**FULL-LENGTH ORIGINAL RESEARCH**

**Glyoxalase 1 and its substrate methylglyoxal are novel regulators of seizure susceptibility**

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**SUMMARY**

**Purpose:** Epilepsy is a complex disease characterized by a predisposition toward seizures. There are numerous barriers to the successful treatment of epilepsy. For instance, current antiepileptic drugs have adverse side effects and variable efficacies. Furthermore, the pathophysiologic basis of epilepsy remains largely elusive. Therefore, investigating novel genes and biologic processes underlying epilepsy may provide valuable insight and enable the development of new therapeutic agents. We previously identified methylglyoxal (MG) as an endogenous \( \gamma \)-aminobutyric acid (GABA\( _A \)) receptor agonist. Here, we investigated the role of MG and its catabolic enzyme, glyoxalase 1 (GLO1), in seizures.

**Methods:** We pretreated mice with MG before seizure induction with picrotoxin or pilocarpine and then assessed seizures behaviorally or by electroencephalography (EEG). We then investigated the role of GLO1 in seizures by treating mice with a pharmacologic inhibitor of GLO1 before seizure induction with pilocarpine and measured subsequent seizure phenotypes. Next, we explored the genetic relationship between GLO1 expression and seizures. We analyzed seizure phenotypes among C57BL/6J × DBA/2J (BXD) recombinant inbred (RI) mice with differential GLO1 expression. Lastly, we investigated a causal role for GLO1 in seizures by administering picrotoxin to transgenic (Tg) mice that overexpress GLO1.

**Key Findings:** Pretreatment with MG attenuated pharmacologically-induced seizures at both the behavioral and EEG levels. GLO1 inhibition, which increases MG concentration in vivo, also attenuated seizures. Among BXD RI mice, high GLO1 expression was correlated with increased seizure susceptibility. Tg mice overexpressing GLO1 displayed reduced MG concentration in the brain and increased seizure severity.

**Significance:** These data identify MG as an endogenous regulator of seizures. Similarly, inhibition of GLO1 attenuates seizures, suggesting that this may be a novel therapeutic approach for epilepsy. Furthermore, this system may represent an endogenous negative feedback loop whereby high metabolic activity increases inhibitory tone via local accumulation of MG. Finally, GLO1 may contribute to the genetic architecture of epilepsy, as GLO1 expression regulates both MG concentration and seizure severity.

**KEY WORDS:** Seizure, Pilocarpine, Picrotoxin, GABA\( _A \) receptors, Methylglyoxal, Glyoxalase 1.
activate γ-aminobutyric acid (GABA_\text{A}) receptors (Distler & Palmer, 2012; Distler et al., 2012). GABA_\text{A} receptors are the primary regulators of fast inhibitory synaptic transmission in the central nervous system (Macdonald et al., 2010). Abundant clinical and experimental evidence has demonstrated that mutations in GABA_\text{A} receptor–encoding genes can perturb GABA_\text{A} receptor signaling and cause epileptic seizures (Galanopoulou, 2010; Briggs & Galanopoulou, 2011). Furthermore, several well-established AEDs activate or potentiate GABA_\text{A} receptors, including benzodiazepines and barbiturates (Perucca & Tomson, 2011). Given the prominent role of GABA_\text{A} receptors in epilepsy and the action of MG at GABA_\text{A} receptors, we hypothesized that MG would protect against epileptic seizures. MG is metabolized by glyoxalase 1 (GLO1); therefore, we predicted that differences in Glo1 expression and activity would affect seizure susceptibility through regulating endogenous MG concentrations in the brain.

In the present study, we investigated whether direct administration of MG would inhibit epileptic seizures induced by the GABA_\text{A}-receptor antagonist, picrotoxin, and the muscarinic cholinergic agonist, pilocarpine. We also investigated whether changes in Glo1 expression or activity would affect seizure susceptibility and severity. Inhibition of GLO1 may potentiate an endogenous negative feedback loop, whereby high metabolic activity could increase inhibitory tone via GABA_\text{A} receptors. As an antiepileptic therapy, GLO1 inhibition might have a different, perhaps more favorable side effect profile than current AEDs.

