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GLO1 inhibitors for neuropsychiatric and anti-epileptic drug development

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Abstract

Many current pharmacological treatments for neuropsychiatric disorders, such as anxiety and depression, are limited by a delayed onset of therapeutic effect, adverse side effects, abuse potential or lack of effect in many patients. These off-target effects highlight the need to identify novel mechanisms and targets for treatment. Recently, modulation of glyoxalase 1 (GLO1) activity was shown to regulate anxiety-like behavior and seizure susceptibility in mice. These effects are likely mediated through the regulation of methylglyoxal (MG) by GLO1, as MG acts as a competitive partial agonist at $GABA_A$ receptors ($GABA_ARs$). Thus, modulation of MG by GLO1 represents a novel target for treatment. Here, we evaluate the therapeutic potential of indirectly modulating MG concentrations through GLO1 inhibitors for the treatment of neuropsychiatric disorders.

Keywords

GLO1 Inhibitor; Glyoxalase 1; Methylglyoxal; neuropsychiatric disorders; epilepsy; treatment

Introduction

Anxiety and depressive disorders affect one in four adults at some point in their lifetime, while epilepsy affects one in fifty[1,2]. Although a variety of pharmaceuticals are available to treat these neuropsychiatric disorders, illness remains refractory in a significant portion of patients and many currently used drugs have adverse side effects and high abuse potential.

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Thus, identification of new biological targets and novel pharmaceuticals remains an important goal in treating these disorders [3,4].

Recent studies have identified glyoxalase 1 (GLO1) as a new target for neurological and psychiatric conditions. Increased *Glo1* gene-expression is associated with anxiety- and depression-like behavior as well as seizure susceptibility in mice[5–9]. GLO1 is a ubiquitous cytosolic enzyme that catalyzes the reaction between glutathione and acyclic α-oxoaldehydes, particularly methylglyoxal (MG)[10–13]. MG is formed as a byproduct during photosynthesis, protein and fatty acid catabolism and glycolysis; principally by the non-enzymatic degradation of acetone, aminoacetone and the glycolytic intermediates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate[14]. *In vitro* studies have demonstrated a critical role for GLO1 in clearing MG; indeed, overexpression of *Glo1* prevents MG accumulation, while GLO1 inhibition results in MG accumulation [10–13].

Historically, most research on GLO1 has focused on the importance of detoxification of MG to prevent cellular damage due to the glycation of proteins and nucleic acids[15,16]. These studies have implicated high concentrations of MG and/or low GLO1 activity in the etiology of metabolic disorders, such as diabetes and in the development of cellular pathologies including aging[13,17]. Thus strategies to reduce MG concentrations and/or enhance GLO1 activity have therapeutic potential. In contrast, many cancers exhibit enhanced GLO1 activity; it has been suggested that inhibition of GLO1 would therefore have anticancer properties [15,18–20].

In addition, recent studies from several labs indicated that modulation of MG concentrations and GLO1 activity can alter anxiety, depression, seizure, sleep, and pain phenotypes in mice [6,7,21–23]. Therefore, increasing MG concentrations by inhibiting GLO1 may also represent a novel strategy for the treatment of neuropsychiatric and epileptic disorders. In this review, we will focus on evaluating the therapeutic potential of utilizing GLO1 inhibitors to indirectly modulate neurophysiology by reducing the rate of MG clearance in the CNS.

Glo1 and methylglyoxal in neuropsychiatric disorders and epilepsy

In mice, a positive correlation between *Glo1* expression and anxiety-like behavior was first reported among a panel of inbred mouse strains, and has since been corroborated by numerous studies[24–28]. Subsequent studies confirmed a causal role for *Glo1* in anxiety-like behavior using viral vectors and transgenic mice to show that *Glo1* overexpression increased anxiety-like behavior, while knockdown decreased anxiety-like behavior[24]. However, human genetic studies have yielded discrepant results regarding the association between *Glo1* and anxiety[29,30]. Interpretation of these data in humans is limited by small sample sizes and potential population stratification. Larger, well-controlled human genetic studies are required to elucidate the role of *Glo1* in human anxiety disorders.

