



Carbamazepine inhibits distinct chemoconvulsant-induced seizure-like activity in *Dugesia tigrina*

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ABSTRACT

Planaria, non-parasitic flatworms, were recently shown to be a simple yet sensitive model for investigating the pharmacology of convulsants and anticonvulsants. The present findings show that three distinct chemoconvulsants, (–)-nicotine, picrotoxin, and *N*-methyl-*D*-aspartate (NMDA), induce dose-dependent seizure-like paroxysms in the planarian *Dugesia tigrina*. Carbamazepine and oxcarbazepine, iminodibenzyl derivatives, exhibit anticonvulsive effects mediated mainly through the inactivation of voltage-gated sodium channels. Apart from these primary molecular targets, both carbamazepine and oxcarbazepine are known to activate γ -aminobutyric acid type A (GABA_A) receptors and inhibit NMDA activated glutamate receptors and neuronal nicotinic acetylcholine receptors (nAChRs). The present study shows that in *D. tigrina* both carbamazepine and oxcarbazepine inhibit chemoconvulsant-induced seizure behaviors in a dose-dependent manner. Carbamazepine (100 μ M) decreased by ~65% the cumulative mean planarian seizure-like activity (pSLA) observed in the presence of (–)-nicotine (10 μ M), picrotoxin (5 mM), or NMDA (3 mM), whereas oxcarbazepine (1 μ M) decreased by 45% the cumulative mean pSLA induced by (–)-nicotine (10 μ M). The results demonstrate, for the first time, the anti-seizure pharmacology of carbamazepine and oxcarbazepine in an invertebrate seizure model.

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1. Introduction

Planaria, commonly defined as free-living flatworms of the phylum Platyhelminthes, possess a bilaterally symmetrical central nervous system (CNS) composed of neurons similar to those of humans, and a body plan common to all vertebrates and many invertebrates (Agata et al., 1998; Cebria et al., 2002; Sarnat and Netsky, 1985). The presence of a well-characterized and moderately simple CNS was recently demonstrated based on electroencephalography (EEG) recordings (Aoki et al., 2009), and allows for ease of manipulation and an understanding of the effects of drugs on planaria. Planaria possess genes and neurotransmitters corresponding to all the major neurotransmission systems found in the vertebrate brain (Buttarelli et al., 2000; Eriksson and Panula, 1994; Farrell et al., 2008; Nishimura et al., 2008; Rawls et al., 2006, 2007b; Ribeiro et al., 2005; Saitoh et al., 1996, 1997). The effects of drugs acting on cholinergic, glutamatergic, dopaminergic, and serotonergic CNS neural transmission have been examined in behavioral pharmacological studies of planaria (Farrell et al., 2008; Passarelli et al., 1999; Raffa and Valdez, 2001; Rawls et al., 2007a). The earlier studies and the results presented here show that planaria are emerging as a promising experimental model organism for investigating biochemical and functional interactions between

different neurotransmitter-receptor systems and also the pharmacological action of diverse drugs.

Seizure activity is associated with an imbalance in excitatory and/or inhibitory neurotransmission in the brain. γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS and there is extensive evidence implicating the impairment of GABAergic inhibition in seizure disorders (Purves, 2008). Similarly, there is considerable support for the role of the excitatory neurotransmitters glutamate and *N*-methyl-*D*-aspartate (NMDA) in the pathophysiology of seizure disorders (Purves, 2008). Individual planaria contain both GABA and glutamate (Eriksson et al., 1995; Eriksson and Panula, 1994; Rawls et al., 2006, 2007b) and bioinformatics suggest the possible presence of mammalian ionotropic GABA_A and glutamate receptor-like proteins (Ribeiro et al., 2005). Recently, Rawls et al. (2009) and Raffa et al. (2010) reported, for the first time, that *Dugesia dorotocephala* displays dose-dependent, seizure-like paroxysms in the presence of the excitatory neurotransmitters NMDA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and *L*-glutamate, but no seizure activity in the presence of the inhibitory neurotransmitter glycine. Furthermore, they reported that topiramate, an antiepileptic drug known to mediate its anticonvulsant activity through the inhibition of ionotropic glutamate receptors, inhibits NMDA-, AMPA-, and *L*-glutamate-induced seizure-like behaviors in planaria. Neuronal nicotinic acetylcholine receptors (nAChR) play an excitatory role in the brain and nicotine, a cholinergic agonist, induces hyperkinesia in

