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Vortioxetine, a novel antidepressant with multimodal activity: Review of preclinical and clinical data

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ABSTRACT

Vortioxetine, a novel antidepressant for the treatment of major depressive disorder (MDD), is 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 serotonin (5-HT) transporter (SERT) inhibitor. Here we review its preclinical and clinical properties and discuss translational aspects. Vortioxetine increases serotonergic, noradrenergic, dopaminergic, cholinergic, histaminergic and glutamatergic neurotransmission in brain structures associated with MDD. These multiple effects likely derive from its interaction with 5-HT-receptor-mediated negative feedback mechanisms controlling neuronal activity. In particular, 5-HT_3 receptors may play a prominent role, since their blockade *i*) increases pyramidal neuron activity by removing 5-HT_3 receptor-mediated excitation of GABA interneurons, and *ii*) augments SSRI effects on extracellular 5-HT. However, modulation of the other 5-HT receptor subtypes also likely contributes to vortioxetine's pharmacological effects. Preclinical animal models reveal differences from SSRIs and SNRIs, including antidepressant-like activity, increased synaptic plasticity and improved cognitive function. Vortioxetine had clinical efficacy in patients with MDD: 11 placebo-controlled studies (including one in elderly) with efficacy in 8 (7 positive, 1 supportive), 1 positive active comparator study plus a positive relapse prevention study. In two positive studies, vortioxetine was superior to placebo in pre-defined cognitive outcome measures. The clinically effective dose range (5–20 mg/day) spans ~50 to >80% SERT occupancy. SERT and 5-HT_3 receptors are primarily occupied at 5 mg, while at 20 mg, all targets are likely occupied at functionally relevant levels. The side-effect profile is similar to that of SSRIs, with gastrointestinal symptoms being most common, and a low incidence of sexual dysfunction and sleep disruption possibly ascribed to vortioxetine's receptor modulation.

Contents

Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); ACh, acetylcholine; ATC, Anatomical Therapeutic Classification; CGI-I, Clinical Global Impression-Improvement; CGI-S, Clinical Global Impression-Severity; CYP, cytochrome P450; DA, dopamine; DSST, Digit Symbol Substitution Test; EMA, European Medicine Agency; FDA, Food and Drug Administration (US); GABA, gamma-aminobutyric acid; HA, histamine; HAM-A, Hamilton Anxiety Rating scale; HAM-D, Hamilton Depression Rating scale; LC, locus coeruleus; LOCF, last observation carried forward; LTP, long-term potentiation; MADRS, Montgomery–Åsberg Depresssion Rating Scale; MDD, major depressive disorder; MMSE, Mini-Mental State Examination; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NE, norepinephrine; NMDA, N-methyl-D-aspartate; OC, observed cases; PCP, phencyclidine; PCPA, 4-chloro-DL-phenylalanine methyl ester HCl; PET, positron emission tomography; qEEG, quantitative electroencephalography; RAVLT, Rey Auditory Verbal Learning Test; SDS, Sheehan Disability Scale; SERT, serotonin transporter; sIPSCs, spontaneous inhibitory post-synaptic currents; SNRI, serotonin noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TEAEs, treatment-emergent adverse events; vHIP, ventral hippocampus.