In addition to anxiety, there is strong evidence that *Glo1* regulates other neuropsychiatric phenotypes in mice, including epilepsy, depression and neuropathic pain. For example, increased seizure susceptibility was associated with high *Glo1* expression among recombinant inbred mice and transgenic mice overexpressing *Glo1*[6]. Also, there is a clear,

positive correlation between GLO1 protein levels and depression-phenotype as assessed by the tail-suspension test (TST)[9]. While some studies have also suggested a role for Glo1 in human neuropsychiatric diseases the evidence is usually less compelling and is limited by small sample size and a lack of replication. For example, one study reported a negative correlation between *Glo1* expression and depression; additional studies have reported negative correlation between *Glo1* expression and neuropathic pain, as well as associations between *Glo1* expression and autism, schizophrenia, and restless legs syndrome[21,31–44]. At this time, rigorous analysis to determine the impact of *Glo1* expression levels, copy number variants or polymorphisms on the etiology or pathogenesis of human neuropsychiatric disorders is lacking.

Mechanism of action - GABA receptors and MG

We recently reported that physiological levels of MG (low μM) are anxiolytic in mice by a simple mechanism: MG is a specific, partial, reversible agonist of $GABA_A$Rs in central neurons[7]. $GABA_A$Rs are pentameric, ligand-gated ion channels, and are comprised of two α-subunits (α_{1-6}), two β-subunits (β_{1-4}) and one γ_{1-4}, δ, ε, θ, π or ρ_{1-3} subunit. The namesake ligand for $GABA_A$Rs is γ-aminobutyric acid (GABA). In the adult brain GABA serves as an inhibitory neurotransmitter. Binding of GABA to specific pockets at the interface of α and β-subunits opens a channel in the center of $GABA_A$Rs, this hyperpolarizes the membrane potential by passing Cl^- ions. $GABA_A$Rs are present both at synapses and on the soma of neurons, and produce phasic and tonic currents, respectively[45–47]. Application of MG to cerebellar granule (CGN) or hippocampal neurons (HN) evokes Cl^- currents that modulate the membrane potential and are blocked by the $GABA_A$ specific antagonist SR-95531[7]. MG evoked currents are ~ ⅓of the magnitude of those evoked by GABA in the same cells and co-application with GABA is competitive, not additive, suggesting that both ligands act at the same binding site[7]. Importantly, the concentration of MG required to evoke currents in neurons is in the physiological range and the EC_{50} measured from the concentration-response relationship is ~10 μM, suggesting that small changes in concentration of MG will produce marked effects in the current magnitude. Based on these observations, MG can be described as an endogenously produced competitive partial agonist at $GABA_A$Rs at physiologically relevant concentrations (Figure 1A).

Alterations in GABAergic signaling are implicated in numerous neurological and psychiatric disorders, including depression, panic, schizophrenia, Huntington's, Parkinson's, Alzheimer's, epilepsy, sleep, and chronic pain syndromes[45]. Many commonly prescribed anxiolytic agents, such as the benzodiazepine, midazolam, target extrasynaptic $GABA_A$Rs with the aim of augmenting tonic inhibition[47,48]. Extracellular $GABA_A$Rs frequently contain α_5/α_6 and δ subunits; assemblies that are prominent in hippocampal and neocortical pyramidal neurons ($\alpha_5\beta\gamma_2$) and CGN ($\alpha_6\beta\delta$)[49]. The action of MG at extrasynaptic $GABA_A$Rs may be of particular relevance to pathophysiology because the concentration of GABA at extrasynaptic receptors is low (<μM), while MG has been measured at ~5 μM in mouse brain[7,46].

Benzodiazepines are positive allosteric modulators of $GABA_A$Rs, augmenting inhibitory currents when GABA binds[47]. Two such benzodiazepines (midazolam and diazepam) also augment GABAergic Cl^- currents when MG binds to $GABA_A$R in HNs. Similarly, the effects of MG are augmented by zolpidem, a non-benzodiazepine, imidazopyridine-based positive allosteric modulator of $GABA_A$Rs[7] (Figure 1B). It is not yet known whether the activity or efficacy of benzodiazepines at specific $GABA_A$R subtypes differs between MG- and GABA-induced activation. However, the studies described above suggest that MG can activate $GABA_A$Rs that contain diazepam- and midazolam-sensitive $\alpha_{1\text{-}3}$ and α_5 subunits as well as receptors with zolpidem- sensitive α_1 and γ_2 subunits. This array of subunits is common in areas of the brain associated with anxiety and depressive disorders, including hippocampal and cortical interneurons ($\alpha_1\beta_2\gamma_2$ receptors) and the limbic system ($\alpha_2\beta_X\gamma_1$ receptors).