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planaria (Buttarelli et al., 2000). In addition, the molecular target for nicotine-induced seizures, the $\alpha 7$ nAChR, was reported to be present in flatworms (Ribeiro et al., 2005).

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide) is an anticonvulsant commonly used for treating complex partial and tonic-clonic seizures. Carbamazepine and its structural derivative oxcarbazepine (10,11-dihydro-10-oxo carbamazepine) have anti-epileptic effects in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) by inhibiting different subtypes of human nAChRs (Di Resta et al., 2010). Both carbamazepine and oxcarbazepine potentiate GABA_A receptor currents (Zheng et al., 2009), and carbamazepine also inhibits both NMDA-activated membrane currents in cultured mouse spinal cord neurons (Lampe and Bigalke, 1990) and NMDA-, AMPA-, and kainate-mediated inward currents in rat hippocampal slices (Giustizieri et al., 2008). To test our hypothesis that alteration of excitatory or inhibitory neurotransmission will induce seizure-like behaviors in planaria and that carbamazepine will attenuate this convulsant-induced seizure activity, in the present study we investigated the seizure-like paroxysms in *Dugesia tigrina* exposed to (–)-nicotine, NMDA, or picrotoxin (a GABA_A antagonist) in the absence and in the presence of carbamazepine. The anticonvulsant effect of oxcarbazepine on (–)-nicotine induced seizure-like activity was also investigated to determine if the anticonvulsant-induced attenuation of convulsant-induced pSLA displayed any structure–activity relationship.

2. Materials and methods

2.1. Animals and drugs

Brown *D. tigrina* were purchased from Wards Natural Science (Rochester, NY). NMDA and picrotoxin were obtained from Ascent Scientific (Princeton, NJ), and (–)-nicotine, carbamazepine, oxcarbazepine, and dimethylsulfoxide (DMSO) from Sigma-Aldrich (St. Louis, MO). AmQuel® Plus (Kordon LLC) was purchased from a local pet store (St. Cloud, MN). Other laboratory supplies and chemicals were purchased from ISC Bioexpress (Kaysville, UT).

2.2. General procedures

Upon arrival, the planaria were transferred to a petri dish containing Artificial Pond Water (APW; 6 mM NaCl; 0.1 mM NaHCO₃; 0.6 mM CaCl₂; pH 7.3) (Pagán et al., 2006) and allowed to acclimate at room temperature for 24 h (12 h light/dark cycle) before the experiments were performed. Planaria 1.0–1.5 cm in length were used for experiments within 3 days of arrival and were not fed at any time. Stock solutions of drugs were prepared on the day of the experiment and diluted to desired concentrations with APW. Carbamazepine is insoluble in water and the stock solution was, therefore, prepared in DMSO and diluted with APW to the desired concentrations. The final DMSO concentration was kept to $\leq 0.1\%$ (Pagán et al., 2006). Due to the limited solubility of picrotoxin in aqueous solutions, the drug was dissolved in APW by sonication.

