Published in final edited form as:

Neuropharmacology. 2020 February ; 163: 107865. doi:10.1016/j.neuropharm.2019.107865.

Activation of small conductance calcium-activated potassium channels suppresses seizure susceptibility in the genetically epilepsy-prone rats

Padmini Khandai¹, Patrick A Forcelli^{2,3,4}, Prosper N'Gouemo^{1,2,4}

¹Departments of Pediatrics, Georgetown University Medical Center, Washington, USA

²Department of Pharmacology and Physiology, Georgetown University Medical Center, Washington DC, USA

³Department of Neuroscience, Georgetown University Medical Center, Washington DC, USA

⁴Interdisciplinary Program in Neuroscience, Georgetown University Medical Center, Washington DC, USA

Abstract

Small conductance calcium-activated potassium (SK) channels dampen neuronal excitability by contributing to slow afterhyperpolarization (AHP) that follows a series of action potentials, and therefore may represent an intrinsic inhibitory mechanism to prevent seizures. We have previously reported that susceptibility to acoustically evoked seizures was associated with downregulation of SK1 and SK3 subtypes of SK channels in the inferior colliculus of the moderated seizure severity strain of the genetically epilepsy-prone rats (GEPR-3s). Here, we evaluated the effects of 1-ethyl-2-benzimidazolinone (1-EBIO), a potent activator of SK channels, on acoustically evoked seizures in both male and female adult GEPR-3s at various time points post-treatment. Systemic administration of 1-EBIO at various tested doses suppressed seizure susceptibility in both male and female GEPR-3s; however, the complete seizure suppression was only observed following administration of relatively higher doses of 1-EBIO in females. These findings indicate that activation of SK channels results in anticonvulsive action against generalized tonic-clonic seizures in both male and female GEPR-3s, with males exhibiting higher sensitivity than females.

Address correspondence to: Prosper N'Gouemo, Georgetown University Medical Center, Department of Pediatrics, 3900 Reservoir Rd, NW, Washington DC 20057. Tel: +1 (202) 687-8464; pn@georgetown.edu.

Conflict of interest

None

PN designed and supervised the experiments with input from PK and PAF. PK and PN performed the experiments and statistical analyses. PN and PAF obtained funding. PN wrote the manuscript with input from PK and PAF. All authors edited the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

1-ethyl-2-benzimidazoline (1-EBIO); audiogenic seizures; calcium-activated potassium channel; inherited epilepsy; hyperexcitability

1. Introduction

Epilepsy is a chronic neurological disease characterized by spontaneous and recurrent seizures that affects approximately 60 million people worldwide (Neligan et al., 2012; Zack & Kobau 2017). Despite the large number of antiepileptic drugs available to treat this debilitating neurological condition, approximately 30% of patients developed therapy-resistant epilepsy to the current pharmacological treatments (Bailer et al 2017; Hawkins and Gidal 2017; Pierzchala, 2010). Thus, there is an urgent need to develop new therapeutic approaches based on the pathogenesis of seizures, with emphasis on enhancing antiepileptic drug (AED) efficacy, reducing side effects of AEDs, and decreasing the need for combination therapy with multiple AEDs to control seizures. Multiple lines of evidence indicated that pretreatment with inhibitors of voltage-gated Ca^{2+} channels (VGCCs) suppressed acoustically evoked seizures in genetically epilepsy-prone rats (GEPRs) and DBA/2 mice (De Sarro et al., 1990, 1992, 2001). These findings suggested a remodeling of VGCCs at least in the inferior colliculus (IC), the initiation site of acoustically evoked seizures. Accordingly, we found an upregulation of VGCCs in the IC of the GEPR-3s, the moderated seizure severity strain of the GEPR, suggesting that facilitated VGCC currents may play an important role in the pathogenesis of seizures in the GEPR-3s (N'Gouemo et al., 2009a). The increased VGCC currents are suggestive of a massive Ca^{2+} influx resulting in abnormal levels of intracellular Ca^{2+} and disturbance of Ca^{2+} homeostasis, which in turn can alter Ca^{2+} -induced Ca^{2+} release from intracellular Ca^{2+} stores and deregulated Ca^{2+} -dependent mechanisms including K^{+} channels. Ca^{2+} -activated K^{+} channels comprised the large (BK) and small (SK) conductance Ca^{2+} -activated K^{+} channels. SK channels are exclusively activated by intracellular Ca^{2+} and contribute to spike frequency adaptation, slow afterhyperpolarization (AHP) conductances and synaptic plasticity (Anderson et al., 2006; Blank et al., 2004; Garduno et al., 2005; Gu et al., 2018; Pedarzani et al., 2008; Stackman et al., 2002; Xia et al., 1998; Wei et al., 2005; Zhang and McBain, 1995). Activation of SK channels therefore may help dampened neuronal hyperexcitability that leads to seizures (Huang et al. 2018; Oliveira et al., 2010; Zhang et al. 2012). Accordingly, reduced spike frequency adaptation and slow AHP conductances were found in CA1 and CA3 neurons of the GEPR-9, the most severe seizure severity strain of the GEPRs (Verma-Ahuja and Pencek, 1994; Verma-Ahuja et al., 1995). Furthermore, we found a reduced protein expression of SK1 and SK3 subtype of SK channels in the IC of the GEPR-3s; no change was found in the protein expression of BK channels (N'Gouemo et al 2009b). In a model of temporal lobe epilepsy, a long-lasting downregulation of SK3 channels, but a transient reduction in the protein expression of SK1 and SK2 channels were reported in the hippocampus (Oliveira et al 2010). The chronic downregulation of SK channels may therefore contribute to neuronal hyperexcitability that give rise to increased seizure susceptibility in a model of temporal lobe epilepsy and the GEPR-3s. In line with this hypothesis, activation of SK channels using 1-ethyl-2-benzimidazolinone (1-EBIO), a potent

