RESEARCH ARTICLE

Brexpiprazole Alters Monoaminergic Systems following Repeated Administration: an in Vivo Electrophysiological Study

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Abstract

Background: Brexpiprazole was recently approved as adjunctive therapy for depression and treatment of schizophrenia in adults. To complement results from a previous study in which its acute effects were characterized, the present study assessed the effect of repeated brexpiprazole administration on monoaminergic systems.

Methods: Brexpiprazole (1 mg/kg, subcutaneous) or vehicle was administered once daily for 2 and 14 days. Single-unit electrophysiological recordings from noradrenaline neurons in the locus coeruleus, serotonin neurons in the dorsal raphe nucleus, dopaminergic neurons in the ventral tegmental area, and pyramidal neurons in the hippocampus CA3 region were obtained in adult male Sprague-Dawley rats under chloral hydrate anesthesia within 4 hours after final dosing.

Results: Brexpiprazole blunted D2 autoreceptor responsiveness, while firing activity of ventral tegmental area dopaminergic neurons remained unaltered. Brexpiprazole increased the firing rate of locus coeruleus noradrenaline neurons and increased noradrenaline tone on α2-adrenergic receptors in the hippocampus. Administration of brexpiprazole for 2 but not 14 days increased the firing rate of serotonin neurons in the dorsal raphe nucleus. In the hippocampus, serotonin1A receptor blockade significantly disinhibited pyramidal neurons after 2- and 14-day brexpiprazole administration. In contrast, no significant disinhibition occurred after 24-hour washout or acute brexpiprazole.

Conclusions: Repeated brexpiprazole administration resulted in a marked occupancy of D2 autoreceptors, while discharge activity of ventral tegmental area dopaminergic neurons remained unaltered. Brexpiprazole enhanced serotonergic and noradrenergic tone in the hippocampus, effects common to antidepressant agents. Together, these results provide further insight in the neural mechanisms by which brexpiprazole exerts antidepressant and antipsychotic effects.

Keywords: brexpiprazole, single unit electrophysiological recordings, serotonin, norepinephrine, dopamine

Introduction

Brexpiprazole (Rexulti) was recently shown to be clinically efficacious in the treatment of schizophrenia (Kane et al., 2015) and as an augmentation strategy in treatment of depression (Thase et al., 2015a, 2015b). Similarly to aripiprazole, brexpiprazole is a partial dopamine (DA) D2 receptor agonist, a pharmacological feature that distinguishes these agents from other...
antipsychotics, which are D<sub>2</sub> receptor antagonists (Stark et al., 2007). Although D<sub>3</sub> receptor antagonism effectively reduces positive symptoms in schizophrenia (Seeman and Lee, 1975; Rao and Remington, 2013), blockade of the D<sub>3</sub>-receptor-mediated signal might be undesirable for management of negative and/or cognitive symptoms. Indeed, aripiprazole improves negative and cognitive symptoms in schizophrenia and schizoaffective disorder (Stip and Tourjman, 2010), effects partly explained by its combined D<sub>2</sub> receptor partial agonism, 5-HT<sub>1A</sub> receptor agonism, and 5-HT<sub>2A</sub> receptor antagonism (Hirose et al., 2004). The degree of D<sub>2</sub> receptor activation could be of crucial importance to the antipsychotic properties of partial D<sub>2</sub> receptor agonists. Indeed, the clinical failure of bifeprunox has, in part, been ascribed to its excessive agonism at D<sub>2</sub> receptors and hence limited effects on positive symptoms (Stahl, 2008). Compared with aripiprazole, brexpiprazole has a higher in vitro affinity yet lower intrinsic activity at D<sub>2</sub> receptors (Maeda et al., 2014b). Indeed, unlike aripiprazole, acute in vivo administration of brexpiprazole did not attenuate the firing activity of ventral tegmental (VTA) DA neurons (Oosterhof et al., 2014). Furthermore, it reversed the inhibitory effect of D<sub>2</sub> autoreceptor agonism on these neurons more potently than aripiprazole (Dahan et al., 2009; Oosterhof et al., 2014). In line with functional D<sub>2</sub> receptor antagonism in animal models (Maeda et al., 2014a), brexpiprazole was recently shown to be clinically effective in the treatment of acute schizophrenia (Kane et al., 2015).

