exhibited strain-dependent differences in cardiac function. Mice from the *Kcna1* strain exhibited faster HR with less HRV than mice from the *Kcnq1* strain (Fig. 3A–D and Table 1). In response to 10 mg·kg^{-1} RTG injection, WT and mutant mice of both strains exhibited trends towards a transient decrease in HR. However, the degree and duration of this HR reduction varied by strain and genotype (Fig. 3A, B, E, F). Whereas Tac:N:NIHS-BC WT mice from the *Kcna1* strain exhibited relatively mild (~15%) and short-lived (~15 min) HR reductions (Fig. 3E), C57BL/6 WT mice from the *Kcnq1* strain exhibited more profound (~40%) and long-lasting (~30 min) HR reductions (Fig. 3F). RTG responses differed in mutant mice of both strains: *Kcna1*$^{-/-}$ mice exhibited ~5% reductions in HR that lasted about 5 min (Fig. 3E) compared to *Kcnq1*$^{A340E/A340E}$ mice, which showed HR reductions of ~45% that lasted up to ~90 min (Fig. 3F).

To assess RTG-induced modification of autonomic control of the heart, HRV was analyzed at 30-min post-injection using the following time domain measures: the standard deviation of the beat-to-beat intervals (SDNN), an index of the total autonomic variability; and the

root mean square of the successive beat-to-beat differences (RMSSD), an index of parasympathetic tone. Following VEH injection, mice from the *Kcnq1* strain exhibited 2- to 4-fold higher SDNN and RMSSD values relative to *Kcna1* strain mice, suggesting higher parasympathetic tone at baseline (Table 1). Mutant mice from both strains exhibited HRV values that were approximately similar to their WT counterparts (Table 1). However, baseline RMSSD was nearly twice as high in $Kcna1^{-/-}$ mice compared to their Tac:N:NIHS-BC WT controls, consistent with a previous study showing elevated HRV measures of parasympathetic tone in these animals.[4] Upon 10 mg·kg^{-1} RTG administration, SDNN ($t = 3.488$, $P = 0.025$, paired t-test) and RMSSD ($t = 3.373$, $P = 0.028$, paired t-test) significantly increased by 4-fold and 8-fold, respectively, in $Kcnq1^{A340E/A340E}$ mice compared to VEH (Table 1), suggesting dysregulation of autonomic control of the heart in response to the drug. In contrast, $Kcna1^{-/-}$ mice showed no change in HRV following RTG injection (Table 1).

Next, we examined whether acute RTG administration affects the frequency of interictal skipped heart beats, which we use as a biomarker of abnormal cardiac function indicative of potential autonomic dysregulation. Previous studies have shown that $Kcna1^{-/-}$ mice exhibit an increased incidence of skipped heart beats due to vagally-driven AV conduction blocks.[3] Similarly, $Kcnq1^{A340E/A340E}$ mice have also been reported to have increased susceptibility to AV blocks in ECG recordings.[9] Acute RTG (10 mg·kg^{-1}) treatment did not significantly alter the frequency of skipped beats in $Kcna1^{-/-}$ and Tac:N:NIHS-BC $Kcna1^{+/+}$ mice (Fig. 4A, B). However, this dose of RTG induced a dramatic increase in the rate of skipped beats in mice of the *Kcnq1* strain, especially $Kcnq1^{A340E/A340E}$ mice which exhibited an increase of ~200–800 per h (equivalent to a ≥ 20-fold increase; $t = 2.891$, $P = 0.045$, paired t-test), suggesting drastic augmentation of parasympathetic tone (Fig. 4C, D).

Kcnq2 mRNA levels are increased in $Kcna1^{-/-}$ mouse brain

To test whether the observed differential drug effects were associated with strain- or genotype-based differences in the expression of the main molecular targets of RTG, qPCR was performed to measure the mRNA levels of *Kcnq2* and *Kcnq3* in the hippocampi and cortices of *Kcna1* and *Kcnq1* mice (n=5–6/genotype per brain region). Regional qPCR revealed that the levels of *Kcnq2* mRNA, but not *Kcnq3*, were significantly higher in both the hippocampus ($F = 4.815$, $P = 0.012$, one-way ANOVA) and cortex ($F = 4.275$, $P = 0.017$, one-way ANOVA) of $Kcna1^{-/-}$ mice compared to *Kcnq1* mice (Fig. 5).

DISCUSSION

In this study, the efficacy of the KCNQ channel opener RTG and its potential for severe adverse behavioral or cardiac effects was profoundly affected by genetic background, suggesting KCNQ activation may not be an optimal strategy to combat SUDEP. RTG treatment has been used as an effective adjunctive therapy for the management of drug-resistant partial-onset seizures, but its production was recently discontinued due to limited usage and continued decline in patient initiation. However, RTG is simply one drug that targets KCNQ channels and many other KCNQ activators are currently under development for controlling disorders of neuronal hyperexcitability.[17,18] Another factor in the demise of RTG as a clinical pharmaceutical was likely the occurrence of unwanted side effects in a

subset of patients, as is seen for most anti-seizure drugs. The most common adverse events associated with RTG in patients include central nervous system effects, increased risk of urinary retention, and altered skin and retinal pigmentation.[19,20] In rodents, the main side-effect of RTG is a transient reduction in locomotor activity described as flat body posture and some muscle relaxation[16], as was observed in WT and *Kcnq1*$^{A340E/A340E}$ mice in this study. We also observed an additional and previously unreported response to KCNQ activation that was specific to mice with the *Kcna1*$^{-/-}$ mutation, namely behavioral hyperexcitability that was characterized by myoclonus, hopping, and vocalization. These behaviors may be the rodent correlate of the tremors that occur in 9% of patients at higher doses of RTG (900 mg·d^{-1}).[19] In one case report, a patient overdosed on the KCNQ opener flupirtine, leading to intermittent myoclonus and active and resting tremor of the extremities reminiscent of the behavior seen in *Kcna1*$^{-/-}$ mice.[21] Although the EEGs in *Kcna1*$^{-/-}$ mice showed some abnormal waveforms following RTG administration, these were more consistent with movement artifacts due to hopping rather than increased cortical excitability (Fig. S2). The hyperexcitable behavioral response to RTG in *Kcna1*$^{-/-}$ mice provides one possible pharmacogenetic explanation for the adverse behavioral effects of RTG in some patients and suggests that future KCNQ activator drugs should be evaluated for potential behavioral hyperexcitability side effects.