GABA analogues have also been considered as potential therapeutics, particularly for acute conditions, such as seizure or mania. However, this strategy has been hampered by significant challenges; principally, that GABA is highly polar and flexible and activates $GABA_B$ and $GABA_C$ receptors in addition to $GABA_A$Rs. In contrast to GABA, MG does not activate neuronal $GABA_B$Rs; the effects of MG at $GABA_C$Rs have yet to be characterized. MG can easily cross the blood-brain barrier[7]; thus, MG precursors or MG bioisosteres might be clinically useful compounds.

In summary, activation of $GABA_A$Rs by MG is a promising approach for treatment of neuropsychiatric disorders and other diseases linked to GABA signaling. Possible approaches include GLO1 inhibition or administration of MG precursors or bioisosteres.

Therapeutic potential of GLO1 inhibitors

Current drug-therapies for depression are limited by negative side effects, including sexual dysfunction, weight gain and insomnia, and require several weeks to produce their full therapeutic effect. Similarly, anxiolytic and anti-epileptic drugs are limited by their sedating effects and abuse potential. Identification of novel molecular targets may provide alternatives with fewer or different side effects. Additionally, the identification of targets with applications in multiple disorders is particularly beneficial as drug development is time consuming and expensive. Given its role in multiple neuropsychiatric disorders, agents that modulate MG levels might be of benefit as next generation treatments.

However, MG is highly bioreactive, modifying arginine and lysine residues in proteins and has been shown to be directly toxic to cells *in vitro*, inducing apoptosis when applied at concentrations > 100 μM. Thus, instead of direct administration of MG, an alternative and perhaps more promising strategy is to raise MG levels by inhibiting the GLO pathway. Application of a GLO1 inhibitor is expected to potentiate the activity of $GABA_A$Rs by reducing the degradation of MG to augment basal levels in the brain (Figure 2). This mechanism of action is fundamentally different to the action of commonly prescribed GABAergic drugs because it depends on the local accumulation of a competitive partial agonist rather that positive allosteric modulation of $GABA_A$Rs. Therefore, GLO1 inhibition is likely to cause anatomically and pharmacologically distinct responses to those observed

following treatment with benzodiazepines and barbiturates. Early studies already support a role for GLO1 inhibition in modulating behavioral phenotypes. For instance, GLO1 inhibition by S-bromobenzylglutathione cyclopentyl diester (BrBzGCp2) increased MG concentration and reduced anxiety-like behavior in mice [7] (Figure 3A–D). Similarly, BrBzGCp2 attenuated epileptic seizures in mice [6](Figure 3E). To date we have not observed undesirable side effects following treatment with anxiolytic and anti-epileptic doses of BrBzGCp2 (unpublished data), however more work is needed to address this obvious concern.

Using the TST, Benton and colleagues reported a positive correlation between *Glo1* expression and depression-like behavior in mice[27,50]. This observation appears surprising in light of the link between Glo1, MG and GABA, since no other $GABA_AR$ agonists (e.g. barbiturates and benzodiazepines) reduce immobility on the TST[50]. While GLO1 inhibitors have not been evaluated for their efficacy in depression-like behaviors, these data suggest that GLO1 inhibition may have antidepressant activity, likely by increasing MG levels. While anxiolytic drugs that modulate GABAergic signaling, such as benzodiazepines, are not effective for treatment of depression, recent evidence shows that co-administration of the serotonin-selective reuptake inhibitor fluoxetine with eszopiclone (a partial agonists at $GABA_ARs$ that contain α_1, α_2 or α_3 subunits) has a greater antidepressant effect than fluoxetine alone, suggesting a role for GABAergic drugs in the treatment of depression[51,52]. In conjunction with the correlation between *Glo1* and depression-like behavior on the TST, these data reflect a role for $GABA_ARs$ in the treatment of depression and highlight the potential utility of GLO1 inhibition versus classical anxiolytics for regulating GABAergic signaling.

