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**Toxicity and mode of action of the aporphine plant alkaloid liriodenine on the insect GABA
receptor**

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ABSTRACT

Liriodenine is a biologically active plant alkaloid with multiple effects on mammals, fungi, and bacteria, but has never been evaluated for insecticidal activity. Accordingly, liriodenine was applied topically in ethanolic solutions to adult female *Anopheles gambiae*, and found to be mildly toxic. Its lethality was synergized in mixtures with dimethyl sulfoxide and piperonyl butoxide. Recordings from the ventral nerve cord of larval *Drosophila melanogaster* showed that liriodenine was neuroexcitatory and reversed the inhibitory effect of 1 mM GABA at effective concentrations of 20-30 μ M. GABA antagonism on the larval nervous system was equally expressed on both susceptible and cyclodiene-resistant rdl preparations. Acutely isolated neurons from *Periplaneta americana* were studied under patch clamp and inhibition of GABA-induced currents with an IC₅₀ value of about 1 μ M were observed. In contrast, bicuculline did not reverse the effects of GABA on cockroach neurons, as expected. *In silico* molecular models suggested reasonable structural concordance of liriodenine and bicuculline and isosteric hydrogen bond acceptor sites. This study is the first assessing of the toxicology of liriodenine on insects and implicates the GABA receptor as one likely neuronal target, where liriodenine might be considered an active chemical analog of bicuculline.

Keywords

Anopheles gambiae, bicuculline, *Drosophila melanogaster*, GABA antagonist, insecticide

Highlights

- Topical insecticidal activity was low, but synergized by DMSO and PBO
- Liriodenine excited insect ventral cord and reversed effects of applied GABA
- Liriodenine blocked GABA receptors in patch clamped cockroach neurons
- Molecular modeling suggests it is a functional analog of bicuculline in insects

Abbreviations: area under the curve (AUC); central nervous system (CNS); piperonyl butoxide (PBO)

1. Introduction

Mosquitoes are vectors for numerous diseases, and control of vector populations via insecticides, as well as personal protection methods, remain key for reducing the impact of mosquito-borne diseases. New active compounds are required to overcome existing field resistance and its future development, with the additional goal of limiting environmental impact of chemical control. The inspiration for new chemical classes of molecules may come from different sources, such as modification of pre-existing insecticides (Li et al., 2013; Sun et al., 2011), receptor docking simulation leading to *de novo* drug synthesis (Mutunga et al., 2019; Rocher and Marchand-Geneste, 2008), or screening compound libraries of natural compounds produced by fauna and flora. With this in mind, secondary metabolites synthesized by plants are a rich potential source of molecules for discovery of new insecticidal chemistry. Secondary plant metabolites are a primary way for plants to survive attack by insect predators, bacteria, and fungi. Millions of years of evolution provide us with a repository of novel chemistry that may be useful for insect control. Among the secondary plant metabolites, over 27,000 alkaloids have been described, having useful analgesic, psychotropic, anti-arrhythmic, and insecticidal properties, with nicotine as a classical botanical insecticide (Wink, 2015).

Liriodenine (Fig. 1) is an aporphine alkaloid that can be isolated from an abundance of plants belonging to different families. It can be extracted from the bark, twigs or seeds with methanol or chloroform solvents, and major sources of liriodenine include the Annonaceae family, including species such as *Fissistigma glaucescens* (Chang et al. 1996), *Goniothalamus tapisoides* (Kim et al. 2012), *Annona foetida* (Costa et al., 2011), *Mitrephora vulpine* (Moharam et al., 2010), and

Enicosanthellum pulchrum (Nordin et al., 2015). Liriodenine has been tested for anti-tumor (Chen et al., 2012; Nordin et al., 2015) and antiarrhythmic effects (Chang et al., 1996), in addition to studies of its antifungal properties (Clark et al., 1987; Cruz-Chacón et al., 2014; Wu et al., 2012). Its antiarrhythmic activity in mammals results from action on multiple targets, such as voltage-gated sodium, potassium and calcium channels (Chang et al., 1996), and in other studies it was found to possess a blocking action on muscarinic (Lin et al., 1994) and nicotinic (Still et al., 2013) acetylcholine receptors. Although it has been shown to possess trypanocidal activity (Costa et al., 2011), liriodenine has not been tested for insecticidal properties, which was the aim of the present study, along with investigation of its mode(s) of action. Accordingly, we evaluated its mosquitocidal activity and its action on GABA receptors due to the structural similarity between liriodenine and bicuculline (Fig. 1).