**Methods**

**Animals**

All studies were approved by the Institutional Animal Care and Use Committee at The University of Chicago or Emory University. Studies with MG and S-bromobenzylglutathione cyclopentyl diester (BrBzGCp2) were performed with C57BL/6J (B6) mice purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A.). Transgenic (Tg) mice were generated on an FVB/NJ (FVB) background as previously described (Distler et al., 2012) and were tested in parallel with their wild-type (WT) littermates. Mice from two FVB Tg lines were tested and pooled into a common Tg group. All studies used male mice to reduce variability arising from the effects of the estrous cycle on seizure phenotypes (Foldvary-Schaefer et al., 2004; Scharfman & Gray, 2007; Veliskova, 2007).

**Reagents**

Picrotoxin (item P1675), pilocarpine hydrochloride (item P6503), and methylglyoxal (item M0252) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Atropine methyl-nitrate (item 417-102A) was obtained from Chem Service (West Chester, PA, U.S.A.). BrBzGCp2 was synthesized at the Beckman Research Institute of the City of Hope (Duarte, CA, U.S.A.) as described previously (Distler et al., 2012).

**Drug administration**

For pretreatment, MG (50 or 200 mg/kg at a volume of 10 ml/kg body weight) or vehicle (0.9% saline at a volume of 10 ml/kg body weight) was administered by intraperitoneal (i.p.) injection 10 min before the seizure-inducing agent. For treatment after seizure onset, MG (200 mg/kg at a volume of 10 ml/kg body weight) or vehicle (0.9% saline at a volume of 10 ml/kg body weight) was administered by i.p. injection 10 min after seizure onset. For Glo1 inhibition, BrBzGCp2 (50 mg/kg at a volume of 5 ml/kg body weight) or vehicle (8% dimethyl sulfoxide and 18% Tween-80 at a volume of 5 ml/kg body weight) was administered by i.p. injection 2 h before seizure induction.

**Seizure induction for behavioral scoring**

For behavioral analysis of picrotoxin-induced seizures, 5 mg/kg of picrotoxin in 0.9% saline was administered by i.p. injection at a volume of 10 ml/kg body weight. Seizures were scored for 1 h after picrotoxin administration. For behavioral analysis of pilocarpine-induced seizures, mice were pretreated with atropine (5 mg/kg in 0.9% saline at a volume of 10 ml/kg body weight) by i.p. injection to reduce the peripheral effects of pilocarpine. Thirty minutes after atropine administration, pilocarpine was administered by i.p. injection at a volume of 10 ml/kg body weight. B6 mice were treated with 250 mg/kg pilocarpine in 0.9% saline, and FVB mice (WT and Tg) were treated with 300 mg/kg pilocarpine in 0.9% saline. The B6 and FVB mice were given slightly different doses of pilocarpine (250 and 300 mg/kg, respectively) due to strain differences in seizure susceptibility (Schauwecker, 2011, 2012). Seizures were scored for 1.5 h after pilocarpine administration.

Picrotoxin-induced seizures were scored as the presence of generalized convulsions as well as the latency to and duration of generalized convulsions.

Pilocarpine-induced seizures were scored as previously reported (Winawer et al., 2011):

**Stage 1:** Immobility/lying low.

**Stage 2:** Partial (limbic) seizures; noncontinuous twitching/tremor/shaking of tail/head/body/limbs, forelimb and/or tail extension, rigid posture, repetitive movement, head bobbing.

**Stage 3:** Partial status epilepticus; continuous tremor/clonic seizures of body and tail while retaining posture.

**Stage 4:** Generalized seizures; rearing/hyperexcitability/running/falling, tonic extension/clonic seizures with loss of posture.