activator of SK channels has been reported to suppress electrically evoked and chemically evoked tonic-clonic and clonic seizures, respectively (Anderson et al., 2006). Furthermore, 1-EBIO reduced the incidence of handling-induced convulsions in alcohol dependent mice (Mulholland et al., 2011). Whether 1-EBIO also suppressed tonic-clonic seizures in a model of inherited epilepsy remains unknown. Here, we sought to evaluate the effects of 1-EBIO, a potent activator of SK channels, on acoustically evoked seizure susceptibility in both male and female, adult GEPR-3s, a model of inherited epilepsy used to evaluate potential anticonvulsant drugs (see De Sarro et al., 2017 for review).

2. Materials and methods

2.1 Animals

The GEPR-3s were obtained from our animal colony at Georgetown University. Ten week-old, male and female GEPR-3s (250-350 g) were housed in standard polycarbonate cages under controlled ambient temperature ($22 \pm 1^\circ\text{C}$) with a 12 hours dark/light cycle; the animals have free access to water and chow food. All experiments were carried out between 10:00 h and 13:00 h and effort was made to minimize the number of animals used in these experiments. All experimental procedures were approved by Georgetown University Animal Care and Use Committee, and were conducted in accordance with the National Institutes of Health Guide for The Care and Use of Laboratory Animals (National Research Council, U.S., 2011).

2.2 Assessment of 1-EBIO anticonvulsant effects

1-EBIO (1-ethyl-2-benzimidazolinone, Alomone Labs, Jerusalem, Israel) was freshly prepared (dissolution in a mixture of 0.9% normal saline and 0.2% dimethyl sulfoxide) before each experiment. The GEPR-3s were placed in a Plexiglas chamber that is enclosed in a sound attenuating cubicle equipped with a ventilation fan, light, sound generator and video monitoring system (Med Associates, St. Albans, VT, USA). Following intraperitoneal injection of the vehicle solutions or 1-EBIO, the GEPR-3s were closely monitored for the occurrence of behavioral abnormalities such as loss of righting reflex, straub tail, sedation, lethargy and ataxia; the monitoring also occurred during and after exposure to the acoustic stimuli. The GEPR-3s were first tested for acoustically evoked seizures at 0.5-h after administration of the vehicle (control testing conditions). The GEPR-3s that exhibited seizures were administered 1-EBIO 1.5-h later in a volume of 0.2 mL/kg body weight, at a dose of 5, 10 or 20 mg/kg; doses of 1-EBIO were determined from our previously conducted preliminary experiments. Seizure testing occurred at 0.5, 1, 2 and 24 hours following 1-EBIO administration based on previous studies (Cho et al., 2017). To induce seizure, an acoustic stimulus consisted of pure tones or a mixed sound (bell) at 100-110 decibels sound pressure levels at intensity 12 kHz, were presented until the GEPR-3 displayed ictal activity or sixty seconds elapsed without any seizure activity. In this study, GEPR-3s exhibited acoustically evoked seizures consisted of wild running seizures (WRSSs) that evolved into generalized tonic-clonic seizures (GTCSs) characterized by tonic dorsiflexion of the neck, tonic flexion of the shoulders and bouncing tonic-clonic seizures (or clonus, i.e., tonic-clonic seizures while animal is lying in ventral position). Thus, seizure severity was classified into four stages as followed: stage 0: no seizure activity; stage 1: one episode of wild running

seizures (WRSs); stage 2: two episodes of WRSs; stage 3: one episode of WRSs followed by GTCSs; and stage 4: two or more episodes of WRSs following by GTCSs. At the conclusion of the experiments, all 1-EBIO treated GEPR-3s were euthanized with Euthasol (100mg/kg; i.p.).

2.4 Statistical analysis

Only GEPR-3s that displayed seizures under control condition (pre-1-EBIO treatment) were included in the study and data was analyzed in a blinded fashion. Following pharmacological intervention with 1-EBIO and acoustic stimulus exposure, GEPR-3s that did not display seizures within the sixty seconds were considered to be protected. For each group, the occurrence of WRSs and GTCSs components of acoustically evoked seizures were recorded. Seizure latency was determined as the time interval between the start of exposure to acoustic stimulus to initial display of WRS, and was analyzed using the paired *t* test. The prevalence of WRSs and GTCSs were analyzed using the McNemar's test that compares categorical data when subjects serve as their own controls. Wilcoxon signed-rank test was used to analyze the seizure severity as it compares the subject's original data from pre-treatment and post-treatment. Probability (*p*) values less than 0.05 ($P < 0.05$) were considered statistically significant and no multiple comparisons and adjustments were performed. Data are presented as mean $\pm$ S.E.M for seizure latency, median score $\pm$ median average deviation (M.A.D.) for seizure severity, and percentage $\pm$ standard error of proportion for the prevalence of WRSs and GTCSs.