KCNQ activation also produced novel genotype- and strain-dependent effects on HR suggesting KCNQ2-5 channels play a role in autonomic control of chronotropy, which varies based on genetic background. Whereas RTG had no significant cardiac effects in *Kcna1* mice, *Kcnq1* mice exhibited transient HR decreases that were most pronounced in *Kcnq1*$^{A340E/A340E}$ mutants, which exhibited a prolonged fall in HR lasting ≥60 min coupled with 4- to 8-fold increases in HRV. Cardiovascular roles for KCNQ2-5 channels have been described in visceral sensory neurons and the vasculature, but less is known about their potential roles in regulating cardiac function.[22] Heteromeric KCNQ2/3 channels are expressed in superior cervical ganglion (SCG) neurons where they regulate release of noradrenaline (NA) onto the heart.[23] Unlike KCNQ1, KCNQ2-5 channels are not expressed at significant levels in the heart[24]; however, their potential compensatory expression in the absence of functional KCNQ1 cannot be ruled out. Previously, in a co-culture preparation of rat SCG sympathetic neurons and mouse cardiomyocytes, RTG abolished the increase in cardiomyocyte constriction rate caused by nicotine-induced stimulation of SCG neurons; however, RTG application to cardiomyocytes cultured alone had no effect.[25] These results suggest that RTG induces up-regulation of KCNQ2/3-mediated M-current preventing NA release from SCG cells, which could lead to a bradycardic effect due to sympathetic inhibition consistent with our observations.

Although RTG has been shown to have broad spectrum anti-seizure activity, our results show that its efficacy depends on the mouse strain and testing paradigm in which it is evaluated. RTG normally exhibits potent anti-seizure effects, especially when administered prior to seizure induction, in a variety of *in vitro* and *in vivo* rodent models of epilepsy.[16,26] However, to our knowledge, this is the first study to examine the anti-seizure efficacy of RTG in the flurothyl seizure model in mice. We found that while RTG could effectively increase flurothyl-induced seizure latency similar to other chemically-induced seizure models, this anti-seizure effect was limited to mice from the *Kcnq1* strain, suggesting an

influence of genetic background of the mouse strain. Interestingly, a 10 mg·kg^{-1} dose of RTG still significantly reduced the occurrence of spontaneous EEG seizures in *Kcna1*$^{-/-}$ mice despite having no effect on flurothyl seizure latency. A similar dissociation has been observed in flurothyl-kindled DBA/2J mice. Following exposure to eight flurothyl-induced seizures, DBA/2J mice exhibit spontaneous seizures without a significant change in flurothyl seizure threshold from pre-epileptic levels.[27] The generalized running bouncing seizures that are evoked by prolonged flurothyl exposure involve activation of brainstem centers, whereas the spontaneous seizures in *Kcna1*$^{-/-}$ mice show evidence of limbic origin.[27–29] Therefore, RTG could have contrasting effects on flurothyl-induced and spontaneous seizures due to differences in the neuronal networks underlying onset or propagation of the two types of seizures.

Quantitative PCR analyses revealed high *Kcnq2* transcript levels in the hippocampus and cortex of *Kcna1*$^{-/-}$ mice that could be partly due to a neuronal homeostatic control response that is the product of the expression and functional complementarity between Kv1.1 and KCNQ2 channels. Kv1.1 and KCNQ2 channels share widespread expression throughout many common brain areas including cortex, hippocampus, thalamus, and cerebellum.[30,31] At the compartmental level, Kv1.1 and KCNQ2 channels show strong expression in axons, including at the AIS.[32,33] Prior studies of axonal and nerve excitability in animal models have uncovered a potential functional synergy between Kv1.1 and KCNQ2 channels. Peripheral nerves from *Kcna1*$^{-/-}$ mice exhibit abnormal nerve activity that is amplified by non-specific KCNQ blockade with TEA and ameliorated by KCNQ activation with flupirtine.[1,34] More recently, functional cooperativity between Kv1.1 and KCNQ2 channels was demonstrated in the AISs of the avian cochlear nucleus, which, following deprivation of afferent inputs by removing the cochlea, switch their dominant Kv channel composition from Kv1.1 to KCNQ2 to maintain homeostasis of auditory circuits.[38] The ability of Kv1.1 and KCNQ2 channels to partially compensate for one another is facilitated by their similar electrophysiological properties, including similar low-voltage activation characteristics and the ability to directly affect action potential threshold according to their different activation kinetics.[35,36] In patients, gene mutations in either *Kcna1* or *Kcnq2* lead to similar functional consequences including epilepsy or peripheral nerve hyperexcitability.[8,37] In addition to potential homeostatic compensation, *Kcnq2* transcripts may also be elevated in *Kcna1*$^{-/-}$ mice partly due to genetic background of the mouse strain since the *Kcna1* strain exhibits slightly elevated basal levels of *Kcnq2/3* expression. Epilepsy in *Kcna1*$^{-/-}$ mice could also play a role, leading to seizure-induced increases in the transcription of *Kcnq2*, which could alter the effects of RTG in accordance with the target hypothesis of pharmacoresistance, which states that epilepsy-related changes in the properties of drug targets themselves may result in altered drug sensitivity.[38] Since expression changes at the mRNA level do not always correlate with alterations in protein abundance, future studies will be needed to address whether the changes in *Kcnq2* mRNA expression correlate with changes in KCNQ2 protein.

