Acute Effects of Brexpiprazole on Serotonin, Dopamine, and Norepinephrine Systems: An In Vivo Electrophysiologic Characterization

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ABSTRACT

Brexipiprazole, a compound currently undergoing investigation for the treatment of depression and schizophrenia, has been shown to possess a unique spectrum of effects on serotonergic and dopaminergic systems. In the present study, the acute effects of brexpiprazole on serotonin (5-HT) receptor subtypes 5-HT1A, 5-HT1B, and 5-HT2A; dopamine (DA) D2 autoreceptors, and α2-adrenergic receptors were compared with those of aripiprazole, a drug in wide clinical use. In the dorsal raphe nucleus (DRN) and in the ventral tegmental area (VTA), brexpiprazole displayed an inhibitory effect on 5-HT1A autoreceptors and 5-HT2A receptor agonist DOI (2,5-dimethoxy-4-iodoamphetamine) was more potent than aripiprazole (ED50 = 110 μg/kg), demonstrating 5-HT2A antagonistic action. Brexpiprazole reversed the inhibitory effect of the DA agonist apomorphine on VTA DA neurons (ED50 = 61 μg/kg), whereas it was ineffective when administered alone, indicating partial agonistic action on D2 receptors. Compared with aripiprazole, which significantly inhibited the firing activity of VTA DA neurons, brexpiprazole displayed less efficacy at D2 receptors. In the hippocampus, brexpiprazole acted as a full agonist at 5-HT1A receptors on pyramidal neurons. Furthermore, it increased 5-HT release by terminal α2-adrenergic heteroceptor but not 5-HT1B autoreceptor antagonism. In the lateral geniculate nucleus, brexpiprazole displayed α1B-adrenergic receptor antagonistic action. Taken together, these results provide insight into the in vivo action of brexpiprazole on monoamine targets relevant in the treatment of depression and schizophrenia.

Introduction

Brexipiprazole (OPC-34712) is a compound currently undergoing investigation for the treatment of depression and schizophrenia. Antipsychotic medications of the first and second generations are efficacious antagonists at dopamine (DA) D2 receptors. Indeed, D2 receptor antagonism is an effective strategy for treatment of positive symptoms in schizophrenia (Seeman and Lee, 1975; Rao and Remington, 2013). However, it is accompanied by unwanted motor side effects leading to extrapyramidal symptoms, at least in part by decreasing dopaminergic transmission in the striatum (Glazer, 2000; Kapur et al., 2000). These side effects are dampened when combined with antagonism of the serotonin (5-HT) receptor subtype 5-HT2A, a defining pharmacologic characteristic of second-generation antipsychotics (Stockmeier et al., 1993) that may be of therapeutic benefit in the treatment of both schizophrenia and mood disorders (Blier and Szabo, 2005; Kuroki et al., 2008).

Most second-generation antipsychotics have higher in vitro affinity for 5-HT2A than D2 receptors; brexpiprazole and aripiprazole are different in this regard (5-HT2A Ki = 0.47 and 4.7 nM and D2 Ki = 0.30 and 0.87 nM, respectively; Maeda et al., 2014b). Whereas most other atypical antipsychotics are D2 receptor antagonists, in vitro data indicate that brexpiprazole is a D2 partial agonist with lower intrinsic activity at D2 receptors than aripiprazole (Maeda et al., 2014b). Partial D2 receptor agonism is thought to have a buffering action on DA neurotransmission by stimulating D2 receptors under low DA conditions, while dampening their activation when DA levels are high (Burris et al., 2002). Indeed, in vivo systemic administration of aripiprazole has been shown to decrease the firing activity of ventral tegmental area (VTA) DA neurons to

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~70% of baseline, whereas complete inhibition of these neurons by the DA receptor agonist apomorphine was reversed by brexpiprazole to a similar degree (Dahan et al., 2009). In contrast, acute haloperidol and clozapine administration increases firing of VTA DA neurons by D2 autoreceptor antagonism (Hand et al., 1987). Despite a different action on D2 autoreceptors, brexpiprazole improved behavioral measures predictive of antipsychotic efficacy, such as apomorphine-induced stereotypy and the conditioned avoidance response (Maeda et al., 2014a). Furthermore, brexpiprazole reduced head twitches induced by the preferential 5-HT2A receptor agonist DOI (2,5-dimethoxy-4-iodoamphetamine) and restored phenylcaine-induced cognitive impairment to a greater extent than the 5-HT2A receptor antagonist M100907 [(R) (+)-α-(2,3-dimethoxyphenyl)-1-(2-(4-fluorophenyl)ethyl)-4-pipidinemethanol], indicating in vivo antagonistic action at 5-HT2A receptors (Maeda et al., 2014a).