3. Results

Administration of 1-EBIO at the tested doses did not alter the gross behavior of the GEPR-3s; no behavioral abnormalities such as loss of righting reflex, straub tail, sedation, lethargy and ataxia were observed at any time during this study. In this study, most male and female GEPR-3s tested for seizure susceptibility under control condition exhibited WRSs that evolved into GTCSs. First, we evaluated the effects of acute 1-EBIO treatment at the dose of 5 mg/kg on acoustically evoked seizure susceptibility in the GEPR-3s. In the control testing condition (pre-1-EBIO treatment), all male ($n=8$) and female ($n=8$) GEPR-3s experienced WRSs (Fig. 1, panels A and B), while $87.5 \pm 11.7\%$ of the males and 100% of the females exhibited GTCSs (Fig. 1, panels C and D). Quantification showed that 1-EBIO reduced the prevalence of WRSs in both male and female GEPR-3s. Distinctly, in males, 1-EBIO reduced the prevalence of WRS by 63% ($\chi^2=60.02$, $df=1$, $P<0.0001$), 63% ($\chi^2=60.02$, $df=1$, $P<0.0001$), 50% ($\chi^2=48.02$, $df=1$, $P<0.0001$), and 25% ($\chi^2=23.04$, $df=1$, $P<0.0001$) at 0.5-, 1-, 2-, and 24-hour post-treatment time points, respectively when compared with the control testing condition. In females, however, 1-EBIO reduced the prevalence of WRSs by 37% ($\chi^2=35.03$, $df=1$, $P<0.0001$) at 0.5-, 1-, and 2-hour post-treatment time points, when compared with the control testing conditions; no change was observed 24-hour post-treatment. Quantification also showed that pretreatment with 1-EBIO significantly reduced the prevalence of GTCSs in male GEPR-3s by 75% ($\chi^2=55.31$, $df=1$, $P<0.0001$) at 0.5-, 1-, and 2-hour post-treatment time points, and 21% ($\chi^2=11.76$, $df=1$, $P<0.001$) 24-hour post-1-EBIO treatment, when compared with the control testing conditions. In female GEPR-3s, the prevalence of GTCSs was reduced by 37% ($\chi^2=35.03$, $df=1$, $P<0.0001$), 75% ($\chi^2=73.01$,

df=1, $P<0.0001$), 75% ($\chi^2=73.01$, df=1, $P<0.0001$), and 25% ($\chi^2=11.76$, df=1, $P<0.0001$) 0.5-, 1-, 2-, and 24-hour post-1-EBIO treatment time points, respectively, when compared with the control testing conditions.

In addition to the prevalence of WRSs and GTCSs, we also evaluated the effects of 1-EBIO's treatment on the seizure latency and seizure severity. In the control conditions, the latency to seizure was 23.25 ± 1.75 s (n=8) and 24 ± 2.31 s (n=8) in male and female GEPR-3s, respectively (Fig. 1, panels E and F). Quantification showed that, in males, 1-EBIO significantly delayed the onset of seizures at 0.5-hour ($t=4.71$, df=7, $P<0.002$), 1-hour ($t=4.40$, df=7, $P<0.003$), and 2-hour ($t=4.30$, df=7, $P<0.004$) but not 24-hour post treatment time points (Fig. 1, panel E). In females, 1-EBIO significantly delayed the onset of seizures at 1-hour ($t=2.85$, df=7, $P<0.025$) and 2-hour ($t=2.87$, df=7, $P<0.024$) but not at 0.5- and 24-hour post treatment time points (Fig. 1, panel F). Furthermore, 1-EBIO completely suppressed seizure severity in male GEPR-3S at 0.5-hour ($z=2.33$, $P<0.016$), 1-hour ($z=2.33$, $P<0.016$), and 2-hour ($z=2.31$, $P<0.016$) post-treatment time points when compared to the control testing conditions; however, the seizure susceptibility returned to control levels 24-hour post-treatment (Fig. 1, panel G). In female GEPR-3s, 1-EBIO significantly suppressed and reduced the seizure severity at 1-hour ($z=2.16$, $P<0.032$) and 2-hour ($z=2.12$, $P<0.035$) post-treatment time points, respectively when compared with the control testing conditions; no changes in seizure severity was observed at 0.5- and 24-hour post-treatment (Fig. 1, panel H).