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1. Introduction

After having successfully introduced the selective serotonin (5-HT) reuptake inhibitor (SSRI) antidepressants, many pharmaceutical companies were inspired by preclinical and clinical research in the mid-90s indicating that serotonin transporter (SERT) inhibition combined with antagonism of serotonin$_{1A}$ (5-HT$_{1A}$) somatodendritic autoreceptors resulted in an earlier and significantly larger increase of extracellular 5-HT levels in the rodent brain and in increased clinical efficacy and/or a shorter time to a clinical antidepressant effect compared to SSRIs (Hjorth, 1993; Artigas et al., 1994; Blier et al., 1997). The somatodendritic 5-HT$_{1A}$ autoreceptors in the raphe nucleus play a critical role in the negative feedback regulation of 5-HT neurotransmission, and microdialysis and electrophysiology studies of SSRIs in rats suggested that desensitization of these receptors was responsible for the observed increase in 5-HT neurotransmission after chronic SSRI treatment compared to the effect of an acute dose. Thus, a drug that inhibited SERT, antagonized 5-HT$_{1A}$ autoreceptors or accelerated their desensitization through direct receptor stimulation, and stimulated postsynaptic 5-HT$_{1A}$ receptors was expected to produce an enhanced 5-HT neurotransmission compared to an SSRI and consequently show an enhanced clinical response. This clinical effect could not be obtained with a silent 5-HT$_{1A}$ receptor antagonist, since the benefit of enhancing presynaptic 5-HT function was canceled by the simultaneous blockade of postsynaptic 5-HT$_{1A}$ receptors (Scorza et al., 2012). However, the search for drugs with combined activity at the SERT and 5-HT$_{1A}$ receptors turned out to be challenging in terms of defining an optimal potency ratio between the two targets and an optimal functional activity at the 5-HT$_{1A}$ receptor. Vilazodone, a SERT inhibitor and 5-HT$_{1A}$ receptor partial agonist is the only antidepressant with this target combination that has made it to the market; it was approved by the Food and Drug Administration (FDA) in 2012 for the treatment of major depressive disorder (MDD).

The drug discovery program that led to the discovery and development of vortioxetine (1-[2-(2,4-dimethylphenyl-sulfanyl)-phenyl]-piperazine, Lu AA21004) (Fig. 1) had its origins in the hypothesis (Artigas, 1993) derived from studies of combined SERT inhibition and 5-HT$_{1A}$ receptor modulation (Bang-Andersen et al., 2011). However, during the drug discovery phase of the project, the target profile was redirected toward a combination of SERT inhibition, 5-HT$_{1A}$ receptor agonism and 5-HT$_3$ receptor antagonism (Bang-Andersen et al., 2011). Combined 5-HT$_{1A}$ receptor stimulation and SERT inhibition have been hypothesized to lead to rapid desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors and an enhanced antidepressant effect through activation of post-synaptic 5-HT$_{1A}$ receptors (Blier et al., 1997; Blier and Ward, 2003). It was also thought that 5-HT$_3$ receptor antagonism might reduce the incidence of nausea observed during treatment with SSRIs and serotonin and noradrenaline reuptake inhibitors (SNRIs) (Bang-Andersen et al., 2011). Furthermore, in the course of the drug discovery program, it was found that 5-HT$_3$ receptor antagonism also potentiated the increase in extracellular 5-HT produced by SERT blockade (Mork et al., 2012). Vortioxetine was approved by the FDA in September 2013 and the European Medicines Agency (EMA) in October 2013 for the treatment of MDD.

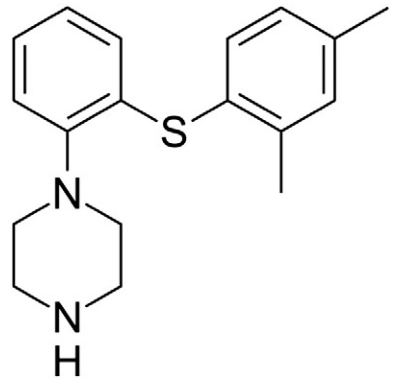

Fig. 1. Chemical structure of vortioxetine. The figure shown is the chemical structure of vortioxetine: (1-[2-(2,4-dimethylphenyl-sulfanyl)-phenyl]-piperazine).

