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# 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 μM) decreased by ~65% the cumulative mean planarian seizure-like activity (pSLA) observed in the presence of (−)-nicotine (10 μM), picrotoxin (5 mM), or NMDA (3 mM), whereas oxcarbazepine (1 μM) decreased by 45% the cumulative mean pSLA induced by (−)-nicotine (10 μM). 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 serotoninergic 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 α7 nAChR, was reported to be present in flatworms (Ribeiro et al., 2005).

Carbamazepine (5H-dibenz[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 $NaHCO_3$; 0.6 mM $CaCl_2$; 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 ≤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 ± 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 ± 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 hyperkinesis. 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 hyperkinesis when exposed to 5 mM picrotoxin. Planaria in the presence of low concentrations of (−)-nicotine (0.1 to 10 μM) displayed C-like and screw-like hyperkinesises. However, planaria exposed to ≥50 μM (−)-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 μM (−)-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 hyperkinesis exhibited by a planarian in the presence of 10 μM (−)-nicotine. For the three convulsant drugs investigated in the present study, the cumulative pSLA/5 min measurements included only hyperkinesises. The distinct hypokineses, such as the walnut-position hypokinesis (WNP) behaviors observed predominantly in the presence of high concentrations of (−)-nicotine, were not included in the pSLA determination.


**Fig. 1.** A) Planarian normal gliding movement in APW; B) snake-like and C) screw-like pSLA displayed by planarians in 3 mM NMDA; D) screw-like pSLA exhibited by planarians in 5 mM picrotoxin; E) walnut position hypokinesia displayed by planarians in 50 M (−)-nicotine; and F) C-like pSLA exhibited by planarians upon exposure to 10 μM (−)-nicotine.

### *3.1. Effect of carbamazepine and oxcarbazepine on (−)-nicotine-induced pSLA*

Fig. 2 shows the cumulative mean ± S.E.M. pSLA exhibited by planaria upon exposure to the pro-convulsant (−)-nicotine. The pSLA increased in a concentration-dependent manner until 10 μM (−)-nicotine. At a concentration of ≥50 μM (−)-nicotine, the planaria began to freeze, with minimal movement after the first minute. As the (−)-nicotine concentration was increased to ≥50 μM, the planaria began exhibiting the WNP, resulting in the organisms staying in a fixed position. Planaria exposed to 10 μM (−)-nicotine displayed the greatest number of pSLA, at 17 ± 1.41 over a 5 min observation interval, which was ~550% more pSLA than the control. The pSLA was greatest during the second and third minutes with 4–5 seizure like movements. The duration of each individual seizure lasted approximately 1 s. The planaria displayed multiple seizures during the first 3 min (~4/min), and then throughout the remaining 2 min, reduced seizure behaviors (~2/min). Because the pSLA was greatest at a concentration of 10 μM (−)-nicotine and was statistically significant ($p<0.05$; see Fig. 2), the effect of increasing concentrations of carbamazepine was tested on the pSLA induced by this concentration of (−)-nicotine (see left inset in Fig. 2). As the carbamazepine concentration was increased, the pSLA significantly decreased. In the presence of 10 μM (−)-nicotine and 100 μM carbamazepine, the pSLA is the lowest, approximately 6 ± 0.58 over a 5 min observation period. During the first 3 min of exposure to 10 μM (−)-nicotine with 100 μM carbamazepine, the planaria displayed ~2 pSLA/min. During the last 2 min, the pSLA decreased to almost none (~0.4/min). The co-application of 100 μM carbamazepine and 10 μM (−)-nicotine decreased the overall pSLA by ~65%. Due to the limited solubility of carbamazepine in aqueous solution and the need to keep the DMSO concentration below 0.1% to avoid toxicity (Pagán et al., 2006), the effect of carbamazepine at concentrations greater than 100 μM on