Even though we observed a complete suppression of GTCSs at a dose of 5 mg/kg of 1-EBIO in males but not female GEPR-3s, we further evaluated the efficacy of the drug at a higher dose (10 mg/kg) to determine if it has a potent anticonvulsant effect (e.g., complete seizure suppression) in females. In the control testing conditions (pre-1-EBIO treatment), all male (n=7) and female (n=8) GEPR-3s experienced WRSs (Fig. 2, panels A and B), while $85.71\pm13.2\%$ of the males and $75\pm15.3\%$ of females exhibited GTCSs (Fig. 2, panels C and D). Quantification showed that 1-EBIO significantly reduced the prevalence of WRS in both male and female GEPR-3s. More precisely, in the males, 1-EBIO at a dose of 10 mg/kg, significantly reduced the prevalence of WRSs by 71% ($\chi^2=69.01$, df=1, $P<0.0001$), 71% ($\chi^2=69.01$, df=1, $P<0.0001$), and 86% ($\chi^2=84.01$, df=1, $P<0.0001$) at 0.5-, 1-, and 2-hour post-treatment time points, respectively, when compared with control testing conditions; the prevalence of WRSs returned to control levels at 24-hour post-treatment (Fig. 2, panel A). Similarly, in the females, 1-EBIO reduced the prevalence of WRSs by 50% ($\chi^2=48.02$, df=1, $P<0.0001$), 50% ($\chi^2=48.02$, df=1, $P<0.0001$), 25% ($\chi^2=23.04$, df=1, $P<0.0001$), and 13% ($\chi^2=10.08$, df=1, $P<0.001$) at 0.5-, 1-, 2-, and 24-hour post-treatment time points, respectively, when compared with the control testing conditions (Fig. 2, panel B). Pretreatment with 10 mg/kg 1-EBIO also significantly reduced the prevalence of GTCSs in male GEPR-3s by 83% ($\chi^2=54.38$, df=1, $P>0.0001$) at 0.5-, 1-, and 2-hour post-treatment time points, when compared with the control testing conditions; no anticonvulsant effect was observed at 24-hour post-treatment (Fig. 2, panel C). In female GEPR-3s, the prevalence of GTCSs was reduced by 50% ($\chi^2=24.01$, df=1, $P<0.0001$) at 0.5-hour and, 63% ($\chi^2=33.22$, df=1, $P<0.0001$), at 1- and 2-hour, post-1-EBIO treatment time points, respectively, when compared with the control testing conditions; no anticonvulsant effect was observed at 24-hour post-treatment.

We also found that 1-EBIO pretreatment significantly delayed the onset of seizure in male GEPR-3S at 1-hour ($t=2.93$, $df=6$, $p<0.026$), 2-hour ($t=4.28$, $df=6$, $P<0.005$) and 24-hour ($t=12.52$, $df=6$, $P<0.0001$) post-treatment time points compared to control testing conditions (Fig. 2, panel E). In female GEPR-3s, 1-EBIO pretreatment significantly delayed the onset of seizure at 0.5-hour ($t=3.91$, $df=7$, $P<0.006$), 1-hour ($t=4.97$, $df=7$, $P<0.001$), 2-hour ($t=6.00$, $df=7$, $P<0.001$), and 24-hour ($t=9.74$, $df=7$, $P<0.0002$) post-treatment time points, compared with control testing conditions (Fig. 2, panel F). Lastly, at the dose of 10 mg/kg, 1-EBIO suppressed seizure susceptibility in male GEPR-3s at 0.5-hour ($Z=2.16$, $P<0.031$), 1-hour ($Z=2.16$, $P<0.031$), and 2-hour ($Z=2.22$, $P<0.031$) post-treatment time points, when compared with the control testing conditions (Fig. 2, panel H). No change in seizure severity was observed 24-hour post 1-EBIO treatment (Fig. 2, panel H). In female GEPR-3s, the seizure severity significantly decreased 0.5 hour ($Z=2.16$, $P<0.031$) and 1-hour ($Z=2.33$, $P<0.016$), and suppressed 2-hour ($Z=2.37$, $P<0.016$) post-treatment time points, when compared with the control testing conditions (Fig. 2, panel H).

To further evaluate the anticonvulsant effects of 1-EBIO, we assessed the extent to which 1-EBIO acute treatment at the dose of 20 mg/kg completely suppresses seizure susceptibility in both male and female GEPR-3s, and whether this anticonvulsant effect lasted 24 hours. In the control testing conditions (pre-1-EBIO treatment), all male ($n=8$) and female ($n=7$) GEPR-3s experienced WRSs, while 100% of the males and 86% of the females exhibited GTCs. Quantification showed that the prevalence of WRSs in male GEPR-3s was reduced by 75% 0.5-, 1- and 2-hour post-treatment time points ($\chi^2=73.01$, $df=1$, $P<0.0001$), and 25% at 24-hour post-treatment ($\chi^2=23.04$, $df=1$, $P<0.0001$), when compared with control testing conditions (Fig.3 panel A). Similarly, in the female GEPR-3s, 1-EBIO reduced the prevalence of WRSs by 72% ($\chi^2=69.01$, $df=1$, $P<0.0001$), 86% ($\chi^2=84.01$, $df=1$, $P<0.0001$), 57% ($\chi^2=55.02$, $df=1$, $P<0.0001$), and 14% ($\chi^2=12.07$, $df=1$, $P<0.0001$) at 0.5-, 1-h, 2-h, and 24-hour post-treatment time points, respectively, when compared with the control testing conditions (Fig.3 panel B). Pretreatment with 1-EBIO also significantly reduced the prevalence of GTCs in male GEPR-3s by 100% ($\chi^2=98.01$, $df=1$, $P<0.0001$), 87% ($\chi^2=85.01$, $df=1$, $P<0.0001$), 87% ($\chi^2=85.02$, $df=1$, $P<0.0001$), and 37% ($\chi^2=23.04$, $df=1$, $P<0.0001$) 0.5-, 1-, 2-, and 24-hour post-treatment time points, respectively, when compared with the control testing conditions (Fig. 3, panel C). In female GEPR-3s, the prevalence of GTCs was reduced by 84% ($\chi^2=50.41$, $df=1$, $P<0.0001$) at 0.5- and 1-hour post-treatment time points when compared with the control testing conditions (Fig. 3, panel D). Interestingly, 1-EBIO completely suppressed the occurrence of GTCs at 2-hour post-treatment ($\chi^2=63.38$, $df=1$, $P<0.0001$); this anticonvulsant effect was reversed 24 hours later ($\chi^2=5.56$, $df=1$, $P<0.03$), when compared with control testing conditions (Fig. 3, panel D).