2. Materials and methods

2.1. Chemicals

Liriodenine was provided by the SONAS laboratory (UA, Angers, France), and was dissolved in DMSO for electrophysiological studies or 100% ethanol for topical assays on mosquitoes. It was extracted from dried, powdered, *Stephania venosa* leaves (Menispermaceae) with cold ethanol as described by Pharadai et al. (1985). Its structure was confirmed by mass spectrometry and ¹H nuclear magnetic resonance, and purity was assessed by high pressure liquid chromatography and absorbance at 210 (77 %) and 280 nm (99 %). DMSO, PBO, and GABA of analytical grade were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Bicuculline methiodide (≥ 99%) was obtained from Tocris (Minneapolis, Minnesota, USA). Other chemicals for buffers, etc. were purchased from a variety of commercial suppliers.

2.2. Topical application bioassay

The insecticide-sensitive G3 strain of *Anopheles gambiae* was reared from eggs provided by the Centers for Disease Control, Atlanta, Georgia, USA, and maintained according to previously described methods (Démarets et al., 2018). Liriodenine was dissolved in ethanol, and adult female mosquitoes (up to 5 days old) were dosed with a micro-applicator (Hamilton, Reno, Nevada, USA). Treatment was 200 nL of liriodenine (5 mg/ml), either by itself, or co-applied with PBO at 0.5 mg/ml in two different solvent mixtures: 100% ethanol, or 75% ethanol:25% DMSO. Negative controls were assessed at the same time point, and included 100% ethanol; PBO alone in 100% ethanol; 75% ethanol:25% DMSO; and PBO alone in 75% ethanol:25% DMSO. Knockdown was defined as uncontrollable walking/flight or a complete inhibition of flight, and was assessed 1 and 4 hours after treatment. Mortality was defined as no visible movement, 24 hours after treatment.

2.3. Ventral ganglia recording

Recordings of peripheral nerve discharge were performed on ganglia from 3rd-instar larvae of *Drosophila melanogaster* by recently modified procedures (Swale et al., 2018) derived from a similar method (Bloomquist, 2003). Experiments were performed on both the wild-type, insecticide susceptible CSOR strain, as well as the cyclodiene-resistant *rdl* 1675 strain carrying a A301S mutation in a GABA subunit gene (ffrench-Constant, 1994). Briefly, larvae were immersed in buffer containing (in mM) NaCl (157), KCl (3), CaCl₂ (2), HEPES (4), with pH = 7.25. The ventral ganglia and its output nerves were dissected free and the CNS was transected just posterior to the cerebral lobes. One nerve was then drawn into a glass pipette fabricated with a micropipette puller (model P-1000, Sutter Instrument, Novato, California, USA), having a tip diameter of 20-50 μm. Signals were acquired with an AC/DC amplifier (model 3000, A-M

systems, Sequim, Washington, USA) and digitized with a PowerLab 4/26 running LabChart 8 Pro (ADInstruments, Dunedin, New Zealand). Line frequency noise (60 Hz) was reduced with a Humbug™ (Quest Scientific, Vancouver, British Columbia, Canada). Spontaneous bursting frequency above baseline noise was recorded for 10 min or until reaching a stable level prior to drug treatment. Spontaneous ganglionic activity after drug treatment was then recorded for 30 minutes. In other experiments, the CNS preparation was pre-exposed to 1 mM GABA before challenge with lirioidenine or bicuculline in DMSO, up to 0.7% final.