Brexiprazole had potent antagonistic action on 5-HT<sub>2A</sub> receptors both in vitro and acute in vivo (Maeda et al., 2014b; Oosterhof et al., 2014), a defining pharmacological quality of atypical antipsychotics thought to underlie a lower incidence of side effects on motor function relative to typical antipsychotics (Kuroki et al., 2008). Blockade of 5-HT<sub>2A</sub> receptors is also known to prevent the dampening effect on the noradrenaline (NE) system of sustained selective serotonin reuptake inhibitors (Dremencov et al., 2007a; 2007b; Chernoloz et al., 2009), providing a neural mechanism by which coadministration of low-dose atypical antipsychotics improves the therapeutic efficacy of antidepresants in treatment-resistant patients (Blier and Szabo, 2005). In addition to blocking inhibitory input of the 5-HT system on NE, the inhibitory effect on 5-HT release mediated by terminal α<sub>2</sub>-adrenergic heteroreceptor activation was potently reduced by brexpiprazole (Oosterhof et al., 2014), an effect potentially preventing attenuated 5-HT release when NE neurotransmission is elevated (Mongeau et al., 1993; Chernoloz et al., 2012).

Both in vitro and in vivo studies demonstrated more potent 5-HT<sub>2A</sub> receptor agonism by brexpiprazole compared with aripiprazole (Maeda et al., 2014b; Oosterhof et al., 2014). Interestingly, acute brexpiprazole—but not aripiprazole—improved phenycyclide-induced cognitive impairments in the novel object recognition test (Maeda et al., 2014b). Coadministration of the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100.635 prevented this behavioral effect of brexpiprazole, suggesting that 5-HT<sub>1A</sub> receptor activation was involved in the restorative effects of brexpiprazole on cognitive-like measures (Maeda et al., 2014b; Yoshimi et al., 2014). Furthermore, since chronic administration of all antidepressants enhances tonic activation of 5-HT<sub>1A</sub> receptors (Haddjeri et al., 1998), the potent in vivo agonism of brexpiprazole on these receptors could be of therapeutic relevance in the treatment of mood disorders (Blier and Ward, 2003; Blier and El Mansari, 2013).

Antidepressant and antipsychotic medications gradually induce neural adaptations, which presumably underlie their delayed onset of action. To characterize such adaptations within monoamine systems, the present study aimed to determine the effects of 2- and 14-day brexpiprazole administration (1 mg/kg/d, s.c., a dose resulting in clinically relevant blood plasma levels) on the discharge activity of VTA DA, locus coeruleus (LC) NE, and dorsal raphe nucleus (DRN) 5-HT neuron populations. In these brain regions, the functional status of D<sub>2</sub>, α<sub>2</sub>-adrenergic, 5-HT<sub>1A</sub>, and 5-HT<sub>2A</sub> receptors was assessed. In the CA3 region of the hippocampus, the effect of sustained brexpiprazole administration on the activity of the 5-HT and NE transporters (SERT and NET, respectively), the status of terminal 5-HT<sub>1A</sub> auto- and α<sub>2</sub>-adrenergic heteroreceptors, sensitivity of postsynaptic 5-HT<sub>1A</sub> and α<sub>2</sub>-adrenergic receptors, and degree of tonic activation of 5-HT<sub>1A</sub>-α<sub>2</sub>- and α<sub>2</sub>-adrenergic receptors was assessed using electrophysiological and pharmacological strategies.

### Methods

#### Animals

Experiments were carried out in chloral hydrate anesthetized male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) weighing 275 to 450 g housed under standard laboratory conditions. All experiments were carried out in accordance with local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, Ontario, Canada).

#### Compounds and Dosing

Brexiprazole (Maeda et al., 2014b) was dissolved in a 0.5% lactic acid solution in distilled water; pH was adjusted to 4.8 by addition of NaOH. Brexpiprazole (1 mg/kg, s.c.) or vehicle was administered acutely for 2 or 14 days. Electrophysiological recordings occurred within 30 minutes to 4 hours after the last injection. Within this time frame, a blood sample was obtained after recordings.

The D<sub>2</sub>-receiver antagonist haloperidol (200 μg/kg) and the DA receptor antagonist apomorphine (40 μg/kg) were dissolved in a 0.5% lactic acid solution in distilled water; pH of the solution was adjusted to 4.5 by addition of NaOH. The 5-HT<sub>2A</sub> receptor antagonist WAY 100907 was dissolved in Tween 80 (0.2%) in distilled water. The 5-HT<sub>2A</sub> receptor agonist flesinoxan (100 μg/kg), the 5-HT<sub>2A</sub> receptor antagonist WAY 100.635 (100 μg/kg), the preferential 5-HT<sub>2A</sub> receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI, 100 μg/kg), the α<sub>2</sub>-adrenoceptor antagonist prazosin (100 μg/kg), the α<sub>2</sub>-adrenoceptor agonist clonidine (5 μg/kg), and the α<sub>2</sub>-adrenoceptor antagonist idazoxan (1000 μg/kg) were dissolved in distilled water. Brexpiprazole was provided by Lundbeck A/S (Valby, Denmark); all other compounds were purchased from Sigma Aldrich (Oakville, ON, Canada).