According to the Anatomical Therapeutic Classification (ATC) system of the World Health Organization, vortioxetine belongs to the "N06AX Other, Antidepressants" class, which includes antidepressants not fitting in to the established classes of SSRIs, tricyclic antidepressants and monoamine oxidase inhibitors. According to a new classification system for psychotropic drugs proposed by a Task Force under the European College of Neuropsychopharmacology (ECNP), vortioxetine is an antidepressant with a multimodal mechanism of action that combines modulation of 5-HT receptor activity with inhibition of the SERT and vilazodone is classified as a serotonin partial agonist reuptake inhibitor (SPARI) (Zohar et al., 2013). SSRIs and SNRIs are antidepressants with a unimodal mechanism of action according to this suggested classification system, as they act via one target class (monoamine transporter inhibition) (Nutt, 2009). In this paper, we review the preclinical and clinical profile of vortioxetine in the context of its novel multimodal mechanism of action and discuss the putative contribution of its combined action on 5-HT receptors and SERT to its preclinical and clinical profile.

2. Preclinical profile

2.1. In vitro target profile

Vortioxetine was characterized in various in vitro binding and functional assays using recombinant cell lines expressing human and rat targets. In these assays, vortioxetine is a 5-HT$_3$, 5-HT$_7$ and 5-HT$_{1D}$ receptor antagonist, a 5-HT$_{1B}$ receptor partial agonist and a 5-HT$_{1A}$ receptor agonist and SERT inhibitor (Table 1) (Bang-Andersen et al., 2011; Mork et al., 2012; Westrich et al., 2012). While the binding affinities and functional activities of vortioxetine in rats are generally similar to those in humans, its affinities for the rat 5-HT$_7$ and rat 5-HT$_{1A}$ receptors are 10- to 15-fold weaker (Table 1). Why there are species differences at these two receptors and whether they can be ascribed to vortioxetine binding to different domains at the human and rat 5-HT$_7$ and rat 5-HT$_{1A}$ receptors remain unknown. Future studies should provide further insights into vortioxetine's interactions with its biological targets. A standard CEREP screening panel revealed no significant activities of vortioxetine at a concentration of 1000 nM at 70 receptors, ion channels, enzymes, kinases and transporters (Bang-Andersen et al., 2011).

Preclinical studies of vortioxetine's metabolites indicate that none of them are likely to contribute to its therapeutic activity. The major metabolite, 3-methyl-4-(2-piperazine-1-yl-phenylsulfanyl)-benzoic acid, Lu AA34443, (Areberg et al., 2012b) (see Section 3.1) has been extensively studied in in vitro cellular assays including all therapeutic targets of vortioxetine and the 70-target CEREP screening panel.

Table 1
In vitro binding affinities and functional activities of vortioxetine at human and rat targets expressed in recombinant cell lines. VOR: vortioxetine, IA: intrinsic activity.

Target	Function	Human				Rat			
		Binding affinity Ki (nM)		Functional potency IC_{50}/EC_{50} (nM) (IA, %)		Binding affinity Ki (nM)		Functional potency IC_{50}/EC_{50} (nM)	
		VOR	Reference	VOR	Reference	VOR	Reference	VOR	Reference
5-HT_3	Ant	3.7[a]	MDL 72222: 4.6[b]	12[a]	Ondansetron: 0.09[b]	1.1[b]	MDL 72222: 14[b]	0.18[a]	Ondansetron: 0.16[b]
5-HT_7	Ant	19[a]	5-HT: 0.2[b]	450[c]	Methiothepine: 56	200[c]	Methiothepine: 2.8[b]	2080[c]	Methiothepine:2.0
5-HT_{1D}	Ant	54[d]	5:HT: 2.9	370	Methiothepine: 152	3.7[d]	5-HT: 0.59	260	Methiothepine: 370
5-HT_{1B}	Part ago	33[a]	5-HT: 1.2[b]	120 (55)[c]	Ziprazodone: 40 (51)[b]	16[c]	5-HT: 6.1[b]	340 (40)	SB216641: 15(30)
5-HT_{1A}	Ago	15[a]	Metergoline: 1.6[b]	200 (96)[b]	5-HT: 9.7[b]	230[b]	8-OH-DPAT: 1.3[b]	ND	–
SERT	Inh	1.6[a]	Escitalopram: 1.0[b]	5.4[a]	Escitalopram: 1.6[b]	8.6[d]	Escitalopram: 1.9	5.3[a]	Citalopram: 7.7[b]

