Modulation of the Antidepressant-Like Effects of Sustained Administration of Carisbamate and Lamotrigine on Monoaminergic Systems: Electrophysiological Studies in the Rat Brain

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Received February 27, 2013; accepted August 27, 2013

ABSTRACT

Carisbamate and lamotrigine are anticonvulsants that act on neuronal voltage-gated sodium channels. Carisbamate has shown antidepressant-like effects in animal models of depression, and lamotrigine is a mood stabilizer with a therapeutic effect in depressive episodes of patients with bipolar disorder. This study examined the effects of carisbamate and lamotrigine on monoaminergic transmission in rodents, which could contribute to their antidepressant action. In vivo electrophysiological recordings were carried out in rats after 2 and 14 days administration of vehicle, carisbamate (60 mg/kg daily), or lamotrigine (25 mg/kg daily). Overall firing activity of the dorsal raphe nucleus (DRN) serotonin (5-HT), locus coeruleus norepinephrine, and ventral tegmental area dopamine (DA) neurons was decreased with carisbamate. Lamotrigine also decreased 5-HT neuronal firing, and this effect was abolished by lesion of the prefrontal cortex. Despite these decreases in firing activity after their prolonged administration, both anticonvulsants exhibited a significant increase in tonic activation of hippocampus 5-HT1A receptors, as shown by a disinhibition of the firing activity of pyramidal neurons in response to the selective antagonist WAY-100635 (N-[2-[4(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-[2-pyridyl] cyclohexanecarboxamide trihydrochloride). This finding reveals an increase in the 5-HT level that may be attributed to a desensitization of the terminal 5-HT1B autoreceptors. This study demonstrates that sustained carisbamate and lamotrigine administration decreases 5-HT firing in the DRN but nevertheless enhances 5-HT transmission in the forebrain. This serotonergic effect may be associated with an antiglutamatergic action and may contribute to the antidepressant-like effect of carisbamate in the forced swim test and the antidepressant properties of lamotrigine.

Introduction

Depressive disorders are the most frequent psychiatric comorbidity in patients with epilepsy, as both disorders share a similar pathogenic mechanism involving reduced serotonergic transmission and excessive glutamate transmission (Cramer et al., 2003; Mazarati et al., 2008). Hence, reduction of glutamate transmission may be the final common step of action in which antidepressant drugs exert their therapeutic effect (Sanacora et al., 2012). Thus, the use of antiglutamatergic drugs as an alternate treatment of major depressive disorder has become of interest. Anticonvulsants are known to act as mood stabilizers in bipolar disorder and have also been shown to have beneficial antidepressant effects in the clinic (Reid et al., 2013). There is an important mechanism of action of some anticonvulsants through inhibition of a class of voltage-gated sodium channels (VGSCs) that would inhibit the excessive firing of neurons that is characteristic of seizures, thus blocking the release of excitatory amino acids (Rogawski, 2006).

Carisbamate [(S)-2-O-carbamoyl-1-o-chlorophenyl-ethanol] is a phenyl monocarbamate that has been shown to block presynaptic VGSCs and repetitive action potential firing in a use-dependent manner in rat hippocampal neurons, which may underlie some of its anticonvulsant activity (Liu et al., 2009; Lee et al., 2011). Carisbamate was shown to inhibit the Na+1.2 isoform of VGSCs, which is highly expressed in the hippocampus (Goldin, 2001; Liu et al., 2009). Its mechanism of action in epilepsy is related to an antiglutamatergic effect, as reductions in glutamate transmission have been observed in the granule cell of the dentate gyrus (Lee et al., 2011).
Indeed, it has been shown to display neuroprotective properties by dose dependently reducing neuronal loss in the hippocampus, amygdala, and thalamus after status epilepticus in rats (François et al., 2011). Another research group demonstrated that carisbamate had an antidepressant-like effect in the forced swim test (FST) model of depression in rodent models, with no significant affinity for 5-hydroxytryptamine (5-HT) or dopamine (DA) receptors. Therefore, it appears to have potential as an antidepressant, perhaps through an antiglutamatergic mechanism via sodium channel blockade.

Lamotrigine (Lamictal) is a widely used anticonvulsant that has been shown to have well tolerated and beneficial antidepressant efficacy in the depressed phase of bipolar disorder, notably in more severely depressed patients (Calabrese et al., 1999; Reid et al., 2013). With a principal mechanism of action similar to carisbamate, lamotrigine has been shown to inhibit VGSCs in rat hippocampal neurons, particularly during repetitive action potential firing (Xie et al., 1995; Kuo and Lu, 1997). Its mechanism is also related to an antiglutamatergic effect, as decreased glutamate transmission has been observed in the dentate gyrus and hippocampus (Lee et al., 2008). Indeed, lamotrigine has been shown to block the release of glutamate induced by the sodium channel opener veratrine and to interfere with the release of nitric oxide in forebrain regions, thus reducing excitotoxicity and imposing a neuroprotective effect (Leach et al., 1986; Lizasoain et al., 1995). A preclinical study by Bourin and others (2009) demonstrated that the antidepressant-like effect of lamotrigine in the FST was reversed by veratrine. In the modified FST, lamotrigine exerted an antidepressant-like effect that was shown to be related to a serotonergic effect, which suggests that its clinical antidepressant effect may be linked to an alteration of monoaminergic transmission on glutamate neurons (Codagnone et al., 2007).