#### In Vivo Electrophysiological Recordings

Electrophysiological recordings were performed as described previously (Oosterhof et al., 2015). Briefly, chloral hydrate-anesthetized animals were mounted in a stereotaxic apparatus; body temperature was maintained at 37°C utilizing a thermistor-controlled heating pad. A catheter was inserted in a lateral tail vein for systemica i.v. injection of agents.

Single-barrel glass micropipettes (Stoelting, Spencerville, MD) preloaded with 2 M NaCl (impedance 2-6 MΩ) were used to record the electrical activity of VTA DA, DRN 5-HT, and LC NE neurons, as described previously (Vandermaelen and Aghajanian, 1983; Grace and Bunney, 1984). For VTA DA and LC NE neurons, the start of a burst was defined as the occurrence of an interspike interval (ISI) <80 ms; end of a burst was defined as an ISI >160 ms (Grace and Bunney, 1984; Chenu et al., 2013).
Bursts in 5-HT neurons were defined as an ISI <20 ms (Hajós et al., 2007). The dose-response effects of systemic flesinoxan, clonidine, DOI, and apomorphine injections were quantified to determine the status of 5-HT₁a autoreceptors on DRN 5-HT neurons, α₂-adrenergic autoreceptors and 5-HT₁a receptors on LC NE neurons, and D₂ autoreceptors on VTA DA neurons, respectively.

Hippocampal CA3 neurons were recorded with 5-barrel micropipettes (impedances: central barrel 2–5 MΩ, side barrels 20–30 MΩ). The central barrel, used for unitary recordings, and one side barrel, used for automatic current balancing, were filled with 2 M NaCl; the other barrels were filled with 5-HT creatinine sulfate (10 mM in 0.2 M NaCl, pH 4), NE bitartrate (10 mM in 0.2 M NaCl, pH 4), or quisqualalic acid (1.5 mM in 0.2 M NaCl, pH 4). 5-HT and NE were ejected as cations (+2 to +20 nA) and retained with a positive current; quisqualate was ejected as an anion (-3 to +1 nA) and retained with a positive current. CA3 neurons were activated within their physiological range (10 to 15 Hz; Ranck 1973) with quisqualate. The inhibitory response to 50-second microiontophoretic application of NE and 5-HT, respectively, expressed as spikes inhibited/nA, was used to determine the status of 5-HT₁a and α₂-adrenergic receptors on these neurons (de Montigny and Aghajanian, 1978; Curet and de Montigny, 1988). The recovery time to 50% of baseline firing (RT₂₀) following iontophoretic application of 5-HT and NE was used as an index of SERT and NET activity (de Montigny et al., 1980; Piñeyro et al., 1994) To determine the degree of tonic activation of postsynaptic 5-HT₁a receptors, firing activity of CA3 pyramidal neurons was decreased to ~4 Hz. A physiological saline injection was administered followed by 4 consecutive injections of the 5-HT₁a receptor antagonist WAY 100.635 (25 µg/kg, 2-minute intervals). The average firing rate (spikes/s) of neurons during the second half of the 120-second period following each WAY 100.635 injection was quantified and expressed as percentage change from baseline. Using the same paradigm, degree of tonic activation of postsynaptic α₁- and α₂-adrenoceptors was quantified as the disinhibitory effect of prazosin (100 µg/kg, i.v.) and idazoxan (1000 µg/kg, i.v.), respectively, on CA3 pyramidal neurons.

To assess the status of 5-HT₁a autoreceptors and α₂-adrenergic heteroreceptors on 5-HT neurons, 5-HT afferents were electrical stimulated (0.5 ms, 300 µA, 1 and 5 Hz) with a bipolar electrode (NE-110, David Kopf, Tujanga, CA) connected to a stimulator (S8800, Grass instruments, Quincy, MA), causing endogenous release of 5-HT, which activation of postsynaptic 5-HT₁a receptors briefly suppresses firing of CA3 pyramidal neurons (Chaput et al., 1986). The inhibitory effect of 200 pulses delivered at 1 and 5 Hz was plotted in a peristimulus time histogram. Duration of silence (DOS, in milliseconds) was defined as the period from the first bin showing a 50% reduction relative to baseline to the first subsequent bin containing a 90% recovery (Chaput et al., 1986).

Increasing the stimulation frequency from 1 to 5 Hz results in greater activation of terminal 5-HT₁a receptors and, consequently, decreased 5-HT release. Therefore, a greater DOS following 1-Hz compared with 5-Hz stimulations is indicative for intact function of terminal 5-HT₁a autoreceptors (Chaput et al., 1986; Blier et al., 1989). The activity of α₂-adrenergic heteroreceptors on 5-HT terminals is known to be frequency dependent (Blier et al., 1989). Therefore, a reduced DOS at 1 Hz indicates enhanced activation of α₂-adrenergic receptors (Mongeau et al., 1993), while a normal or DOS in presence of enhanced NE neurotransmission demonstrates α₂-adrenergic heteroreceptor blockade (Chernoloz et al., 2012).