In the control conditions, the latency to seizures was 26.63 ± 3.82 s ($n=8$) and 20.1 ± 1.8 s ($n=7$) in male and female GEPR-3s, respectively (Fig. 3., panels E and F). Quantification showed that 1-EBIO pretreatment significantly delayed the onset of seizures at 0.5- hour ($t=6.21$, $df=7$, $P<0.0004$), 1-hour ($t=5.58$, $df=7$, $P<0.0001$), 2-hour ($t=5.07$, $df=7$, $P<0.001$) but not 24-hour post-treatment in male GEPR-3s, when compared with control testing conditions (Fig. 3, panels E and F). In female GEPR-3s, the onset of seizures was delayed at 0.5-hour ($t=6.21$, $df=6$, $P<0.001$), 1-hour ($t=4.09$, $df=6$, $P<0.006$), 2-hour ($t=6.31$, $df=6$, $P<0.001$), and 24-hour ($t=2.84$, $df=6$, $P<0.03$) post-treatment time points, when compared

with the control testing conditions (Fig. 3 panel F). Quantification also showed that 1-EBIO pre-treatment completely suppressed seizures in male GEPR-3s at 0.5-hour ($z=2.57$, $P<0.008$), 1-hour ($Z=2.44$, $P<0.016$) and 2-hour ($Z=2.44$, $P<0.016$), but not 24-hour post-treatment time points, when compared with control testing conditions (Fig. 3, panel G). Similarly, 1-EBIO pretreatment also suppressed seizure susceptibility in female GEPR-3s at 0.5-hour ($z=2.22$, $P<0.031$), 1-hour ($Z=2.22$, $P<0.031$) and 2-hour ($Z=2.33$, $P<0.016$), but not 24-hour post-treatment time points, when compared with control testing conditions (Figure 3, panel H).

4. DISCUSSION

The main finding of this present study is that acute pretreatment with 1-EBIO, a potent activator of SK channels, suppressed acoustically evoked seizure susceptibility in both male and female GEPR-3s. The seizure suppression was observed in males at all tested doses of 1-EBIO, whereas only relatively higher doses were effective in suppressing the seizure susceptibility in females suggesting that male GEPR-3s are more sensitive to the anticonvulsive effect of 1-EBIO treatment; the underlying biological mechanism for this differential sex effect is yet unknown. Since 1-EBIO activates SK channels, our findings suggest that activation of these channels in selected brain sites may have a potent anticonvulsant activity against tonic-clonic seizures in the GEPR-3s. The neural networks of acoustically evoked seizures include the IC in the GEPRs. Interestingly, single unit recordings in awake and behaving GEPRs revealed that acoustically evoked seizure activity is initiated in the IC (Faingold, 1999; N'Gouemo and Faingold, 1998). Thus, the suppression of acoustically evoked seizures following 1-EBIO treatment suggests a remodeling of SK channels, at least in the IC, may play an important role in inherited seizure susceptibility in the GEPR-3s. Interestingly, SK channels are found in the IC and other brain sites including amygdala, hippocampus, thalamus, substantia nigra and cerebellum (N'Gouemo et al., 2009; Wei et al., 2005). Although these brain sites are of interest in the pathogenesis and pathophysiology of seizures and epilepsies, only activation of the IC can trigger brainstem GTCSs.

The potential mechanisms underlying the anticonvulsant action of 1-EBIO may be the result of its ability to stabilize the calcium-calmodulin interaction on SK channels, thereby deactivating the channels, and thus prolonging the open probability of the channel and increasing K^+ efflux (Pedarzani et al., 2001; Xia et al., 1998). By increasing the repolarization phase, 1-EBIO reduces neuronal hyperexcitability by prolonging the resting period between regular electrical discharges, thus allowing for spike frequency adaptation, which protects the neuron from excessive impulse activity.