2.4. Whole-Cell Patch Clamp Recordings

Thoracic ganglia of adult *Periplaneta americana* were dissected in calcium-free saline containing (in mM): NaCl (215), KCl (3.1), MgCl₂ (4), glucose (50), HEPES (15), final pH = 7.4, as adapted from Zhao et al. (2003). After collecting and triturating the ganglia, cells were transferred to a saline solution supplemented with Ca²⁺ (5 mM), FBS (5%), and penicillin-streptomycin (0.5%), and then placed on coverslips treated with poly-L-lysine (0.005%) in a petri dish. Dissociated cells were allowed to settle for 30 minutes at room temperature and then incubated at 28 °C and used over the next 2 to 24 hr. The extracellular buffer contained (in mM) NaCl (167), KCl (3.1), CaCl₂ (5), MgCl₂ (4), sodium gluconate (33), HEPES (10), with a final pH = 7.4. The patch pipette was filled with buffer containing (in mM) NaCl (15), KCl (170), CaCl₂ (0.5), MgCl₂ (1), EGTA (10), Mg-ATP (3), HEPES (20), with final pH = 7.4. Stock solutions of bicuculline and lirioidenine in DMSO were diluted into extracellular buffer (0.1% final DMSO), giving concentrations in the range of 0.1 μM to 10 μM. GABA, dissolved in extracellular buffer at 5 mM, was used to evoke Cl⁻ currents from activation of GABA receptors. The dissociated cells were clamped at -60 mV and the drug perfusing pipette was placed close to the cell (within about 1 mm). Inhibition of GABA-evoked currents was assessed after 3-min

perfusion with increasing concentrations of each compound. Currents were elicited by microperfusing GABA for 10 seconds, after a 3-min preincubation of each liriodenine concentration. The amplitude of the recorded current was expressed relative to the current amplitude of the cell recorded with GABA alone. Current signals were recorded with an Axon 200B amplifier, sampled at 2 kHz, and analyzed with pClamp 10.7 software (both from Molecular Devices, San Jose, California, USA).

2.5. Molecular Modeling

Structures of liriodenine and bicuculline were downloaded from PubChem and validated using MarvinView from ChemAxon, which was also used for calculating the chemical parameters logP and logS. Shape and electrostatic similarity were determined using vROCS 3, (Hawkins et al., 2007) after a library of conformers of each compound was generated with OMEGA2 (Hawkins et al., 2010). Molecular similarity between the compounds was determined using the chemistry nodes of KNIME (<https://www.knime.com>), with the MoSS maximum common substructure (MCSS) molecular similarity node.

2.6. Statistical analysis

Statistical analysis was performed in GraphPad Prism (GraphPad Software, San Diego, CA USA). Data are presented as mean \pm SEM. Two-way repeated measures ANOVA assessed the effect of time and treatment on knockdown and mortality rates, with post-*hoc* Bonferroni or Tukey multiple comparisons post-test (Fig. 2). For CNS recordings, the average percentage of control firing rate was calculated per minute of drug exposure and plotted at 1 min or 3 min intervals. Data were again subjected to two-way ANOVA to determine effect of time and concentration (Figs. 3 and 4A). Non-linear concentration-response relationships were assessed from the AUC of firing rate recordings, with the GABA pretreated signals as baseline and

expressed as percentage of untreated control (Fig. 4B). EC₅₀ value was determined with, using a nonlinear regression fit: $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogIC}_{50} - X) * \text{Hill Slope}))}$).

3. Results

3.1. Topical application effects

In topical assays, there was a significant effect of observation time (two-way ANOVA, $F_{2,48} = 17.2$, $df = 2$, $p < 0.001$) and chemical treatment (two-way ANOVA, $F_{7,24} = 51.8$, $df = 7$, $p < 0.001$) on the results (Fig. 2). After one hour, there was no significant difference in knockdown between the control group (100 % ethanol solvent), all other negative controls, and liriodenine alone. Only the mosquitoes treated with liriodenine + 25% DMSO or with liriodenine + PBO + 25 % DMSO showed significantly increased knockdown levels compared to control. These treatments showed 29.2 ± 3.2 % ($p \leq 0.0002$) and 49.0 ± 1.0 % ($p \leq 0.0001$) knockdown, respectively, compared to 4.2 ± 2.4 % control knockdown. Although they were different from controls, the 1 h knockdown response of mosquitoes treated with liriodenine + PBO and liriodenine + PBO + 25 % DMSO were not significantly different.

After four hours, the levels of knockdown from all treatments increased (Fig. 2). The negative control treatment PBO + 25% DMSO had significantly higher knockdown than the control ethanol group, 30.3 ± 1.7 % compared to 6.2 ± 2.1 % ($p \leq 0.0004$), but was not statistically different from the other negative controls, the mosquitoes treated with liriodenine alone, nor the group treated with liriodenine + PBO (Fig. 2). Once more, the mosquitoes treated with liriodenine + DMSO 25 %, and with liriodenine + PBO + DMSO 25 % showed significantly increased knockdown levels compared to all the other treatment groups, and were different from each other.