Bioanalysis of Brexpiprazole in Plasma

Brexiprazole concentrations were determined in plasma using ultrasensitive liquid chromatography followed by tandem mass spectrometry detection. 150 µL acetonitrile containing isotope-labeled internal standard was added to 25 µL of calibration standards and test samples. After centrifugation, 100 µL supernatant from each sample was mixed with 100 µL 0.1% formic acid, centrifuged, and placed in the autosampler. Chromatography was performed on a Waters C18SB HSS column (30 x 2.1 mm, 1.8 µm particles) using a mobile phase gradient of 0.1% formic acid in water and acetonitrile. MS/MS detection was done with an Applied Biosystems Sciex API 4000 instrument in positive-electrospray ionization mode. Brexpiprazole was detected at a parent > daughter mass to charge ratio of 434.2 > 273.1. The peak area correlated linearly with the plasma concentration in the range of 0.5 to 1000 ng/mL. All individually measured plasma concentrations of brexpiprazole obtained at varying time points were extrapolated to a 2-hour postdose time point.

Data Analysis / Statistics

Electrophysiological recordings were filtered from artifacts by waveform analysis in Spike2 software version 6.17 (Cambridge Electronic Design, Cambridge, UK). Firing and burst analysis of DRN 5-HT, LC NE, and VTA DA neurons was performed using burstiDAtor software (Oosterhof and Oosterhof, 2013).

For dose-response effects, data from 2- and 14-day vehicle-administered animals were pooled. The dose-response effects of flesinoxan and clonidine were analyzed with linear regression analysis. Effect of acute brexpiprazole administration on LC NE neurons was analyzed with 1-way ANOVA. Effects on the firing and burst activity of DRN 5-HT, LC NE and VTA DA, dose-response effects of apomorphine and DOI, degree of tonic activation on α-adrenergic receptors, and 5-HT afferent stimulation were analyzed with 2-way ANOVA and Bonferroni posthoc analysis; alphas were corrected for multiple comparisons when appropriate. Body weight gain and degree of 5-HT₁a receptor activation was assessed using repeated measures (RM) ANOVA and Bonferroni posthoc analysis. All data were analyzed with Graphpad Prism (Version 5.01, Graphpad software, La Jolla, CA). Data are presented as mean, error bars represent SEM, and P < .05 was considered statistically significant.

Results

Brexiprazole Plasma Levels

Two days of brexpiprazole administration resulted in mean plasma concentrations of 62 ± 8 ng/mL (n = 23), and its administration for 14 days resulted in a concentration of 131 ± 11 ng/mL (n = 27; data extrapolated to a 2-hour postdose time point). These plasma concentrations reflect the values around Cmax following oral dosing (Maeda et al., 2014b). As the elimination half-life is around 2 hours in rats (Maeda et al., 2014b), drug exposure profiles are thus expected to fluctuate between the 24-hour dosing intervals.

Body Weight

Administration of brexpiprazole for 14 days had no effect on body weight (Fᵢ,ᵢ = 1.8, P > .05; Figure 1).
VTA DA Neurons

Brexpiprazole administration for 2 and 14 days did not alter the VTA DA neuron population or burst activity (Table 1). The firing rate of VTA DA neurons used for D_2 receptor status assessment did not differ between vehicle, 2-day and 14-day brexpiprazole-administered animals (4.3 ± 0.5, 4.3 ± 0.5, and 4.3 ± 0.5 Hz, respectively, P > .05). Saline administration had no effect on their firing rate (P > .05). In vehicle-administered animals, apomorphine (40 µg/kg, i.v.) completely inhibited the firing activity of all VTA DA neurons tested (Figure 2B). Following 2- and 14-day brexpiprazole administration, 40 µg/kg of apomorphine had a significantly smaller inhibitory effect compared with vehicle-administered animals (1-way ANOVA, F_{1,16} = 77, P < .001, data from 7, 6, and 5 neurons, respectively). Up to a dose of 80 µg/kg, apomorphine inhibited the firing of VTA DA neurons by approximately 30%, and not a single neuron was inhibited completely (Figure 2C).

Results: 5-HT System

Brexpiprazole administration for 2 but not 14 days increased the firing activity of 5-HT neurons in the DRN (F_{2,20} = 9.8, P < .002) (Figure 5A). No effect of brexpiprazole was detected on burst parameters (P > .05, data not shown). At baseline, the firing rate of 5-HT neurons used for determining 5-HT_1A autoreceptor sensitivity did not differ between vehicle, 2-day, and 14-day brexpiprazole-administered animals (1.2 ± 0.3, 1.4 ± 0.4, and 1.2 ± 0.3 Hz, respectively). Administration of saline did not alter firing activity relative to baseline (P > .05), and responsiveness to flesinoxan was unaltered after 2- and 14-day brexpiprazole administration (F_{2,15} = 0.18, P > .05; Figure 5B).