In summary, from a clinical perspective, this study demonstrates the principle that treatment strategies in channelopathy may have unexpected outcomes. Our findings show that activation of KCNQ channels does not prevent neurocardiac dysfunction, at least in the mouse models tested. Furthermore, KCNQ activation led to unexpected adverse strain- and

genotype-dependent effects in mice that suggest this class of drugs may be contraindicated in patients with *Kcna1* or *Kcnq1* mutations. The differential effects of KCNQ activation may be partly due to underlying differences in *Kcnq2/3* channel expression, which could alter drug target levels. Our findings emphasize the importance of an individual's genomic profile in determining drug responsiveness, toxicity, and sensitivity, providing a clear example of pharmacogenetic interactions in epilepsy which could contribute to pharmacoresistance. To achieve effective therapeutic rebalancing of channel defects will require improved understanding of channel interactions at the circuit and tissue levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

4-AP	4-aminopyridine
AIS	axon initial segment
ANOVA	analysis of variance
ASD	anti-seizure drug
AV	atrioventricular
BG/Th	basal ganglia/thalamus
BS	brain stem
Cbl	cerebellum
Ctx	cortex
ECG	electrocardiography
EEG	electroencephalography
Hipp	hippocampus
HR	heart rate
HRV	heart rate variability
ip	intraperitoneal
iv	intravenous

KO	knockout
LQT	long QT syndrome
NA	noradrenaline
qPCR	quantitative polymerase chain reaction
RMSSD	root mean square of successive differences
RTG	retigabine
SCG	superior cervical ganglion
SDNN	standard deviation of the RR intervals
SUDEP	sudden unexpected death in epilepsy
TEA	tetraethylammonium
VEH	vehicle
WT	wildtype

References

1. Glasscock E, Qian J, Kole MJ, et al. Transcompartmental reversal of single fibre hyperexcitability in juxtaparanodal Kv1.1-deficient vagus nerve axons by activation of nodal KCNQ channels. J Physiol. 2012; 590:3913–26. [PubMed: 22641786]
2. Qi Y, Wang J, Bomben VC, et al. Hyper-SUMOylation of the Kv7 Potassium Channel Diminishes the M-Current Leading to Seizures and Sudden Death. Neuron. 2014; 83:1159–71. [PubMed: 25189211]
3. Glasscock E, Yoo JW, Chen TT, et al. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. J Neurosci. 2010; 30:5167–75. [PubMed: 20392939]
4. Mishra V, Karumuri BK, Gautier NM, et al. Scn2a deletion improves survival and brain-heart dynamics in the Kcna1-null mouse model of sudden unexpected death in epilepsy (SUDEP). Hum Mol Genet. 2017; 26:2091–103. [PubMed: 28334922]
5. Moore BM, Jerry Jou C, Tatalovic M, et al. The Kv1.1 null mouse, a model of sudden unexpected death in epilepsy (SUDEP). Epilepsia. 2014; 55:1808–16. [PubMed: 25377007]
6. Smart SL, Lopantsev V, Zhang CL, et al. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. Neuron. 1998; 20:809–19. [PubMed: 9581771]
7. Klassen TL, Bomben VC, Patel A, et al. High-resolution molecular genomic autopsy reveals complex sudden unexpected death in epilepsy risk profile. Epilepsia. 2014; 55:e6–12. [PubMed: 24372310]
8. Zuberi SM, Eunson LH, Spauschus A, et al. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. Brain. 1999; 122:817–25. [PubMed: 10355668]
9. Goldman AM, Glasscock E, Yoo J, et al. Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. Sci Transl Med. 2009; 1:2ra6.
10. Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet. 1996; 12:17–23. [PubMed: 8528244]
11. Anderson JH, Bos JM, Cascino GD, et al. Prevalence and spectrum of electroencephalogram-identified epileptiform activity among patients with long QT syndrome. Heart Rhythm. 2014; 11:53–7. [PubMed: 24103226]

12. Auerbach DS, McNitt S, Gross RA, et al. Genetic biomarkers for the risk of seizures in long QT syndrome. Neurology. 2016; 87:1660–8. [PubMed: 27466471]
13. Gunthorpe MJ, Large CH, Sankar R. The mechanism of action of retigabine (ezogabine), a first-in-class K(+) channel opener for the treatment of epilepsy. Epilepsia. 2012; 53:412–24. [PubMed: 22220513]
14. Casimiro MC, Knollmann BC, Yamoah EN, et al. Targeted point mutagenesis of mouse Kcnq1: phenotypic analysis of mice with point mutations that cause Romano-Ward syndrome in humans. Genomics. 2004; 84:555–64. [PubMed: 15498462]
15. Friedel HA, Fitton A. Flupirtine. A review of its pharmacological properties, and therapeutic efficacy in pain states. Drugs. 1993; 45:548–69. [PubMed: 7684675]
16. Rostock A, Tober C, Rundfeldt C, et al. D-23129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. Epilepsy Res. 1996; 23:211–23. [PubMed: 8739124]
17. Kalappa BI, Soh H, Duignan KM, et al. Potent KCNQ2/3-specific channel activator suppresses in vivo epileptic activity and prevents the development of tinnitus. J Neurosci. 2015; 35:8829–42. [PubMed: 26063916]
18. Kumar M, Reed N, Liu R, et al. Synthesis and Evaluation of Potent KCNQ2/3-Specific Channel Activators. Mol Pharmacol. 2016; 89:667–77. [PubMed: 27005699]
19. Brodie MJ, Lerche H, Gil-Nagel A, et al. Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. Neurology. 2010; 75:1817–24. [PubMed: 20944074]
20. French JA, Abou-Khalil BW, Leroy RF, et al. Randomized, double-blind, placebo-controlled trial of ezogabine (retigabine) in partial epilepsy. Neurology. 2011; 76:1555–63. [PubMed: 21451152]
21. Hoffmann O, Gommert LR, Egert M. Paradoxical cerebral cortical hyperexcitability following flupirtine overdose. J Toxicol Clin Toxicol. 2004; 42:913–6. [PubMed: 15533031]
22. Mackie AR, Brueggemann LI, Henderson KK, et al. Vascular KCNQ potassium channels as novel targets for the control of mesenteric artery constriction by vasopressin, based on studies in single cells, pressurized arteries, and in vivo measurements of mesenteric vascular resistance. J Pharmacol Exp Ther. 2008; 325:475–83. [PubMed: 18272810]
23. Hernandez CC, Zaika O, Tolstykh GP, et al. Regulation of neural KCNQ channels: signalling pathways, structural motifs and functional implications. J Physiol. 2008; 586:1811–21. [PubMed: 18238808]
24. Brown DA, Passmore GM. Neural KCNQ (Kv7) channels. Br J Pharmacol. 2009; 156:1185–95. [PubMed: 19298256]
25. Zaika O, Zhang J, Shapiro MS. Functional role of M-type (KCNQ) K+ channels in adrenergic control of cardiomyocyte contraction rate by sympathetic neurons. J Physiol. 2011; 589:2559–68. [PubMed: 21486761]
26. Kay HY, Greene DL, Kang S, et al. M-current preservation contributes to anticonvulsant effects of valproic acid. J Clin Invest. 2015; 125:3904–14. [PubMed: 26348896]
27. Kadiyala SB, Ferland RJ. Dissociation of spontaneous seizures and brainstem seizure thresholds in mice exposed to eight flurothyl-induced generalized seizures. Epilepsia Open. 2017; 2:48–58. [PubMed: 28825051]
28. Gautier NM, Glasscock E. Spontaneous seizures in Kcna1-null mice lacking voltage-gated Kv1.1 channels activate Fos expression in select limbic circuits. J Neurochem. 2015; 135:157–64. [PubMed: 26112121]
29. Samoriski GM, Applegate CD. Repeated generalized seizures induce time-dependent changes in the behavioral seizure response independent of continued seizure induction. J Neurosci. 1997; 17:5581–90. [PubMed: 9204939]
30. Cooper EC, Harrington E, Jan YN, et al. M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. J Neurosci. 2001; 21:9529–40. [PubMed: 11739564]
31. Wang H, Kunkel DD, Schwartzkroin PA, et al. Localization of Kv1.1 and Kv1.2, two K channel proteins, to synaptic terminals, somata, and dendrites in the mouse brain. J Neurosci. 1994; 14:4588–99. [PubMed: 8046438]