**Stage 5:** Generalized status epilepticus (continuous stage 4 seizures) resulting in death.
Seizure induction for EEG recording

Electroencephalography (EEG) recording of mice treated with picrotoxin was performed at Emory University. Under isoflurane anesthesia, mice were surgically implanted subcutaneously with four sterile stainless-steel screw electrodes for EEG recordings, and fine-wire electrodes were inserted into the neck muscle for electromyography (EMG), as described previously (Martin et al., 2007, 2010; Papale et al., 2010; Dutton et al., 2012). After a minimum of 10 days of recovery, each mouse was placed into an acrylic glass box (15 × 15 × 15 cm) and was attached to the EEG cable. Digital video/EEG/EMG recordings were amplified, filtered (0.3–35 Hz bandpass for the EEG and 10–70 Hz bandpass for the EMG), and digitized at a sampling rate of 200 Hz by Stellate Harmonic amplifier and software (Natus Medical, Inc., San Carlos, CA, U.S.A.). The mice were injected i.p. with 7 mg/kg picrotoxin or vehicle (0.9% saline) at a volume of 10 ml/kg body weight. This dose of picrotoxin (7 mg/kg) was slightly higher than that used for behavioral scoring of seizures (5 mg/kg) at the University of Chicago, because it maximized seizure outcomes by EEG under the experimental conditions at Emory University. EEG and EMG data were collected for 1 h. Seizures were characterized by the onset of sharp, highly synchronous spike discharges that increased in frequency and achieved amplitudes that were at least two times the background amplitude, with detection in all cortical EEG channels and attenuation of the background rhythm. Simultaneous video recordings allowed the behavior of the mouse to be observed during the seizure. These data were used to assess latency to seizure onset, seizure duration, and number of seizures. Three mice failed to show seizures by EEG (two pretreated with vehicle and one pretreated with MG) and were excluded from data analysis.

Gene network

Data on seizure susceptibility and Glo1 expression in C57BL/6J × DBA/2J (BXD)-recombinant inbred (RI) lines were obtained from and analyzed using WebQTL at www.genenetwork.org (Wang et al., 2003). The Record ID for hippocampal Glo1 expression was 1424109_a_at from the Hippocampus Consortium M430v2 (Jun06) PDNN database. The Record ID for seizure susceptibility was 10388, representing data reported by McCall and Frierson (1981). Data were retrieved on January 28, 2012.

Measurement of MG concentration

MG concentration was measured in the brains of WT and Tg FVB mice by high-performance liquid chromatography (HPLC) as described previously (see supplemental data in Distler et al., 2012).

Statistical analyses

All statistical analyses were performed using StatView for Windows (SAS Institute, Inc., Cary, NC, U.S.A.). All behavioral and EEG seizure outcomes were assessed using nonparametric tests, because they were not normally distributed. Two-group comparisons were made using Mann-Whitney U tests. Three-group comparisons were made using Kruskal-Wallis tests, and post-hoc comparisons were made using Mann-Whitney U tests. The relationship between Glo1 expression and seizure phenotypes in BXD RI lines was assessed using Pearson correlations.

RESULTS

MG treatment reduces the severity and duration of picrotoxin-induced seizures

First, we investigated whether MG could prevent or attenuate seizures by administering exogenous MG (50 or 200 mg/kg) or vehicle to mice before seizure induction. We previously demonstrated that this treatment dose-dependently increases MG concentration in the brain (Distler et al., 2012). Treatment with 50 and 200 mg/kg MG is expected to increase the concentration of MG in the brain by approximately 16% and 43%, respectively, and does not cause cytotoxicity (Distler et al., 2012). After pretreatment with MG, we induced seizures using picrotoxin. Picrotoxin is a GABAA-receptor antagonist that we selected based on its ability to induce seizures in mice (Fisher, 1989) and the role of MG as a GABAA-receptor agonist (Distler et al., 2012). Pretreatment with MG dose-dependently attenuated generalized convulsions induced by 5 mg/kg picrotoxin. Specifically, MG treatment delayed seizure onset (Fig. 1A), reduced seizure duration (Fig. 1B), and reduced the percentage of animals undergoing generalized convulsions (Fig. 1C) at the behavioral level.