Aripiprazole and brexpiprazole are in vitro 5-HT1A receptor agonists (Ki = 1.3 and 0.12 nM, respectively; Maeda et al., 2014b), a relevant pharmacologic characteristic in the treatment of mood disorders and schizophrenia (Blier and Ward, 2003; Newman-Tancréd, 2010). For aripiprazole, in vivo electrophysiology studies showed acute agonistic action at 5-HT1A autoreceptors in the dorsal raphe nucleus (DRN; Stark et al., 2007; Dahan et al., 2009). Interestingly, these autoreceptors were desensitized after only 2 days of aripiprazole administration (Chernoloz et al., 2009). Desensitization of 5-HT1A receptors could increase 5-HT neurotransmission, a common effect of long-term antidepressant administration thought to be therapeutically beneficial in the treatment of mood disorders (Blier and El Mansari, 2013). The recent demonstration that the restorative effect of both acute and chronic brexpiprazole on impaired cognitive function was lost by 5-HT1A receptor blockade further suggests in vivo 5-HT1A receptor agonism by this compound (Maeda et al., 2014a; Yoshimi et al., 2014).

Antagonism of α-adrenoceptors is a pharmacologic feature that may have therapeutic implications for schizophrenia and depression (Fawcett and Barkin, 1998; Arnsten, 2004; Marcus et al., 2010). In vitro, brexpiprazole has selective affinity for α1B-adrenoceptors (Ki = 0.17 nM) over α1A (Ki = 3.8 nM) and α1D (Ki = 2.6 nM) subtypes (Maeda et al., 2014b). Furthermore, it may have antagonistic properties on α2-adrenoceptors (Maeda et al., 2014b), a pharmacologic characteristic known to increase neurotransmission of norepinephrine (NE) (Ghanbari et al., 2011; Chernoloz et al., 2012) and 5-HT (Mongeau et al., 1993; Haddjeri et al., 1998) by blockade of terminal α2-adrenergic autoreceptors and heteroreceptors, respectively.

Compared with aripiprazole, brexpiprazole has a 3- to 4-fold-higher in vitro affinity for the 5-HT transporter (SERT; IC50 = 29 nM) and NE transporter (NET; IC50 = 140 nM) (Maeda et al., 2014b). Blockade of these transporters is known to enhance 5-HT and NE neurotransmission (El Mansari et al., 2005; Chernoloz et al., 2012). As agents that block these transporters are currently first-line treatments for mood disorders, monoamine reuptake–blocking properties could contribute to the clinical efficacy of brexpiprazole.

To complement and extend insight into its action on therapeutically relevant targets, the present study used electrophysiologic techniques to determine the in vivo activity of brexpiprazole on 5-HT1A, 5-HT1B, 5-HT2A, and D2 receptors, on α1- and α2-adrenoceptors, and on the SERT and NET.

Materials and Methods

Animals. Experiments were carried out in male Sprague-Dawley rats (Charles River Laboratories, St. Constant, QC, Canada) weighing 275–325 g housed under standard laboratory conditions (12-hour light/dark cycle with food and water ad libitum). In vivo extracellular unitary recordings were carried out in chloral hydrate–anesthetized rats (400 mg/kg i.p.) that were mounted in a stereotaxic apparatus. Body temperature was maintained at 37°C throughout the experiment utilizing a thermistor-controlled heating pad. If applicable, prior to the electrophysiologic recordings, a catheter was inserted in a lateral tail vein for systemic intravenous injection of pharmacologic agents. At the end of experiments, animals were euthanized by a lethal dose of chloral hydrate (4% solution i.p.). All experiments were carried out in accordance with the Canadian Council on Animal Care and the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, ON, Canada).

Compounds. Brexpiprazole (200 µg/kg; Maeda et al., 2014b), aripiprazole (200 µg/kg), and the DA agonist apomorphine (40 µg/kg) were dissolved in a 0.5% lactic acid solution in distilled water; the pH of the solution was adjusted to 4.5 by addition of NaOH. The preferential 5-HT2A receptor agonist DOI (100 µg/kg), the α2-adrenoceptor antagonist yohimbine (10 and 400 µg/kg), and the 5-HT1A receptor antagonist WAY 100.635 ([N-(2-[4-(2-methoxyphenyl)-1-piperidinyl]ethyl)-(2-pyridyl)cyclohexanecarboxamide) were dissolved in distilled water. The selective α1A-adrenoceptor antagonist (Wetzel et al., 1995) SNAP 5089 ([5-[[4-(4-diphenyl-1-piperidinyl)propyl]amino]carbonyl]-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylic acid methyl ester hydrochloride; 1 mg/kg) was dissolved in 20% β-cyclodextrin in distilled water. Brexpiprazole and aripiprazole were provided by Lundbeck A/S (Valby, Denmark); all other compounds were purchased from Sigma-Aldrich (Oakville, ON, Canada).

In Vivo Electrophysiologic Recordings. Extracellular recordings of neurons in the VTA, DRN, and locus coeruleus (LC) were carried out with a single-barrel glass micropipette (Stoelting, Spencerville, MD) preloaded with 2 M NaCl and with impendence between 2 and 6 MΩ. Neurons in the dorsal raphe (DRN; Stark et al., 2007; Dahan et al., 2009). Interestingly, these autoreceptors were desensitized after only 2 days of aripiprazole administration (Chernoloz et al., 2009). Desensitization of 5-HT1A receptors could increase 5-HT neurotransmission, a common effect of long-term antidepressant administration thought to be therapeutically beneficial in the treatment of mood disorders (Blier and El Mansari, 2013). The recent demonstration that the restorative effect of both acute and chronic brexpiprazole on impaired cognitive function was lost by 5-HT1A receptor blockade further suggests in vivo 5-HT1A receptor agonism by this compound (Maeda et al., 2014a; Yoshimi et al., 2014).

