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Glutamate carboxypeptidase II (GCPII) inhibitor displays anti-glutamate and anti-cocaine effects in an invertebrate assay

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Summary

Glutamate carboxypeptidase II (GCPII) inhibitors are promising anti-glutamatergic and anti-addictive agents. We hypothesized that a GCPII inhibitor 2 (phosphonomethyl)pentanedioic acid (2-PMPA) would display anti-stereotypical activity in planarians. Experiments revealed that 2-PMPA displayed no overt behavioral activity by itself but attenuated stereotypical counts (C-shape hyperkinesias) elicited by four compounds (2-PMPA rank order potency: glutamate > NMDA > pilocarpine > cocaine). These data suggest GCPII inhibitors display broad-spectrum efficacy against behavioral activity produced by glutamatergic and non-glutamatergic compounds in an invertebrate assay.

Keywords

GCPII; glutamate; 2-PMPA; cocaine; planaria; NMDA; pilocarpine; stereotypy

Introduction

Glutamate carboxypeptidase II (GCPII) is an astrocytic protein expressed in human and rat brain that converts N-acetylaspartylglutamate (NAAG), a peptide neurotransmitter and metabotropic glutamate receptor 3 agonist, into glutamate and N-acetylaspartate. GCPII inhibitors attenuate neuropathic pain in rats, prolong survival in a mouse model of amyotrophic lateral sclerosis, and are neuroprotective in models of ischemic brain injury (Neale et al., 2005). GCPII inhibitors also attenuate cocaine-induced reinstatement of drug seeking behavior, behavioral sensitization, conditioned place preference, and seizure activity in mammals (Xi et al., 2010; Slusher et al., 2001; Witkin et al., 2002). What remains unclear is the extent to which the pharmacological effects of GCPII inhibitors are conserved across different species and extend to non-glutamatergic systems. We used an invertebrate assay (planarians) to investigate the effects of a GCPII inhibitor, 2 (phosphonomethyl)pentanedioic acid (2-PMPA), on stereotypical activity produced by glutamatergic agents (glutamate, NMDA), a stimulant (cocaine), and a cholinergic agonist (pilocarpine) (Rawls et al., 2010). Planarians are flatworms that possess a centralized nervous system and display mammalian-like behaviors during exposure to proconvulsants and addictive substances, including enhanced stereotypical activity, behavioral sensitization, and conditioned place preference (Kusayama and Watanabe, 2000; Raffa and Valdez, 2001;

Rawls et al., 2009; Palladini et al., 1996; Pagan et al., 2008; Rowlands and Pagán, 2008). We now report that a GCPII inhibitor displays anti-stereotypical effects in planarians.

Methods

Planarians (*Dugesia dorotocephala*) purchased from Carolina Biological Supply were acclimated to room temperature (21 °C) and tested within 3 days of receipt. L-glutamic acid, NMDA, pilocarpine hydrochloride, and 2-PMPA were purchased from Tocris Biosciences. Cocaine hydrochloride was provided by the National Institute on Drug Abuse. Solutions were prepared daily in tap water containing AmQuel® water conditioner.

Individual planarians were placed into a plastic petri dish (5.5 cm diameter) containing a drug or drug combination and activity counts - defined as the number of C-like hyperkinesias - were quantified during 5 min of exposure (Rawls et al., 2009). In control experiments, planarians were exposed to drug-free water or 2-PMPA (0.01, 0.1, 1, 10, 100 μ M) for 5 min. In a second series of experiments, planarians were exposed for 5 min to a combination of 2-PMPA (0, 0.01, 0.1, 1, 10, 100 μ M) and a fixed concentration of glutamate (10 mM); NMDA (5 mM); pilocarpine (50 mM); or cocaine (1 mM). Concentrations of glutamate, NMDA, pilocarpine, and cocaine were based on prior work in planarians (Rawls et al., 2009, 2010). Comparisons of group means ($\pm$ S.E.M.) were evaluated by one-way ANOVA followed by a Dunnett's *post-hoc* analysis. Values of $p < 0.05$ were considered statistically significant. Approximate IC_{50} values for 2-PMPA against each proconvulsant were determined using GraphPad Prism (GraphPad Software). For each concentration (0.01, 0.1, 1, 10, 100 μ M) of PMPA, the percentage of control response was determined and plotted against log PMPA concentration [PMPA]. The best-fit linear regression was then determined for each group.