32. Goldberg EM, Clark BD, Zagha E, et al. K+ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. Neuron. 2008; 58:387–400. [PubMed: 18466749]

33. Pan Z, Kao T, Horvath Z, et al. A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. J Neurosci. 2006; 26:2599–613. [PubMed: 16525039]

34. Zhou L, Messing A, Chiu SY. Determinants of excitability at transition zones in Kv1.1-deficient myelinated nerves. J Neurosci. 1999; 19:5768–81. [PubMed: 10407018]

35. Kuba H, Yamada R, Ishiguro G, et al. Redistribution of Kv1 and Kv7 enhances neuronal excitability during structural axon initial segment plasticity. Nat Commun. 2015; 6:8815. [PubMed: 26581625]

36. Johnston J, Forsythe ID, Kopp-Scheinpflug C. Going native: voltage-gated potassium channels controlling neuronal excitability. J Physiol. 2010; 588:3187–200. [PubMed: 20519310]

37. Dedek K, Kunath B, Kananura C, et al. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K+ channel. Proc Natl Acad Sci U S A. 2001; 98:12272–7. [PubMed: 11572947]

38. Remy S, Beck H. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. Brain. 2006; 129:18–35. [PubMed: 16317026]

KEY POINTS

- Effects of KCNQ channel activation in *Kcna1* and *Kcnq1* mutant mice were evaluated by behavior, electrophysiology, and gene expression
- KCNQ channel activation caused adverse behavioral and cardiac responses and variable anti-seizure effects depending on genotype
- Levels of *Kcnq2* mRNA were increased in *Kcna1*$^{-/-}$ mice, possibly contributing to their abnormal drug responses
- Pharmacogenetic interactions may limit KCNQ modulation as a strategy to prevent deleterious neurocardiac dysfunction in epilepsy
- Treatment strategies in channelopathy may have unexpected outcomes highlighting the complexity of rebalancing channel defects