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100 µg/kg i.v.) were administered to reverse the inhibitory effect of DOI. The reversing effect of brexpiprazole was quantified relative to baseline firing activity.

**Recording of VTA DA Neurons.** Putative DA neurons were recorded by positioning single-barrel glass micropipettes at the following coordinates (in millimeters from lambda): AP, 3.8–4.2; ML, 4.0–4.2; and DV, 4.5–5.5. They were characterized by flashing light in the eye of the rat, which is a brisk excitatory response in these neurons (Curet and de Montigny, 1988). Because adrenoceptors of the 1B subtype are densely expressed in the dorsal LGN, whereas α₁B-adrenoceptors are absent and there is minimal presence of α₁A-adrenoceptors (Day et al., 1997), the LGN was chosen to assess the effect of brexpiprazole on α₁B-adrenoceptors. Neuronal responsiveness to the iontophoretic application of NE before and after administration of brexpiprazole was assessed by determining the total number of spikes produced, corrected for baseline activity. Baseline activity was defined as the average firing rate 30 seconds pre- and post-ejection. For all data points, the average number of spikes excited was obtained by averaging the neural response to at least two consecutive injections of NE separated by an interval of at least 100 seconds.

**Recording of Pyramidal Neurons in the CA3 Region of the Hippocampus.** CA3 pyramidal neurons were recorded by positioning multibarrel micropipettes at the following coordinates (in millimeters from lambda): AP, 3.8–4.2; ML, 4.0–4.2; and DV, 3.5–4.5. Because most CA3 pyramidal neurons are not spontaneously active in chloral hydrate–anesthetized rats, a small ejection current (1–10 nA) was applied to the quisqualate barrel to activate them within their physiologic firing range (10–15 Hz) (Ranck 1973). The current and duration of 5-HT and NE ejection were kept constant before and after each intravenous injection of brexpiprazole. Neuronal responsiveness to the iontophoretic application of NE was assessed by determining the total number of spikes suppressed from start of ejection to recovery to 80% of baseline firing rate. Activity of the NET was assessed by determining the recovery time to 50% of the maximal inhibitory effect following a high-current ejection of NE (10–20 nA) and was expressed as RT50 (De Montigny et al., 1980). Neuronal responsiveness to 5-HT was assessed by determining the total number of spikes inhibited during a 50-second ejection divided by the ejection current of 5-HT (2–5 nA). Activity of the SERT was assessed by determining the time (in seconds) from the end of a 50-second ejection of 5-HT (20 nA) that fully inhibited the neuron to 50% recovery of baseline firing and was expressed as RT50 (Piaseyro et al., 1994). Partial or full agonism of brexpiprazole on 5-HT1A receptors was assessed by comparing the inhibitory effect of ejection of 5-HT alone to the inhibitory effect of concomitant ejection of 5-HT and brexpiprazole, following restoration of the firing rate to the same level as before ejection brexpiprazole by increasing quisqualate ejection. In this paradigm, coapplication of a partial agonist reduces the inhibitory effect of 5-HT, whereas coapplication of a full agonist does not change the inhibitory effect of 5-HT (Dong et al., 1998; Chambri et al., 2010). To test whether the inhibitory effect of 5-HT and brexpiprazole was mediated by 5-HT1A receptors, the inhibitory effect of iontophoretic 5-HT and brexpiprazole application was compared before and after administration of the selective 5-HT1A receptor antagonist WAY 100,655 (100 µg/kg i.v.).

**Electrical Stimulation of Afferent 5-HT Projections to Hippocampus.** A bipolar electrode (NE-110; David Kopf, Tujunga, CA) was inserted at the following coordinates (in millimeters from lambda): AP, 1.0; ML, 0.0; and DV, 7.8–8.2—to electrically stimulate 5-HT afferents while recording a CA3 pyramidal neuron using a multibarrel pipette (see above). A stimulator (S8800; Grass Technologies, Warwick, RI) was used to deliver 200 square pulses (0.5 millisecond, 1 or 5 Hz) at 300 µA. Duration of inhibition per stimulation was plotted in a peristimulus time histogram with a 2-millisecond bin size. The inhibitory effect of 5-HT fiber bundle stimulation on CA3 neurons was expressed as duration of suppression of firing (DOS; in milliseconds), defined as the period from the first bin showing a 50% reduction in the number of events per bin from the prestimulation value and the first subsequent bin attaining a 90% recovery of the number of events per bin from prestimulus values (Chaput et al., 1986). Electrical stimulation of 5-HT afferents causes endogenous release of 5-HT and briefly suppresses firing of CA3 pyramidal neurons by activating postsynaptic 5-HT1A receptors, an effect previously shown to be independent of 5-HT reuptake inhibition (Chaput et al., 1986). To determine the action of brexpiprazole on α₂-adrenoceptors on 5-HT terminals, we determined whether brexpiprazole (500 µg/kg i.v.) could prevent and, in a subsequent experiment, reverse the decreasing effect on DOS of a high dose of the α₂-adrenoceptor agonist clonidine (400 µg/kg i.v.). To validate this paradigm, a low dose of clonidine (10 µg/kg i.v.) was administered before the high dose of clonidine. Indeed, it is well established that a low dose of clonidine primarily activates α₂-adrenoceptor autoreceptors on NE terminals and so decreases NE tone on α₂-adrenoceptors located on 5-HT terminals, resulting in an increased DOS. A subsequent high dose of clonidine activates α₂-adrenoceptors on 5-HT terminals, thereby producing a decrease in DOS (Mongeau et al., 1994).