Abbreviations — inh: inhibition; ant:antagonist; ago: agonist, SERT: serotonin transporter, 5-HT: 5-hydroxytryptamine; 8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)-tetralin.
[a] Bang-Andersen et al. (2011);
[b] Sanchez et al. (2012);
[c] Mork et al. (2012);
[d] Westrich et al. (2012).

In addition to low blood–brain barrier penetration (Areberg et al., 2012b), no significant pharmacological activities were detected at any of these targets at concentrations up to 1000 nM. Thus, the pharmacological activities of vortioxetine are believed to be mediated entirely by the parent compound and the in vitro characterization of vortioxetine predicts that its pharmacological activities are mediated through a combination of direct 5-HT receptor modulation and inhibition of the SERT.

2.2. In vivo profile

Vortioxetine's pharmacological profile in rodents is summarized in Table 2. In general, the doses used in the preclinical pharmacology studies were chosen to be equivalent to clinical doses based on the level of SERT occupancy determined by ex vivo autoradiography (Fig. 2 and discussed in more detail in Section 2.2.1). In rats, vortioxetine preferentially occupies the 5-HT_3 receptor and the SERT at the lower end of the dose spectrum (0.3–10 mg/kg, s.c.) whereas the other 5-HT receptors are occupied at the higher end of the dose spectrum (Fig. 2). Vortioxetine's lower affinity for 5-HT_{1A} and 5-HT_7 receptors in rats compared to humans may mean that the net pharmacodynamic effects of vortioxetine mediated through these receptors is underestimated in the rat.

2.2.1. Neurochemistry and in vivo electrophysiology

In line with the hypothesis that receptor-mediated modulation of neuronal feedback systems can increase neurotransmitter release (Artigas, 2013), an acute dose of vortioxetine increased extracellular 5-HT levels to more than twice those obtained with an SSRI in microdialysis studies of the rat ventral hippocampus (vHIP) (Table 2) (Pehrson et al., 2013b). 5-HT_3 receptor antagonism by vortioxetine may contribute to the enhanced extracellular 5-HT levels seen in terminal areas, since co-administration of an SSRI and a 5-HT_3 receptor antagonist increased extracellular levels of 5-HT in the medial prefrontal cortex (mPFC) and vHIP to higher levels than the SSRI alone (Mork et al., 2012). 5-HT_{1B} autoreceptors may also contribute to the enhanced extracellular 5-HT levels, since electrophysiological recordings of CA3 hippocampal pyramidal neurons showed that vortioxetine is a functional antagonist at 5-HT_{1B} autoreceptors, decreasing their function under high, but not low levels of activation (Lecours et al., 2012). Furthermore, vortioxetine's antagonism of 5-HT_{1D} presynaptic autoreceptors may contribute, since these receptors also mediate inhibition of 5-HT release. Thus, antagonism of 5-HT_{1D} receptors has been shown to potentiate SSRI-induced increases of extracellular 5-HT (Pullar et al., 2004). Likewise, vortioxetine's 5-HT_7 receptor antagonism may also contribute to its net effect on extracellular 5-HT levels, as combining a 5-HT_7 receptor antagonist and an SSRI increased extracellular 5-HT levels in terminal areas more than the SSRI alone (Bonaventure et al., 2012). While a 5-HT_{1A} receptor agonist will acutely suppress 5-HT neuron firing in the dorsal raphe nucleus (DRN) and thereby reduce the release of 5-HT in terminal areas (e.g., mPFC and vHIP), repeated dosing of a 5-HT_{1A} receptor agonist will lead to desensitization of somatodendritic but not post-synaptic 5-HT_{1A} receptors (Haddjeri et al., 1999). The enhanced level of extracellular 5-HT with vortioxetine compared to an SSRI was sustained under steady-state conditions after 3 days of dosing via an osmotic minipump inserted subcutaneously (s.c.) (Pehrson et al., 2013b). Single unit recordings from 5-HT neurons in the DRN showed that vortioxetine acutely reduces the firing rate. However, vortioxetine produced a more rapid recovery of the firing rate than the SSRI fluoxetine (24 h vs. >7 days) when both drugs were continuously administered via osmotic minipumps (Betry et al., 2013). Vortioxetine's 5-HT_3 receptor antagonism appears to contribute to this difference, as co-administration of vortioxetine and a 5-HT_3 receptor agonist, SR57227, attenuated vortioxetine's acute suppression of DRN firing and delayed the recovery of neuronal firing. In conclusion, vortioxetine appears to increase extracellular levels of 5-HT through a combined inhibition of the SERT and the modulation of inhibitory feedback systems involving several 5-HT receptor subtypes. At this time, however, the contribution of the individual receptors to the net effect of vortioxetine is not fully understood.