Results

Control experiments revealed that planarians exposed to drug-free water or 2-PMPA (0.01, 0.1, 1, 10, 100 μ M) did not display C-like hyperkinesias (*i.e.*, stereotypical counts). Prior work has also demonstrated that planarians exposed to drug-free water do not display significant stereotypical activity (Rawls et al., 2009, 2010). Planarians exposed to glutamate (10 mM), NMDA (5 mM), pilocarpine (50 mM), and cocaine (1 mM) displayed 41.8 ± 1.9 , 27.3 ± 1.2 , 24.6 ± 1.3 , and 24.8 ± 1.2 stereotypical counts, respectively (Fig. 1). For combination experiments with 2-PMPA and glutamate, one-way ANOVA indicated a significant main effect [$F(5, 42) = 51.75$, $p < 0.0001$] (Fig. 1). Each 2-PMPA concentration (0.01, 0.1, 1, 10, 100 μ M) inhibited stereotypy elicited by glutamate. Similar effects were observed in combination experiments with 2-PMPA and NMDA, as one-way ANOVA revealed a significant main effect [$F(5, 42) = 31.78$, $p < 0.0001$] (Fig. 1). Each concentration of 2-PMPA reduced the number of stereotypical counts induced by NMDA. One-way ANOVA for combination studies with 2-PMPA and pilocarpine revealed a significant main effect [$F(5, 42) = 8.944$, $p < 0.0001$] (Fig. 1). Pilocarpine-evoked stereotypical counts were lower in planarians co-treated with 2-PMPA concentrations of 1 μ M, 10 μ M, or 100 μ M. For combination experiments with 2-PMPA and cocaine, one-way ANOVA revealed a significant main effect [$F(5, 42) = 4.762$, $p < 0.01$] (Fig. 1). Cocaine-induced stereotypical counts were lower in planarians co-treated with 2-PMPA concentrations of 1 μ M, 10 μ M, or 100 μ M. Approximate IC_{50} values for PMPA were (proconvulsant, IC_{50}): (glutamate, 0.69 μ M with 95% confidence limits of 0.10 to 3.70); (NMDA, $> 10 \mu$ M); (pilocarpine, $> 100 \mu$ M); and (cocaine, $> 100 \mu$ M).

Discussion

We provide the first evidence that a GCPII inhibitor (2-PMPA) displays efficacy in planarians, the simplest animal to possess a body plan common to all vertebrates, and most invertebrates, and a model organism for studying drug action (Raffa and Rawls, 2008). 2-PMPA decreased stereotypical activity elicited by a group of pharmacologically diverse compounds that target the glutamatergic, cholinergic, and dopaminergic systems. The rank order potency of 2-PMPA was glutamate > NMDA > pilocarpine > cocaine. The strength of the 2-PMPA effect against glutamate- and NMDA-induced stereotypical activity is consistent with the documented anti-glutamate mechanism of 2-PMPA in mammals and is supported by planarian studies demonstrating that compounds which reduce glutamate transmission, specifically glutamate transporter activators and NMDA/AMPA receptor antagonists, attenuate stereotypical activity induced by glutamate agonists (Rawls et al., 2009, 2010; Raffa et al., 2010). 2-PMPA also antagonized stereotypical activity produced by pilocarpine and cocaine albeit the inhibition was less pronounced.

The most effective concentrations of 2-PMPA in our study ranged from 1 – 100 μ M. Our results are consistent with the prior demonstration that GCPII inhibition by 2-PMPA rescues neurons from neurotoxicity elicited by the HIV glycoprotein gp120III (Thomas et al., 2009). In the Thomas et al. (2009) study, 2-PMPA protected hippocampal neurons from gp120IIIB-induced apoptosis in a concentration-dependent manner, with concentrations between 1 and 10 μ M producing maximal protection. Efficacious concentrations of 2-PMPA in both planarians and hippocampal neurons were previously reported to be inactive in more than 100 different receptor, transporter, ion channel, and enzyme assays (Slusher et al., 1999).

Planarians synthesize neurotransmitters targeted by the compounds used here, including glutamate, glycine, GABA, acetylcholine, and dopamine (Vyas et al., 2011; Eriksson and Panula, 1994; Nishimura et al., 2010). A specific GCPII isoform has not yet been identified in planarians, but variants of mammalian GCPII are expressed in non-mammalian species (e.g. earworms, crayfish). On the basis of findings in the crayfish, one explanation for the 2-PMPA effects observed here is that GCPII is activated in response to enhanced glutamatergic and cholinergic transmission (Urazaev et al., 2005). In the case in which planarians are co-exposed to 2-PMPA and a glutamatergic or cholinergic agonist, the resulting GCPII block attenuates the stereotypical response normally caused by increased glutamatergic or cholinergic transmission. Although it is likely that 2-PMPA acted through a glutamatergic mechanism to elicit anti-stereotypical effects in planarians, it is conceivable that a non-glutamatergic mechanism also contributed to its effects. For example, an *in vivo* microdialysis study conducted in rats suggests that 2-PMPA displays anti-allodynic activity through a mechanism that does not involve NAAG or mGluR3 receptors (Nagel et al., 2006).

In summary, the ability of 2-PMPA to attenuate stereotypy induced by glutamatergic, cholinergic, and dopaminergic agonists suggests that GCPII inhibitors display efficacy against a broad range of pharmacologically diverse compounds in planarians and that certain aspects of GCPII pharmacology may be conserved across non-mammalian and mammalian species. Future work in planarians will determine if GCPII inhibitors display efficacy against additional behavioral endpoints in planarians (e.g. behavioral sensitization, abstinence-induced withdrawal, conditioned place preference) and investigate the exact mechanism of 2-PMPA as more knowledge about the planarian genome becomes available.

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Fig. 1. Effects of 2-PMPA (0.01, 0.1, 1, 10, 100 μ M) on stereotypical counts produced by glutamate (10 mM), NMDA (5 mM), pilocarpine (50 mM), or cocaine (1 mM) in planarians. Data are expressed as mean stereotypical counts $\pm$ S.E.M. over the 5-min observation interval. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to group treated with glutamate, NMDA, pilocarpine, or cocaine by itself. N = 8 planarians per group.