To determine the effect on the activity of terminal 5-HT1B autoreceptors, the effect of three doses of brexpiprazole (500 µg/kg i.v.) on the DOS following low- and high-frequency (1 and 5 Hz, respectively) stimulations in the same neuron was compared. Previous in vivo and in vitro studies showed that increasing the stimulation from 1 to 5 Hz results in greater activation of terminal 5-HT1B receptors and, consequently, decreased 5-HT release. Therefore, a longer DOS following 1 Hz compared with 5-Hz stimulation is indicative of functional terminal 5-HT1B autoreceptors (Chaput et al., 1986; Blier et al., 1989).

**Data Analysis/Statistics.** Electrophysiologic recordings were made, and filtered from artifacts by waveform analysis, using Spike2 software version 6.17 (Cambridge Electronic Design, Cambridge, UK). Quantification of firing activity was performed using Spike2, with the exception of firing and burst analysis of VTA DA neurons, for which burstiDAtor (https://github.com/nno/burstiDAtor) software was used. In experiments in which no competitive exogenous ligand was present, linear regression analysis was used to determine ED₅₀ values and to compare the slope and intercept when comparing the effect of aripiprazole and brexpiprazole. In the presence of a competitive exogenous ligand, nonlinear curve fitting was used to obtain ED₅₀ values. Experiments with <3 observations within the same subject were analyzed with a paired t test; experiments with ≥3 observations within the same subject were analyzed with repeated-measures analysis of variance followed by a Tukey post hoc test. All data were analyzed with GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA). Data are presented as mean ± S.E.M.; P < 0.05 was considered significant.
Results

Effect of Brexpiprazole and Aripiprazole on VTA DA Neurons. Brexpiprazole at cumulative doses of 200, 400, and 800 μg/kg did not significantly alter firing rate ($F_{1,39} = 3.15, n = 11, P = 0.083$; Fig. 1C) or bursting activity ($F_{1,29} = 1.61, n = 10, P = 0.21$; Fig. 1D) of VTA DA neurons from baseline activity. Aripiprazole, administered at these same doses, significantly decreased firing activity ($F_{1,22} = 11.93, n = 6, P = 0.0023$; Fig. 1C) and bursting activity of VTA DA neurons ($F_{1,18} = 7.58, n = 5, P = 0.013$; Fig. 1D). For an illustrative trace of the effect of brexpiprazole and aripiprazole, see Fig. 1, A and B, respectively.

Effect of Brexpiprazole on D2 Autoreceptors on VTA DA Neurons. Following a 60-second inhibition period of putative DA neurons in the VTA by the DA receptor agonist apomorphine (40 μg/kg i.v.), brexpiprazole administration (25–100 μg/kg) reversed the effect of apomorphine to ∼65% of baseline firing. Sigmoidal curve fitting ($n = 9$) yielded an ED$_{50}$ value of 61 μg/kg for brexpiprazole on this effect (Fig. 2B; for an illustrative trace, see Fig. 2A).

Effect of Brexpiprazole and Aripiprazole on 5-HT$_{1A}$ Autoreceptors. In the DRN, brexpiprazole at cumulative doses of 100 and 200 μg/kg inhibited the firing of 5-HT

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Fig. 1. Effect of systemic brexpiprazole and aripiprazole administration on VTA DA neurons. (A and B) Illustrative trace of DA neurons and response to intravenous administration of brexpiprazole (A, black arrows) and aripiprazole (B, gray arrows). (C and D) Brexpiprazole ($n = 11$) did not change firing activity of VTA DA neurons, whereas aripiprazole ($n = 6$) significantly reduced both firing and bursting activity of these neurons. Data were analyzed using linear regression and are presented as mean ± S.E.M. Aripiprazole had a significant effect in comparison with saline administration. **$P < 0.01$ for slope; ##$P < 0.01$ for intercept.