The present in vivo electrophysiological study was aimed at determining the effects of both short- and long-term administration of carisbamate on monoaminergic transmission in both presynaptic and postsynaptic regions of the brain in relation to its antidepressant-like properties. After characterization, a comparison of carisbamate with lamotrigine was carried out to determine whether its efficacy in animal models could be translated to a potential antidepressant effect in a clinical setting.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) weighing 250–350 g at the time of the experiments were used. Animals were housed two per cage under standard laboratory conditions (12:12 hour light/dark cycle; light cycle start at 7:00 AM; temperature 21°C, 40–50% relative humidity) with access to food and water ad libitum. All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care and the local animal care committee of the University of Ottawa, Institute of Mental Health Research (Ottawa, ON, Canada).

Experimental Preparations. For short- and long-term drug administration, carisbamate (Molecular formula: C9H10ClNO3; RWJ-333689) was administered via subcutaneous implanted osmotic minipumps (Alzet; Durect Corporation, Cupertino, CA) for 2 and 14 days at a dose of 60 mg/kg daily; the dose used in a previous study (Francois et al., 2011); control rats received the vehicle (40% propylene glycol: 30% ethanol). The minipumps remained in situ throughout the recordings to ensure steady-state levels of the drug to mimic clinical conditions. Lamotrigine was administered via i.p. injections for 2 and 14 days at a dose of 25 mg/kg daily; control rats received the vehicle (35% propylene glycol: 7% ethanol). This dose of lamotrigine was used previously (Bourin et al., 2005).

The extracellular unitary recordings of the monoaminergic neurons were carried out using a single-unit glass micropipette filled with 2 M NaCl solution and impedance ranging between 2 and 4 MΩ. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses of chloral hydrate (50–100 mg/kg i.p.) were given to prevent any noxious reaction to tail or hind paw pinch. A burr hole was drilled at specific stereotaxic coordinates for each defined region for recordings, and neurons were identified by their spike shape, duration, and frequency. Neuronal activity was recorded in real time using the Spike2 software (Cambridge Electronic Design, Cambridge, UK), which was also used for analysis offline of the electrophysiological characteristics of the neurons. Body temperature was maintained at 37°C throughout the experiments by using a thermistor-controlled heating pad. A catheter was inserted in the lateral tail vein for intravenous injections of pharmacological agents.

Electrophysiological Recording of Dorsal Raphe Nucleus 5-HT Neurons. 5-HT neurons were recorded reliably at the following coordinates (in millimeters from lambda): anterior–posterior (A-P) 1–1.2, medial–lateral (M-L) 0, dorsal–ventral (D-V) 5.0–7.0. They were identified by their slow, regular firing rate (0.5–2.5 Hz) and their long spike duration, which is between 2 and 5 milliseconds (Besson et al., 2000).

Electrophysiological Recording of Locus Coeruleus Norepinephrine Neurons. Norepinephrine (NE) neurons were reliably recorded at the following coordinates (in millimeters from lambda): A-P –1.0 –1.2, M-L 1.0–1.3, D-V 5.0–7.0. They were identified by their regular firing rate (1–5 Hz) and their positive action potential duration, which is between 0.8 and 1.2 milliseconds. They can also be identified by a brief neuronal excitation to a noiceptive pinch of the contralateral hind paw followed by a short period of inhibition (Szabo et al., 2000).

Electrophysiological Recording of Ventral Tegmental Area DA Neurons. DA neurons were reliably recorded at the following coordinates (in millimeters from lambda): A-P 2.3–3.6, M-L 0.9–1.1, D-V 7.0–9.0. They were identified by their established electrophysiological criteria (Freeman et al., 1985; Ungless and Grace, 2012), including the following: 1) spontaneous firing rate between 2 and 90 spikes per second, exhibiting bursting activity or irregular firing; 2) biphasic or triphasic waveforms with an initial positive deflection (usually notched followed by prominent negative phase); 3) long duration potential (2–4 milliseconds); and 4) low pitch sound when monitored by an audioamplifier. A duration >1.1 millisecond from the start of the action potential to the center of the negative trough was also used to determine the DA neuron identity (Ungless and Grace, 2012).

Burst Analysis. The firing patterns of the monoaminergic neurons were analyzed by interspike interval (ISI) burst analysis. The onset of a burst was signified by the occurrence of two spikes with ISI <0.08 second for NE and DA neurons and ISI <0.01 second for 5-HT neurons. The termination of a burst was defined as an ISI >0.16 second for NE and DA neurons, and ISI >0.01 second for 5-HT neurons (Grace and Bunney 1983; Hajo and Sharp, 1996; Dawe et al., 2001).

