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SHORT COMMUNICATION

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

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Abstract 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. 2010a, b; 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.

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Individual planarians were placed in 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 μM) 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 μM) 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 (±SEM) 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 μM) 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 μM) 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 μM) 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, 10, or 100 μM. 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, 10, or 100 μM. Approximate IC_{50} values for PMPA were (proconvulsant, IC_{50},): (glutamate, 0.69 μM with 95% confidence limits of 0.10–3.70), (NMDA >10 μM), (pilocarpine >100 μM), and (cocaine >100 μM).

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,

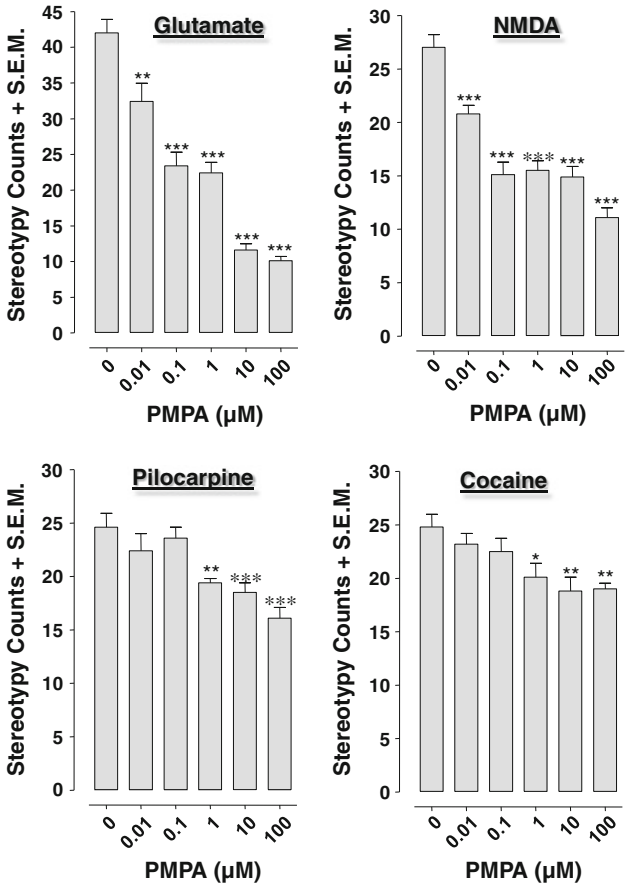

Fig. 1 Effects of 2-PMPA (0.01, 0.1, 1, 10, 100 μM) on stereotypical counts produced by glutamate (10 mM), NMDA (5 mM), pilocarpine (50 mM), or cocaine (1 mM) in planarians. Data are expressed as mean stereotypical counts ± SEM over the 5 min observation interval. *p <0.05, **p <0.01, ***p <0.001 compared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

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). 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 μM. 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 μM 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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References

Eriksson KS, Panula P (1994) gamma-Aminobutyric acid in the nervous system of a planarian. J Comp Neurol 345:528–536

Kusayama T, Watanabe S (2000) Reinforcing effects of methamphetamine in planarians. Neuroreport 11:2511–2513

Nagel J, Belozertseva I, Greco S, Kashkin V, Malyshkin A, Jirgensons A, Shekunova E, Eilbacher B, Bespalov A, Danysz W (2006) Effects of NAAG peptidase inhibitor 2-PMPA in model chronic pain-relation to brain concentration. Neuropharmacology 51:1163–1171

Neale JH, Olszewski RT, Gehl LM, Wroblewska B, Bzdega T (2005) The neurotransmitter *N*-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy, CNS injury and schizophrenia. Trends Pharmacol Sci 26:477–484

Nishimura K, Kitamura Y, Taniguchi T, Agata K (2010) Analysis of motor function modulated by cholinergic neurons in planarian *Dugesia japonica*. Neuroscience 168:18–30

Pagán OR, Rowlands AL, Azam M, Urban KR, Bidja AH, Roy DM, Feeney RB, Afshari LK (2008) Reversal of cocaine-induced planarian behavior by parthenolide and related sesquiterpene lactones. Pharmacol Biochem Behav 89:160–170

Palladini G, Ruggeri S, Stocchi F, De Pandis MF, Venturini G, Margotta V (1996) A pharmacological study of cocaine activity in planaria. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 115:41–45

Raffa RB, Rawls SM (2008) A model for drug action and abuse. Landes Bioscience, Austin

Raffa RB, Valdez JM (2001) Cocaine withdrawal in planaria. Eur J Pharmacol 430:143–145

Rawls SM, Thomas T, Adeola M, Patil T, Raymondi N, Poles A, Loo M, Raffa RB (2009) Topiramate antagonizes NMDA- and AMPA-induced seizure-like activity in planarians. Pharmacol Biochem Behav 93:363–367

Rawls SM, Karaca F, Madhani I, Bhojani V, Martinez RL, Abou-Gharbia M, Raffa RB (2010) β-lactamase inhibitors display anti-seizure properties in an invertebrate assay. Neuroscience 169:1800–1804

Rowlands AL, Pagán OR (2008) Parthenolide prevents the expression of cocaine-induced withdrawal behavior in planarians. Eur J Pharmacol 583:170–172

Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Harukuni I, Bhardwaj A, Traystman RJ, Robinson MB, Britton P, Lu XC, Tortella FC, Wozniak KM, Yudkoff M, Potter BM, Jackson PF (1999) Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. Nat Med 5:1396–1402

Slusher BS, Thomas A, Paul M, Schad CA, Ashby CR Jr (2001) Expression and acquisition of the conditioned place preference

response to cocaine in rats is blocked by selective inhibitors of the enzyme *N*-acetylated-alpha-linked-acidic dipeptidase (NAALADASE). Synapse 41:22–28

Thomas AG, Bodner A, Ghadge G, Roos RP, Slusher BS (2009) GCP II inhibition rescues neurons from gp120IIIB-induced neurotoxicity. J Neurovirol 15:449–457

Urazaev AK, Grossfeld RM, Lieberman EM (2005) Regulation of glutamate carboxypeptidase II hydrolysis of *N*-acetylaspartylglutamate (NAAG) in crayfish nervous tissue is mediated by glial glutamate and acetylcholine receptors. J Neurochem 93:605–610

Vyas CA, Rawls SM, Raffa RB, Shackman JG (2011) Glutamate and aspartate measurements in individual planaria by rapid capillary electrophoresis. J Pharmacol Toxicol Methods 63:199–222

Witkin JM, Gasior M, Schad C, Zapata A, Shippenberg T, Hartman T, Slusher BS (2002) NAALADase (GCP II) inhibition prevents cocaine-kindled seizures. Neuropharmacology 43:348–356

Xi ZX, Li X, Peng XQ, Li J, Chun L, Gardner EL, Thomas AG, Slusher BS, Ashby CR Jr (2010a) Inhibition of NAALADase by 2-PMPA attenuates cocaine-induced relapse in rats: a NAAG-mGluR2/3-mediated mechanism. J Neurochem 112:564–576

Xi ZX, Kiyatkin M, Li X, Peng XQ, Wiggins A, Spiller K, Li J, Gardner EL (2010b) *N*-acetylaspartylglutamate (NAAG) inhibits intravenous cocaine self-administration and cocaine-enhanced brain-stimulation reward in rats. Neuropharmacology 58:304–313