Fig. 2. Effect of systemic brexpiprazole administration on VTA DA neurons inhibited by the dopamine agonist apomorphine. (A) Illustrative trace of a DA neuron and response to intravenous administration of apomorphine and brexpiprazole. (B) Brexpiprazole reversed the inhibitory effect of apomorphine ($n = 9$). The ED$_{50}$ value was obtained with a sigmoidal curve fit; data are presented as mean ± S.E.M.
neurons \((n = 11)\), an effect not reversed by administration of the NE reuptake inhibitor desipramine (5 mg/kg), whereas the selective 5-HT1A receptor antagonist WAY 100.635 reversed this inhibition \((n = 3)\); for an illustrative trace, see Fig. 3A). Similarly, an inhibitory effect of aripiprazole was observed on 5-HT neurons \((n = 15)\) that was reversed by WAY 100.635 but not desipramine \((n = 3)\). For aripiprazole, the experiment was initially conducted by administering cumulative doses of 200 \(\mu g/kg\) \((n = 6)\), based on a previous study (Dahan et al., 2009). As the inhibitory effect of these injections did not fully inhibit 5-HT neurons up to a cumulative dose of 600 \(\mu g/kg\), aripiprazole was administered at 500 \(\mu g/kg\) \((n = 9)\) in a subsequent experiment. Data from these experiments were pooled, as statistical analysis demonstrated that the dose-responsiveness of 5-HT neurons to aripiprazole at increments of 200 and 500 \(\mu g/kg\) was not different (slope: \(F_{1,45} = 0.04, P > 0.05\); intercept: \(F_{1,46} = 0.08, P > 0.05\)). Statistical analysis demonstrated a significantly lower ED50 value for brexpiprazole than for aripiprazole (230 and 700 \(\mu g/kg\), respectively; \(F_{1,78} = 31.51, P < 0.0001\); Fig. 3B).

**Effect of Brexpiprazole on Postsynaptic 5-HT1A Receptors.** In the hippocampus, systemic administration of brexpiprazole up to a dose of 1500 \(\mu g/kg\) did not change the number of spikes suppressed per nanoampere following iontophoretic application of 5-HT on CA3 pyramidal neurons \((F_{1,30} = 2.72, P > 0.05; \text{data not shown})\).

Iontophoretic application of both brexpiprazole and 5-HT had an inhibitory effect on CA3 pyramidal neurons \((n = 12)\); for an illustrative trace, see Fig. 4A). When brexpiprazole and 5-HT were ejected concomitantly, the neuronal inhibition of CA3 pyramidal neurons did not differ from when 5-HT was ejected alone \((P > 0.05, n = 12; \text{Fig. 4D})\). This inhibitory effect of both agents was significantly dampened after systemic administration of WAY 100.635 \((P < 0.05\) and \(P < 0.01, \text{respectively}; \text{Fig. 4, B and C})\).

**Effect of Brexpiprazole on the SERT.** In the hippocampus, the RT50 value (a measure for SERT activity) following iontophoretic application of 5-HT on CA3 pyramidal neurons was not changed by administration of brexpiprazole up to a dose of 1500 \(\mu g/kg\) \((F_{1,32} = 0.47, P > 0.05; \text{data not shown})\).

**Effect of Brexpiprazole on Terminal 5-HT1B Autoreceptors.** The DOS produced on the same neuron by electrical stimulation of 5-HT afferents with stimulation frequencies of 1 and 5 Hz was increased by brexpiprazole at doses of 500, 1000, and 1500 \(\mu g/kg\) (Fig. 7B). For 5-Hz stimulations, the corresponding DOS at these doses were 38 ± 3, 48 ± 5, and 49 ± 4, while the DOS at baseline was 27 ± 2 milliseconds. Statistical analysis demonstrated that under control conditions and after cumulative doses of brexpiprazole, the DOS after 1-Hz stimulation remained significantly greater than the DOS after 5-Hz stimulation (slope: \(F_{1,36} = 0.91, P > 0.05\); intercept: \(F_{1,37} = 13.96, P = 0.0006\)).

**Effect of Brexpiprazole on 5-HT2A Receptors in the LC.** Following a 60-second inhibition period of NE neurons in the LC by the 5-HT2A agonist DOI (100 \(\mu g/kg\) i.v.), brexpiprazole administration (50–400 \(\mu g/kg\)) reversed NE
neural firing to ~80% of baseline firing, with an ED$_{50}$ value estimated at 110 $\mu$g/kg (Fig. 5B; for an illustrative trace, see Fig. 5A).

**Effect of Brexpiprazole on Postsynaptic $\alpha_{1B}$-Adrenoceptors in the LGN.** In the LGN, acute administrations of brexpiprazole at a dose of 500 and 1000 $\mu$g/kg significantly decreased the excitatory action of exogenous NE on LGN neurons by 44% and 77%, respectively ($F_{2,5} = 20.10, P = 0.0034, P < 0.05$, and $P < 0.001$ for these respective doses; Fig. 6B), with an ED$_{50}$ value estimated at 630 $\mu$g/kg. The excitatory effect of NE ejection was not changed by prior administration of the $\alpha_{1A}$-adrenoceptor antagonist SNAP 5089 (1 mg/kg i.v.), whereas brexpiprazole administration still decreased neuronal excitability in the LGN after SNAP 5089 administration ($n = 2$; for an illustration, see Fig. 6A).

**Effect of Brexpiprazole on Postsynaptic $\alpha_{2}$-Adrenoceptors.** In the hippocampus CA3 region, systemic administration of brexpiprazole up to a dose of 1500 $\mu$g/kg did not change the number of spikes inhibited per nanoampere following iontophoretic application of NE on pyramidal neurons ($F_{1,28} = 0.95, P > 0.05$; data not shown).