For 24 h mortality rates, the results were similar to 4 h knockdown. The negative control groups PBO alone and the 25 % DMSO-amended ethanol were not significantly different from the ethanol control group, liriodenine alone, or liriodenine + PBO. Mortality in the control group PBO + 25% DMSO was greater and of similar magnitude to mosquitoes treated with liriodenine + DMSO (respectively, 36.4 % and 37.1 % mortality). The highest mortality rate ($67.7 \pm 5.2\%$) was observed when the three components were applied together (viz., liriodenine + PBO + DMSO 25 %), which was significantly different from all the other treatment groups (Fig. 2).

3.2. Ventral ganglia recording

When applied directly onto the CNS of *D. melanogaster* larvae, liriodenine by itself showed an increase in the average rate of firing that was concentration-dependent (Fig. 3).

Neuroexcitation was significantly elevated above control at both 40 and 50 μM , but not at 10 μM . Maximal excitation developed within three min at 50 μM , and six min at 40 μM . At these concentrations, the elevated discharge continued for several minutes, but declined thereafter (Fig. 3). Concentrations showing less excitatory effect, and eventually leading to fast block without neuroexcitation. For example, 70 μM liriodenine (in 0.7% DMSO suspension) showed no firing increase during the first 9 min but only block of nerve discharge; however, nerve block was incomplete, with a minimum of 40% of original firing rate still occurring (data not shown). Higher concentrations could not be tested due to solubility limitations.

Given its resemblance to bicuculline and to further explore its mode of action, liriodenine was applied to CNS preparations pretreated for 5 minutes with 1 mM GABA (Fig. 4A). GABA treatment stopped neuron firing almost completely after the first 5 minutes of exposure, and nerve discharge did not resume over 30 minutes of recording (Fig. 4A). When the CNS was exposed to liriodenine after the GABA preincubation, the CNS resumed firing and the effect was

concentration dependent. There was no effect of 15 μM liriodenine above that of DMSO, and the increased firing observed at 20 μM was not statistically significant. However, a significant increase in firing occurred at concentrations $\geq 30 \mu\text{M}$ (Fig. 4A). For concentration-response analysis (Fig. 4B), the response to liriodenine was quantified as the area under the curve (AUC) over the 30-min liriodenine exposure and expressed as the percent of the matched AUC of the same preparation before GABA treatment. This analysis gave an $\text{EC}_{50} = 22 \pm 3.5 \mu\text{M}$ and Hill slope of 5.8. Finally, the performance of liriodenine in this assay was compared between the wild-type CSOR and *rdl* strains (Fig. 5), and there was no difference observed in the ability of liriodenine to reverse GABA-dependent inhibition.

3.3. Whole-cell patch clamp recordings

Dissociated *P. americana* thoracic neurons were tested for their sensitivity to 5 mM GABA before incubation with increasing concentrations of bicuculline and liriodenine (Fig. 6). After perfusion of bicuculline methiodide at either 1 or 10 μM , the GABA-mediated currents were not significantly different from control (Fig. 6A, C). When perfused with liriodenine at 0.1 μM , the peak current elicited by GABA was slightly increased ($106 \pm 4 \%$), but not significantly different from control (Fig. 6B, C). However, GABA currents were significantly decreased to $51.6 \pm 2.5\%$ ($p < 0.01$) and $30.5 \pm 5.1\%$ ($p < 0.0001$), when perfused at 1.0 μM and 10 μM liriodenine, respectively (Fig. 6B, C). The GABA-elicited current after rinsing the last tested concentration were partially recovered, up to $84.4 \pm 7.6\%$ (Fig. 6C), and this value was not significantly different from control ($p = 0.48$).

3.4. Molecular similarity of liriodenine and bicuculline

A variety of modeling approaches were used to analyze the structural similarity between liriodenine and bicuculline (Fig. 1A). The shape and electrostatic similarity overlay of

liriodenine and bicuculline are shown in Figure 1B and C. Liriodenine has a rigid cyclic structure, while bicuculline has torsional freedom of the dioxole ring system, showing an out-of-plane orientation compared to liriodenine, while both showed similar hydrogen bond acceptor points. The molecular similarity MCSS calculated in KNIME was 0.92 suggesting a high degree of similarity (Pradeep et al. 2017). The logP calculated for liriodenine was 3.06, and bicuculline 2.88, while the logS calculated for liriodenine was -3.6, and -4.9 for bicuculline (Fig. 1A). These properties are important for solvent/membrane access for compounds interacting with proteins in phospholipid membranes.