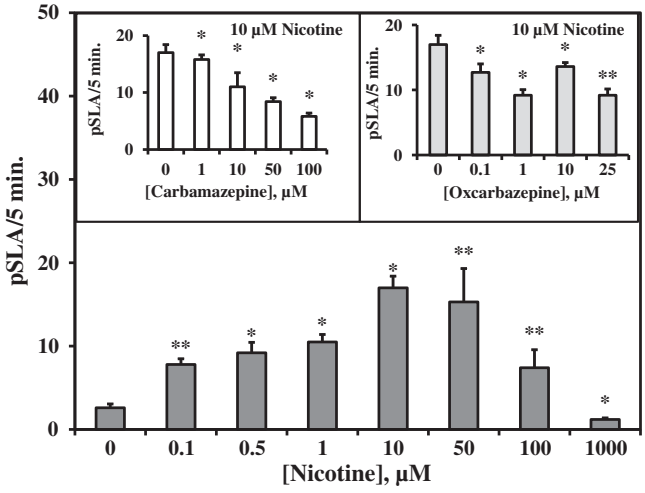


**Fig. 2.** (−)-Nicotine- (0.1–1000 μM) induced concentration-dependent pSLA, expressed as the cumulative mean (± S.E.M.) over a 5 min observation period. * $p<0.05$ or ** $p<0.01$ compared to control (planarians tested in APW). The effect of carbamazepine (1–100 μM; open bars; left inset figure) or oxcarbazepine (0.1–25 μM; filled bars; right inset figure) on 10 μM (−)-nicotine-induced pSLA, expressed as the cumulative mean (± S.E.M.) over a 5 min observation. Carbamazepine or oxcarbazepine significantly decreased 10 μM (−)-nicotine-induced SLA in planaria. * $p<0.05$ or ** $p<0.01$ compared to pSLA recorded in the presence of 10 μM (−)-nicotine. The sample size was N = 10 planarians for control or for each concentration of drugs tested.

nicotine-induced pSLA was not tested. Planaria exposed to carbamazepine (1–100 μM) alone displayed no significant pSLA. The observed pSLA ranged from 1 to 2 per 5 min in planaria exposed to 1–100 μM carbamazepine. In comparison with the control planaria, which displayed 2.6 ± 0.40 paroxysms per 5 min in APW, the planaria treated with 1–100 μM carbamazepine exhibited marginally fewer (~1 pSLA) paroxysms over the 5 min observation period.

Oxcarbazepine, a structural derivative of carbamazepine, also decreased nicotine-induced paroxysms in a concentration-dependent manner (see right inset in Fig. 2). As the oxcarbazepine concentration was increased from 0 to 1 μM the mean cumulative pSLA decreased by nearly 45%. Although the pSLA was marginally higher when 10 μM oxcarbazepine was co-applied with 10 μM (−)-nicotine, the pSLA decreased again by ~40% when 25 μM oxcarbazepine was tested (see right inset in Fig. 2). Due to the limited solubility of oxcarbazepine in DMSO, the effect of oxcarbazepine at concentrations greater than 25 μM on 10 μM (−)-nicotine-induced pSLA was not tested.

### 3.2. Effect of carbamazepine on picrotoxin-induced pSLA

Fig. 3 shows that as the concentration of picrotoxin was increased from 0.01 to 5.0 mM, the pSLA significantly increased. At the lowest picrotoxin concentration (0.01 mM), 10 ± 0.58 cumulative pSLA occurred during the 5 min observation. At the highest picrotoxin concentration (5 mM), the cumulative pSLA was 40 ± 3.69 per 5 min. The effects of picrotoxin concentrations greater than 5 mM were not tested because of the limited solubility of picrotoxin in water (see Section 2.2). The rate of 5 mM picrotoxin-induced pSLA remained fairly constant during the 5 min observation (~8/min.). Because, over the range tested, 5 mM picrotoxin induced the highest pSLA, this concentration was chosen for testing the effect of carbamazepine on pSLA induced by picrotoxin. Fig. 3 inset shows the mean cumulative pSLA ± S.E.M. at a fixed concentration of picrotoxin (5 mM) with or without the addition of increasing concentrations of carbamazepine. As the concentration of carbamazepine was increased, the picrotoxin-induced pSLA significantly decreased (see inset in Fig. 3). When the planaria were placed in the test solution containing 100 μM carbamazepine and 5 mM picrotoxin, the mean cumulative pSLA was 14 ± 0.51. During the 5 min observation period, the effect of 100 μM carbamazepine on the rate of 5 mM picrotoxin-induced pSLA remained fairly constant (~3/min). Co-application of 100 μM carbamazepine and 5 mM picrotoxin inhibited by ~65% the 5 mM picrotoxin-induced pSLA.