**Effect of Brexpiprazole on the NET.** In the hippocampus CA3 region, the RT$_{50}$ value following iontophoretic application of NE on CA3 pyramidal neurons was not changed by administration of brexpiprazole up to a dose of 1500 $\mu$g/kg ($F_{1,30} = 0.01, P = 0.05$; data not shown).

**Effect of Brexpiprazole on Terminal $\alpha_{2}$-Adrenoceptors on 5-HT Terminals.** Clonidine at a dose of 10 $\mu$g/kg significantly increased the DOS value following 1-Hz stimulation compared with baseline, an effect completely reversed by a subsequent injection of clonidine at a dose of 400 $\mu$g/kg ($F_{3,5} = 13.63, n = 6, P < 0.0001$; Fig. 7A). Following these doses of clonidine, the DOS increased with cumulative administration of brexpiprazole (500, 1000, and 1500 $\mu$g/kg i.v.) in a dose-dependent fashion (Tukey post hoc test, $P < 0.01$, and $P < 0.01$ for these doses, respectively).

Administration of brexpiprazole alone also increased the DOS value in a dose-dependent manner ($F_{3,4} = 4.16, n = 5, P = 0.02$; Fig. 7B). Tukey post hoc testing revealed a significant effect of brexpiprazole on DOS at a dose of 1000 and 1500 $\mu$g/kg ($P < 0.01$ and $P < 0.01$, respectively). Clonidine administration (10 and 400 $\mu$g/kg) had no altering effect on the

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**Fig. 4.** Effect of iontophoretically applied brexpiprazole, 5-HT, and concomitant ejection of 5-HT and brexpiprazole on pyramidal neurons in the hippocampus CA3 region. (A) Illustrative trace of discharge activity of a pyramidal neuron in the CA3 region of the hippocampus, and effects of iontophoretically applied agents before and after systemic WAY 100.635 administration. (B, C) The inhibitory response to iontophoretic application of brexpiprazole and 5-HT is significantly reduced after WAY 100.635 administration. (D) There was no different inhibitory effect of ejection of brexpiprazole and 5-HT compared with 5-HT ejection alone. The numbers of neurons (n) and animals (a) are presented in the histograms; data were analyzed with a paired t test and are presented as mean ± S.E.M. *$P < 0.05$; **$P < 0.01$ for effect of WAY 100.635 administration on the inhibitory effect of brexpiprazole and 5-HT.
DOS when it was administered after brexpiprazole ($P > 0.05$; Fig. 7B).

### Discussion

**Brexpiprazole: Effect on VTA DA Neurons.** Brexpiprazole reversed the inhibitory action of the DA receptor agonist apomorphine on neural firing of VTA DA neurons, thus demonstrating antagonistic action at $D_2$ autoreceptors (Fig. 2). Interestingly, brexpiprazole by itself did not significantly change the firing rate or bursting activity of DA neurons (Fig. 1). In contrast, the classic $D_2$ receptor antagonist haloperidol is known to increase firing and burst activity of VTA DA neurons when administered acutely, an effect attributable to blockade of endogenous DA inhibitory tone on $D_2$ autoreceptors (Pucak and Grace, 1994). Because brexpiprazole did not alter firing and bursting activity of VTA DA neurons, this result demonstrates that, in vivo, it acts neither as a pure antagonist nor as an agonist with high intrinsic activity on $D_2$ autoreceptors. Aripiprazole, similarly to brexpiprazole, was previously shown to reverse neuronal inhibition of VTA DA neurons by apomorphine, although less potently (Dahan et al., 2009). In the present study, aripiprazole significantly reduced the firing and bursting activity of VTA DA neurons to a similar degree as reported previously (Fig. 1; Dahan et al., 2009). The difference in effects on DA neurons of aripiprazole and brexpiprazole is likely due to a lower intrinsic activity of the latter agent at $D_2$ receptors, in line with their in vitro profiles (Maeda et al., 2014b). Notably, the present study also demonstrated more potent in vivo agonism of brexpiprazole on 5-HT1A receptors than aripiprazole (discussed below). Because activation of prefrontal 5-HT1A receptors is known to excite VTA DA neurons (Arborelius et al., 1993; Díaz-Mataix et al., 2005; Gronier, 2008), a difference in degree of 5-HT1A receptor agonism could contribute to the distinct effects of brexpiprazole and aripiprazole on the firing activity of DA neurons.

**Brexpiprazole: Effect on the 5-HT System.** Brexpiprazole dose-dependently decreased the firing rate of 5-HT neurons in the DRN (Fig. 3). This inhibitory effect was due to agonism at 5-HT1A receptors on these neurons, as the selective 5-HT1A receptor antagonist WAY 100.635, but not the NE reuptake inhibitor desipramine, reversed the inhibitory action of brexpiprazole (Fig. 3A). Compared with aripiprazole, brexpiprazole was significantly more potent at activating 5-HT1A autoreceptors (Fig. 3B), a finding in line with the in