Extracellular Recordings and Microiontophoresis of Dorsal Hippocampus CA3 Pyramidal Neurons. Microiontophoresis with a five-barreled micropipette was used to record dorsal hippocampus CA3 pyramidal neurons at A-P 3.8–4.5, M-L 4.2–4.5, D-V 3–4.5. They were identified by their large-amplitude (0.5–1.2 mV) with long-duration (0.8–1.2 millisecond) single-action potentials, alternating with complex spike discharge (Haddjeri et al., 1998). The central barrel of the micropipette was used for extracellular unitary recording and filled with 2 M NaCl. Side barrels were loaded with 5-HT creatinine sulfate (15 mM in 200 mM NaCl, pH 4), NE bitartrate (10 mM in 200 mM NaCl, pH 4), 3 M saline (automatic current balancing), and quisqualic acid (α-amino-3-hydroxy-5-methyl-4-isoxazolalprionic acid, AMPA; 1.5 mM in 200 mM NaCl, pH 8) to activate the neurons.

Neuronal responsiveness of the CA3 pyramidal neurons was determined by 50-second microiontophoretic applications of 5-HT and dopamine (DA) receptors. Therefore, it appears to have potential as an antidepressant, perhaps through an antiglutamatergic mechanism via sodium channel blockade.
NE and measured by the number of spikes suppressed per nanomole. To determine the relative degree to which the 5-HT or NE transporters were blocked, the 50% recovery time (RT50) was measured by determining the time (in seconds) after a 50-second ejection period of 5-HT or NE to recover 50% of the initial firing rate (de Montigny et al., 1980; Pineyro and Blier 1999).

**Tonic Activation of the Postsynaptic 5-HT1A Receptors on CA3 Pyramidal Neurons.** After the long-term regimen, to assess the degree of activation of the postsynaptic 5-HT1A receptors exerting an inhibitory effect on the firing activity of CA3 pyramidal neurons, the selective 5-HT1A receptor antagonist WAY-100635 N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl)-N-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride (25–100 µg/kg) was administered (intravenously) to disinhibit the hippocampal neurons, resulting in enhanced firing activity. It is most desirable to determine the disinhibition when the neurons are not firing at a high rate; therefore, their discharge rate was reduced to about 3–5 Hz by decreasing the ejection current of quisqualate, and WAY-100635 was then injected (Haddjeri et al., 1998) in incremental doses of 25 µg/kg at intervals of 2 minutes. To avoid residual drug effects, only one cell is studied per rat.

Any changes in the firing activity of hippocampus pyramidal neurons administered idazoxan (1 mg/kg) and prazosin (100 µg/kg) were intravenously given. After reducing and maintaining a steady firing rate of quisqualate, and WAY-100635 was then injected (intravenously) to disinhibit the hippocampal neurons, resulting in enhanced firing activity. It is most desirable to determine the disinhibition when the neurons are not firing at a high rate; therefore, their discharge rate was reduced to about 3–5 Hz by decreasing the ejection current of quisqualate, and WAY-100635 was then injected (Haddjeri et al., 1998) in incremental doses of 25 µg/kg at intervals of 2 minutes. To avoid residual drug effects, only one cell is studied per rat.

**Tonic Activation of Postsynaptic α1-Adrenergic Receptors.** The degree of tonic activation of postsynaptic α2- and α1-adrenoceptors was assessed using the selective antagonists idazoxan and prazosin, respectively. After reducing and maintaining a steady firing baseline, idazoxan (1 mg/kg) and prazosin (100 µg/kg) were intravenously administered to determine the disinhibition effects in control rats (vehicle-treated) and in rats administered carisbamate or lamotrigine for 14 days. Using this same paradigm in a recent study, suprion was shown to increase the tonic activation of postsynaptic α2- and α1-adrenoceptors by endogenous NE (Ghanbari et al., 2011).

**Sensitivity of Terminal 5-HT1B Autoreceptors.** The CA3 region of the hippocampus receives extensive projections from the median and DRN 5-HT neurons. The ascending 5-HT pathway afferent to the hippocampus was electrically stimulated by a bipolar electrode (NE-100; David Kopf, Tujunga, CA), which was implanted 1 mm anterior to lambda on the midline with a 10-degree backward angle in the ventromedial tegmentum and 8.0 ± 0.2 mm below the cortical surface of the brain. Two hundred square pulses with a duration of 0.5 ms and frequency delivered by a stimulator (S48; Grass Instruments, West Warwick, RI) at an intensity of 300 µA and at frequencies of 1 and 5 Hz. Stimulation of the 5-HT pathway induces a brief period of suppression resulting from the release of 5-HT in the synapse, exerting an inhibitory effect on the CA3 hippocampal pyramidal neurons, which corresponds to the duration of suppression (DOS, in milliseconds) value. Peristimulus time histograms of CA3 pyramidal neurons were generated to determine the suppression of firing measured as the DOS, which is defined as the time interval initiated by a 50% decrease in the number of spikes per bin from the mean prestimulation probability of firing to the time it returned to 90% of that same prestimulation value (Chaput et al., 1986). Stimulation of 1 and 5 Hz on the same neuron were used to determine the function of terminal 5-HT1B autoreceptors (Chaput et al., 1986). These two series of stimulations were used because previous studies showed that activation of terminal 5-HT1B autoreceptors decreases the 5-HT release in the terminal areas and that increasing the frequency of stimulation from 1 to 5 Hz induces a greater degree of activation of the autoreceptors, thus resulting in a greater negative feedback on the release of 5-HT (Chaput et al., 1986). As a result, the smaller release of neurotransmitter in the synapse obtained at 5 Hz induces a smaller DOS value compared with that of the 1-Hz stimulation. If the autoreceptor is desensitized, the difference between the effectiveness of the two rates of stimulation will be significantly reduced.