Current GLO1 inhibitors, such as BrBzGCp2, are frequently based on the glutathione scaffold and have been patented for a variety of disorders[53–57]. Flavonoids, curcumin and other non-peptidic reagents have also been evaluated for their GLO1 inhibitory activity[58–62]. Although these compounds generally inhibit GLO1 activity with therapeutically useful K_i, utilizing native structures such as glutathione as a scaffold *a priori* increases the risk of interaction with other signaling pathways and could result in undesired off-target effects or limited bioavailability [58,62,63]. Poor cell permeability has also hampered the utility of some glutathione analogs and flavonoids *in vitro*, while poor absorption and bioavailability have limited the success of curcumin in human trials [60,62,63]. Further, many existing inhibitors of GLO1 were intended as anti-tumor agents and as such, have frequently been evaluated *in vitro* for their ability to inhibit cellular proliferation and induce apoptosis in tumor cells at high concentrations [58–61]. Thus, a key question is whether doses of GLO1 inhibitors can be identified produce therapeutic effects without also producing undesired effects such as increases in neuropathic pain. Identification or synthesis of novel GLO1 inhibitors could address the limitations of current inhibitors by reducing off target effects and minimizing side effects. Ultimately, the therapeutic viability of GLO1 inhibitors requires the identification of an inhibitor with excellent oral availability, a favorable pharmacokinetics and dynamics and negligible toxicity after chronic treatment.

Although mounting evidence shows that GLO1 inhibitors may have applications in the treatment of anxiety, depression and epilepsy, a negative correlation was observed between

Glo1 copy number and sensitivity to neuropathic pain in diabetic mice[64,65]. Subsequent mechanistic studies demonstrated that overexpression of human GLO1 reduced hyperalgesia in diabetic mice[21]. Although a correlation between a SNP in *Glo1* and diabetic neuropathy among type 2 diabetics has been reported in humans, the effect was not statistically significant when corrected for multiple comparisons [34]. Recent work has demonstrated decreased GLO1 activity in patients with painful diabetic neuropathy as compared to those with painless diabetic neuropathy, suggesting a role for GLO1 in pain [33]. The mechanism of MG-induced hyperalgesia has been attributed to protein modification and activation of TRPA1 receptors [21,66]. Such studies underscore the need to assess the potential cytotoxic consequences of GLO1 inhibition and suggest that GLO1 inhibitors may be contraindicated in diabetic patients[21,66].

Conclusions

While effective in many cases, current drug therapies for neuropsychiatric disorders and epilepsy are plagued by confounding off-target effects and often carry a risk for addiction in patients, generating the need for novel pharmaceuticals to treat these debilitating disorders. Therapeutic treatment by GLO1 inhibition/MG accumulation would provide a pharmacological avenue for anxiety and other mental health issues that is fundamentally distinct from the current pharmacopeia, such as positive allosteric modulators of $GABA_A$Rs. The neuroanatomical distribution of MG production will influence the effects of GLO1 inhibition as local MG production would dictate regions of accumulation. MG is cell-permeable, and as such, it is possible that MG preferentially acts at extrasynaptic $GABA_A$Rs where concentrations of GABA are low. Insufficient inhibition of neuronal excitability in amygdala-prefrontal cortex circuitry could explain GLO1/MG control over anxiety and depression-like behavior and would suggest a role for GLO1/MG in the regulation of behaviors associated with anxiety and depression.

Thus, GLO1 inhibition has the potential to improve efficacy, reduce side effects and ultimately treat multiple highly comorbid disorders. While evidence in mice suggests that GLO1 inhibition alters behavior, concerns about neuropathic pain and cytotoxicity mandate further exploration and characterization of lead compounds to properly evaluate the therapeutic efficacy of GLO1 inhibitors for the treatment of neuropsychiatric disorders and epilepsy.

Abbreviations

GLO1	Glyoxalase 1
MG	Methylglyoxal
GABA	γ-aminobutyric acid
TST	tail suspension test
CGN	cerebellar granule
HN	hippocampal neurons

BrBzGCp2 S-bromobenzylglutathione cyclopentyl diester

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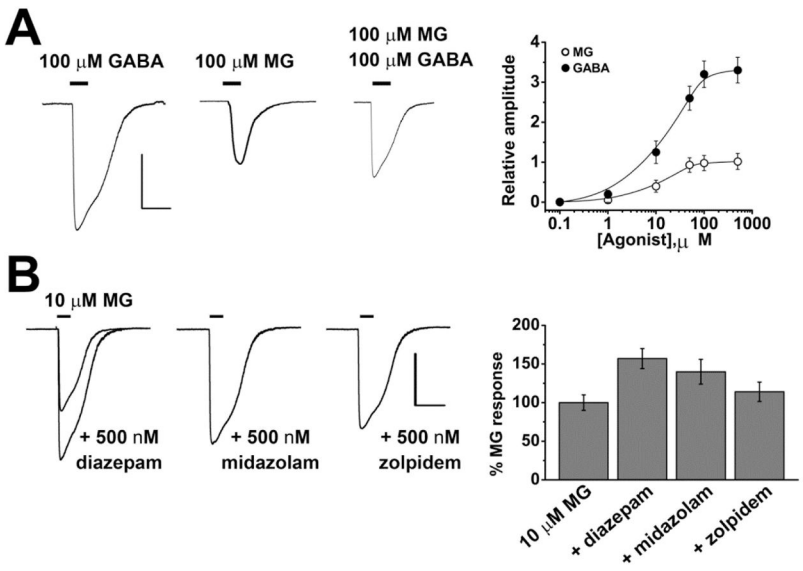

