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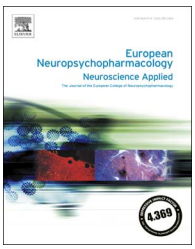

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Chronic vortioxetine treatment in rodents modulates gene expression of neurodevelopmental and plasticity markers

Jessica A. Waller[a], Joseph A. Tamm[b], Aicha Abdourahman[b], Alan L. Pehrson[a], Yan Li[a], Manuel Cajina[c], Connie Sánchez[a,*]

[a]External Sourcing and Scientific Excellence, Lundbeck Research USA, Paramus, NJ 07652, USA
[b]In Vitro Biology, Lundbeck Research USA, Paramus, NJ 07652, USA
[c]Molecular Pharmacology, Lundbeck Research USA, Paramus, NJ 07652, USA

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Abstract
The multimodal antidepressant vortioxetine displays an antidepressant profile distinct from those of conventional selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) and possesses cognitive-enhancing properties in preclinical and clinical studies. Recent studies have begun to investigate molecular mechanisms that may differentiate vortioxetine from other antidepressants. Acute studies in adult rats and chronic studies in a middle-aged mouse model reveal upregulation of several markers that play a central role in synaptic plasticity. However, the effect of chronic vortioxetine treatment on expression of neuroplasticity and neurodevelopmental biomarkers in naïve rats has not been evaluated. In the present study, we demonstrate that vortioxetine at a range of doses regulates expression of genes associated with plasticity in the frontal cortex, hippocampus, region encompassing the amygdala, as well as in blood, and displays similar effects relative to the SSRI fluoxetine in adult naïve rats. These genes encode immediate early genes (IEGs), translational regulators, and the neurodevelopmental marker *Sema4g*. Similar findings detected in brain regions and in blood provide a potential translational impact, and vortioxetine appears to consistently regulate signaling in these networks of neuroplasticity and developmental markers.

1. Introduction

Evolving preclinical evidence reveals antidepressants play a role in various neuroplasticity processes in brain regions implicated in major depressive disorder (MDD) (Castren and Hen, 2013; Pehrson et al., 2015; Russo and Nestler, 2013).

*Corresponding author.
E-mail addresses: wallerjessica14@gmail.com (J.A. Waller), connie_sanchez@clin.au.dk (C. Sánchez).

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The beneficial effects of antidepressant treatments may be partly mediated by these plasticity mechanisms and alterations in neuronal circuitry. Identification of molecular mechanisms eliciting these effects and translational studies to determine levels of plasticity-related biomarkers in response to antidepressant treatments may aid in a better understanding of their therapeutic capacity. Due to lack of accessibility of CNS tissue in patients, blood biomarkers that show similar regulation in brain regions implicated in MDD may provide a useful diagnostic in patient populations.

The multimodal-acting antidepressant vortioxetine displays a differentiated antidepressant profile relative to other conventional antidepressants in preclinical studies and possesses robust pro-cognitive properties (Sanchez et al., 2015). In addition to its activity as a serotonin (5-HT) transporter (SERT) inhibitor, it is an antagonist at 5-HT_{1D}, 5-HT_3, and 5-HT_7 receptors, agonist at 5-HT_{1A} receptors, and partial agonist at 5-HT_{1B} receptors (Bang-Andersen et al., 2011; Sanchez et al., 2015). Several clinical studies revealed vortioxetine enhances performance in tests of verbal learning and memory and processing of speed (Katona et al., 2012; Mahableshwarkar et al., 2015; McIntyre et al., 2014). At the behavioral level, numerous preclinical studies have demonstrated that vortioxetine augments working memory, including visuospatial and recognition memory (du Jardin et al., 2014; Jensen et al., 2014; Li et al., 2015a; Mork et al., 2013; Wallace et al., 2014). At the functional level, vortioxetine enhances gamma and theta power, related to memory encoding, sensory processing, and attention (Basar et al., 2000; Kaiser and Lutzenberger, 2005; Ward, 2003), pyramidal cell firing in the frontal cortex, and long-term potentiation or synaptic strengthening in hippocampal slices *in vitro* (Dale et al., 2014; Leiser et al., 2014; Riga MS et al., 2013). Vortioxetine showed differentiation from the selective serotonin reuptake inhibitors (SSRIs) fluoxetine and escitalopram and serotonin-norepinephrine reuptake inhibitor (SNRI) duloxetine on these various measures. The localization of the various vortioxetine receptor targets on glutamatergic and GABAergic neurons may be related to its effect on enhanced glutamatergic signaling (Pehrson and Sanchez, 2014). However, the underlying molecular mechanisms by which vortioxetine can enhance memory performance, cognition, and synaptic plasticity remain incompletely understood.