The stimulation pulses and the firing activity were analyzed by computer using Spike 2.

**Lesion of the Prefrontal Cortex.** Rats were anesthetized with an intraperitoneal injection of equithesin, a mixture of chloral hydrate, magnesium sulfate, and pentobarbital. Using a bipolar electrode (NE-100; David Kopf, Tujunga, CA), bilateral electrolytic lesions were made by passing a 1-mA for 10-second current via a 0.3-mm electrode, insulated except for the tip. A constant anodic current DC source was used. The coordinates were 3.4 mm anterior to bregma, 0.5 mm lateral to midline, and 4 mm ventral to dura (Joel et al., 1997). Control animals underwent the same surgical procedure but without the insertion of the electrode. Animals were left to recover in their home cages for 4 to 5 days before the start of 2-day injections of lamotrigine, followed by electrophysiological recordings. Histologic verification of lesion location was performed after electrophysiological recordings.

**Statistical Analysis.** All results are reported as mean values ± S.E.M. Statistical comparisons were carried out using the two-tailed Student's t test. Two-way analysis of variance (ANOVA) was applied for the statistical analyses of the data with pretreatment (prefrontal cortex [PFC]-lesioned versus -nonlesioned rats) and treatment (vehicle versus lamotrigine) as main factor, followed by Fisher's post hoc test. For tonic activation, results were analyzed using one-way repeated measures ANOVA, followed by Fisher's post hoc test when a parameter was studied in vehicle and treated rats. Statistical significance was taken as P < 0.05.

**Drugs.** Carisbamate was provided by Ortho-McNeil Janssen Scientific Affairs, LLC (Titusville, NJ) and dissolved in 40% propylene glycol:30% ethanol. Lamotrigine was purchased from LKT Laboratories, Inc. (St. Paul, MN) and dissolved in 35% propylene glycol:7% ethanol. WAY-100635 was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in distilled water.

**Results**

**Effects of 2- and 14-Day Administration of Carisbamate on the Firing Activity of DRN 5-HT, Locus Coeruleus NE, and Ventral Tegmental Area DA Neurons.** Compared with the vehicle group, the 2-day administration of carisbamate resulted in a significant reduction of the DRN firing rate by 28% (P < 0.001; unpaired Student’s t test; Fig. 1A). This result may be attributed to either a significant decrease in the number of bursting versus nonbursting neurons by 14% (P < 0.05; Fisher’s exact test; Table 1) or to a significant decrease in the burst rate by 47% (P < 0.05; unpaired Student’s t test; Table 1). The significant reduction in firing activity persisted after the 14-day carisbamate administration (Fig. 1B) to a similar degree of 24% (P < 0.01; unpaired Student’s t test) but with no change in the burst activity (Table 1).

The 2-day administration of carisbamate yielded no change in the locus coeruleus (LC) NE neurons firing activity (Fig. 1C) or in the burst activity (Table 1). The 14-day carisbamate administration resulted in a significant reduction of NE neurons firing rate by 34% (P < 0.001; unpaired Student’s t test; Fig. 1D). This result may be due to a significant decrease in the number of bursting versus nonbursting neurons by 19% (P < 0.01; Fisher’s exact test; Table 1).

The 2-day administration of carisbamate yielded no change in the ventral tegmental area (VTA) DA neurons firing activity (Fig. 1E) or in the burst parameters. The 14-day carisbamate administration significantly decreased the firing activity of VTA DA neurons by 26% (P < 0.001; unpaired Student’s t test; Fig. 1F). This finding may be attributed to...
Fig. 1. Effects of 2- and 14-day carisbamat administration on monoaminergic firing activity. Mean (± S.E.M.) of the firing rate of DRN 5-HT (A and B), LC NE (C and D), VTA DA (E and F) neurons in vehicle rats and rats treated with carisbamate (60 mg/kg per day s.c.) for 2 days (A, C, and E) and 14 days (B, D, and F). The numbers in the histograms correspond to the number of neurons recorded (4–8 rats tested per group). ***P < 0.001; **P < 0.01.