Figure 1. MG is an endogenous, partial agonist at neuronal GAB_AA receptors

(A) The application of 100 μM MG to hippocampal neurons evokes Cl− currents through $GABA_A$ receptors that are ~ ⅓ the magnitude of those evoked by 100 μM GABA in the same cells. The EC50 of the currents evoked by MG was 9.5 ± 1 μM and the physiological concentration of MG in rodent brain was measured at 5 μM. MG has a similar efficacy when applied to cerebellar granule neurons. **(B)** MG evoked currents in hippocampal neurons are augmented by co-application of classical anxiolytics that act as positive allosteric modulators of $GABA_A$Rs, such as the benzodiazapenes diazepam and midazolam and the imidazopyridine zolpidem. Scale bars represent 1 nA and 10 s. Adapted from Distler et al., (2012)[7].

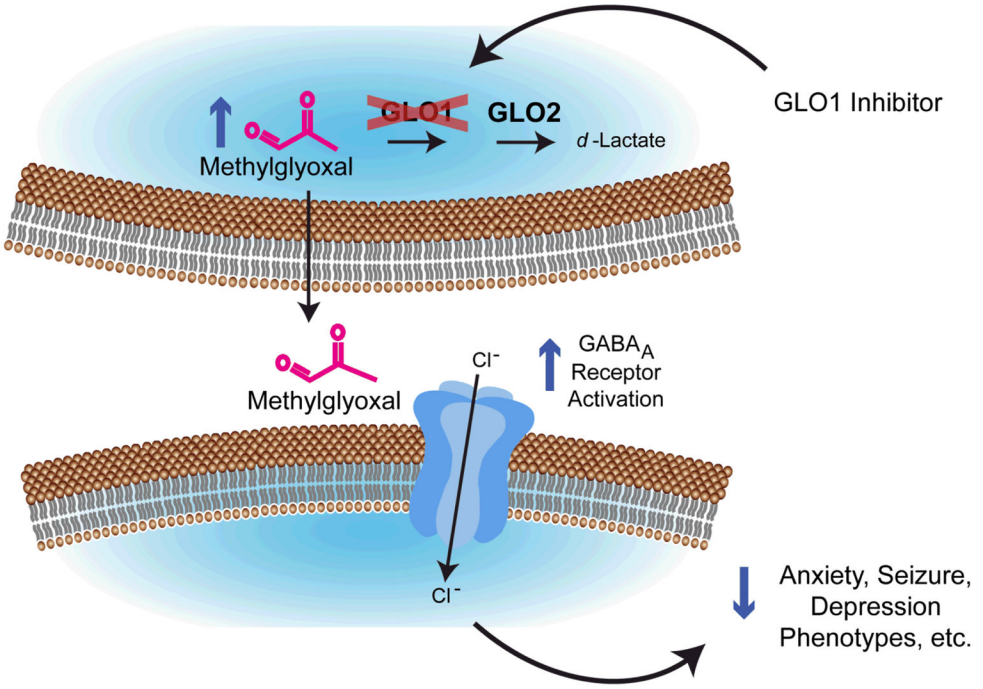

Figure 2. A model for GLO1 Inhibition in the treatment of neuropsychiatric disorders and epilepsy

Treatment with GLO1 inhibitors will increase concentrations of methylglyoxal due to decreased clearance by GLO1. Increased methylglyoxal will result in increased activation of GABAA receptors and subsequently, a decrease in neuropsychiatric disorder phenotypes (ie. reduced anxiety, depression and seizure). Adapted from Distler and Palmer (2013)[67].