Evidence links human epilepsy with defects of Ca^{2+} channel function (see Gambardella A and Labate A, 2014 for review). Interestingly, evidence indicates that facilitated $Ca_v2.3$ currents is a disease mechanism for human epilepsy, as *de novo* gain-of-function *CACNA1E* (gene that encoded $Ca_v2.3$ channels) variants contributes to the pathogenesis of seizures in developmental and epileptic encephalopathies characterized by intractable seizures (Helbig et al., 2019). These findings suggest a critical role of cytosolic Ca^{2+} in the mechanisms of seizures and epilepsy. In the present study, we found that activation of SK channels, which

are solely activated by Ca^{2+} , suppresses seizure susceptibility in the GEPR-3s. The mechanisms underlying this anticonvulsant are unknown but may include increased Ca^{2+} sensitivity of SK channels and/or increased total and/or cell surface expression of these channels. SK channels can be activated by increases in cytosolic Ca^{2+} from one of these multiple sources including Ca^{2+} influx via VGCCs, receptor-operated channels, Ca^{2+} release from internal stores, or a combination of these Ca^{2+} routes. Cytosolic Ca^{2+} in the IC may play a role in the pathophysiology of acoustically evoked seizures in the GEPR-3s, as pharmacological blockade of VGCCs suppressed these seizures (De Sarro et al., 1990, 1992). Accordingly, we reported increases of VGCC currents in IC neurons of the GEPR-3s (N’Gouemo et al. 2009a). Such upregulation of VGCCs may contribute to increase cytosolic Ca^{2+} in IC neurons. We also have reported upregulation of SK2 subtype of SK channels but downregulation of SK1 and SK3 subtypes in the IC of the GEPR-3s (N’Gouemo et al. 2010). Thus, 1-EBIO may increase the Ca^{2+} sensitivity of SK1 and SK3 channels in the IC of the GEPR-3s, leading to seizure suppression. Alternatively, 1-EBIO may activate SK2 channels that are upregulated in IC neurons of the GEPR-3s, resulting in a “gain-of-function” of these channels and increased K^+ efflux, leading to seizure suppression.

In conclusion, the present reveals that activation of SK channels may provide a powerful mechanism for the suppression of GTCSs in the GEPR-3s.

Acknowledgments

This publication was funded by the National Institutes of Health (NIH) Public Health Service grants R01 AA020073 and R21 AA027171 (PN), and R01 NS097762 (PAF) and its contents are the responsibility of the authors and do not necessarily represent the official views of NIH.

Abbreviations:

1-EBIO	1-ethyl-2-benzimidazoline
AED	antiepileptic drug
AHP	afterhyperpolarization
BK	large conductance calcium-activated potassium
GEPR	genetically epilepsy-prone rat
GTCS	generalized tonic-clonic seizures
SK	small conductance calcium-activated potassium
VGCC	voltage-gated calcium channel
WRS	wild running seizure

References

1. Adelman JP, Maylie J, Sah P. 2012 Small-conductance Ca^{2+} -activated K^+ channels: form and function. *Ann Rev Physiol* 74, 245–269. [PubMed: 21942705]

2. Anderson NJ, Slough S, Watson WP, 2006 In vivo characterization of the small-conductance K_{Ca} (SK) channel activator 1-ethyl-2-benzimidazolinone (1-EBIO) as a potential anticonvulsant. *European Journal of Pharmacology* 546, 48–53. [PubMed: 16925994]
3. Blank T, Nijholt I, Kye MJ, Spiess J, 2004 Small conductance Ca^{2+} -activated K^{+} channels as targets of CNS drug development. *Cur Drug Targets- CNS & Neurol Dis* 3, 161–167.
4. Cho SJ, Vaca MA, Miranda CJ, N'Gouemo P, 2018 Inhibition of transient potential receptor vanilloid type 1 suppresses seizure susceptibility in the genetically epilepsy-prone rat. *CNS Neurosci Ther* 24, 18–28. [PubMed: 29105300]
5. De Sarro G, Russo E, Citraro R, Meldrum BS, 2017 Genetically epilepsy-prone rats (GEPRs) and DBA/2 mice: Two animal models of audiogenic reflex epilepsy for the evaluation of new generation AEDs. *Epilepsy Behav*, 165–173.
6. De Sarro G, De Sarro A, Federico F, Meldrum BS, 1990 Anticonvulsant properties of some calcium antagonists on sound-induced seizures in genetically epilepsy prone rats. *Gen Pharmacol* 21, 768–778.
7. De Sarro G, Ascioti C, di Paola ED, Vidal MJ, De Sarro A, 1992 Effects of antiepileptic drugs, calcium channel blockers and other compounds on seizures induced by activation of voltage-dependent L calcium channel in DBA/2 mice. *Gen Pharmacol* 23, 1205–1216. [PubMed: 1283140]
8. De Sevilla Fernández D, Garduño J, Galván E, Buño W., 2006 Calcium-activated afterhyperpolarization regulate synchronization and timing of epileptiform bursts in hippocampal CA3 pyramidal neurons. *J Neurophysiol* 96, 3028–3041. [PubMed: 16971683]
9. Faingold CL, 1999 Neuronal networks in the genetically epilepsy-prone rat. *Adv Neurol* 79, 311–321. [PubMed: 10514823]
10. Gambardella A, Labate A, 2014 The role of calcium channel mutations in human epilepsy. *Prog Brain Res* 213, 87–96. [PubMed: 25194484]
11. Garduño J, Galván E, Fernández de Sevilla D, Buño W, 2005 1-ethyl-2 benzimidazolinone (1-EBIO) suppresses epileptiform activity in vitro hippocampus. *Neuropharmacology* 49, 376–388. [PubMed: 15993438]
12. Helbig KL, Lauerer RJ, Bahr JC, Souza IA, Myers CT, Uysal B, Schwarz N, Gandini MA et al., 2019 *De novo* pathogenic variants in *CACNA1E* cause developmental and epileptic encephalopathy with contractures, macrocephaly, and dyskinesia. *Am J Hum Genet* 103, 666–678, 2019.
13. Hirschberg B, Maylie J, Adelman JP, Marrion NV, 1998 Gating of recombinant small-conductance Ca^{2+} -activated K^{+} channels by calcium. *J Gen Physiol* 111, 565–581. [PubMed: 9524139]
14. Huang Y, Liu X, Wang G, Wang Y, 2018 SK channels participate in the formation of after burst hyperpolarization and partly inhibit the burst strength of epileptic ictal discharges. *Mol Med Rep* 17, 1762–1774. [PubMed: 29257204]
15. Kaur H, Kumar B, Medhi B, 2016 Antiepileptic drugs in development pipeline: A recent update. *eNeurol Sci* 4, 42–51.
16. Löscher W 2011 Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure* 20, 359–368. [PubMed: 21292505]
17. Mulholland PJ, Becker HC, Woodward JJ, Chandler LJ, 2011 SK2 channels regulate alcohol-associated plasticity of glutamatergic synapses. *Biol Psychiatry* 69, 625–632. [PubMed: 21056409]
18. N'Gouemo P, Faingold CL, 1998 Periaqueductal gray neurons exhibit increased responsiveness associated with audiogenic seizures in the genetically epilepsy-prone rat. *Neuroscience* 84, 619–625. [PubMed: 9539230]
19. N'Gouemo P, Faingold CL, Morad M, 2009a Calcium channel dysfunction in inferior colliculus neurons of the genetically epilepsy-prone rat. *Neuropharmacology* 56, 665–675. [PubMed: 19084544]
20. N'Gouemo P, Yasuda RP, Faingold CL, 2009b Protein expression of small conductance calcium-activated potassium channels is altered in inferior colliculus neurons of the genetically epilepsy-prone rat. *Brain Res* 1270, 107–111. [PubMed: 19254702]