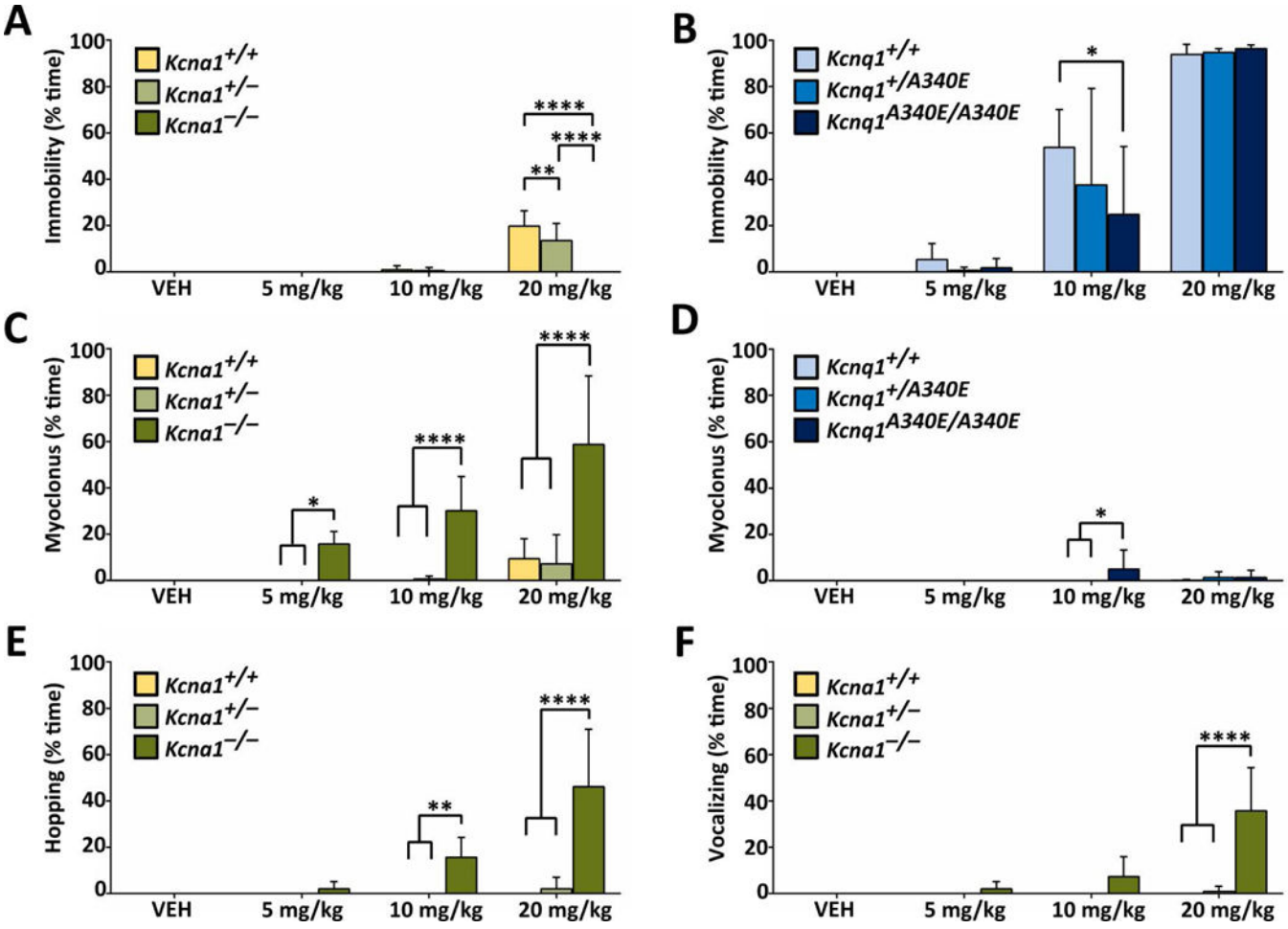

Figure 1.

RTG increases behavioral excitability in *Kcna1*$^{-/-}$ mice. (**A, B**) At 20 mg·kg^{-1}, RTG caused all mice to become immobile and lie with their abdomen and thorax against the floor of the cage, except for *Kcna1*$^{-/-}$ mice which continued to be active. (**C, D**) RTG induced myoclonus in *Kcna1*$^{-/-}$ mice at 5, 10, and 20 mg·kg^{-1} (ip). In the other genotypes, RTG induced myoclonus only sporadically at the highest doses. RTG induced hopping (**E**) and vocalizing (**F**) in *Kcna1*$^{-/-}$ mice. These two behavioral effects were not seen in the *Kcnq1* mice at any dose. Two-way repeated measures ANOVA showed the following statistical results. For (**A**), effect of treatment: $P < 0.0001$, $F_{3,45} = 61.87$; genotype: $P < 0.0001$, $F_{2,15} = 19.85$; interaction between treatment and genotype: $P < 0.0001$, $F_{6,45} = 17.10$). For (**B**), effect of treatment: $P < 0.0001$, $F_{3,30} = 101.8$; genotype: $P = 0.36$, $F_{2,10} = 1.118$; interaction between treatment and genotype: $P = 0.47$, $F_{6,30} = 0.9533$). For (**C**), effect of treatment: $P < 0.0001$, $F_{3,45} = 19.82$; genotype: $P < 0.0001$, $F_{2,15} = 34.09$; interaction between treatment and genotype: $P < 0.0001$, $F_{6,45} = 8.664$). For (**D**), effect of treatment: $P < 0.0001$, $F_{3,30} = 101.8$; genotype: $P = 0.36$, $F_{2,10} = 1.118$; interaction between treatment and genotype: $P = 0.47$, $F_{6,30} = 0.9533$). For (**E**), effect of treatment: $P < 0.0001$, $F_{3,45} = 17.81$; genotype: $P < 0.0001$, $F_{2,15} = 26.69$; interaction between treatment and genotype: $P < 0.0001$, $F_{6,45} = 15.59$). For (**F**), effect of treatment: $P < 0.0001$, $F_{3,45} = 19.86$; genotype: $P < 0.0001$, $F_{2,15} =$

17.29; interaction between treatment and genotype: $P < 0.0001$, $F_{6,45} = 18.33$). *, $P \leq 0.05$; **, $P \leq 0.01$; ****, $P \leq 0.0001$ (two-way repeated measures ANOVA, Tukey's post hoc; n=4–6 per genotype).