**Fig. 5.** Effect of systemic brexpiprazole on LC NE neurons inhibited by the preferential 5-HT2A agonist DOI. (A) Illustrative trace of an NE neuron inhibited by intravenous administration of DOI and reversal of inhibition by brexpiprazole. Between 720 and 740 seconds, the electrical signal of the neuron decreased below the level of the differential amplitude discriminator. Following this decrease, the electrode was quickly lowered by $\sim 20 \mu m$ and the signal was restored to its prior amplitude. Waveform analysis confirmed that the same neuron was recorded before and after this period. (B) Graphic presentation of the reversing effect of brexpiprazole on neurons inhibited by DOI ($n = 10$). The ED50 value was obtained with simple nonlinear regression; data are presented as mean $\pm$ S.E.M.
vitro affinity for 5-HT$_{1A}$ receptors of these agents (Maeda et al., 2014b). To the best of our knowledge, two previous studies assessed the dose-dependent effect of aripiprazole to inhibit DRN 5-HT neurons; Dahan et al. (2009) reported an ED$_{50}$ value of 540 mg/kg, in close similarity to the present findings, while the ED$_{50}$ for aripiprazole in the work by Stark et al. (2007) is estimated at 200–250 mg/kg. The reason for this difference remains to be established, although linear versus logarithmic dose-response estimation could play a role.

In the hippocampus, the activity of the SERT, assessed using the RT$_{50}$ index, remained unaltered following brexpiprazole administration up to a dose of 1500 µg/kg, whereas drugs that occupy SERT to a significant extent in vivo are known to prolong the RT$_{50}$ index (Piñeyro et al., 1994; Ghanbari et al., 2010). Microiontophoretic application of brexpiprazole and 5-HT both inhibited CA3 pyramidal neurons (Fig. 4, A and C), an effect attributable to 5-HT$_{1A}$ activation. Indeed, the inhibitory effect of these agents was blocked after systemic administration of WAY 100.635 (Fig. 4D). This absence of competitive action on 5-HT$_{1A}$ receptors of brexpiprazole with 5-HT indicates that brexpiprazole acted as a full 5-HT$_{1A}$ receptor agonist in vivo. Indeed, previous studies using the same paradigm showed full agonistic action of BAY 3702 (Dong et al., 1998) and trazodone (Ghanbari et al., 2010), whereas 8-OHDPAT (2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene; Hadrava et al., 1996), gepirone (Hadrava et al., 1995), and asenapine (Ghanbari et al., 2009) acted as partial agonists at 5-HT$_{1A}$ receptors in the hippocampus.

Brexpiprazole administration dose-dependently increased the DOS following electrical stimulations of 5-HT afferents at 1 Hz (Fig. 7B), an effect that could theoretically be attributable to 5-HT$_{1A}$ activation. Indeed, the inhibitory effect of these agents was blocked after systemic administration of WAY 100.635 (Fig. 4, B and C). Importantly, the inhibitory effect of 5-HT alone was unaltered when brexpiprazole was coejected (Fig. 4D). This absence of competitive action on 5-HT$_{1A}$ receptors of brexpiprazole with 5-HT indicates that brexpiprazole acted as a full 5-HT$_{1A}$ receptor agonist in vivo. Indeed, previous studies using the same paradigm showed full agonistic action of BAY 3702 (Dong et al., 1998) and trazodone (Ghanbari et al., 2010), whereas 8-OHDPAT (2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene; Hadrava et al., 1996), gepirone (Hadrava et al., 1995), and asenapine (Ghanbari et al., 2009) acted as partial agonists at 5-HT$_{1A}$ receptors in the hippocampus.

Fig. 6. Effect of brexpiprazole on the excitatory effect of iontophoretically applied NE on LGN neurons. (A) Illustrative trace of the electrical activity of an LGN neuron, its excitation by iontophoretically applied NE, ineffectiveness of the selective α1A-adrenergic receptor antagonist SNAP 5089 to alter this excitation, blockade of this excitation by systemic brexpiprazole administration, and brisk excitatory response to light flashes, confirming proper recording electrode positioning. (B) Effect of brexpiprazole on the excitatory response of LGN neurons to iontophoretic application of NE. The number of neurons tested is presented in the histogram; data were analyzed with repeated-measures analysis of variance and Tukey post hoc test and are presented as mean ± S.E.M. *P < 0.05; ***P < 0.001 for effect of brexpiprazole compared with baseline. Note: Data from the neuron recorded in (A) were not included in (B) due to the injection of SNAP 5089 prior to brexpiprazole administration.
to terminal 5-HT_{1B} autoreceptors and/or α_{2}-adrenergic heteroreceptor antagonism (Chaput et al., 1986; Mongeau et al., 1993).

However, brexpiprazole administration did not change the difference in DOS following 1- and 5-Hz stimulations, indicating that terminal 5-HT_{1B} autoreceptor functionality was unaffected by brexpiprazole. In contrast, brexpiprazole acted as a potent α_{2}-adrenergic heteroreceptor antagonist, as shown in two complementary experiments where brexpiprazole reversed (Fig. 7A) and prevented (Fig. 7B) the typical decrease in DOS caused by a high dose of the α_{2}-adrenoceptor agonist clonidine (400 μg/kg i.v.). Such effects have been previously observed following acute administration of the antidepressants mianserin and mirtazapine (Mongeau et al., 1993; Haddjeri et al., 1996).