TABLE 1
Summary of the effect of 2- and 14-day administration of carisbamate (CRS) and lamotrigine (LTG) on the firing and burst activity of dorsal raphe nucleus (DRN) 5-hydroxytryptamine (5-HT), locus coeruleus (LC) norepinephrine (NE), and ventral tegmental area (VTA) dopamine (DA) neurons

<table>
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<tr>
<th>Drug</th>
<th>Area</th>
<th>TX</th>
<th>Veh</th>
<th>TX</th>
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<th>TX</th>
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<tr>
<td>CRS</td>
<td>DRN 5-HT</td>
<td>2-day</td>
<td>1.3 ± 0.07</td>
<td>0.9 ± 0.06***</td>
<td>37</td>
<td>23*</td>
<td>15.8 ± 2.52</td>
<td>8.4 ± 1.74*</td>
<td>21 ± 0.06</td>
<td>21 ± 0.05</td>
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<td></td>
<td></td>
<td>14-day</td>
<td>1.4 ± 0.08</td>
<td>1.1 ± 0.05**</td>
<td>28</td>
<td>28</td>
<td>13.4 ± 2.92</td>
<td>12.1 ± 2.86</td>
<td>21 ± 0.04</td>
<td>21 ± 0.07</td>
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<tr>
<td></td>
<td>LC NE</td>
<td>2-day</td>
<td>1.6 ± 0.09</td>
<td>1.5 ± 0.08</td>
<td>21</td>
<td>28</td>
<td>3.0 ± 0.62</td>
<td>2.6 ± 1.19</td>
<td>21 ± 0.05</td>
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<td></td>
<td></td>
<td>14-day</td>
<td>2.0 ± 0.09</td>
<td>1.3 ± 0.09***</td>
<td>40</td>
<td>21*</td>
<td>2.3 ± 0.35</td>
<td>3.2 ± 0.69</td>
<td>21 ± 0.06</td>
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<td></td>
<td>VTA DA</td>
<td>2-day</td>
<td>3.5 ± 0.18</td>
<td>3.6 ± 0.16</td>
<td>87</td>
<td>94</td>
<td>18.3 ± 2.29</td>
<td>18.5 ± 1.83</td>
<td>27 ± 0.13</td>
<td>29 ± 0.15</td>
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<td></td>
<td></td>
<td>14-day</td>
<td>4.6 ± 0.17</td>
<td>3.4 ± 0.19**</td>
<td>99</td>
<td>96</td>
<td>30.9 ± 2.35</td>
<td>22.7 ± 2.41*</td>
<td>3.3 ± 0.16</td>
<td>2.8 ± 0.12*</td>
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<tr>
<td>LTG</td>
<td>DRN 5-HT</td>
<td>2-day</td>
<td>1.1 ± 0.06</td>
<td>0.9 ± 0.05**</td>
<td>34</td>
<td>29</td>
<td>11.7 ± 1.44</td>
<td>11.6 ± 1.91</td>
<td>21 ± 0.05</td>
<td>2.0 ± 0.04</td>
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<td></td>
<td></td>
<td>14-day</td>
<td>1.0 ± 0.06</td>
<td>0.8 ± 0.05**</td>
<td>39</td>
<td>23</td>
<td>15.7 ± 3.19</td>
<td>11.5 ± 2.74</td>
<td>2.0 ± 0.03</td>
<td>2.1 ± 0.05</td>
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Veh, vehicle; TX, treatment.

*P < 0.05; **P < 0.01; ***P < 0.001.
either a significant reduction in the burst rate by 27% ($P < 0.05$; unpaired Student’s $t$ test; Table 1) or to a significant decrease in the mean number of spikes per burst by 15% ($P < 0.05$; unpaired Student’s $t$ test; Table 1).

Assessment of the Overall Serotonergic Tone after 14-Day Carisbamate Administration on Postsynaptic 5-HT$_{1A}$ Receptors on Dorsal Hippocampus CA3 Pyramidal Neurons. Microiontophoretic application of exogenous 5-HT resulted in no change in the sensitivity of the postsynaptic 5-HT$_{1A}$ receptors, as shown by a lack of significant difference in the number of spikes suppressed per nanoampere [vehicle: $11 \pm 0.7$ spikes suppressed/nA ($n = 10$); carisbamate: $12 \pm 0.7$ spikes suppressed/nA ($n = 11$)]. There was no change in the RT$_{50}$, indicating a lack of effect at the 5-HT transporters [vehicle: $32 \pm 4$ seconds ($n = 10$); carisbamate: $42 \pm 7$ seconds ($n = 9$)].

The 14-day carisbamate administered group displayed enhanced tonic activation of the postsynaptic 5-HT$_{1A}$ receptors by extracellular 5-HT in the hippocampus, as shown by a robust disinhibition of the neuronal firing rate (Fig. 2B) in response to WAY-100635 at $50 \mu g/kg$ (321% baseline), $75 \mu g/kg$ (391%), and $100 \mu g/kg$ (396%) cumulative doses ($P < 0.001$ for the three doses; one-way repeated measures ANOVA; Fig. 2C). Indeed, the last injection of WAY-100635 enhanced the pyramidal neuron firing activity by approximately 3.8-fold compared with vehicle rats, indicating that there is enhanced tonic activation of 5-HT$_{1A}$ receptor, potentially accompanied by an increase in serotonergic transmission in the postsynaptic region. As illustrated in Fig. 2A and C, cumulative intravenous injections of WAY-100635 do not markedly alter the firing activity of dorsal hippocampus CA3 pyramidal neurons in the vehicle group.