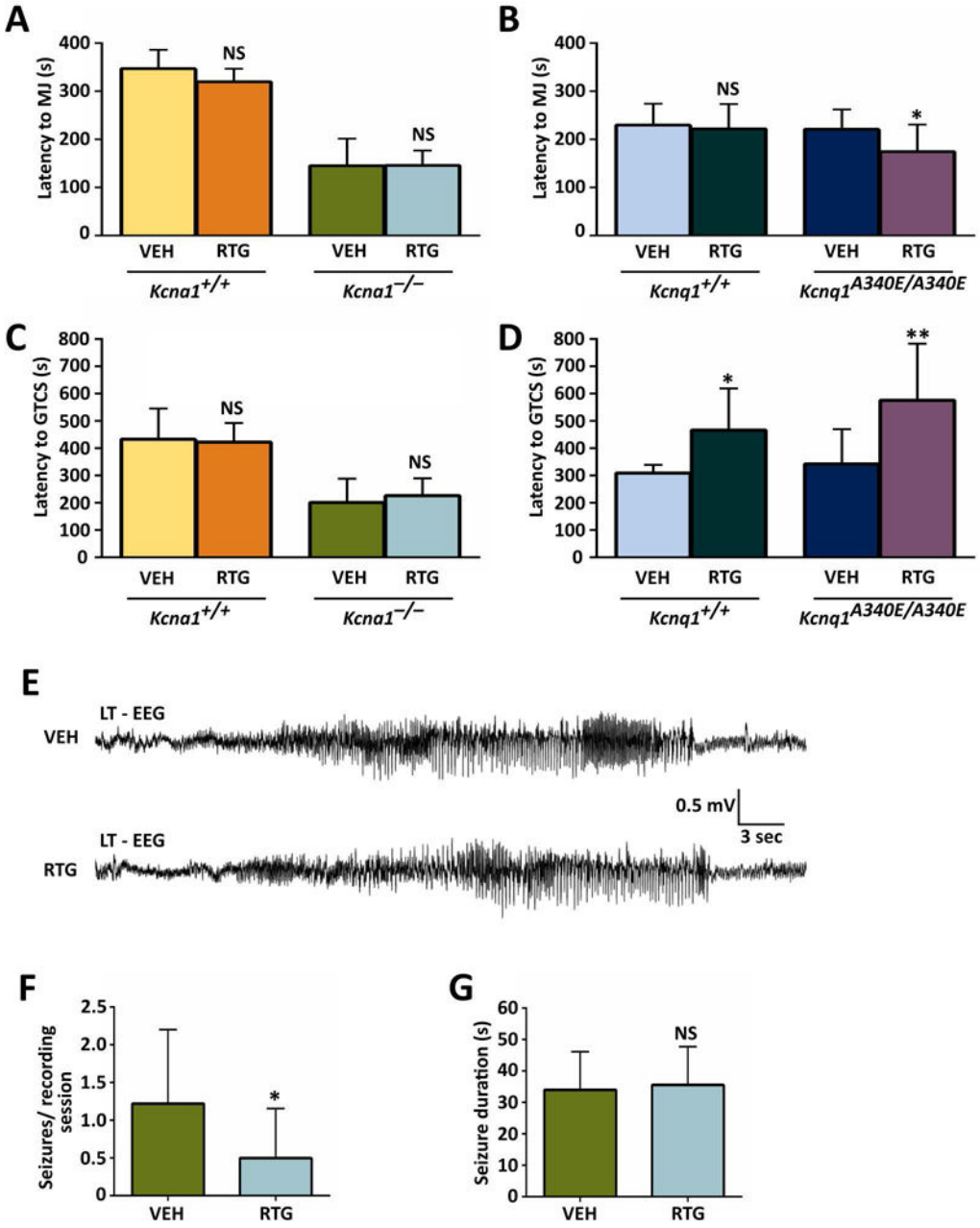

Figure 2.

KCNQ activation increases flurothyl-induced seizure latencies in *Kcnq1* mice and decreases spontaneous seizure frequency in *Kcna1*$^{-/-}$ mice. (**A**) Administration of RTG (10 mg·kg^{-1}, ip) had no significant effect on latency to flurothyl-induced myoclonic jerks (MJ) in Tac:N:NIHS-BC *Kcna1*$^{+/+}$ and *Kcna1*$^{-/-}$ mice. However, RTG decreased latency to myoclonic jerk in *Kcnq1*$^{A340E/A340E}$ mutant mice (**B**). (**C**) Administration of RTG (10 mg·kg^{-1}, ip) had no significant effect on latency to flurothyl-induced generalized tonic-clonic seizures (GTCS) in Tac:N:NIHS-BC *Kcna1*$^{+/+}$ and *Kcna1*$^{-/-}$ mice. However, RTG significantly increased the latency to GTCS in *Kcnq1* strain (C57BL/6) mice (**D**). (**E**) EEG traces showing representative spontaneous seizure activity in *Kcna1*$^{-/-}$ mice after receiving

either VEH or RTG (10 mg·kg^{-1}). (**F**) Analysis of spontaneous seizure frequency in *Kcna1*$^{-/-}$ mice (n = 6) showed a significant decrease after treatment with 10 mg·kg^{-1} RTG. (**G**) Average spontaneous seizure durations were similar in *Kcna1*$^{-/-}$ mice that exhibited seizures after VEH and 10 mg·kg^{-1} RTG administration (n = 3). For (**A**-**D**): *, $P \leq 0.05$; **, $P \leq 0.01$ (two-tailed unpaired Student's *t*-test; n = 6–11 per treatment). For (**F**) and (**G**): *, $P \leq 0.05$ (two-tailed paired Student's *t*-test). Abbreviations: LT, left temporal; VEH, vehicle; RTG, retigabine; NS, not significant compared to VEH control.