In hippocampus, spikes inhibited/nA 5-HT, and RT_{5-HT} did not differ between vehicle- and 14-day brexpiprazole-administered animals (189 ± 11 vs 181 ± 14 spikes inhibited/nA and 33 ± 3 vs 38 ± 3 seconds, respectively, data from 24 and 19 neurons in groups of 8 and 7 animals, P > .05). Baseline firing activity of CA3 pyramidal neurons before assessment of degree of tonic 5-HT_{1A} receptor activation did not differ between 14-day vehicle, acute, 2-day brexpiprazole + 24-hour washout, 2-day brexpiprazole, and 14-day brexpiprazole-administered animals (3.6 ± 0.3, 3.6 ± 1.2, 4.0 ± 0.5, 3.9 ± 0.2, and 3.3 ± 0.2 Hz, respectively, F_{1,24} = 0.7, P > .05). Blockade of 5-HT_{1A} receptors by WAY 100.635 at a dose of 25 µg/kg had a significant overall disinhibiting effect only in 14-day brexpiprazole-administered animals (RM ANOVA with Bonferroni posthoc, P < .05; Figure 6D). At doses of 50, 75, and 100 µg/kg, WAY 100.635 caused a significant disinhibitory effect in 2- and 14-day brexpiprazole-administered animals (RM ANOVA with Bonferroni posthoc, P < .001 for 50, 75, and 100 µg/kg, Figure 6D; for illustrative firing histograms of a neuron in a vehicle, 2- and 14-day brexpiprazole-administered animals, see Figure 6A-C, respectively).

Electrical stimulation of 5-HT afferents caused a shorter DOS at 5 compared with 1 Hz in 14-day vehicle- and brexpiprazole-administered animals (F_{1,34} = 62.0, P < .001) (Figure 6E). The DOS at 1 and 5 Hz did not differ between these groups (P > .05).

Discussion

After 2 and 14 days of administration, brexpiprazole plasma levels were in the clinical range observed in patients taking 1 to 4 mg/d (data on file) and corresponded to striatal D_2 receptor occupancies ranging between 60% and 75% (Maeda et al., 2014b).
Figure 2. Effect of 2- and 14-day brexpiprazole administration on ventral tegmental area (VTA) DA neurons. (A) Firing activity was unaltered by brexpiprazole administration. (B-D) Illustrative firing histograms of the inhibitory effect of apomorphine in a vehicle- (B), 2-day brexpiprazole- (C), and 14-day brexpiprazole-administered animal (D). (E) Graphic presentation of the inhibitory effect of apomorphine in vehicle- and 2-day and 14-day brexpiprazole-administered animals. Error bars represent SEM, numbers in histograms of (A) represent neurons recorded/animals used. In (E), data points were nudged to prevent overlap. #Significant effect of 2-day brexpiprazole administration compared with vehicle; ###P < .001. $Significant effect of 14-day brexpiprazole administration compared with vehicle; $$$P < .001.

Table 1. Discharge parameters of VTA DA neurons

|                  | Bursts/min | % spikes in burst | Spikes/burst | ISI (ms) | % neurons bursting | Neurons per tract |
|------------------|------------|------------------|--------------|----------|--------------------|------------------|
| 2-day vehicle    | 26 ± 3     | 31 ± 4           | 3.1 ± 0.2    | 70 ± 2   | 90                 | 0.9 ± 0.2        |
| 2-day brexpiprazole | 23 ± 3     | 30 ± 4           | 3.0 ± 0.1    | 67 ± 2   | 86                 | 1.1 ± 0.4        |
| 14-day vehicle   | 28 ± 3     | 35 ± 4           | 3.2 ± 0.2    | 71 ± 2   | 80                 | 1.0 ± 0.1        |
| 14-day brexpiprazole | 25 ± 3     | 33 ± 4           | 3.0 ± 0.2    | 72 ± 2   | 92                 | 1.0 ± 0.2        |