To validate these behavioral data, we assessed seizure activity by EEG analysis of mice treated with 7 mg/kg picrotoxin. Again, pretreatment with 200 mg/kg MG delayed seizure onset (Fig. 2A), reduced seizure duration (Fig. 2B), and reduced the number of seizures (Fig. 2C) as measured by EEG. Representative EEG traces are shown in Fig. 2D. These data indicate that MG attenuates seizures induced by GABAA-receptor blockade at both the behavioral and EEG level.

MG treatment reduces the severity and duration of pilocarpine-induced seizures

We next investigated the anti-seizure effects of MG in a mechanistically distinct seizure model. We induced seizures using pilocarpine, a muscarinic cholinergic agonist that induces severe, continuous limbic seizures after acute administration (Curia et al., 2008). We pretreated mice with MG (50 or 200 mg/kg) or vehicle and then induced seizures with 250 mg/kg pilocarpine. This dose of pilocarpine induced partial status epilepticus (stage 3 seizures), but not generalized status epilepticus, in vehicle- and MG-treated mice. Pretreatment with MG (50 and 200 mg/kg) dose-dependently delayed acute seizure onset (Fig. 3A), reduced...
Figure 1.
Exogenous MG attenuates picrotoxin-induced seizures at the behavioral level. B6 mice were pretreated with vehicle or MG (50 or 200 mg/kg) prior to seizure induction with picrotoxin (5 mg/kg). (A) MG pretreatment increased the latency to first generalized convolution; \( p = 0.0002 \) by Kruskal-Wallis test. (B) MG pretreatment reduced the duration of generalized convulsions; \( p = 0.0025 \) by Kruskal-Wallis test. (C) MG pretreatment reduced the percentage of animals undergoing generalized convulsions; \( p = 0.0033 \) by Kruskal-Wallis test. Data are mean ± standard error of the mean (SEM). \( n = 16 \) vehicle, 8 MG (50 mg/kg), 10 MG (200 mg/kg). \* \( p < 0.05 \), ** \( p < 0.005 \) versus vehicle-treated group as determined by Mann-Whitney U tests.

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Figure 2.
Exogenous MG attenuates picrotoxin-induced seizures as assessed by EEG. B6 mice were pretreated with vehicle or MG (200 mg/kg) prior to seizure induction with picrotoxin (7 mg/kg). (A) MG pretreatment increased the latency to first EEG-confirmed seizure. \( p = 0.036 \) by Mann-Whitney U test. (B) MG pretreatment reduced the duration of EEG-confirmed seizures. \( p = 0.014 \) by Mann-Whitney U test. (C) MG pretreatment reduced the number of EEG-confirmed seizures. \( p = 0.028 \) by Mann-Whitney U test. (D) Representative EEG recordings of a mouse during baseline period (prior to any injections), after acute saline and picrotoxin (7 mg/kg) injections, or after acute MG (200 mg/kg) and picrotoxin (7 mg/kg) injections. As shown in B, MG pretreatment reduced the duration of EEG-confirmed generalized seizures (high amplitude and high frequency spike discharges located between arrows). Seizures are associated with high muscle activity, as indicated by the electromyography (EMG) trace. Note: animals from both groups (MG and saline) showed myoclonic jerks associated with high amplitude single spikes (▼). EEG traces correspond to four differential recordings from each of our four subdural electrodes (EEG1 and EEG2, right cortical hemisphere; EEG3 and EEG4, left cortical hemisphere). EMG activity was recorded using two fine wires placed into the neck muscle. Calibration mark: 500 \( \mu \text{V/mm} \) and 1 s. Data are mean ± SEM. \( n = 4 \) vehicle, 5 MG (200 mg/kg). \* \( p < 0.05 \) versus vehicle-treated group as determined by Mann-Whitney U tests.