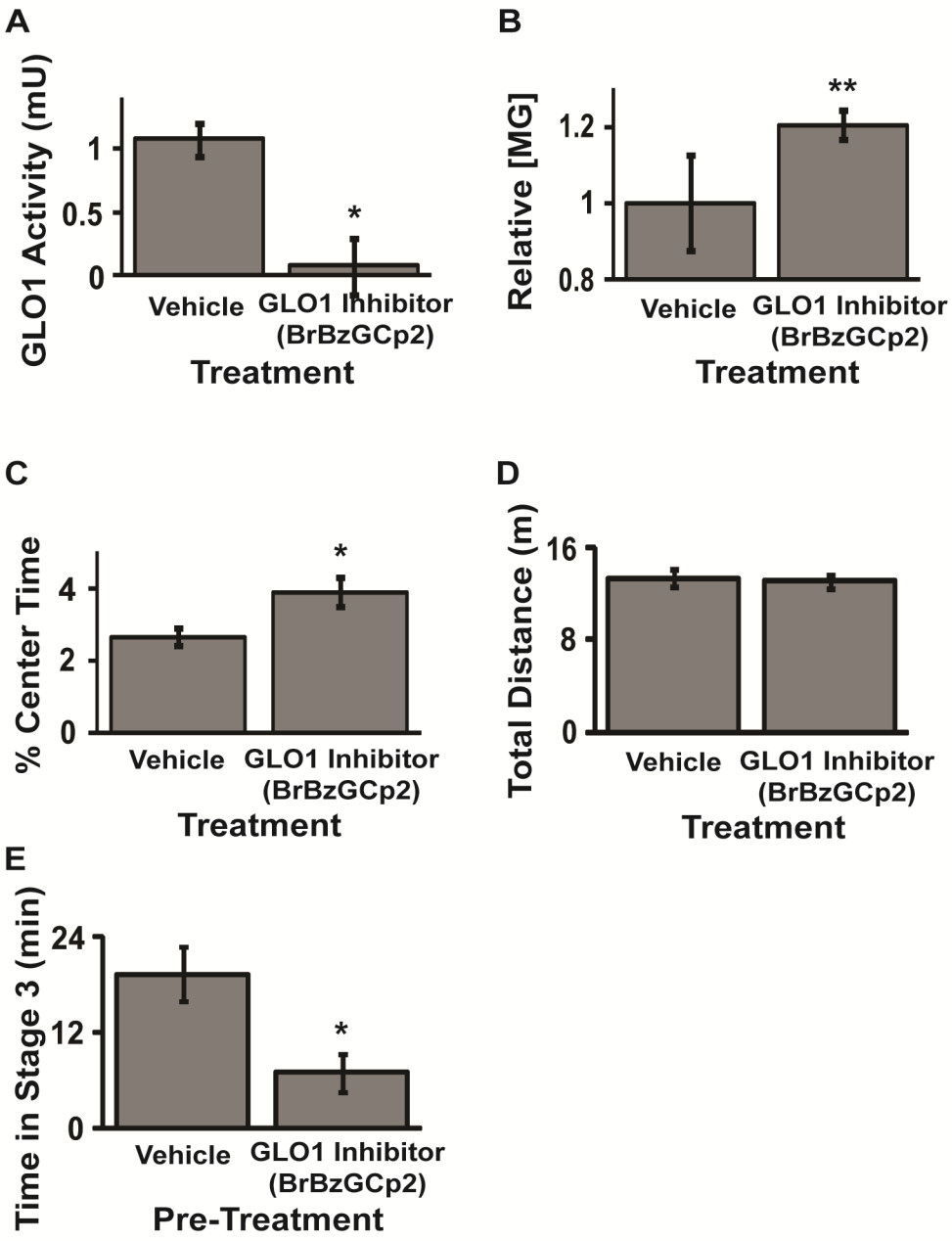

Figure 3. Systemic administration of a GLO1 inhibitor regulates MG concentration, reduces anxiety-like behavior and attenuates seizure in mice

Pharmacological inhibition of GLO1 by BrBzGCp2 **(A)** reduces GLO1 enzymatic activity; **(B)** increases concentrations of MG in whole brain of mice 2 hrs after i.p. treatment; **(C)** reduces anxiety-like behavior in the open field test (C57BL6/J mice) without affecting total distance traveled **(D)**; and **(E)** attenuates pilocarpine-induced seizures (50 mg/kg prior to pilocarpine (250mg/kg)). Adapted from Distler et al. (2012) and (2013)[6,7].