No significant effect on any parameter was detected.
Figure 3. Effect of acute, 2-day, and 14-day brexpiprazole administration on locus coeruleus (LC) noradrenaline (NE) neurons and status of 5-HT_{2A} receptors. (A) Firing activity after acute and (B) after 2 and 14 days of brexpiprazole administration. (C-E) Illustrative firing histograms of the inhibitory effect of the 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) after vehicle (C), 2-day brexpiprazole (D), and 14-day brexpiprazole administration (E). Note the unaltered response to acute administration of the α_{2}-adrenoceptor agonist clonidine after brexpiprazole administration. (F) Firing histogram illustrating no reversal by brexpiprazole of neural inhibition by clonidine in a vehicle-administered animal. (G) Graphic presentation of the inhibitory effect of DOI in control, 2-day, and 14-day brexpiprazole administration. Error bars represent SEM; numbers in histograms of (A-B) represent neurons recorded/animals used. *Significant effect of brexpiprazole administration; **p<0.01, ***p<0.001. #Significant effect of 2-day brexpiprazole administration compared with vehicle; ###p<0.001. $Significant effect of 14-day brexpiprazole administration compared with vehicle; $$$p<0.001.
DA System

Administration of the DA agonist apomorphine (40 µg/kg, i.v.; corresponding to the ED$_{100}$ in controls) reduced the firing activity of VTA DA neurons in 2- and 14-day brexpiprazole-administered animals to ~70% of baseline activity, demonstrating appreciable occupancy of D$_2$ receptor by brexpiprazole (Figures 2C-E). Interestingly, firing, bursting, and population activity of VTA DA neurons remained unaltered by these regimens (Figure 2A, Table 1). These data support and extend insight in different dynamics of agents with antagonistic vs partial agonistic action on D$_2$ receptors (Oosterhof et al., 2014). Acutely, D$_2$ receptor antagonists robustly increase the firing activity of VTA DA neurons by blocking the D$_2$ receptor-mediated autoinhibitory signal of DA (Chiodo and Bunney, 1983; Ghahari et al., 2009). Depending on their degree of intrinsic activity, partial D$_2$ receptor agonists acutely either decrease (e.g., aripiprazole, bifeprunox) or do not alter (brexpiprazole) the firing activity of DA neurons (Dahan et al., 2009; Oosterhof et al., 2014). Chronic D$_2$ receptor antagonism sensitizes D$_2$ autoreceptors and decreases population activity of VTA DA neurons (Vogelsang and Piercey, 1985; Skarsfeldt, 1995). Of particular interest, asenapine partially blocked the inhibitory effect of apomorphine on DA neurons after 2 days of administration, similarly to the effect of 2-day brexpiprazole administration. However, 21-day asenapine administration sensitized D$_2$ autoreceptors (Oosterhof et al., 2015). In contrast, the response of VTA DA neurons to apomorphine was indistinguishably dampened following 2- and 14-day brexpiprazole administration, demonstrating unaltered D$_2$ receptor sensitivity following these drug regimens (Figure 2C-E).

NE System

Acute administration of brexpiprazole increased the firing activity of LC NE neurons (Figure 3A). This firing activity remained elevated after repeated administration (Figure 3B), similarly to the effect of sustained asenapine, clozapine, quetiapine, and olanzapine administration (Ramirez and Wang, 1986; Seager et al., 2005; Chernoloz et al., 2012; Oosterhof et al., 2015). Interestingly, 2- and 14-day aripiprazole administration did not alter the firing activity of LC neurons (Chernoloz et al., 2009), demonstrating distinct effects of aripiprazole and brexpiprazole on the NE system. Brexpiprazole did not cause a blockade...
on \(\alpha_2\)-adrenergic autoreceptors, as an \(ED_{50}\) dose of clonidine (5 \(\mu g/kg, i.v\)) indistinguishably inhibited LC NE neurons in vehicle, 2-day, and 14-day brexpiprazole-administered animals (Figure 3C-F). Although these data do not exclude the possibility that brexpiprazole caused a sensitization of \(\alpha_2\)-adrenergic autoreceptors, this is highly unlikely; first, since such sensitization would decrease—and not increase—the firing rate of LC NE neurons, and second because intravenous brexpiprazole did not reverse the inhibitory effect of clonidine on NE neurons (in vehicle-administered animals; Figure 3F). These observations support the lack of activity of brexpiprazole on \(\alpha_2\)-adrenergic autoreceptors.

Activation of 5-HT\(_{1A}\) receptors located on terminals arising from the hypoglossal nucleus stimulates GABA release on NE neurons, providing a pharmacological node by which the 5-HT system modulates the activity of LC NE neurons (Szabo and Blier, 2001). Indeed, acute administration of the 5-HT\(_{1A}\) receptor agonist DOI inhibited all LC NE neurons in control animals \((ED_{50}=83 \mu g/kg)\), an effect fully reversed by the selective 5-HT\(_{1A}\) receptor antagonist M100907 (Figure 3C,G). In accordance with its acute behavioral and in vivo electrophysiological effects (Maeda et al., 2014b; Oosterhof et al., 2014), brexpiprazole administration for 2 and 14 days potently reduced the inhibitory effect of DOI, thus demonstrating antagonistic action on 5-HT\(_{1A}\) receptors (Figure 3D,E,G). Using the same methodology, antagonistic effects on 5-HT\(_{1A}\) receptors were previously demonstrated following sustained asenapine and quetiapine + norquetiapine administration (Chernoloz et al., 2012; Oosterhof et al., 2015).