21. National Research Council (U.S.), Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.), 2011 Guide for the care and use of laboratory animal, 8th Edition. National Academies Press, Washington DC.
22. Neligan A, Hauser WA, Sander JW, 2012 The epidemiology of the epilepsies. Handbook of Clinical Neurology 107, 113–133. [PubMed: 22938966]
23. Oliveira MS, Skinner F, Arshadmansab MF, Garcia I, Mello CF, Knaus HG, Ermolinsky BS, Otalora LF, Garrido-Sanabria E,R., 2010 Altered expression and function of small-conductance (SK) Ca^{2+} -activated K^{+} channels in pilocarpine-treated epileptic rats. Brain Res 1348, 187–199. [PubMed: 20553876]
24. Pedarzani P, Stocker M, 2008 Molecular and cellular basis of small-and-intermediate-conductance, calcium-activated potassium channel function in the brain. Cell Mol Life Sci 65, 3196–3217. [PubMed: 18597044]
25. Pedarzani P, Mosbacher J, Rivard A, Cingolani LA, Oliver D, Stocker M, Adelman JP, Fakler B 2001 Control of electrical activity in central neurons by modulating the gating of small conductance Ca^{2+} -activated K^{+} channels. J Biol Chem 276, 9762–9769. [PubMed: 11134030]
26. Pierzchala K, 2010 Pharmacoresistant epilepsy–epidemiology and current studies. Polish J Neurol Neurosurg 44, 285–290.
27. Quansah H, N’Gouemo P, 2014 Amiloride and SN-6 suppress audiogenic seizure susceptibility in genetically epilepsy-prone rats. CNS Neurosci Ther 20, 860–866. [PubMed: 24948133]
28. Rogawski MA, Löscher W, 2004 The neurobiology of antiepileptic drugs. Nature Rev Neurosci 5, 553–564. [PubMed: 15208697]
29. Stackman RW, Hammond RS, Linardatos E, Gerlach A, Maylie J, Adelman JP, Tzounopoulos T, 2002 Small conductance Ca^{2+} -activated K^{+} channels modulate synaptic plasticity and memory encoding. J Neurosci 22, 10163–10171. [PubMed: 12451117]
30. Verma-Ahuja S, Pencek TL, 1994 Hippocampal CA1 neurons properties in the genetically epilepsy-prone rats. Epilepsy Res 18, 205–215. [PubMed: 7805642]
31. Verma-Ahuja S, Evans MS, Pencek TL, 1995 Evidence for decreased calcium dependent potassium conductance in hippocampal CA3 neurons of the genetically epilepsy-prone rats. Epilepsy Res 22, 137–144. [PubMed: 8777900]
32. Walia KS, Khan EA, Ko DH, Raza SS, Khan YN, 2004 Side effects of antiepileptics - a review. Pain Practice 4, 194–203. [PubMed: 17173601]
33. Wei AD, Gutman GA, Aldrich R, Chandy KG, Grissmer S, Wulff H, 2005 International Union of Pharmacology. II. Nomenclature and molecular relationships of calcium-activated potassium channels. Pharmacol Rev 57, 463–472. [PubMed: 16382103]
34. Xia XM, Fakler A, Rivard GW, Johnson-Pais T, Keen JE, Ishii T, Hirschberg B, Bond CT, Lutsenko S, Maylie J, Adelman JP, 1998 Mechanism of calcium gating in small-conductance calcium-activated potassium channels. Nature 395, 503–507. [PubMed: 9774106]
35. Zack MM, Kobau R, 2017 National and State Estimates of the Numbers of Adults and Children with Active Epilepsy - United States, 2015. Morb Mortal Wkly Rep 66, 821–825.
36. Zhang L, McBain CJ, 1995 Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurons. J Physiol 488, 661–672. [PubMed: 8576856]
37. Zhang M, Pascal JM, Schumann M, Armen RS, Zhang J, 2012 Identification of the functional binding pocket for compounds targeting small-conductance Ca^{2+} -activated potassium channels. Nat Commun 3, 1021 DOI: 10.1038/ncomms2017. [PubMed: 22929778]