Assessment of the Overall Noradrenergic Tone after 14-Day Carisbamate Administration on Postsynaptic $\alpha_2$-Adrenergic Receptors on Dorsal Hippocampus CA3 Pyramidal Neurons. Microiontophoretic application of exogenous NE did not change the sensitivity of the postsynaptic $\alpha_2$-adrenergic receptors, as shown by a lack of significant difference in the number of spikes suppressed per nanoampere [vehicle: $7 \pm 0.9$ spikes suppressed/nA ($n = 7$); carisbamate: $6 \pm 0.4$ spikes suppressed/nA ($n = 8$)]. There was also no effect on the RT$_{50}$, indicating a lack of effect at the NE transporters [vehicle: $78 \pm 19$ seconds ($n = 6$); carisbamate: $54 \pm 14$ seconds ($n = 7$)].

The long-term administration of carisbamate resulted in no change in the tonic activation of the $\alpha_2$- and $\alpha_1$-adrenergic receptors as shown by a lack of significant disinhibition of the neuronal firing rate in response to the selective antagonists idazoxan and prazosin, respectively (Fig. 3).

Assessment of the Effects of 14-Day Carisbamate Administration on the Function of Terminal 5-HT$_{1B}$ Autoreceptors. The 5-HT fibers afferent to the hippocampus were electrically stimulated, and the frequency of the stimulation was increased from 1 to 5 Hz on the same neuron to assess the function of the terminal 5-HT$_{1B}$ autoreceptor. This resulted in a significantly reduced DOS in the vehicle group by 20% ($P < 0.001$; paired Student’s $t$ test; Fig. 4) stemming from a greater degree of activation of the autoreceptors, thus producing a greater negative feedback on the release of 5-HT at 5 Hz (Chaput et al., 1986). Conversely, the rats treated with carisbamate reduced the difference between the effectiveness of the two stimulation frequencies, indicating that the autoinhibitory role of the terminal 5-HT$_{1B}$ receptor was dampened (Fig. 4).

Effects of 2- and 14-Day Administration of Lamotrigine on the Firing Activity of DRN 5-HT Neurons. Compared with the vehicle group, 2-day administration of lamotrigine induced a significant decrease in the DRN firing rate of 24% ($P < 0.01$; unpaired Student’s $t$ test; Fig. 5A) but with no change in the burst activity (Table 1). Similar to carisbamate, this significant reduction in the firing rate also persisted after 14-day lamotrigine administration (Fig. 5B) to a similar degree, 22% ($P < 0.01$; unpaired Student’s $t$ test), accompanied with a significant decrease in the number of
bursting versus nonbursting neurons by 16% ($P < 0.05$; Fisher’s exact test; Table 1).

This decrease in 5-HT neuron firing may stem from an action of lamotrigine on the facilitatory effect of glutamatergic neurons in the PFC (Celada et al., 2001), suggesting that the lesion of PFC may dampen the effect of lamotrigine. Indeed, the lesions obtained were elongated in the anteroposterior axis and extended A-P from 3.7 to 2.7 mm anterior to bregma. A two-way ANOVA on the firing activity of 5-HT neurons revealed a significant effect of treatment factor [$F(3,351) = 5.42, P = 0.04$] and an interaction between both variables [$F(3,351) = 3.9, P = 0.04$]. Although the lesion of the PFC resulted in a significant decrease in the firing activity of the 5-HT neurons ($P < 0.02$), it abolished this inhibitory effect induced by 2-day administration of lamotrigine ($P > 0.05$; Fig. 6).

Assessment of the Overall Serotonergic Tone after 14-Day Lamotrigine Administration on Postsynaptic 5-HT$_{1A}$ Receptors on Dorsal Hippocampus CA3 Pyramidal Neurons. Microiontrophic application of exogenous 5-HT resulted in no significant difference in the sensitivity of the postsynaptic 5-HT$_{1A}$ receptors, as shown by a lack of change in the number of spikes suppressed per nanoampere [vehicle: 9 ± 0.5 spikes suppressed/nA ($n = 9$); lamotrigine: 8 ± 0.7 spikes suppressed/nA ($n = 6$)]. There was no change in the RT$_{50}$, indicating a lack of effect at the 5-HT transporters [vehicle: 45 ± 8 seconds ($n = 8$); lamotrigine: 46 ± 8 seconds ($n = 7$)].

Similar to carisbamate, the group administered lamotrigine for 14 days displayed enhanced tonic activation of the postsynaptic 5-HT$_{1A}$ receptor, as shown by a significant disinhibition of the neuronal firing rate (Fig. 7B) in response to WAY-100635 at 75 mg/kg (364% of baseline) and 100 mg/kg (395%) cumulative doses ($P < 0.001$ for both doses; one-way repeated measures ANOVA; Fig. 7C). Indeed, the last injection of WAY-100635 enhanced the pyramidal neuron firing activity by approximately 3-fold compared with vehicle rats. This finding indicates that there is enhanced serotonergic transmission in this postsynaptic region. As illustrated in Fig. 7, A and C, cumulative systemic injections of WAY-100635 did not significantly change the firing activity of dorsal hippocampus CA3 pyramidal neurons in the vehicle group.

