



Neuropharmacology and Analgesia

Topiramate-antagonism of L-glutamate-induced paroxysms in planarians

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ABSTRACT

We recently reported that NMDA (*N*-methyl-D-aspartate) and AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) induce concentration-dependent paroxysms in planarians (*Dugesia dorotocephala*). Since the postulated mechanisms of action of the sulfamate-substituted monosaccharide antiepileptic drug topiramate include inhibition of glutamate-activated ion channels, we tested the hypothesis that topiramate would inhibit glutamate-induced paroxysms in our model. We demonstrate that: (1) L-glutamate (1–10 mM), but not D-glutamate, induced dose-related paroxysms, and that (2) topiramate dose-relatedly (0.3–3 mM) inhibited L-glutamate-induced paroxysms. These results provide further evidence of a topiramate-sensitive glutamate receptor-mediated activity in this model.

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1. Introduction

Topiramate is an anticonvulsant drug that is used as mono- or adjunctive therapy and in a wide spectrum of other CNS disorders (Maryanoff et al., 1987). Topiramate initially was believed to produce these effects by inhibition of carbonic anhydrase (Shank et al., 1994, 2005, 2006; Dodgson et al., 2000). However, following additional extensive study it is currently believed that several mechanisms contribute to topiramate's anticonvulsant activity, either individually or in concert, including an action on ionotropic (including glutamate) receptors (reviewed in Shank and Maryanoff, 2008). The sugar subunit in topiramate adopts a 'twist-boat' conformation that is conducive to its overall spectrum of pharmacologic activities; the sulfamate moiety (OSO_2NH_2) is an essential component for its anticonvulsant activity (Maryanoff et al., 1987). An effect of topiramate on glutamate in vivo was demonstrated in a study using double mutant spontaneous epileptic rats. These rats have basal hippocampal extracellular levels of glutamate about 2–3-fold higher than do normal Wistar rats (Kanda et al., 1996). Topiramate (20–40 mg/kg, i.p.) dose-relatedly reduces the extracellular levels of glutamate in these rats, but not normal Wistar rats, over a time course similar to its active suppression of tonic seizures (Nakamura et al., 1994). A recent comprehensive review summarizes topiramate's clinical use in epilepsy (Lyseng-Williamson and Yang, 2007).

Planarians express mammalian-like neurotransmitter and 2nd-messenger systems and provide a simple model of mammalian pharmacologic effects (reviewed in the recent monograph Raffa and

Rawls, 2008). Individual planarians contain glutamate levels of at least 323 ± 44 pmol/mg-animal (Rawls et al., 2006) and express the gene products for at least two types of ionotropic glutamate receptors that share high sequence similarity with mouse and human neural-specific genes (Cebrià et al., 2002).

In a previous study we demonstrated that seizure-like paroxysms are induced in a concentration-dependent manner by the excitatory ligands NMDA and AMPA, but not the inhibitory amino acid glycine (Rawls et al., 2009). Topiramate attenuated the pSLA induced by NMDA and AMPA. The results of that study suggested an inhibitory action of topiramate on glutamate activity. The present study demonstrates this activity directly.

2. Methods

2.1. Animals and drugs

All planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA). They were acclimated to ambient room temperature (21 °C) and they were tested within 2 days of receipt. L- and D-glutamate were purchased from Tocris Biosciences (St Louis, MO) or Sigma Chemical Co (St Louis, MO). Topiramate (catalog no. T540250) was purchased from Toronto Research Chemicals (North York, Ontario).

2.2. Behavioral experiments

Planarians were placed individually into round Petri dishes (5.1 cm diameter) containing water or test compound(s) and were observed for the occurrence of presumptive seizure-like activity, defined as paroxysms resulting in disruption of normal spontaneous

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locomotor activity. The planarians were observed for 5 min for the occurrence of paroxysms (the number of events counted). Separate planarians were photographed to illustrate the behavioral response, but the results were not included in the quantification analyses.

Prior to testing, each planarian was individually pretreated by 15-min exposure to either water or topiramate. The test groups were (pretreatment-test): (1) water → water, (2) water → L-glutamate (1, 3, and 10 mM), (3) water → D-glutamate (3 mM), (4) water → topiramate (3 mM), (5) topiramate (3 mM) → water, and (6) water → [glutamate (3 mM) + topiramate (0.3, 1, 3 mM)].

Each planarian was used only one time. $N=8$ planarians per treatment or control group. The ED₅₀ for L-glutamate-induction and topiramate-antagonism of paroxysms was determined with InStat® (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. L-Glutamate induces paroxysms

Consistent with normal behavior, planarians tested in water displayed a constant-velocity spontaneous locomotor activity and no paroxysms (representative photographs shown in Fig. 1). In contrast, planarians exposed to L-glutamate displayed recurrent paroxysms (shown for 10 mM glutamate in Fig. 2). The onset of paroxysms following L-glutamate exposure was rapid, beginning within 10 s following exposure. The duration of each individual behavior was approximately 1 s. The effect of L-glutamate was concentration-dependent, as planarians that were exposed to 1, 3, or 10 mM L-glutamate displayed an increasing amount of paroxysms with nearly 100% responding at 10 mM. In previous work we showed that planarians exposed to a variety of classes of other compounds do not display paroxysms (Rawls et al., 2009). Locomotor activity, although not specifically quantified here, was reduced in relation to concentration of L-glutamate. The possibility that low pH of the L-glutamate solutions (~3.5) contributed to induction of paroxysms was investigated. Planarians exposed to D-glutamate did not display paroxysms. The concentration-related L-glutamate-induced paroxysms observed qualitatively in the photographs were quantified in subsequent behavioral assay (by counts) (ED₅₀ = 2.1 mM) and are shown in Fig. 3.