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seizure duration (Fig. 3B), and reduced the highest seizure stage reached (Fig. 3C) in response to pilocarpine.

We then investigated whether MG could stop or reduce the severity of ongoing seizures. We induced seizures with pilocarpine and then administered MG (200 mg/kg) 10 min after seizure induction. We selected this time point, because vehicle-treated mice first reach stage 3 seizures approximately 10 min after pilocarpine administration (Fig. 3A). MG treatment reduced seizure duration in mice undergoing pilocarpine-induced seizures (Fig. 3D). Neither vehicle- nor MG-treated mice returned to normal behavior during the observation period. Therefore, we conclude that this dose of MG reduced time spent in partial status epilepticus but did not eliminate seizure activity altogether. We did not explore higher doses of MG, because their potential cytotoxic effects could confound the interpretation of the results.

**GLO1 inhibition reduces seizure severity**

Next, we investigated the antiseizure effects of BrBzGCp2 (Vince et al., 1971; Thornalley et al., 1996), a pharmacologic inhibitor of GLO1. We previously demonstrated that administration of BrBzGCp2 increased MG concentration in the brain by approximately 20% (Distler et al., 2012). We pretreated mice with BrBzGCp2 or vehicle 2 h before administration of pilocarpine. Mice treated with BrBzGCp2 had shorter seizure durations than those treated with vehicle (Fig. 4). However, GLO1 inhibition did not significantly affect seizure latency or highest seizure stage reached (data not shown). These data demonstrate that increasing endogenous levels of MG reduces seizure duration. Furthermore, they indicate that GLO1 inhibition is a potential therapeutic intervention for seizures.

**Differential Glo1 expression affects seizure susceptibility and severity**

Finally, we explored whether Glo1 expression affects epileptic seizures. This may provide a link between the complex genetic architecture underlying epilepsy and MG, a novel mediator of seizures in mice. We focused on Glo1, because this gene negatively regulates MG concentration in the brain (Distler et al., 2012). We hypothesized that mice with increased Glo1 expression would display increased seizure susceptibility and severity.

We utilized data from BXD RI lines (Williams et al., 2001; Peirce et al., 2004; Shifman et al., 2006), which are derived from intercrosses between B6 and DBA/2J (D2) inbred strains. The D2 strain carries a genomic duplication of Glo1 on chromosome 17, whereas the B6 strain does not; BXD lines that inherit the duplication show an approximately twofold increase in Glo1 expression (Williams et al., 2009). We used tools at Gene Network (www.gene.network.org) to assess the correlation between Glo1 expression and published seizure phenotypes in BXD RI lines. A locus on chromosome 17 was significantly associated with seizure susceptibility at high atmospheric pressure among BXD RI lines (McCall & Frierson, 1981; Plomin et al., 1991). In this model, mice exposed to increasing pressure in a helium-oxygen atmosphere have progressive convulsive
seizures (Lever et al., 1971; Mansfield et al., 1980; McCall & Frierson, 1981). This model may be clinically relevant, because patients with epilepsy have an increased susceptibility to seizures at high atmospheric pressure (Doherty et al., 2007). We found that the locus for susceptibility to seizures at high atmospheric pressure co-localized with that of Glo1 expression (Fig. 5A,B), which we previously attributed to the Glo1 duplication (Williams et al., 2009). Indeed, BXD RI lines with the Glo1 duplication displayed a significant reduction in seizure threshold compared to those without the duplication (Fig. 5C). Furthermore, there was a significant inverse correlation between Glo1 expression and seizure threshold (Fig. 5D). This is consistent with our hypothesis that increased Glo1 expression increases seizure susceptibility. Therefore, naturally occurring differences in Glo1 expression may regulate MG concentration and thus seizure sensitivity in mice.