Vortioxetine increased extracellular norepinephrine (NE) levels significantly in the mPFC and vHIP of rats after acute dosing and at steady-state conditions after 3 days s.c. dosing via osmotic minipumps (Table 2) (Mork et al., 2012; Pehrson et al., 2013b). Since vortioxetine does not significantly inhibit NE reuptake (Bang-Andersen et al., 2011), it is likely that its effects on NE neurotransmission are mediated through its activity at 5-HT receptors, given the strong reciprocal interactions between both systems. Furthermore, unlike an SNRI, an acute dose of vortioxetine did not increase NE in the nucleus accumbens (NAc) (Muneoka et al., 2009; Pehrson et al., 2013b), and only affected locus coeruleus (LC) firing at very high i.v. doses (Pehrson et al., 2013b), unlike the reduction in firing rates produced by acute treatment with an SSRI. The absence of an effect of vortioxetine on LC firing may involve 5-HT_3 receptor antagonism, since stimulation of 5-HT_3 receptors has been found to increase NE release in the LC and thereby decrease LC neuronal firing via stimulation of $alpha_2$ adrenergic receptors (Fernandez-Pastor et al., 2013). Hence, vortioxetine's 5-HT_3 receptor antagonism appears to be involved in its enhancing effects on NE neurotransmission. However, other receptor activities beyond 5-HT_3 receptor antagonism might be involved, since the minimal effective dose of vortioxetine (5 mg/kg, s.c.) promoting NE release is higher than a dose that fully occupies 5-HT_3 receptors and the SERT (i.e., ED_{80} is 0.016 and 1.5 mg/kg for 5-HT_3 receptor and SERT occupancy, respectively, calculated from ED_{50} values in Table 2) (Fig. 2). Microdialysis

Table 2
Summary of preclinical in vivo and ex vivo findings for vortioxetine. * Route of administration is s.c. if not stated otherwise; i.v.: intravenous; i.p.: intraperitoneal; p.o.: per oral by gavage; dietary: administered in the chow. –: no effect; MED (mg/kg): minimal effective dose; ED_{50} (mg/kg): dose that produces 50% of the maximum effect; DRN: dorsal raphe nucleus; SD: Sprague-Dawley; FSL: Flinders Sensitive Line; LE: Long-Evans; NSF: novelty suppression of feeding.