Recent evidence reveals vortioxetine can promote expression of various genes that play a role in synaptic plasticity. Following acute treatment in adult rats, vortioxetine promotes expression of targets that play a role in glutamatergic signaling and dendritic spine morphology (du Jardin et al., 2013). Moreover, chronic vortioxetine treatment in middle-aged mice induces expression of a variety of plasticity targets including those involved in transcription, synaptic signaling, and maintenance of dendritic spine structure (Li et al., 2015a). A bioinformatics analysis revealed a common biology modulated by acute and chronic vortioxetine in rats and mice, respectively (Waller et al., submitted for publication). In support of its effect on expression of cytoskeletal and dendritic spine markers, vortioxetine promotes dendritic branching (Chen et al., 2015; Guilloux et al., 2013), increases spine density *in vivo* (Chen et al., 2015), and promotes a transition to a mature-like, potentially stable, spine morphology and an increase in the number of spines forming presynaptic contacts *in vitro* in hippocampal neurons (Waller et al., 2016).

A major goal has been to determine if treatment-induced alterations in gene expression in various brain regions leaves a transcriptional signature in blood, which would permit testing in patient populations and provide a potential translational link. Thus, we examined a wide array of plasticity targets in brain regions implicated in depression including the frontal cortex (Koenigs and Grafman, 2009), hippocampus (Sapolsky, 2001), region encompassing the amygdala (Hamilton et al., 2008; Yang et al., 2010), as well as in blood, following chronic vortioxetine treatment in adult rats. We also examined the effect of chronic exposure to the SSRI fluoxetine in parallel. We proposed that similar classes of targets would be regulated in response to vortioxetine in both the brain and blood.

2. Experimental procedures

2.1. Animals and dosing

All animal studies were performed in accordance with Lundbeck IACUC institutional and NIH guidelines for the care and use of laboratory animals. Adult male Sprague-Dawley rats, ages 8–12 weeks, from Charles River (Wilmington, MA, USA) were pair-housed under a 12 h light/dark cycle and given ad libitum access to food and water. Following 1 week acclimatization, vortioxetine was administered in food for 1 month, beginning at 8 weeks, at a range of clinically-relevant doses, including 0.22 g/kg of food weight, corresponding to ~50% rSERT occupancy, 0.6 g/kg, corresponding to full SERT occupancy and ~50% occupancy at the r5-HT_{1B} receptor, and a high dose of 1.8 g/kg that achieves full occupancy at SERT and ~90% occupancy at r5-HT_{1B}. Fluoxetine was administered in drinking water for 1 month at a dose of 0.16 g/L, corresponding to full SERT occupancy. Control animals received the same formulation of food without vortioxetine and same drinking water without the fluoxetine.

2.2. Open array (brain regions)

Following chronic, 1-month dosing, rats were sacrificed and the frontal cortex, hippocampus, and region encompassing the amygdala were rapidly dissected in RNAlater (Ambion, Life Technologies, Carlsbad, CA, USA) on ice and stored at -20 °C prior to mRNA processing. Tissue was homogenized on ice in 1 ml of lysis buffer (Ambion RNAqueous 96 kit) using an Autogizer (Tomtec, Hamden, CT, USA). Total RNA was extracted from an aliquot of the lysate using the Ambion RNAqueous 96 automated kit according to the manufacturer's protocol. Following RNA elution from the column, a second DNase digestion was added to eliminate any residual genomic DNA in the samples. The total RNA was evaluated with an Agilent Bioanalyzer 2100 to determine RNA concentration and integrity. The average RNA integrity number (RIN) values were 6.7 for the cortex and 6.3 for the hippocampus. RNA concentration was normalized to 20 ng/μl and reverse transcription was performed using 200 ng of RNA and Superscript VILO (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. The Quant-It dye intercalation assay (Life Technologies, Carlsbad, CA, USA) was used to determine cDNA yield, and the samples were normalized to a concentration of 3 ng/μl.

Pre-amplification of the samples was accomplished using 12 cycles of PCR in a reaction containing 10 ng of cDNA, 112 primer sets exactly matching targets on the OpenArray chip, and 2 ×

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