4. Discussion

Topical toxicity experiments showed that liriodenine alone does not have much efficacy for killing mosquitoes, and that PBO does not greatly increase its effect (Fig. 2). In contrast, 25% DMSO increased significantly the efficacy of liriodenine on mortality rates, as well as increasing the toxicity of PBO, and the co-application of all three compounds showed greatest efficacy (Fig. 2). To assess whether these effects were synergistic or just additive, we numerically added the toxicity values of liriodenine, PBO, and DMSO alone for comparison to the toxicity observed when all three were applied together. Assumed additivity gave 1 hr knockdown (mean \pm SEM) of $29 \pm 3\%$, 4 hr knockdown of $51 \pm 4\%$, and 24 mortality of $22 \pm 3.4\%$. Statistical analysis was then performed to determine if the actual effects of the experimental ternary treatment were significantly different at the three time points. There was a significant effect of observation time (two-way ANOVA, $F_{2,30} = 27.3$, $df = 2$, $p < 0.001$) and all three calculated values (Bonferroni posttest, $p < 0.01$). Thus, the combined actions of liriodenine, DMSO, and PBO had a synergistic effect on mosquito mortality, probably due to DMSO facilitating the cuticular uptake of

liriodenine and PBO along with increased bioavailability within the mosquito by increasing half-life, *in vivo*.

Liriodenine caused hyper-excitation of the CNS (Fig. 3), and a GABA-blocking mode of action was hypothesized by comparing the structure of liriodenine to bicuculline (Fig. 1). Although it functions as a competitive GABA antagonist in mammals (Johnston et al., 1972), bicuculline is generally regarded to be inactive on insect GABA receptors. For example, Benson (1988) demonstrated that responses to GABA in voltage-clamped *Locusta migratoria* neurons were insensitive to 100 μ M bicuculline. Exposure of *D. melanogaster* CNS to liriodenine resulted in hyper-excitation, which could be indicative of GABA antagonism, although a multitude of other modes of action could also have this effect. To test the GABA hypothesis in the CNS assay, it was first pre-treated with 1 mM GABA solution, and application of increasing liriodenine concentrations restored firing in a concentration-dependent manner. To confirm that liriodenine is targeting directly the GABA receptor, current responses to GABA on neurons isolated from the thoracic ganglia of *Periplaneta americana* was evaluated after incubation of the preparation with increasing concentrations of liriodenine or bicuculline. Bicuculline up to 10 μ M was not able to antagonize significantly the currents induced by GABA, whereas liriodenine at both 1 and 10 μ M significantly blocked the effect of GABA (Fig. 6). These results confirm that liriodenine blocks the GABA receptors in a concentration-dependent manner and this could account for the observed hyperexcitation. Finally, the lack of cross resistance to liriodenine in CNS responses in the *rdl* strain of *D. melanogaster* (Fig. 5), suggests that liriodenine antagonizes GABA action by binding to a site unaffected by the *rdl* mutation.

To explore possible differences in protein interactions of liriodenine and bicuculline, we utilized *in silico* molecular modeling approaches. We found that the shape and electrostatic

properties of the two compounds to be quite similar, although liriodenine presents with a rigid core structure, whereas the methylenedioxyphenyl group of bicuculline fused to the lactone is rotated out of plane (Fig. 1). The planarity of liriodenine and higher lipophilic character as compared to bicuculline may explain the difference seen in pharmacological profiles. Additional mode of action studies along with molecular modeling are needed to understand more fully the actions of liriodenine with the insect GABA receptor.

5. Conclusions

These studies demonstrated that liriodenine exhibited moderate topical toxicity to mosquitoes, that was synergized by DMSO and PBO. As contact exposure might not be the most adequate to readily affect mosquitoes, ingestion might be better route of exposure, but that remains to be explored. Liriodenine showed potent activity in GABA assays at the tissue and cellular levels with no cross resistance from mutant GABA receptors. Molecular modeling studies suggested a structural basis for the differences in activity of bicuculline and liriodenine, although more study is required to verify this supposition. Altogether, the data present strong evidence that liriodenine affects the insect GABA receptor, but it also has documented ability to interact with ion channels (Chang et al., 1996) and these possible sites of action remain to be explored in insects, such as other chloride channels.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure Captions