Test	Dosing regimen*	Effect	Ref
Microdialysis, rat			
mPFC	Acute, 50–120 min	MED: 5-HT (<0.31); NE (5); DA (5); ACh (5), HA (2.5)	a, j
vHIP	Acute, 50–120 min	MED: 5-HT (<2.5); NE (10), DA (10)	a
NAc	Acute, 50–150 min	MED: 5-HT (<2.5), NE (–), DA (–)	a
mPFC	3 days minipump	MED (mg/kg/day): 5-HT (10); NE (28), DA (28)	a
vHIP	3 days minipump	MED (mg/kg/day): 5-HT (19), NE (28), DA (–)	a
In vivo and slice electrophysiology, rat (anesthetized)			
Suppression of DRN firing	Acute, i.v.	ED_{50}: 0.4	b
Recovery of DRN firing	Minipump: 5 h, 10 h, 1 day, 3 days, 7 days, 14 days	5 mg/kg/day: Full recovery in 1 day Fluoxetine 10 mg/kg/day: Full recovery at 14 days	b
Suppression of LC firing	Acute i.v.	ED_{50}: 7.5	a
Suppression of VTA firing	Acute i.v.	No effect	a
CA3 hippocampal pyramidal neuron firing	Acute i.v.	6 mg/kg: Vortioxetine did not potentiate the effect of electric stimulation (1 Hz) of ascending 5-HT bundle, but prevented the decrease of effectiveness when stimulation frequency was increased from 1 to 5 Hz and reversed the inhibitory effect of 5-HT_{1B} receptor agonist, CP-94253	g
	Minipump 14 days	5 mg/kg/day: Vortioxetine increased tonic activation of post-synaptic 5-HT_{1A} receptors, measured as effect of the 5-HT_{1A} receptor antagonist, WAY 100,635, to microiontophoretically applied 5-HT compared to vehicle controls	
Theta burst LTP; hippocampus slices	Bath application	Vortioxetine (20 μM) significantly potentiated LTP; escitalopram (10 μ) inactive	c
sIPSC, rat hippocampus slices	Bath application	Vortioxetine (20 μM) counteracted 5-HT- and 5-HT_3 receptor agonist-induced sIPSC; escitalopram (10 μM) inactive	c
In vivo LTP	i.p., 20–50 min	Vortioxetine decreased LTP in non-stressed rats but reversed stress-induced decrease of LTP	d
Neurogenesis			
Cell proliferation in SD rat dentate gyrus	Minipump	5 mg/kg/day: Significant increase in cell proliferation after 1 day	d
Cell proliferation, maturation and survival, in dentate gyrus of 129/sv mice	p.o. 14 days, 21 day	14 days: Vortioxetine 20 significantly increased dendritic length and number of dendrite intersections; fluoxetine 18 no effect 21 days: Vortioxetine 5 and fluoxetine 18: increased cell proliferation, maturation and survival	e
Depression and anxiety, behavior			
Forced swim test, FSL rats	Acute, 30 min	Vortioxetine MED: 7.8; imipramine MED: 15	f
Forced swim test, progesterone withdrawn female LE rats		Vortioxetine 10 and amitriptyline 20 active; fluoxetine 16 and duloxetine 20 inactive	i
	Acute, i.p. 14 days, p.o. 14 days, i.p.	Vortioxetine 10 and amitriptyline 20 active, fluoxetine 16 inactive Vortioxetine 10 active; duloxetine 20 inactive	
Forced swim test, BALB/c mice	1 h, p.o.	Vortioxetine MED: 5; fluoxetine MED: 18	e
Forced swim test, BALB/c mice	21 days, p.o.	Vortioxetine MED: 5; fluoxetine 18 no effect	e
Forced swim test, 12 m C57Bl female mice	1 m, p.o.	Vortioxetine 10 active; fluoxetine 16 no effect	l
Social interaction, SD rats	1 h, p.o.	Vortioxetine MED: 2; chlordiazepoxide MED: 5	f
Conditioned fear-induced vocalization, SD rats	30 min	Vortioxetine MED: 3.9; buspirone MED: 1; duloxetine: no effect	f
NSF, 129/sv mice	1 h, p.o.	Vortioxetine MED: 5; diazepam MED: 1.5 s.c.; fluoxetine 18 p.o. no effect	e
NSF, 129/sv mice	14 days and 21 days p.o.	Vortioxetine MED: 20 (14 days) and 5 (21 days); fluoxetine 18 no effect	e
Open field, BALB/c mice	1 h, p.o.	Vortioxetine MED: 2.5; diazepam MED: 1.5 s.c.; fluoxetine 18 p.o. no effect	e
Open field, BALB/c mice	21 days, p.o.	Vortioxetine MED: 5; fluoxetine 18 p.o. no effect	e
Chronic mild stress, Wistar rats	5w, i.p.	Vortioxetine inactive 5 and 10; imipramine 10 active	h
Cognitive function, behavior			
Contextual fear conditioning, SD rats	Acute	Memory acquisition 10 Memory consolidation 5	j
Novel object recognition, SD rats	Acute	MED: 10	j
Novel object recognition, PCPA treated female LE rats	Acute 23 days dietary	Vortioxetine MED: 0.1; ondansetron MED: 0.001; flesinoxan MED: 1; escitalopram 0.5 no effect; duloxetine 15 no effect Doses corresponding to >80% SERT, approx. 60% 5-HT_{1B} receptor occupancy: active	k, l
Y-maze spontaneous alternation, PCPA treated female LE rats	Acute 23 days dietary	Vortioxetine MED: 3; ondansetron no effect; flesinoxan MED: 1 Doses corresponding to >80% SERT, approx. 60% 5-HT_{1B} receptor occupancy: not active	k
Object placement in 12 m old C57Bl female mice	1 m, dietary	Vortioxetine 10 active; fluoxetine 16 no effect	m
Attentional set shifting, sub-chronic PCP, SD rats	Acute	Vortioxetine MED: 3	n
Attentional set shifting, PCPA treated male SD rats	Acute, i.p. 3 days, i.p.	Vortioxetine 10 active Vortioxetine 10 active	o
Attentional set shifting, chronic intermittent cold stress male SD rats	18 days dietary	Doses corresponding to 53% SERT and 22% 5-HT_{1B} receptor occupancy and 82% SERT and 36% 5-HT_{1B} receptor occupancy both active	o

