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Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by behavioural and neurogenesis outcomes in mice☆

Jean-Philippe Guilloux [a], Indira Mendez-David [a], Alan Pehrson [b], Bruno P. Guiard [a], Christelle Repérant [a], Sophie Orvoën [a], Alain M. Gardier [a], René Hen [d,e], Bjarke Ebert [c], Silke Miller [b,1], Connie Sanchez [b], Denis J. David [a,*]

[a] Univ Paris-Sud, EA3544, Faculté de Pharmacie, Châtenay-Malabry F-92296, France
[b] Lundbeck Research USA, 215 College Rd, Paramus, NJ 07652, USA
[c] H. Lundbeck A/S, Medical Affairs Mood & Anxiety, Ottiliavej 9, DK-2500 Valby, Denmark
[d] Department of Neuroscience, Columbia University, New York, NY 10032, USA
[e] Department of Psychiatry, Columbia University, New York, NY 10032, USA

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ABSTRACT

Vortioxetine (Lu AA21004) is an investigational novel antidepressant with multimodal activity that functions as a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of the 5-HT transporter *in vitro*. Here we explore its anxiolytic and antidepressant potential in adult mice. Vortioxetine was assessed in BalB/cJ@RJ mice using the open-field and forced-swim tests (acute: p.o. 1 h, repeated: daily p.o. 21 days), and in 129S6/SvEvTac mice using the novelty suppressed feeding paradigm (acute: p.o. 1 h, sustained: daily p.o. 14 or 21 days). Fluoxetine and diazepam were controls. Acute and repeated dosing of vortioxetine produced more pronounced anxiolytic- and antidepressant-like activities than fluoxetine. Vortioxetine significantly increased cell proliferation and cell survival and stimulated maturation of immature granule cells in the subgranular zone of the dentate gyrus of the hippocampus after 21 days of treatment. After 14 days, a high dose of vortioxetine increased dendritic length and the number of dendrite intersections, suggesting that vortioxetine accelerates the maturation of immature neurons. Vortioxetine displays an antidepressant and anxiolytic profile following repeated administration associated with increased neurogenesis at several stages. Vortioxetine effects were observed at low levels of 5-HT transporter occupancy, suggesting an alternative mechanism of action to 5-HT reuptake inhibition.

1. Introduction

Depression is a major psychiatric disease, with a ≈17% lifetime prevalence (Kessler et al., 2005). Rates of response to initial pharmacotherapy can vary from 30 to 60% depending on the studies while remission rates in the first step of the STAR*D study was ≈37% (Guilloux et al., 2012; Rush et al., 2006). Side effects with Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly reported during chronic treatment, notably insomnia, somnolence, dizziness, akathisia, and long-term sexual dysfunction (e.g., decreased libido, delayed ejaculation) (Hamon and Bourgoin, 2006). The modest efficacy of conventional antidepressants such as the selective serotonin (5-HT) reuptake inhibitors (SSRIs) calls for novel approaches to treat depression and anxiety disorders. Combinatorial pharmacological therapies, such as additional blockade of aminergic receptors in addition to monoamine transporter inhibition, have earlier been proposed to shorten the time to antidepressant effect and/or to increase efficacy in clinical studies (Artigas et al., 2006; Kennedy et al., 2011).

The 5-HT_{1A} and the 5-HT_{1B} receptors were the first serotonergic receptors targeted to treat anxiety and depression due to their

* Corresponding author. Tel.: +33 1 46 83 59 68; fax: +33 1 46 83 53 55.
E-mail addresses: jean-philippe.guilloux@u-psud.fr (J.-P. Guilloux), indira.david@u-psud.fr (I. Mendez-David), APEH@Lundbeck.com (A. Pehrson), bruno.guiard@u-psud.fr (B.P. Guiard), christelle.reperant@u-psud.fr (C. Repérant), sophie.orvoen@gmail.com (S. Orvoën), alain.gardier@u-psud.fr (A.M. Gardier), rh95@columbia.edu (R. Hen), BJEB@Lundbeck.com (B. Ebert), silkem@amgen.com (S. Miller), cs@Lundbeck.com (C. Sanchez), denis.david@u-psud.fr (D.J. David).
[1] Present address: Amgen Inc., One Amgen Center Dr., MS 29-2-B, Thousand Oaks, CA 91320, USA.