Figure 1. Two dimensional structures of liriodenine and bicuculline (A), with the physiochemical properties logP and solubility logS. B) Wireframe (left), shape and fill (middle) and 3D structure of liriodenine with hydrogen bond acceptor sites (right). C) Wireframe electrostatic overlay of bicuculline on liriodenine, indicating structural similarity (left), with fill and key hydrogen bond acceptor sites that overlap (middle). Planar projection of the two compounds shows significant similarity, with a major difference being the benzo[1,3]dioxole system, which is out of plane (C, right hand figure). **(2 column width)**

Figure 2. Knockdown and lethality after topical application of liriodenine to *Anopheles gambiae* females. Knockdown was assessed at one hour and four hours after treatment, and mortality was determined after 24 hours. Bars are means \pm SEM. Within each time point, bars not labeled by the same letter indicate significant differences between groups (from post-hoc multiple comparison analyses) at $p < 0.05$. **(2 column width)**

Figure 3. Effects of the liriodenine on the CNS, showing time-dependent effects on firing frequency of the CNS, shown at 1 min intervals for 1-6 min post-treatment and at 3 min intervals thereafter. At T=0, application of liriodenine ranged from 0 (DMSO 0.7% control) to 50 μ M. Symbols are mean \pm SEM of $n = 8$ replicates of each concentration on individual CNS preparations. Horizontal lines for the 50 μ M (dotted line) and 40 μ M (solid line) treatments indicate time spans of data points that are significantly different from DMSO controls (2-Way ANOVA with Dunnett's post-test, $p < 0.05$). None of the responses at 10 μ M were different from controls. **(1 column width)**

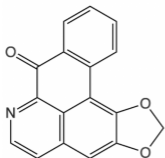
Figure 4. Effects of the liriodenine on GABA pre-treated CNS, showing time-dependent effects on firing frequency at 3 min intervals after GABA pretreatment. A) The points at T=0 reflect residual nerve activity after several min incubation in 1 mM GABA. Treatments at T =0 ranged from 0 (0.7% DMSO control) to 50 μ M liriodenine. Horizontal lines for the 50 μ M (dotted line), 40 μ M (solid line), and 30 μ M (dashed line) treatments indicate spans of data points that are significantly different from DMSO controls (2-Way ANOVA with Dunnett's post-test, $p < 0.05$). None of the responses at 15 or 20 μ M liriodenine were different from controls. B) Concentration-response analysis of liriodenine for reversing the effects of applied GABA. Effect is quantified as the area under the curve (AUC) during the 30-min drug exposure and compared to that before GABA treatment, and expressed as percentage increase above control AUC. Symbols are mean \pm SEM with N=3 to 5 replicates per treatment. **(2 column width)**

Figure 5. Effects of 40 μ M liriodenine on the GABA pre-treated *Dm*CNS of the wild type susceptible CSOR strain and the cyclodiene-resistant *rdl* strain. After 9 min of 1 mM GABA exposure, the preparation was challenged at T = 0 min with 40 μ M liriodenine in 0.1% DMSO. Symbols are mean \pm SEM. Statistical analysis of the liriodenine responses in the CSOR and *rdl* strains to matched DMSO controls found that liriodenine reversed significantly the effect of GABA in both strains, as indicated by the asterisks (t-test, $p < 0.05$). **(1 column width)**

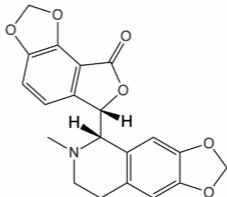
Figure 6. Blocking effect of bicuculline and liriodenine on GABA-evoked currents. After three-minute perfusion of saline (control) or drug, a challenge with 5 mM GABA was applied (horizontal line). A) Superimposed evoked currents recorded for GABA alone (Control, black line) and GABA + 1 or 10 μ M bicuculline (grey lines). B) Evoked currents recorded for GABA

alone (Control, black line) and GABA + 0.1, 1, or 10 μ M liriodenine (grey lines). C) Quantitation and statistical analysis of drug effects compared to the control amplitude of the first GABA current after saline perfusion. Bars are means \pm SEM. The horizontal dashed line indicates 50% inhibition. Only liriodenine at 1.0 μ M and 10 μ M concentrations produced a significant block of GABA currents when compared to control by ANOVA and Dunnett's post-test (*** $p < 0.0001$).