3.2. Topiramate inhibits L-glutamate-induced paroxysms

Topiramate (3 mM) did not induce paroxysms. However, planarians that were exposed to a combination of L-glutamate (3 mM) and topiramate (0.3, 1, 3 mM) displayed a dose-related reduction in paroxysms induced by L-glutamate (3 mM) (ED₅₀ = 0.3 mM) (Fig. 4).

4. Discussion

Glutamate, the most abundant excitatory amino acid in the mammalian brain, is released in large quantities during seizures and triggers ion fluxes that are mediated or modulated by glutamate receptor subtypes. Fast excitatory glutamate neurotransmission involves ionotropic glutamate receptors (NMDA, AMPA, and kainate), whereas slow responses involve metabotropic G protein-coupled glutamate receptors (mL-GluR₁ to mL-GluR₈) (see Bazan et al., 2002).

Excitatory glutamate activity has been linked to the etiology, maintenance, or sequelae of epilepsy. Pathophysiology might not require excess glutamate *per se*, but merely an imbalance between excitatory (glutamate) and inhibitory (γ -aminobutyric acid (GABA)) influences on neurotransmission. As summarized in the comprehensive review by Dalby and Mody (2001): glutamate release increases during kindling epileptogenesis and during and after status epilepticus; chronically elevated glutamate levels caused by a lack of glutamate transporters (GLT-1 and GLAST) render mice more susceptible to seizures; NMDA receptor levels are increased in epileptic tissue; the gain of NMDA receptor-mediated transmission in temporal lobe epilepsy is also effectively enhanced by post-translational phosphorylation and redox modifications of NMDA receptor subunits; and the enhanced glutamate-induced Ca²⁺ influx through AMPA receptors in genetically modified mice expressing a Q/R unedited GluR-B subunit renders the mice significantly more susceptible than their wild type counterparts to temporal lobe epilepsy.

Planarians express gene products for at least two types of ionotropic glutamate receptors that share a high sequence similarity to neural-specific mouse and human gene products (Cebrià et al., 2002). Previous studies that used neurochemical, molecular, and behavioral approaches revealed that planarians have endogenous glutamate and express glutamate-like receptors. We demonstrated the presence of glutamate and aspartate in individual planarians using a fluorescence high-pressure liquid chromatography (HPLC) method after a perchloric acid based extraction and filtration (Rawls et al., 2006).

Topiramate is used as medication for the treatment of a variety of CNS disorders associated with excess glutamatergic activity. In mammals, topiramate exerts an indirect inhibitory effect on glutamate receptor subtypes (e.g., Gibbs et al., 2000; Gryder and Rogawski, 2003; Qian and Noebels, 2003; Angehagen et al., 2004), possibly via an allosteric modulatory mechanism (Angehagen et al., 2004; Shank and Maryanoff, 2008).

In our previous study, planarians exposed to NMDA or to AMPA displayed pSLA. The effects of both NMDA and AMPA were antagonized by topiramate (Rawls et al., 2009). In the present study topiramate antagonized pSLA induced by L-Glu. While this inhibitory

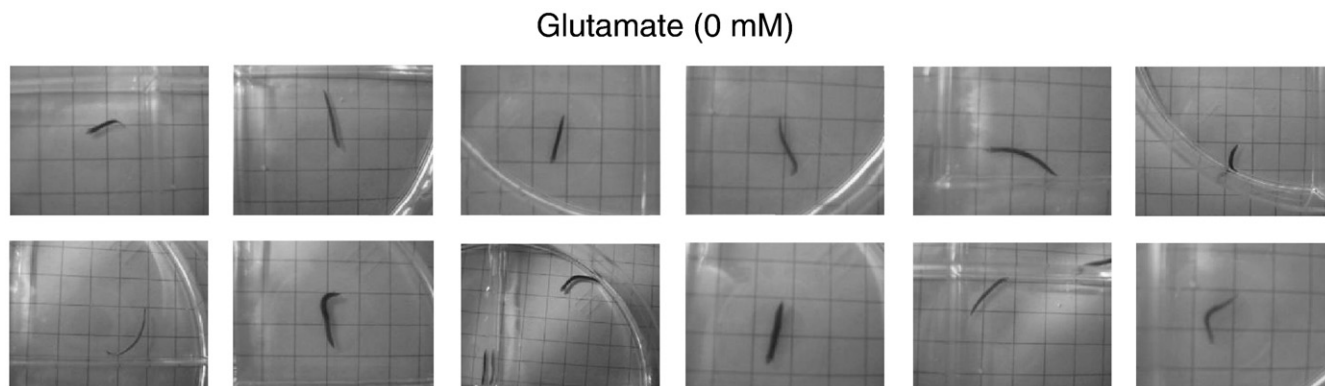


Fig. 1. Representative photographs of the spontaneous locomotor activity displayed by planarians tested in water. When quantified as rate of gridlines crossed, locomotor activity is nearly linear for at least 10 min (see refs in Raffa and Rawls, 2008).

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