**Brexpiprazole: Effect on the NE System.** In the LC, NE neurons are innervated by inhibitory GABA neurons that express excitatory 5-HT_{2A} receptors; activation of 5-HT_{2A} receptors enhances GABA neurotransmission and inhibits NE neurons (Szabo and Blier, 2001). Indeed, systemic administration of the preferential 5-HT_{2A} receptor agonist DOI strongly inhibited NE neurons. Because this effect was potently reversed by brexpiprazole, antagonistic action of this agent on 5-HT_{2A} receptors was clearly demonstrated (Fig. 5). In the present study, the ED_{50} value of brexpiprazole for 5-HT_{2A} receptor antagonism was ∼2-fold higher than for D_{2} receptor antagonism (110 versus 61 μg/kg, respectively), which is qualitatively in line with its in vitro profile (Maeda et al., 2014b). Notably, caution should be taken when comparing in vitro and in vivo data for agents in their effectiveness in reversing the action of receptor agonists, as ED_{50} values are relative to the affinity, intrinsic activity, and dose of the agonist. Additionally, the multiple receptor activity of brexpiprazole could affect more than one neuronal substrate in vivo.

To illustrate this point, the in vitro affinity of asenapine is 1 order of magnitude greater for 5-HT_{2A} than for D_{2} receptors (Shahid et al., 2009), whereas the in vivo ED_{50} value for D_{2} receptors was found to be ∼2-fold higher than for 5-HT_{2A} receptors (Ghanbari et al., 2009).

In the hippocampus CA3 region, brexpiprazole did not modify the inhibitory response to iontophoretic application of NE or the RT_{50} index, indicating no action on postsynaptic α_{2}-adrenoceptors and the NET, respectively (De Montigny et al., 1980; Curet and De Montigny, 1988). In the LGN, neurons are known to almost exclusively express α_{1}-adrenergic receptors of the 1B subtype (Day et al., 1997). In this brain region, the antagonistic effect of brexpiprazole on these receptors was assessed by quantifying neuronal excitation following iontophoretically applied NE, a response known to be mediated by α_{1B}-adrenoceptors (Rogawski and Aghajanian, 1980, 1982; Menkes et al., 1981). Neuronal excitation decreased with brexpiprazole administration, strongly suggesting antagonistic action at α_{1B}-adrenergic receptors (Fig. 6B). Because there is no expression of α_{1A}-adrenoceptors yet minimal expression of α_{1A}-adrenoceptors (Day et al., 1997), the excitatory effect of NE after administration of the selective α_{1A}-adrenoceptor antagonist SNAP 5089 was assessed (Wetzel et al., 1995). The excitatory effect of iontophoretic application of NE on LGN neurons was not altered by SNAP 5089 administration, whereas consecutive administrations of brexpiprazole largely blocked this excitatory effect (Fig. 6A). Taken together, these results suggest antagonistic properties of brexpiprazole predominantly at α_{1B}-adrenoceptors, consistent with its 20-fold-higher affinity for this receptor subtype over α_{1A}- and α_{1D}-adrenoceptors (Maeda et al., 2014b).

**Conclusion.** The present results show acute in vivo action of brexpiprazole at all three monoamine (5-HT, NE, and DA) systems. Similarly to aripiprazole, brexpiprazole acted as a partial D_{2} receptor agonist in vivo but is relatively less efficacious at this receptor subtype. Clinically, D_{2} receptor partial agonism is thought to buffer fluctuations in DA transmission (Burris et al., 2002; Shapiro et al., 2003), in line with the behavioral effects of brexpiprazole in animal models of schizophrenia (Maeda et al., 2014a). Furthermore, the potent in vivo agonistic action of brexpiprazole on 5-HT_{1A} receptors could be a relevant pharmacologic feature in treatment of both mood disorders and schizophrenia (Blier and Ward, 2003; Newman-Tancredi, 2010). Acute brexpiprazole administration reduced inhibition of two important interaction nodes between the 5-HT and NE systems. First, it blocked...
5-HT\textsubscript{2A} receptors, a receptor type that dampens LC NE firing when 5-HT neurotransmission is enhanced (Dremencov et al., 2007; Chernoloz et al., 2009). Second, brexpiprazole blocked \(\alpha\)-adrenoreceptors on 5-HT terminals, a receptor type that dampens 5-HT release when NE neurotransmission is elevated (Mongeau et al., 1993). As the therapeutic effect of potent 5-HT\textsubscript{2A} receptor antagonism in combination with 5-HT reuptake inhibitors is well recognized (Nelson and Papakostas, 2009), the present data support the use of brexpiprazole as an augmentation strategy. This notion is strengthened by the recent demonstration of clinical efficaciousness of brexpiprazole as an adjunct to antidepressants in major depressive disorder (Thase et al., 2014). In addition, combined but not separate administration of brexpiprazole and NE or 5-HT reuptake inhibitors had an antidepressant-like effect in rodents (Hirose et al., 2014). Following this in vivo pharmacologic characterization, and because brexpiprazole will be administered on a long-term basis in the clinic, it will be crucial to investigate the effect of sustained brexpiprazole administration on therapeutically relevant monoamine targets.

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References


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