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localization at the pre- and post-synaptic levels. Both receptors modulate serotonergic neurotransmission (Gingrich and Hen, 2001; Guilloux et al., 2011). For instance, pindolol, a beta adrenoceptor blocker with 5-HT_{1A} receptor partial agonism, has shown some efficacy in augmentation strategies with SSRIs; however, the low doses used in clinical studies, its antagonistic action on 5-HT_{1B} heteroreceptors combined with its effects on post-synaptic 5-HT_{1A} receptors limits its efficacy (Guilloux et al., 2006; Whale et al., 2010; Martiny et al., 2012).

A link between the activity of antidepressant drugs and 5-HT_3 receptor function has been suggested since 5-HT_3 receptor antagonists administered alone exert antidepressant- and anxiolytic-like effects in preclinical settings (Costall and Naylor, 2004). Moreover, pretreatment with the 5-HT_3 receptor antagonist ondansetron potentiates the effects of antidepressant drugs in preclinical models (Redrobe and Bourin, 1997; Ramamoorthy et al., 2008). However, the preclinical observations were not confirmed in the few clinical studies that have been conducted. Thus, there is currently a weak support of 5-HT_3 antagonism alone or in combination with SSRI in the treatment of depression. Furthermore, whereas selective 5-HT_3 receptor antagonists are used routinely to attenuate nausea associated with chemotherapy, irradiation or cisplatin treatment, there are only few studies conducted showing that 5-HT_3 receptor antagonism reduces nausea in patients being treated with SSRIs (Bailey et al., 1995).

Early indications of an involvement of 5-HT_7 receptors in mood disorders came from a study showing down-regulation of 5-HT_7 receptor expression after chronic treatment with various antidepressants (Mullins et al., 1999; for a review, see Mnie-Filali et al., 2011). Recent studies further support a role for 5-HT_7 receptors in treating depression. Hence, SB-269970, a 5-HT_7 receptor antagonist, decreased immobility in both the tail suspension and forced swim tests (Guscott et al., 2005; Hedlund et al., 2005; Faure et al., 2006; Wesolowska et al., 2006a, 2006b) and enhanced the antidepressant-like effect of citalopram (Bonaventure et al., 2007; Sarkisyan et al., 2010). In agreement with these pharmacological data, 5-HT_7 receptor knockout mice showed reduced immobility in both the forced swim and the tail suspension tests (Hedlund et al., 2005). Thus, it appears that the efficacy of SSRIs may be enhanced by blocking feedback systems and modulating relevant receptors.

Vortioxetine (Lu AA21004; 1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is a novel investigational antidepressant with multimodal activity. Vortioxetine acts as an inhibitor at the 5-HT transporter (SERT, Ki = 1.6 nM) in recombinant cells expressing human receptors or SERT and as a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist (Ki = 3.7, 19 and 54 nM, respectively), a partial agonist at the 5-HT_{1B} receptor (Ki = 33 nM), an agonist at the 5-HT_{1A} receptor (Ki = 15 nM) (Bang-Andersen et al., 2011; Mork et al., 2012; Westrich et al., 2012). In rats the binding affinities are Ki = 1.1, 200, 3.7, 16 and 230 nM, for 5-HT_3, 5-HT_7, 5-HT_{1D}, 5-HT_{1B}, and 5-HT_{1A} receptors, respectively and Ki = 8.6 nM for the SERT (Mork et al., 2012; Westrich et al., 2012). *In vivo*, vortioxetine increases the extracellular levels of 5-HT, noradrenaline (NA), and dopamine (DA) in rat prefrontal cortex and hippocampus (Mork et al., 2012).

Although preclinical findings indicate that, acutely, vortioxetine produces an antidepressant and anxiolytic profile (Mork et al., 2012), the behavioural consequences of chronic administration have not been described. To investigate the effects of chronic vortioxetine treatment, as well as to confirm its anxiolytic- and antidepressant-like activities, we assessed its behavioural effects after acute (1 h) or repeated (14 or 21 days) dosing using the open field (OF) paradigm, the novelty suppressed feeding (NSF) paradigm and the mouse forced swim test (FST). OF and FST studies were conducted in Balb/cJ mice that have been shown to display a high basal anxiety- and depression-like behaviour (Belzung and Griebel, 2001). We confirmed the behavioural effects of 14 or 21 days of treatment with vortioxetine in 129S6/SvEvTac mice in the NSF paradigm. As stimulation of hippocampal neurogenesis has been suggested to underlie the delayed onset of therapeutic efficacy of SSRIs and tricyclic antidepressants (Duman et al., 1999; Malberg et al., 2000; Santarelli et al., 2003), we investigated the effects of vortioxetine dosed for 14 or 21 days on cell proliferation and maturation/survival in the dentate gyrus in 129S6/SvEvTac mice.