### 3.3. Effect of carbamazepine on NMDA-induced pSLA

NMDA induced pSLA in a concentration-dependent manner and significant pSLA was observed when planaria were exposed to 1, 3, or 10 mM NMDA (Fig. 4). The most pSLA occurred during the first minute for nearly every concentration of NMDA that was tested. There was a rapid onset of pSLA upon exposure of the planaria to NMDA; pSLA occurring less than 10 s after the exposure, with each individual behavior lasting less than 1 s. When exposed to 3 mM NMDA, planaria exhibited the largest mean cumulative pSLA (60.4 ± 2.45). During the first 2 min, the rate of 3 mM NMDA-induced pSLA was ~14/min, while during the remaining 3 min it was ~11/min. The effect of increasing concentrations of carbamazepine on pSLA induced by a fixed concentration of NMDA (3 mM) is displayed in the inset to Fig. 4. Increasing carbamazepine concentrations significantly reduced the 3 mM NMDA-induced pSLA. When planaria were exposed to 3 mM NMDA in the presence of 50 μM or 100 μM carbamazepine, the rate of pSLA was ~4.5/min, with an average cumulative pSLA of ~23 ± 1.10 per 5 min. There was no significant difference in the average cumulative pSLA recorded in the presence of 50 or 100 μM carbamazepine; while the addition of either one of these two carbamazepine concentrations in the presence of 3 mM NMDA resulted in ~63% recovery from the 3 mM NMDA-induced pSLA.

## 4. Discussion

In the present study, the observed convulsive effects of (−)-nicotine, picrotoxin, and NMDA in *D. tigrina* provides pharmacological evidence for the presence of cholinergic, GABAergic, and glutamatergic neurotransmission systems in this nonparasitic flatworm. Nicotine is reported to induce seizures due to its binding to nAChRs in the brain (Broide et al., 2002; Di Resta et al., 2010; Narahashi et al., 2000). Based on the mechanism of nicotine-induced seizures, the α7-nAChR subtype was

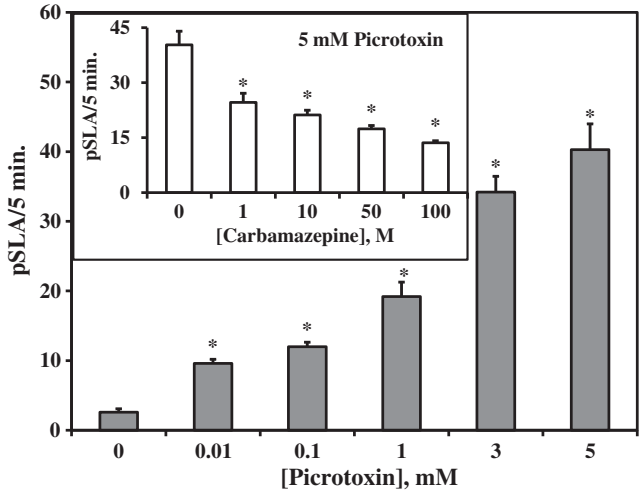


**Fig. 3.** Picrotoxin- (0.01–5 mM) induced concentration-dependent pSLA (filled bars) and carbamazepine (1–100 M) significantly decreased 5 mM picrotoxin-induced pSLA (inset figure; open bars), expressed as the cumulative mean (± S.E.M.) during the 5 min observation interval. * $p<0.05$ or ** $p<0.01$ compared to control [(APW; filled bars) or pSLA measured in the presence of 5 mM picrotoxin (open bars)]. The sample size was N = 10 planarians for control or for each concentration of the drugs tested.