Assessment of the Effects of 14-Day Lamotrigine Administration on the Function of Terminal 5-HT$_{1B}$ Autoreceptors. Increasing the frequency of the stimulation from 1 to 5 Hz resulted in a significantly reduced DOS in the vehicle group by 34% ($P < 0.001$; paired Student’s $t$ test; Fig. 8) as the result of a greater degree of activation of the autoreceptors, thus resulting in less 5-HT being released at 5 Hz (Chaput et al., 1986). Similar to those treated with carisbamate, the rats treated with lamotrigine showed a reduction in the difference between the effectiveness of the two stimulation frequencies, indicating that the autoinhibitory effect of the terminal 5-HT$_{1B}$ receptor was diminished (Fig. 8). The DOS at a stimulation frequency of 1 Hz was significantly increased with lamotrigine by 20% compared with the vehicle group, suggesting that more 5-HT is being released per electrical impulse reaching the terminal ($P < 0.05$; unpaired Student’s $t$ test (Fig. 8).
Clinical studies have shown that lamotrigine has some beneficial antidepressant effects in major depressive episodes (Normann et al., 2002; Barbosa et al., 2003; Barbee et al., 2011), whereas currently there are no clinical studies for the use of carisbamate in mood disorders. However, carisbamate and lamotrigine present some similarities in that their main mechanism of action is at VGSCs, and they both have antidepressant-like effects in the FST model of depression; hence their comparison may allow a better understanding of their preclinical and clinical effects.

Carisbamate and lamotrigine administration for both 2 and 14 days resulted in a decrease in DRN 5-HT neuronal firing activity. The latter result may not be due to an acute increase in 5-HT, as it was reported that lamotrigine (20 mg/kg), when administered systemically, dose-dependently decreases the extracellular 5-HT levels in the DRN (Tanahashi et al., 2012). Alternatively, previous in vitro evidence indicates that ionotropic glutamate receptors are involved in the excitatory control of 5-HT neurons (Pan and Williams, 1989). It was also shown that AMPA/kainate receptors regulate extracellular levels of 5-HT in the raphe and forebrain (Tao et al., 1997). Moreover, using orthodromic stimulation of the medial PFC, it was found that its afferents modulate 5-HT neuron activity in vivo (Celada et al., 2001). It was then suggested in the latter study that endogenous glutamate from stimulated medial PFC afferents, acting on N-methyl-D-aspartate (NMDA) receptors, would induce a sustained facilitation of serotonergic activity. Carisbamate and lamotrigine decreased the discharge activity of 5-HT neurons, which could result from a reduction of excitatory inputs onto DRN because these drugs inhibit glutamate release (Bourin et al., 2009). This possibility was supported in the present study whereby a lesion of the PFC prevented the inhibitory effect of 2-day administration of lamotrigine on 5-HT neuronal firing. Similarly, selective 5-HT reuptake inhibitors (SSRIs), the most prescribed antidepressants, decrease the firing activity of 5-HT neurons after subacute administration. This dampened activity, unlike that produced by carisbamate and lamotrigine, returns to the control level after long-term administration, accompanied by terminal 5-HT1B autoreceptor desensitization and an increased tonic activation of 5-HT1A receptors in the hippocampus (Haddjeri et al., 1998; Pineyro and Blier, 1999).

An increase in tonic activation of 5-HT1A receptors after 14-day administration of carisbamate and lamotrigine was also found in a projection area, namely, the hippocampal CA3 region, despite the decrease in 5-HT firing activity. This disconnect between 5-HT neuronal firing and net transmission level is not uncommon, as a similar result was obtained with 2-day administration of a combination of the α2-adrenoceptor blocker mirtazapine and the SSRI paroxetine: a marked decrease in DRN 5-HT firing activity accompanied with significantly enhanced tonic activation of 5-HT1A receptors in the hippocampus (Besson et al., 2000). Moreover, a study using quetiapine also produced a diminished 5-HT firing activity and an increased tonic activation of the postsynaptic 5-HT1A receptors (Chernoloz et al., 2012). It is important to note that the net increase in 5-HT transmission, observed in the hippocampus, might have been due to enhanced responsiveness of the postsynaptic 5-HT1A receptors, as tricyclic antidepressant and electroconvulsive shocks have been shown to increase the sensitivity of these receptors to 5-HT (de Montigny and Aghajanian, 1978; de Montigny, 1984). However, the present in vivo study did not show the 5-HT1A sensitivity after carisbamate and lamotrigine administration to be a contributing factor. Furthermore, the enhancement in 5-HT transmission in the hippocampus was not due to blockade of 5-HT reuptake with either drug, since the
present results showed no change in the RT50 index, which reflects 5-HT reuptake activity (Pineyro and Blier, 1999). The latter was shown to be effectively blocked in vitro after acute lamotrigine administration in rat brain synaptosomes and human platelets. However, it was established in the same study that the active concentration range of lamotrigine was 4 orders of magnitude greater than that of the SSRI fluoxetine necessary to exert the same effect and that the uptake inhibition was nonselective because NE and DA carriers were also blocked (Southam et al., 1998).