BXD RI lines are a convenient population for investigating the effects of differential Glo1 expression on seizure susceptibility. However, there are numerous genetic differences between the lines, making it impossible to establish a causal relationship with a particular variant. To more

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**Figure 5.**

Glo1 expression is associated with seizure susceptibility. (A) The position along the mouse genome is on the x-axis, and the logarithm of odds (LOD) score is on the y-axis. Pink and blue horizontal lines indicate genome-wide significance thresholds for Glo1 expression and seizure threshold, respectively. Hippocampal Glo1 messenger RNA (mRNA) expression was assessed by microarray (Record ID 1424109_a_at). Seizure threshold data were obtained using Record ID 10388 corresponding to data from McCall & Frierson (1981). The figure was generated by www.genenetwork.org. (B) Data from (A) were replotted for chromosome 17 only. (C) Seizure susceptibility in BXD RI lines with and without the Glo1 duplication based on their genotype at SNP rs3145545. (D) Association between Glo1 expression in the hippocampus (Record ID 1424109_a_at) and seizure threshold (Record ID 10388). Pearson correlation: \( r^2 = 0.59, p = 5.42 \times 10^{-5} \). Data were obtained from www.genenetwork.org on January 28, 2012 using the indicated Record IDs. Glo1 expression was measured as described previously (Overall et al., 2009), and each unit represents a twofold difference in expression level. For seizure threshold, units represent the pressure at which animals seized. For D, black symbols represent BXD RI lines harboring the B6 Glo1 allele (single copy), and gray symbols represent BXD RI lines harboring the D2 Glo1 allele (duplicated) based on their genotype at SNP rs3145545.
directly test Glo1’s effect on seizures, we employed Tg mice that overexpress Glo1 (Distler et al., 2012). Tg mice displayed approximately 15% less MG in the brain than WT mice (Fig. 6A). We administered pilocarpine (300 mg/kg) to WT and Tg mice and measured subsequent seizure activity. Tg mice displayed a nonsignificant trend toward reduced latency to first seizure (Fig. 6B). Tg mice displayed significantly increased seizure duration and seizure severity compared to WT mice (Fig. 6C,D). This suggests that differences in Glo1 expression or activity influence seizures. Therefore, Glo1 polymorphisms may contribute to the genetic underpinnings of epilepsy.

**Discussion**

In the present study, we demonstrated that pretreatment with MG attenuated picrotoxin- and pilocarpine-induced seizures. MG’s efficacy in two seizure models demonstrates its broad antiseizure effects across mechanistically distinct types of seizures. This is consistent with the role of MG in the central nervous system as an agonist at GABA_A receptors, which are responsible for mediating neuronal inhibitory tone (Macdonald et al., 2010). At the behavioral level, MG affected three important measures of seizures: latency to first seizure, seizure duration, and seizure severity. For picrotoxin-induced seizures, pretreatment with MG also reduced the percentage of mice exhibiting convulsive behavior. The behavioral data were corroborated by EEG recordings, demonstrating that MG pretreatment attenuated EEG-confirmed picrotoxin-induced seizures. These data have important implications. First, they demonstrate that MG, an endogenous GABA_A-receptor agonist, protects against seizures. Second, they suggest that endogenous levels of MG may mediate seizure phenotypes in vivo. Third, they open the door for further investigation of MG’s therapeutic potential in the treatment of epilepsy.

We also used a GLO1 inhibitor, BrBzGcp2, to investigate the effects of increasing endogenous MG concentration on seizures. We found that pretreatment with BrBzGcp2 decreased seizure duration. Therefore, our results highlight the potential for GLO1 inhibitors in the pharmacologic treatment of epilepsy. This would represent a novel mechanism of action among AEDs. Future studies should explore whether GLO1 inhibition might also protect against neuronal damage associated with seizures. Current AEDs act mainly through modulating ion channels, including GABA_A receptors (Brodie et al., 2011). GLO1 inhibition, on the other hand, would increase MG concentration in proportion with its endogenous production. This might avoid some of the adverse effects caused by traditional AEDs, a possibility that should be investigated in future studies.