2. Methods

2.1. Animals

One hundred and eighty BALB/cJ@Rj male mice, 7–8 weeks old (25–30 g, Centre d'élevage Janvier, Le Genest-St-Isle, France) were used for the acute and repeated dosing experiments in the OF and FST. Eighty 129S6/SvEvTac male mice, 7–8 weeks old (25–30 g, Taconic Farms, Denmark) were used for the acute and repeated dosing NSF and the cell proliferation and survival/maturation study and Sholl analysis.

Mice were maintained under standard conditions (12/12 h light/dark cycle, lights on at 6AM, 22 ± 1 °C, food and water *ad libitum*, 5 mice/cage). The protocols involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive # 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92-256B to DJD).

2.2. Drugs and treatment

2.2.1. Acute studies

Three doses of vortioxetine (2.5, 5 and 10 mg/kg, free base dissolved in 10% β-cyclodextrin, oral gavage, p.o.,) were used in the OF test, the NSF test and the FST. The effects of vortioxetine were compared to the vehicle control group (10% β-cyclodextrin) and also to a fluoxetine- (18 mg/kg p.o., (David et al., 2007)) and a diazepam-treated group (1.5 mg/kg, s.c. (David et al., 2007)). All doses were corrected for the weight of the salt. All treatments were administered 1 h before testing.

2.2.2. Chronic studies

Two doses of vortioxetine (5 and 20 mg/kg/day, free base dissolved in 10% β-cyclodextrin, oral gavage, p.o.) were tested in mice after 14 days of administration in the NSF and 21 days of administration in the OF test, the NSF test and the FST. The mice were tested 24 h after the last dose. The effects of vortioxetine were compared to a vehicle control group (10% β-cyclodextrin) and also to a fluoxetine-treated group (18 mg/kg/day p.o.).

2.3. Ex vivo SERT and 5-HT_3 receptor occupancy assays

Brains from mice treated with vehicle, fluoxetine, or vortioxetine (1 h after acute administration or 24 h after the 14th or 21st injection) were flash frozen, sectioned coronally using a cryostat, and then mounted on slides and frozen until use. Slices were 20 μm thick, and began at approximately +1.2 mm anterior from bregma for SERT receptor occupancy or −2.7 mm posterior from bregma for 5-HT_3 receptor occupancy determination (Franklin and Paxinos, 2008). Slides were stored for at least 24 h at −20 °C before use in autoradiography experiments.

2.3.1. Assessment of SERT occupancy

Slides were incubated at room temperature for 60 min in buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM KCl, pH = 7.4) containing 4.5 nM [^{3}H]-escitalopram. Nonspecific binding was determined using 1 μM escitalopram. Slides were washed briefly in cold buffer, dried, and exposed in a Beta imager for 16 h. The region of interest (ROI) for the SERT assay included the lateral and medial septum, the nucleus accumbens and the olfactory tubercle. An example image of the ROI for the SERT assay can be found in Supplementary Fig. 2A.

2.3.2. Assessment of 5-HT3 receptor occupancy

Slides were preincubated for 5 min in a buffer consisting of 50 mM Tris and 150 mM NaCl. Slides were dried under a stream of air for 30–45 min. Subsequently, slides were incubated at room temperature for 60 min in buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM KCl, pH = 7.4) containing 1 nM [^{3}H]LY278584 (Perkin-Elmer, USA). Nonspecific binding was determined using 1 μM ondansetron. Slides were washed briefly in cold buffer, dried, and exposed in a Beta imager for 24 h. The ROI for the 5-HT_3 receptor occupancy assay consisted of the hippocampus. An example image for the 5-HT_3 receptor occupancy assay can be found in Supplementary Fig. 2B.