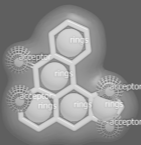
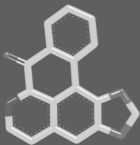
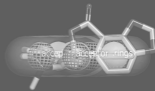
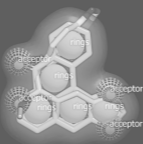
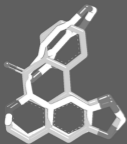
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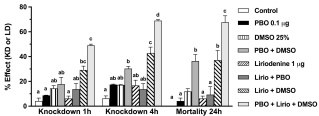
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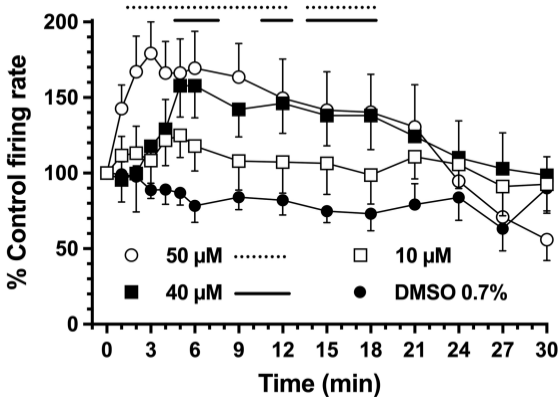
Liriodenine
 logP: 2.88
 logS: -3.6

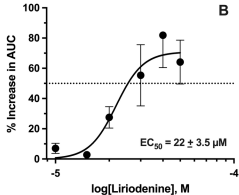
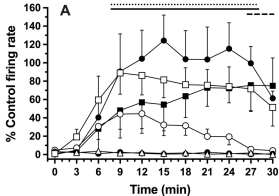


Bicuculline
 logP: 3.06
 logS: -4.06

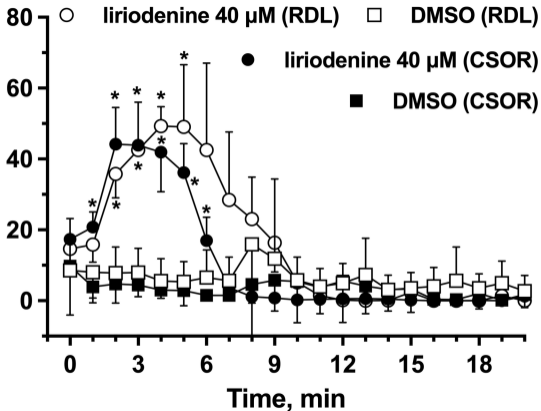
B**C**

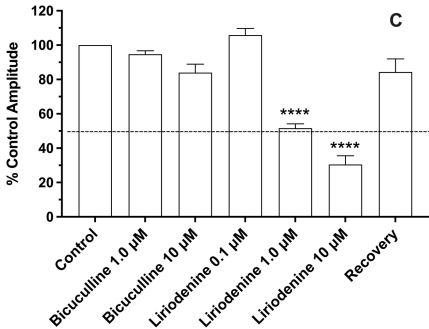
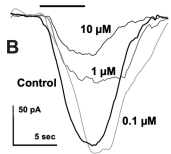
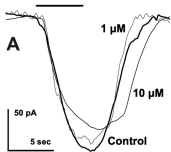




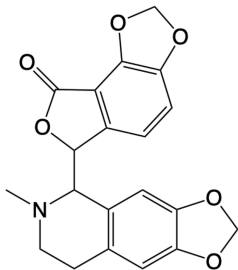
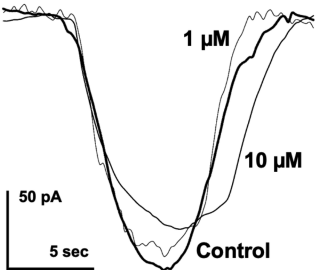


% Control firing rate



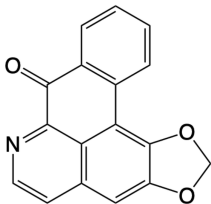
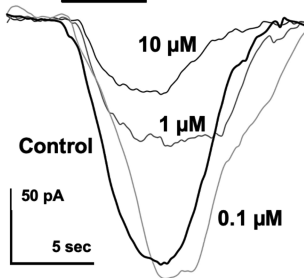


5 mM GABA



bicuculline

5 mM GABA



liriodenine