Notably, the in vitro affinity for 5-HT\(_{1A}\) receptor of asenapine (\(K_i=0.06 nM\); Shahid et al., 2009), brexpiprazole (\(K_i=0.47 nM\); Maeda et al., 2014b), and quetiapine + norquetiapine (quetiapine \(K_i=101 nM\) [Kroese et al., 2003]; norquetiapine \(K_i=58 nM\) [Jensen et al., 2008]) is reflected in their respective complete, partial (Figure 3G), and slight blockade of DOI in vivo (Chernoloz et al., 2012; Oosterhof et al., 2015).

Previous studies showed that sustained antidepressant administration increases 5-HT neurotransmission, causing tonic activation of 5-HT\(_{1A}\) receptors and consequently, decreased NE firing. Under these conditions, 5-HT\(_{1A}\) receptor blockade normalizes NE firing (Dremencov et al., 2007a, 2007b; Chernoloz et al., 2012), providing a rationale for the augmenting effects of coadministering low-dose atypical antipsychotics to an antidepressant regimen (Blier and Szabo, 2005). In accordance, the current demonstration of 5-HT\(_{1A}\) receptor blockade provides one mechanism for the clinical effectiveness of brexpiprazole when administered in adjunct to antidepressants (Thase et al., 2015a, 2015b).

In the hippocampus, brexpiprazole increased the tonic activation of \(\alpha_2\)- but not \(\alpha_1\)-adrenergic receptors on CA3 pyramidal neurons (Figure 4C). Since the activity of the NET and the sensitivity of postsynaptic \(\alpha_2\)-adrenergic receptors remained unaltered in this brain region, increased hippocampal NE transmission was likely due to the excitatory effect of brexpiprazole on LC NE neurons (Figure 3A,B), an effect previously observed following sustained trazodone and quetiapine administration (Chernoloz et al., 2012; Ghanbari et al., 2012). In further resemblance to these agents, brexpiprazole has antagonistic action on \(\alpha_2\)-adrenergic receptors in vivo (Oosterhof et al., 2014), providing a mechanism by which it prevented tonic activation of these receptors. Importantly, brexpiprazole did not increase hippocampal or cortical NE levels following its acute administration (Maeda et al., 2014b), in contrast to other atypical antipsychotics (Westerink et al., 1998), suggesting that its enhancing effect on NE neurotransmission requires delayed neural adaptations.

**5-HT System**

Brexpiprazole administration for 2 days significantly increased the firing activity of DRN 5-HT neurons (Figure 5A), similarly to the effect of 2-day aripiprazole administration (Chernoloz et al., 2009). However, the firing activity of 5-HT neurons returned to control levels after 14-day brexpiprazole administration, while this remained elevated after 14-day aripiprazole administration (Chernoloz et al., 2009). In further contrast to aripiprazole, 2- and 14-day brexpiprazole administration did not alter the responsiveness of 5-HT neurons to acute 5-HT\(_{1A}\) receptor agonist administration, demonstrating that 5-HT\(_{1A}\) autoreceptor desensitization did not occur (Figure 5B). This finding might be surprising, especially since brexpiprazole was previously shown to act as a full 5-HT\(_{1A}\) receptor agonist in the hippocampus (Oosterhof et al., 2014). Indeed, the full 5-HT\(_{1A}\) receptor agonists...
BAY x 3702 and trazodone desensitized 5-HT₁A autoreceptors after long-term administration (Dong et al., 1998; Ghanbari et al., 2010). On the other hand, these agents decreased the firing activity of 5-HT neurons following 2-day administration, in contrast to the effect of brexpiprazole (Figure 5A). The DRN receives excitatory NE projections from the LC (Svensson et al., 1975), and excitatory D₂ receptors are also located on DRN 5-HT neurons (Haj-Dahmane, 2001). Possibly, a combination of these excitatory inputs prevented DRN 5-HT neurons to decrease their firing. Indeed, the excitatory effect of aripiprazole on DRN 5-HT neurons was to a significant degree mediated by partial D₂ receptor agonism (Chernoloz et al., 2009). In addition, since brexpiprazole plasma concentrations were significantly higher after 14 compared with 2 days, it is conceivable that this caused a greater activation of 5-HT₁A autoreceptors, preventing firing of DRN 5-HT neurons to remain elevated after the longer regimen.