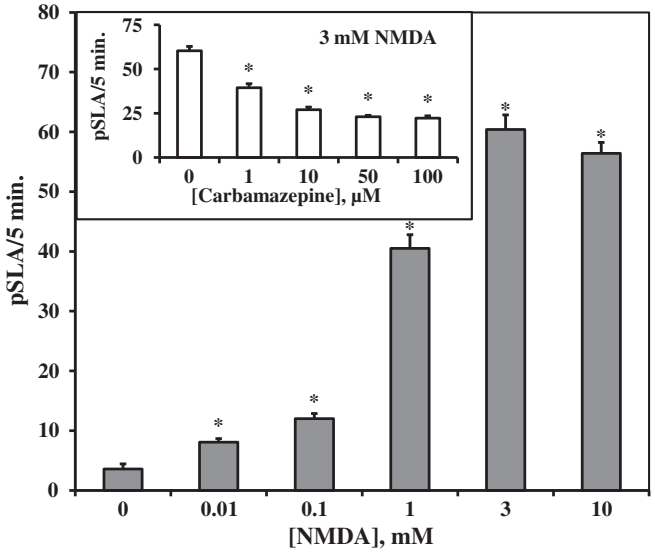


**Fig. 4.** NMDA (0.01–10 mM) induced concentration-dependent pSLA (filled bars) and carbamazepine (1-100 M) effectively decreased 3 mM NMDA-induced pSLA (inset figure; open bars), expressed as the cumulative mean (± S.E.M.) during the 5 min observation period. * $p<0.05$ or ** $p<0.01$ compared to either control [(APW; filled bars) or pSLA in the presence of 3 mM NMDA (open bars)]. The sample size was N = 10 planarians for control or for each concentration of tested drugs.

shown to be the molecular target for the convulsive effects of nicotine (Broide et al., 2002; Damaj et al., 1999; Di Resta et al., 2010; Narahashi et al., 2000). The presence of this particular nAChR subtype in the flatworms was recently demonstrated through gene homology studies (Ribeiro et al., 2005). Previous behavioral pharmacological studies in planaria, carried out in the presence of nicotinic agonists and muscarinic antagonists, confirmed the expression of both muscarinic and nicotinic receptors (Buttarelli et al., 2000; Pagán et al., 2009). Picrotoxin, a convulsant and a noncompetitive antagonist of $GABA_A$ receptors, specifically inhibits the receptor's chloride channel in the brain (Ramakrishnan and Hess, 2005; Singh et al., 2010). The dose-related robust pSLA induced by picrotoxin strongly suggests the presence of $GABA_A$ receptor-like proteins in planaria. This is supported by the molecular evidence for the presence of $GABA_A$ receptor-like proteins in the flatworms and by chromatographic and immunohistochemical studies that demonstrated the presence of GABA in planaria (Nishimura et al., 2008; Rawls et al., 2007b; Ribeiro et al., 2005). In planaria, the presence of endogenous glutamate as well as two types of ionotropic glutamate receptor-like proteins, has been proposed, based on chromatographic and gene sequence homology studies (Cebria et al., 2002; Rawls et al., 2006; Ribeiro et al., 2005; Vyas et al., 2011). Further, NMDA, AMPA, and L-glutamate induced dose-related paroxysms in the planarian *D. dorotocephala,* and mechanistic studies suggested that NMDA may induce seizure activity through both NMDA and non-NMDA receptor activation (Raffa et al., 2010; Rawls et al., 2009).

Carbamazepine is widely used for the treatment of partial and generalized seizures and also in the management of neuropathic pain and psychiatric disorders. In various mice models, carbamazepine decreased seizure activity induced by nicotine, picrotoxin, or NMDA (Kaminski et al., 2004; Teper et al., 2007; Vohora and Pillai, 1999). To the best of our knowledge, this is the first report on the dose-dependent anti-seizure pharmacology of carbamazepine on (−)-nicotine-, picrotoxin-, or NMDA-induced seizure-like paroxysms in a planarian. Oxcarbazepine, a structural derivative of carbamazepine, was also effective in inhibiting (−)-nicotine-induced seizures in the same species. Interestingly, the fact that oxcarbazepine is more potent than carbamazepine suggests a structure–activity relationship. 1 μM oxcarbazepine decreased by ~45% 10 μM (−)-nicotine-induced pSLA, whereas 1 μM carbamazepine decreased it by only 7%. The anticonvulsant effect of oxcarbazepine was not strictly dose-dependent between 1 and 25 μM; however, this structural analog of carbamazepine does exhibit significant anticonvulsive effect against (−)-nicotine-induced pSLA. The limited solubility of oxcarbazepine in DMSO prevented us from testing higher concentrations of this drug.