- Epilepsies are disorders of neuronal excitability
- SK channels dampen neuronal excitability via slow afterhyperpolarization conductance
- 1-EBIO, SK channel activator, markedly suppresses audiogenic seizures in the GEPR-3s
- Male GEPR-3s exhibited higher sensitivity to the effects of 1-EBIO than females

Figure 1.

Effects of acute 1-EBIO pretreatment at a dose of 5 mg/kg on occurrence of acoustically evoked seizures in male and female GEPR-3s. The anticonvulsant effects of 1-EBIO were evaluated at different post-treatment time points of 0.5, 1, 2, and 24 hours. 1-EBIO pretreatment markedly reduced the incidence of WRS in both male (panel **A**) and female (panel **B**) GEPR-3s. 1-EBIO also reduced the prevalence of GTCSs in both male (panel **C**) and female (panel **D**) GEPR-3s. Furthermore, 1-EBIO delayed the onset of seizure in both males (panel **E**) and females (panel **F**). A complete seizure suppression was observed in male (panel **G**) GEPR-3s, while only a reduction of seizure severity occurred in female (panel **H**) GEPR-3s. Data from prevalence of WRS and GTCSs were represented as percentage (%)±standard error of proportion, and McNemar's test was used for statistical

analysis. The seizure latency data was presented as mean $\pm$ S.E.M., and paired t-test was used for analysis. The seizure severity data was represented as median score $\pm$ median average deviation, and the Wilcoxon signed-rank test was used for statistical analysis. Opened and filled bar graphs represent controls (pre-1-EBIO) and 1-EBIO treated GEPR-3s, respectively. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Figure 2.

Effects of acute 1-EBIO pretreatment at a dose of 10 mg/kg on occurrence of acoustically evoked seizures in male and female GEPR-3s. The anticonvulsant effects of 1-EBIO were evaluated on the prevalence and severity of seizures at different post-treatment time points including 0.5-, 1-, 2-, and 24-hour. 1-EBIO at a dose of 10 mg/kg (i.p.) reduced the incidence of WRS in both male (panel **A**) and female (panel **B**) GEPR-3s. 1-EBIO also markedly reduced the prevalence of GTCs in both male (panel **C**) and female (panel **D**) GEPR-3s. Pretreatment with 1-EBIO delayed the onset of seizure onset in either male (panel **E**) or female (panel **F**) GEPR-3s. A complete seizure suppression was observed in male GEPR-3s (panel **G**) up to 2-hour post-treatment, while this effect was seen in female GEPR-3s by 2nd hour post-treatment (panel **H**). The prevalence of WRS and GTCs, seizure

latency, and seizure severity were analyzed as described in Figure 1. Opened and filled bar graph represent controls (pre-1-EBIO) and 1-EBIO treated GEPR-3s, respectively. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.001$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Effects of 1-EBIO pretreatment at a dose of 20 mg/kg on occurrence of acoustically evoked seizures in both male and female GEPR-3s. The anticonvulsant effects of 1-EBIO were evaluated on the prevalence and severity of seizures at different post-treatment time points of 0.5, 1, 2, and 24 hours. 1-EBIO at a dose of 20 mg/kg (i.p.) reduced the incidence of WRS in male (panel **A**) and female (panel **B**) GEPR-3s, respectively. 1-EBIO also suppressed or reduced the prevalence of GTCSs in male (panel **C**) and female (panel **D**) GEPR-3s. Pretreatment with 1-EBIO delayed the onset of seizure onset in both male (panel **E**) and female (panel **F**) GEPR-3s. Time course of the effects of 1-EBIO on seizure severity revealed that 1-EBIO completely suppressed seizures in both male (panel **G**) and female (panel **H**) GEPR-3s. The prevalence of WRSs and GTCSs, seizure latency, and seizure severity were analyzed as described in Figure 1. Opened and filled bar graph represent controls (pre-1-EBIO) and 1-EBIO treated GEPR-3s, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.001$.