In the hippocampus CA3 region, brexpiprazole increased the tonic activation of 5-HT₁A receptors on pyramidal neurons after 14 days of administration (Figure 6D), an effect common to all antidepressant agents tested in this paradigm (Blier and El Mansari, 2013). Since 14-day brexpiprazole did not alter the firing activity of 5-HT neurons, activity of the SERT, and status of 5-HT₁B terminal autoreceptors, it was deemed critical to assess whether this enhanced tonic activation was due to adaptive...
changes or attributable to the full agonistic action of brexpiprazole on postsynaptic 5-HT<sub>1A</sub> receptors (Oosterhof et al., 2014).

Interestingly, 2-day brexpiprazole administration caused a tonic activation on 5-HT<sub>1A</sub> receptors quantitatively comparable to the 14-day regimen. In contrast, 2-day bupropion, mirtazapine, and duloxetine administration all produced a lesser degree of post-synaptic 5-HT<sub>1A</sub> receptor activation, presumably due to adaptive changes following sustained administration (Ruer et al., 1998; Besson et al., 2000; Ghanbari et al., 2011).

Because the strong tonic activation after 2-day brexpiprazole was unexpected, it was decided to assess whether this effect was due to adaptive changes occurring within this short exposure period or full agonistic action of brexpiprazole on 5-HT<sub>1A</sub> receptors. Clearly, adaptive changes after 2-day brexpiprazole administration alone did not lead to increased tonic activation of 5-HT<sub>1A</sub> receptors, as a 24-hour washout prevented this effect. Similarly, full 5-HT<sub>1A</sub> agonism alone was insufficient to produce this effect, as the acute administration of brexpiprazole did not significantly enhance 5-HT<sub>1A</sub> receptor activation, at least at this dose.

Based on these results, it is possible that acute administration resulted in lower brexpiprazole levels and less tonic activation of 5-HT<sub>1A</sub> receptors compared with the 2- and 14-day regimens, where brexpiprazole accumulated with repeated injections. Alternatively, the combination of adaptive changes together with higher levels of brexpiprazole resulted in the marked enhancement of tonic activation on postsynaptic 5-HT<sub>1A</sub> receptors after 2- and 14-day brexpiprazole administration.

**Conclusion**

Repeated administration of brexpiprazole had profound effects on the activity of the DA, NE, and 5-HT monoamine systems, both at their cell body level and in projection areas. Brexpiprazole produced a significant occupation of D<sub>2</sub> autoreceptors while the firing activity of VTA DA neurons remained unchanged, supporting a stabilizing effect of brexpiprazole on DA neurotransmission. This might be particularly relevant in the treatment of schizophrenia, because a striatal hyperdopaminergic state has been commonly associated with positive symptoms, and low DA neurotransmission in prefrontal regions presumably contributes to negative symptoms and/or cognitive impairments (Laruelle et al., 1996; Silfstein et al., 2015). Although the precise effects of antipsychotics with partial D<sub>2</sub> receptor agonism on DA neurotransmission in patients requires further characterization, their therapeutic effect in schizophrenia is in line with a stabilizing effect of brexpiprazole on the DA system (Kane et al., 2015). Repeated brexpiprazole administration caused a blockade of 2 receptor populations mediating inhibitory crosstalk between the 5-HT and NE systems. First, it blocked 5-HT<sub>1A</sub> receptors, a pharmacological target important in the lower incidence of motor side effects produced by second-generation antipsychotics in the treatment of schizophrenia (Tarazi and Stahl, 2012) and in adjunct to serotonin reuptake inhibitors in the treatment of depression (Blier and Szabo, 2005). Secondly, it blocked α<sub>2</sub>-adrenergic heteroreceptors on 5-HT terminals, thereby not attenuating 5-HT release despite enhanced NE neurotransmission. Finally, accumulation of brexpiprazole and/or adaptive changes produced enhanced tonic activation of postsynaptic 5-HT<sub>1A</sub> receptors, a common effect of all agents with antidepressant action (Blier and El Mansari, 2013). With the limitation that the present findings were obtained in naive rats, these data provide insight in the effects of repeated brexpiprazole administration on monoamine systems that might be relevant to symptom domains.

**Acknowledgments**

This research was supported by a Tier 1 chair from the Canadian government and an Endowed chair from the University of Ottawa Institute of Mental Health Research to Pierre Blier. We thank Richard Bélanger for excellent technical support at the animal facility, and Dr. Jarr Arnt, Dr. Arne Merk, and Dr. Tine Bryan Stensel for critical reading and comments on the manuscript.

This work was supported by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) and H. Lundbeck A/S (Valby, Denmark).

**Statement of Interest**

M. El Mansari and C.A. Oosterhof declare no conflict of interest. P. Blier received grants and/or honoraria for giving lectures and/or participating on advisory boards from Astra Zeneca, Bristol-Myers Squibb, Eli Lilly, Janssen, Forest, Labopharm, Lundbeck/Takeda, Merck, Pfizer, Servier, sunovion, and Valeant.

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