2.3. Planaria seizure-like activity measurements

In APW, planaria exhibit normal gliding behavior. Planaria seizure-like activity (pSLA) is defined as asynchronous paroxysms resulting in a sudden disruption of normal spontaneous locomotor activity. As previously reported (Rawls et al., 2009), upon exposure to pro-convulsive drugs planaria display dose-dependent sudden asynchronous convulsive movements, such as C-like, screw-like, and snake-like hyperkinesia, which are very distinct from their normal locomotor activity. The duration of each individual seizure behavior is approximately 1 s. To measure pSLA, individual planaria were placed in a clear polystyrene petri dish (60 × 15 mm) containing APW (control), or APW

solutions of pro-convulsant drug(s) at different concentrations with or without anticonvulsant drug(s). The cumulative pSLA/5 min was calculated as the number of seizure-like behaviors displayed, minute by minute, over the course of 5 min observation. Each planarian was tested only once for determining the effect of a convulsant drug or of a combination of convulsant and anticonvulsant drug.

2.4. Statistical analysis

At least 10 planaria were tested with each drug treatment and the number of pSLA counted per minute for 5 min was averaged to determine the cumulative mean pSLA/5 min and S.E.M., which was calculated using Microsoft® Excel. The cumulative group means $\pm$ S.E.M. for the different drug treatments were evaluated by two-way t-tests, and p values of ≤ 0.05 were considered statistically significant.

3. Results

On average, planaria exhibited 2.6 ± 0.40 (mean $\pm$ S.E.M.) cumulative mean seizure-like movements in APW during the 5 min observation period. AmQuel® Plus is a water purifier that removes nitrates and ammonia from tap water. When we tested the planaria in AmQuel® Plus-treated tap water, the planaria exhibited a cumulative mean pSLA of 2.6 ± 0.81 in the 5 min observation. This is very similar to the result previously reported by Rawls et al. (2009). To our knowledge, there is no literature report on the effects of APW on pSLA and the reason for the observed pSLA activity in APW is not clear. Although a 24 h acclimatization period was allowed, the stress due to transportation might have contributed to this minimal seizure-like behavior. The mean cumulative pSLA did not significantly differ between APW or AmQuel® Plus treated tap water. Further, the mean cumulative pSLA observed during the 5 min observation was not significant in comparison with the pSLA counted in the presence of each of the three chemoconvulsants. Therefore, we carried out all seizure-like activity testing in APW.

With increasing concentrations of convulsant drugs, the planaria exhibited an increasing number of sudden asynchronous convulsive movements, which are very distinct from their normal locomotor activity. Fig. 1 illustrates both the normal movement and examples of asynchronous paroxysms and abnormal hypokinesia exhibited by the planaria. Fig. 1A presents the normal gliding movement of the planaria in APW. Typically, NMDA-exposed *D. tigrina* exhibited C-like, screw-like, and snake-like hyperkinesia. Fig. 1B and C displays the behavior of *D. tigrina* exposed to 3 mM NMDA. In Fig. 1B, the planarian demonstrated a snake-like movement whereas in Fig. 1C it performed a screw-like movement. The pSLA characterized as C-like hyperkinesia and twitching behaviors upon exposure to NMDA were previously observed in *D. dorotocephala* (Rawls et al., 2009). Fig. 1D shows *D. tigrina* exhibiting screw-like hyperkinesia when exposed to 5 mM picrotoxin. Planaria in the presence of low concentrations of (–)-nicotine (0.1 to 10 μ M) displayed C-like and screw-like hyperkineses. However, planaria exposed to $\geq 50 \mu$ M (–)-nicotine had an increasing tendency to undergo longitudinal contraction, resulting in a walnut-like position; once a planarian exhibited this behavior it essentially remained frozen in that behavioral state during the remainder of the observation period. Fig. 1E shows a planarian in the presence of 50 μ M (–)-nicotine displaying a walnut-like position with fixed posture and reduced length; whereas Fig. 1F demonstrates a partial screw-like movement and a C-like hyperkinesia exhibited by a planarian in the presence of 10 μ M (–)-nicotine. For the three convulsant drugs investigated in the present study, the cumulative pSLA/5 min measurements included only hyperkineses. The distinct hypokineses, such as the walnut-position hypokinesia (WNP) behaviors observed predominantly in the presence of high concentrations of (–)-nicotine, were not included in the pSLA determination.

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