The increase in 5-HT transmission could stem from desensitization of the 5-HT1B autoreceptors, as the decreased inhibition of CA3 pyramidal neurons after increased electrical stimulation from 1 to 5 Hz is dampened, as shown in Figs. 4 and 8. This phenomenon has also been observed after treatment with SSRIs, where 5-HT transmission was increased in postsynaptic areas (Pineyro and Blier, 1999). The present experiments showed that the inhibitory capacity of endogenous 5-HT after 5-HT bundle stimulation is increased after long-term lamotrigine administration, suggesting that more 5-HT is being spontaneously released under chronic lamotrigine. Indeed, an increase in 5-HT transmission was reflected in the enhancement of tonic activation of 5-HT1A receptors after lamotrigine and carisbamate, in accordance with microdialysis studies showing that administration of lamotrigine for 14 days increased basal extracellular 5-HT in the ventral hippocampus of freely moving rats (Ahmad et al., 2004).

Evidence that the postsynaptic enhancement of 5-HT is related to a potential antidepressant effect was observed in a study by Bourin and others (2005) demonstrating that the anti-immobility effect of lamotrigine in the FST was potentiated by the postsynaptic agonist 8-OH-DPAT. Activation of postsynaptic 5-HT1A receptors by 8-OH-DPAT has also been implicated in the improvement of seizure-induced depressive deficits in animal models (Pineda et al., 2011). This finding suggests that the mechanism of action of lamotrigine and carisbamate involves activation of these 5-HT1A receptors, which would be beneficial for the treatment of both bipolar and unipolar depression. Moreover, using similar doses to those used in the present study, it has been shown that the behavioral effect of lamotrigine in the modified FST was associated with an increase in swimming, which is a serotonergic-related behavior (Consoni et al., 2006), as also demonstrated with SSRIs (Page et al., 1999). This serotonergic effect may be associated with its antiglutamatergic activity since the increase in swimming was blocked by the sodium channel opener veratrine (Codagnone et al., 2007), which suggests that altered serotonergic transmission in postsynaptic brain areas caused by lamotrigine and carisbamate may be due to their control of glutamate transmission in the hippocampus. The present results are in accordance with the hypothesis that antiglutamatergic drugs exert their antidepressant effect partly through a suppression of glutamatergic activity and an increase in 5-HT transmission, which also can inhibit glutamate release (Consoni et al., 2006).
Although the present study showed no net increase in noradrenergic transmission as measured in the hippocampus, there was a reduction in NE and DA firing activity after the 14-day carisbamate regimen. It was also shown that systemic administration of lamotrigine had no effect on extracellular levels of NE and DA when measured in the medial PFC (Quarta and Lare, 2011), whereas Ahmad et al. (2004) demonstrated a decrease in overflow of DA in the hippocampus. Even though no change in NE transmission was measured in our hands, a previous behavioral study had shown that climbing, which is a measure of the activity dependent on NE, was increased by lamotrigine (Codagnone et al., 2007). The dampening effect of carisbamate on catecholamines is comparable to SSRIs, which are associated with decreased catecholaminergic activity after long-term regimens as a result of increased serotonergic transmission (Szabo et al., 2000; Dremencov et al., 2009). In addition, the decreased firing activity of NE and DA lends support to an antinamic effect of carisbamate and lamotrigine for bipolar disorder, as catecholamine depletion has been shown to attenuate symptoms of mania (Barney et al., 1971).

Postsynaptic enhancement of serotonergic transmission observed with carisbamate may be related to a potential antidepressant effect in a clinical setting. It also supports an antidepressant effect of lamotrigine in the clinic, as it is more typically used for treatment of the depressed phase of bipolar disorder rather than for treatment of mania or hypomania (Reid et al., 2013). It has been suggested that change in serotonergic neurotransmission may provide a common link between epilepsy and depression, as augmenting antidepressant treatments with anticonvulsants improves antidepressant effects (Jobe et al., 1999; Normann et al., 2002; Barbosa et al., 2003). Furthermore, vagus nerve stimulation is effective in both epilepsy and treatment-resistant depression, presumably through an enhancement of postsynaptic serotonergic and noradrenergic transmission (Schlaepfer et al., 2008; Manta et al., 2012). One way to determine whether the therapeutic antidepressant effect of lamotrigine in patients with a major depressive episode is indeed due increased serotonergic transmission would be to determine whether tryptophan depletion causes a relapse in depressive symptoms.

In conclusion, the present study reinforces the hypothesis that the potential antidepressant-like effect of carisbamate and lamotrigine could be due in part to a serotonergic involvement and a direct attenuation of glutamate transmission. The potential of these drugs to rectify the impairments in cellular plasticity and cell survival support the use of carisbamate and lamotrigine as augmentation strategies and may in fact represent a new class of antidepressants in the growing field of glutamatergic-based treatments.

Authorship Contributions

Participated in research design: El Mansari, Blier.
Conducted experiments: Shim.
Performed data analysis: Shim, El Mansari.
Wrote or contributed to the writing of the manuscript: Shim, El Mansari, Blier.

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