Table 2 *(continued)*

Test	Dosing regimen*	Effect	Ref
Studies with translational potential			
Target occupancy, ex vivo autoradiography, rat brain slices	Acute, 1 h	ED_{50}: 5-HT_3: 0.004; SERT 0.38 and 5-HT_{1B} 3.1 mg/kg. 5-HT_{1A} and 5-HT_7 ~ 40% at 10 mg/kg	p
Quantitative EEG, rat	Acute	*Active awake* increase: Vortioxetine MED: 3; flesinoxan MED: 2.5; ondansetron MED: 0.3; and duloxetine MED: 10. No effect: Escitalopram 2 and SB-269970-A 10 no effect *Theta power* increase: Vortioxetine MED: 5; ondansetron 0.3; and SB-269970-A 10. No effect: Flesinoxan 2.5; duloxetine 10, and escitalopram 2 mg/kg *Alpha power* no effect: Vortioxetine 10; flesinoxan 2.5; ondansetron 0.3; SB 269970-A 10 and escitalopram 2. Decrease: duloxetine 10 mg/kg *Gamma power* increase: Vortioxetine MED 5; flesinoxan 2.5. No effect: Ondansetron 0.3; SB 269970-A 10; escitalopram 2, and duloxetine 10 mg/kg	p

a: Pehrson et al. (2013b); b: Betry et al. (2013); c: Dale et al. (in press); d: Haddjeri et al. (2012); e: Guilloux et al. (2013); f: Mork et al. (2012); g Lecours et al. (2012); h: unpublished; i: Li et al. (2013a); j: Mork et al. (2013); k: du Jardin et al. (2014); l: Jensen et al. (2014); m: Li et al. (2013b); n: Pehrson et al. (2013a); o: (Wallace et al.) p: Leiser et al. (in press)