At 100 μM carbamazepine inhibited ~65% of the (−)-nicotine- (10 μM), picrotoxin- (5 mM), or NMDA- (3 mM) induced pSLA. In the present study carbamazepine did not show either apparent specificity or difference in efficacy against (−)-nicotine-, picrotoxin-, or NMDA-induced pSLA and does not provide any insights about the mechanism of action of this anticonvulsant drug in the planarian seizure model tested. The molecular mechanisms of the convulsant effects of the drugs tested are also not clearly understood as the physiological targets for the tested convulsants and the anticonvulsants in planaria have not yet been isolated, cloned and characterized. It is widely accepted that the anti-seizure properties of carbamazepine and its structural derivatives, such as oxcarbazepine, are largely due to their inhibition of voltage-gated sodium channels (McLean and Macdonald, 1986; Schwarz and Grigat, 1989). Furthermore, carbamazepine's action on voltage-gated ion channels alters neuronal excitability by impairing glutamate release (Sitges et al., 2007a,b). Therefore, future behavioral pharmacological studies testing the effect of carbamazepine on pSLA induced by convulsive drugs acting on voltage gated sodium channels, and quantification of levels of glutamate in planaria exposed to carbamazepine, are expected to provide insights as to the mechanisms of seizures and the effects of drugs used to combat them.

The planarian *D. tigrina* exhibited similar paroxysmal behaviors, such as C-like and screw-like movements, in the presence of (−)-nicotine, picrotoxin, and NMDA. In the presence of NMDA, *D. tigrina* also displayed snake-like asynchronous movements. In the present study, when (−)-nicotine concentrations of ≥50 μM were tested, the planaria displayed an increasing tendency to contract longitudinally and assume a walnut-like position. Previously, Buttarelli et al. (2000) and Pagán et al. (2009) observed similar behaviors in planaria exposed to nicotine concentrations from 0.1 to 3 mM, described those behaviors as walnut-position hypokinesia, and attributed this distinct behavior to the likely stimulation of nicotinic receptors.

To summarize, seizure disorders, such as epilepsy, are characterized by pathological brain hyperactivity due to imbalances in excitatory and/or inhibitory neurotransmission (Luszczki, 2009). In spite of the remarkable array of anti-seizure drugs that are currently available for treatment, 30% of seizures are still not controlled by the existing antiepileptic drugs (Rogawski, 2006). Therefore, the need to better understand the underlying neurobiological and molecular mechanisms of epilepsy pathogenesis requires new approaches and novel experimental models of seizure-like states. Although the rodent seizure models continue to serve as the foundation for basic and translational epilepsy research, unconventional vertebrate (zebrafish) and invertebrate (fruit fly and roundworm) models are proving to have greater potential for analyzing the epilepsy phenotype among these genetically tractable organisms (Baraban, 2007). Invertebrate planaria have the genes and neurotransmitters corresponding to the major inhibitory (GABA) and excitatory (glutamate) neurotransmission systems (Agata et al., 1998; Aoki et al., 2009; Eriksson and Panula, 1994; Rawls et al., 2006, 2007b). In the present study, carbamazepine was shown to be effective in decreasing, by as much as ~65%, and in a concentration-dependent manner, the SLA induced in the planarian *D. tigrina* by chemoconvulsants such as, (−)-nicotine, picrotoxin, and NMDA. These results demonstrate, for the first time, the effectiveness of carbamazepine in inhibiting the planarian seizure-like behaviors induced by convulsants acting directly either on excitatory [(−)-nicotine and NMDA] or inhibitory (GABA) neurotransmission. Further, the results presented here support previous findings that the planarian seizure-like activity is a sensitive, quantifiable end-point for studying the anti-seizure pharmacology of small molecular drugs (Raffa et al., 2010; Rawls et al., 2009). Together, the present and previous studies demonstrate the potential usefulness of planaria as experimental models in which to screen for anticonvulsant drugs. Planarian seizure models are even more appealing because they are easy to maintain and handle under laboratory conditions, and are rather inexpensive in comparison with the other existing animal seizure models.

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