MG levels increase under conditions of high metabolic load, positioning MG as an intermediate between metabolic state and neuronal inhibitory tone. Therefore, metabolic interventions that raise endogenous MG levels could be a promising therapeutic approach for epilepsy without the adverse side effects of AEDs (Brodie et al., 2011; Perucca & Tomson, 2011; Rossetti & Lowenstein, 2011). For instance, the ketogenic diet (KD) is a high-fat, low-carbohydrate diet that is administered to patients with epilepsy who do not respond to AEDs (Payne et al., 2011; Rossetti & Lowenstein, 2011). The mechanism by which KD controls seizures is unknown, but it has been hypothesized that the KD might increase MG levels (Beisswenger et al., 2005; Gasior et al., 2007; Hartman et al., 2007; Kalapos, 2007). In addition, other manipulations that increase MG could be investigated as novel methods for controlling seizures.

Finally, we investigated whether genetic polymorphisms regulating MG concentration underlie genetic susceptibility to seizures. Specifically, we examined the effect of Glo1 expression on seizures. We found that that differential expression of Glo1 in BXD RI lines was associated with susceptibility to seizures at high atmospheric pressure. We note that no other seizure phenotype from Gene Network (handling-induced convulsions, pentylenetetrazol-induced seizures, and audiogenic seizures) was significantly associated with Glo1 expression in BXD RI lines. We then used Tg mice overexpressing Glo1 to establish a causal role for Glo1 in increasing seizure susceptibility and severity. Tg mice had reduced MG concentration in the brain and increased susceptibility to pilocarpine-induced seizures (Fig. 6). Therefore, we speculate that genetic variants in Glo1 may contribute to epilepsy.

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Our previous work established a causal role for Glo1 in increasing anxiety-like behavior by reducing the concentration of MG in the brain (Distler & Palmer, 2012; Distler et al., 2012). In those studies, we did not observe hypolocomotion (sedation) or ataxia at the 50 mg/kg dose; however, 100 mg/kg produced sedation and 300 mg/kg produced ataxia. We have not observed sedation or ataxia following any dose of BrBzGCp2. Taken together, these observations suggest a therapeutic window in which antiepileptic effects can be achieved without negative side effects, such as sedation and ataxia. More broadly, the present results emphasize the importance of GLO1 and MG in the central nervous system. This pathway plays an important role in neuronal physiology through regulating neuronal inhibitory tone as well as associated pathophysiological conditions, including anxiety and epilepsy. Finally, our results may provide insight into the high comorbidity between epilepsy and anxiety disorders (Beyenburg et al., 2005; LaFrance et al., 2008; Schmidt, 2009; Titlic et al., 2009). There is a 25–50% prevalence of anxiety disorders among patients with epilepsy, which is twice the prevalence among the general population (LaFrance et al., 2008). The prevalence of comorbid anxiety disorders is especially high in patients with epilepsy who do not respond to AEDs (LaFrance et al., 2008; Schmidt, 2009). This could suggest a convergent pathologic mechanism that is not targeted by current AEDs. Polymorphisms in Glo1 and other genes that affect MG concentration may contribute to both anxiety and epilepsy, providing a common underlying pathway.

In conclusion, the present study demonstrated an important physiologic role for MG in reducing seizure susceptibility and severity. Increasing endogenous MG by GLO1 inhibition had a similar effect and may be a useful therapeutic strategy. Finally, polymorphisms in Glo1 that regulate MG concentration may contribute to the genetic underpinnings of epilepsy and comorbid anxiety disorders.

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Disclosure

None of the authors has any conflict of interest to disclose. 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.

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