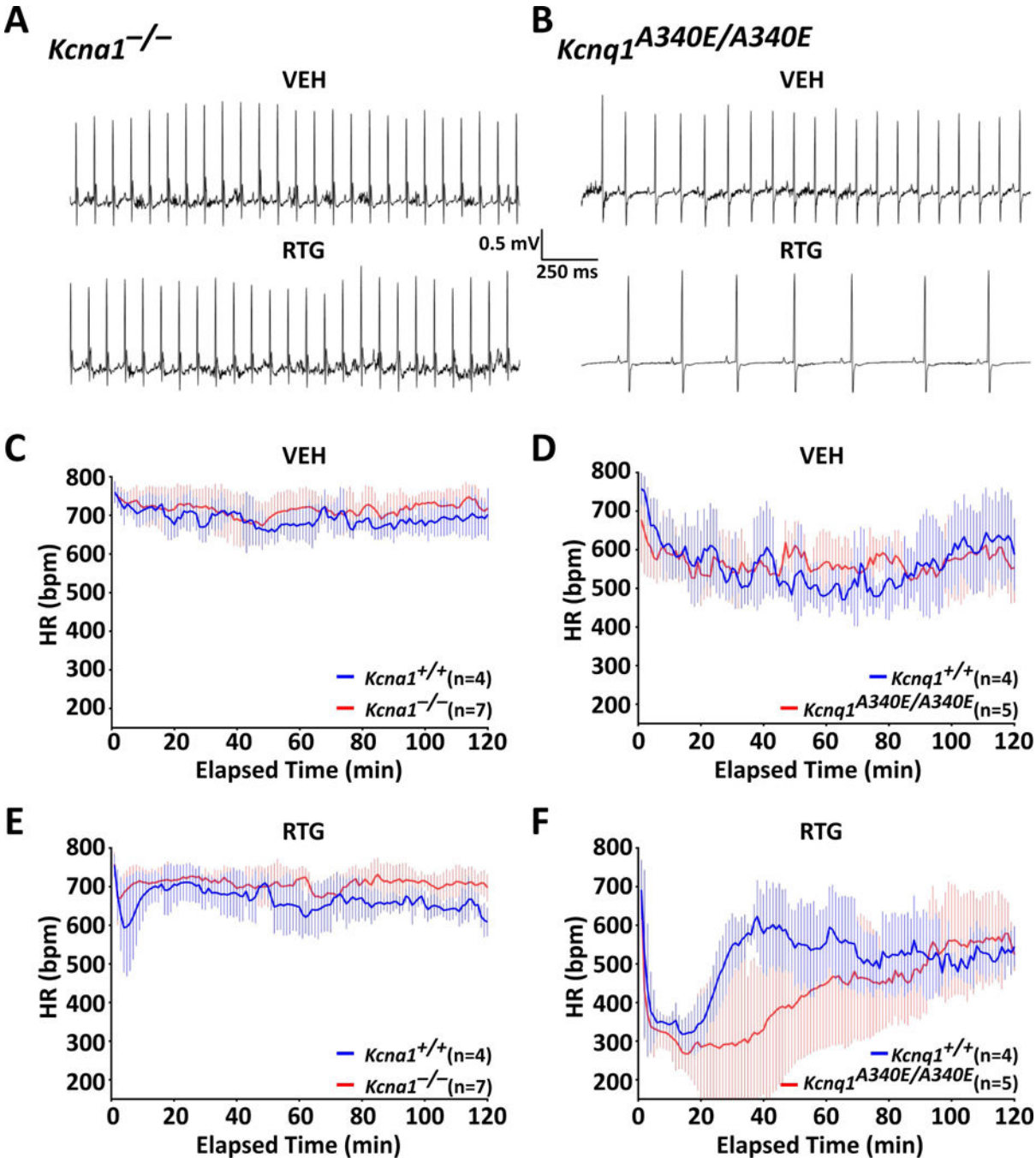

Figure 3.

KCNQ activation transiently decreases heart rate in *Kcna1* and *Kcnq1* mice. Representative 2-s ECG traces taken ~30 min after injection of either VEH or RTG showing HR effects in $Kcna1^{-/-}$ (**A**) and $Kcnq1^{A340E/A340E}$ mice (**B**). Plots showing HR responses for the 2 h immediately following VEH (**C, D**) and 10 mg·kg^{-1} RTG injections (**E, F**) in *Kcna1* and *Kcnq1* mice. Baseline HRs were not significantly different between WTs and mutants of either strain. Following RTG, HR transiently decreased from baseline rates by approximately 50–100 bpm and 250–300 bpm in *Kcna1* (Tac:N:NIHS-BC) and *Kcnq1* (C57BL/6) strain

mice, respectively. At 38 min after RTG administration, HR was significantly different ($P \leq 0.05$) between C57BL/6 *Kcnq1*$^{+/+}$ and *Kcnq1*$^{A340E/A340E}$ mice (effect of time: $P < 0.0001$, $F_{119,833} = 6.664$; genotype: $P = 0.21$, $F_{1,7} = 1.935$; interaction between time and genotype: $P < 0.0001$, $F_{119,883} = 2.634$; two-way repeated measures ANOVA, Sidak's post hoc). HR did not differ significantly between RTG-treated Tac:N:NIHS-BC *Kcna1*$^{+/+}$ and *Kcna1*$^{-/-}$ mice.

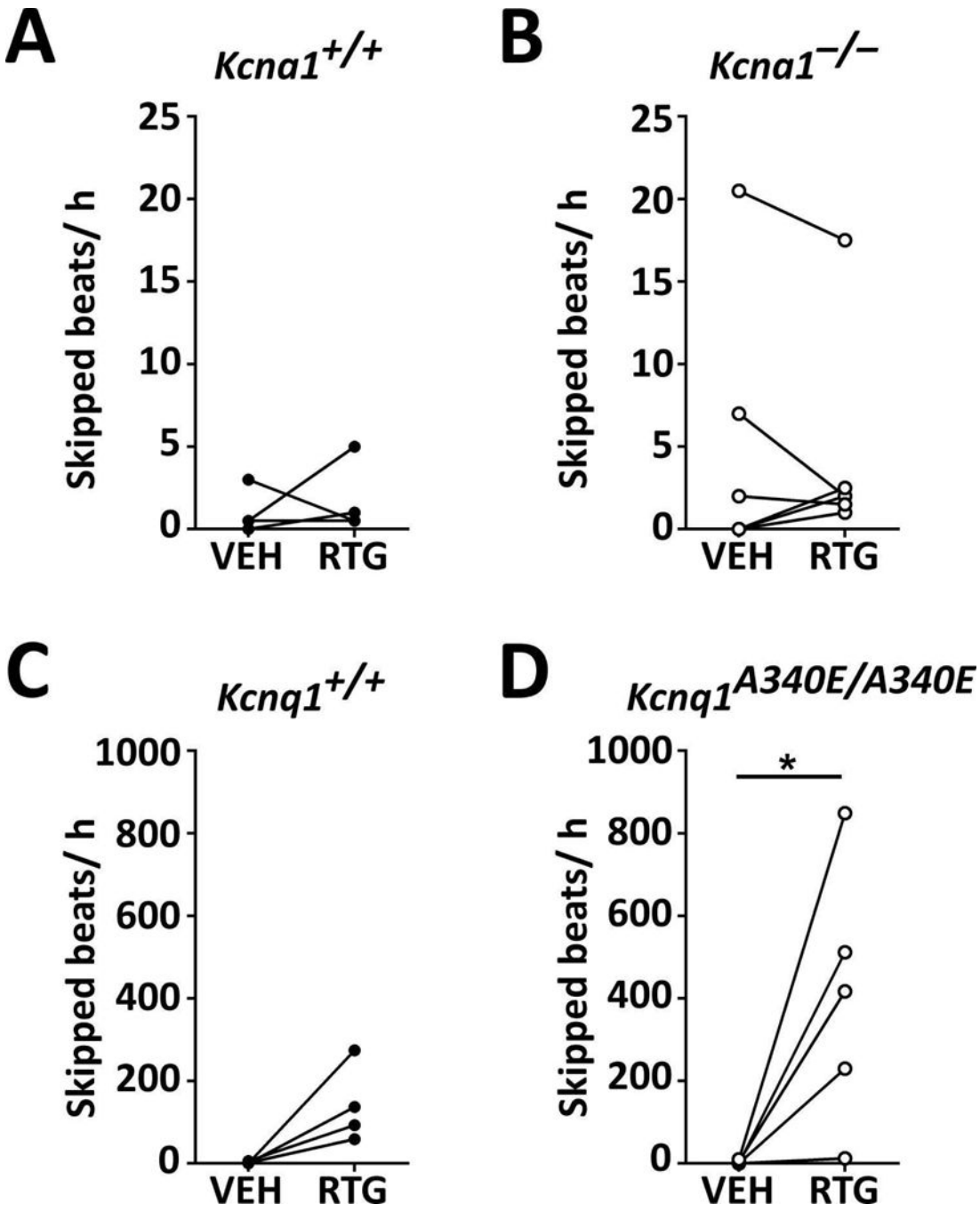

Figure 4.

KCNQ activation increases the frequency of skipped heart beats in *Kcna1*$^{A340E/A340E}$ mice. RTG treatment did not significantly alter the frequency of skipped beats (i.e., AV conduction blocks) in Tac:N:NIHS-BC *Kcna1*$^{+/+}$ (**A**) and *Kcna1*$^{-/-}$ (**B**) mice. However, RTG increased the rate of skipped beats in *Kcnq1* mice (**C**; $P = 0.06$, two-tailed paired Student's *t*-test), especially in *Kcnq1*$^{A340E/A340E}$ animals which exhibited a ≥20-fold increase (**D**). *, $P < 0.05$ (two-tailed paired Student's *t*-test.)

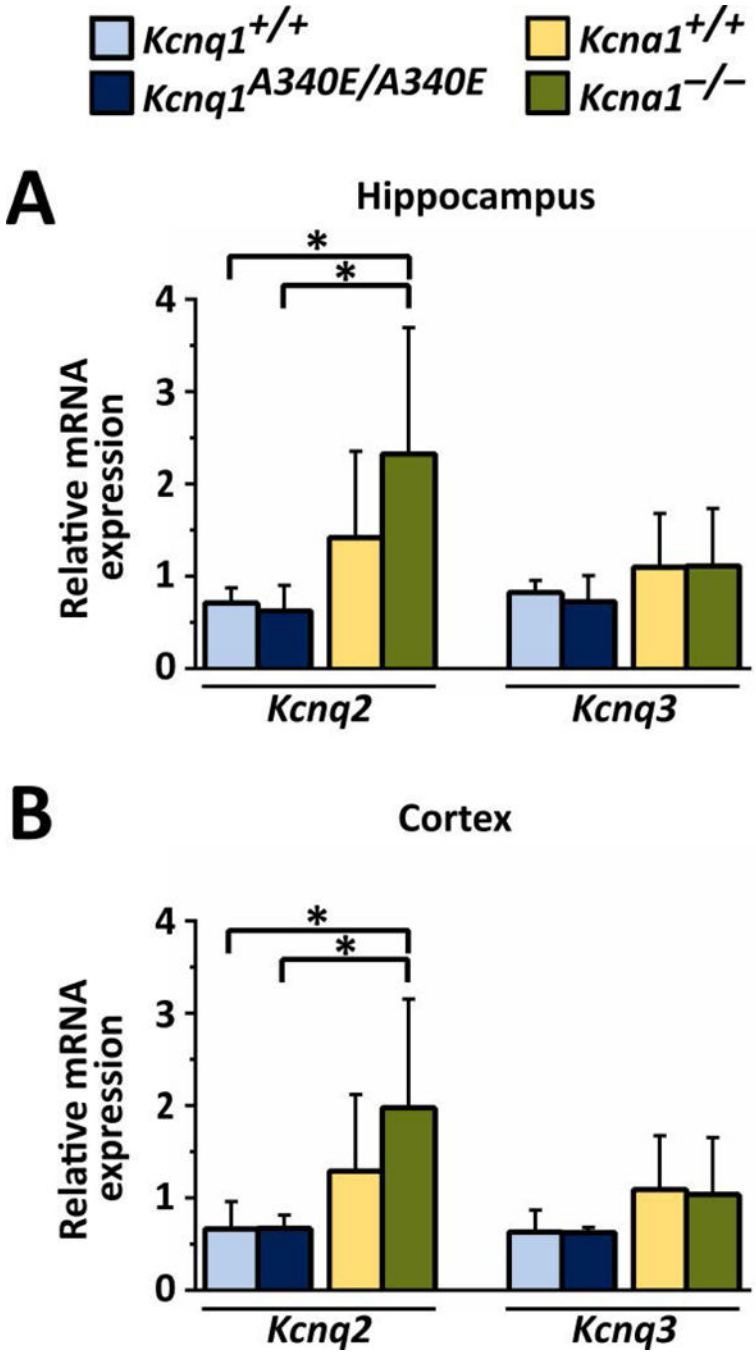

Figure 5.

Kcnq2 mRNA levels are increased in *Kcna1*$^{-/-}$ mouse brain. qPCR analyses of relative *Kcnq2* and *Kcnq3* mRNA expression revealed *Kcnq2* mRNA levels in hippocampus (**A**) and cortex (**B**) were significantly increased in *Kcna1*$^{-/-}$ mice compared to *Kcnq1* strain mice. *, $P \leq 0.05$ (one-way ANOVA, Tukey's post hoc; n=5–6/genotype per brain region).

Table 1

Effects of RTG (10 mg·kg^{-1}) on HRV in *Kcna1* and *Kcnq1* mice at 30-min post-injection.

Gene	Genotype	n	Condition	SDNN (ms)	RMSSD (ms)	HR (bpm)
Kcna1	+/+	4	VEH	2.9 ± 1.3	1.6 ± 0.6	697 ± 25
			RTG	2.6 ± 1.5	1.4 ± 0.7	682 ± 47
	−/−	7	VEH	3.6 ± 1.9	3.0 ± 2.7	700 ± 56
			RTG	3.6 ± 1.2	3.0 ± 2.0	711 ± 21
Kcnq1	+/+	4	VEH	11.1 ± 1.7	5.9 ± 0.4	549 ± 55
			RTG	9.8 ± 2.8	6.3 ± 3.0	593 ± 50
	A340E/A340E	5	VEH	11.0 ± 3.5	7.2 ± 3.5	555 ± 34
			RTG	47.2 ± 26.0 *	57.8 ± 36.8 *	340 ± 190 *

*, $P < 0.05$ (two-tailed paired Student's *t*-test). Abbreviations: HR, heart rate; RMSSD, root mean square of successive beat-to-beat differences; RTG, retigabine; SDNN, standard deviation of the beat-to-beat intervals; VEH, vehicle.