studies showed that 5-HT_{1A} receptor agonists increase extracellular NE levels (Suzuki et al., 1995; Suwabe et al., 2000). Since the minimal effective doses of vortioxetine correspond to those with significant (≈40%) 5-HT_{1A} receptor occupancy, it may be hypothesized that 5-HT_{1A} receptor agonism contributes to the increased extracellular NE levels in the mPFC and vHIP. This pharmacological activity may add to 5-HT_3 receptor blockade to increase NE levels, since the 5-HT_{1A} receptor agonist and SERT inhibitor vilazodone failed to increase NE in these brain regions (Hughes et al., 2005). In conclusion, vortioxetine appears to increase NE levels in forebrain areas through activity at several 5-HT receptors. Due to species differences in receptor affinities, NE elevation via 5-HT_{1A} receptor agonism may be greater in humans than in the rat.

Vortioxetine increased extracellular dopamine (DA) levels in the vHIP after an acute dose; however, the effect was not sustained after 3 days of dosing via osmotic minipumps (Table 2) (Pehrson et al., 2013b). In contrast, vortioxetine increased extracellular DA in the mPFC acutely and after 3 days of administration via osmotic minipumps. In all cases the minimal effective doses corresponded to >90% SERT occupancy (Pehrson et al., 2013b). In contrast, an acute dose of vortioxetine failed to increase extracellular DA in the NAc. These regional differences may be explained by the different origins of DA in mPFC and NAc. Whereas extracellular DA arises exclusively from DA nerve terminals in NAc, DA in mPFC may also be released by NE axons and is sensitive to drugs acting on NE terminals and to LC electrical stimulation (Masana et al., 2011, 2012). Since vortioxetine does not inhibit DA reuptake, its effect on DA neurotransmission is likely to be mediated by its receptor activities. While the role of 5-HT_3 receptors in the regulation of DA neurotransmission is unclear (Bétry et al., 2011), stimulation of post-synaptic 5-HT_{1A} receptors is well known to increase extracellular DA levels in the PFC (Rasmusson et al., 1994; Diaz-Mataix et al., 2005). The effects of vortioxetine on extracellular DA levels are only observed at doses that produce a significant occupancy of 5-HT_{1A} receptors, which suggests the involvement of this 5-HT receptor. Again, species differences indicate that this effect would be underestimated in rats compared to humans. However, since vilazodone failed to produce increases of DA levels in mPFC and vHIP (Hughes et al., 2005), other mechanisms may be involved beyond 5-HT_{1A} receptor activation, as observed for NE.

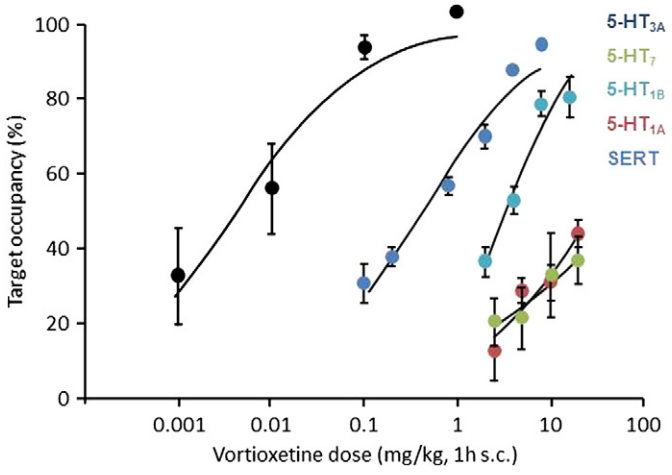

Fig. 2. Multi-target occupancies of vortioxetine in the rat brain as measured by ex vivo binding. The occupancies (% of total binding) of vortioxetine at different doses for multiple targets, 5-HT_3, 5-HT_7, 5-HT_{1B}, 5-HT_{1A} receptors and the SERT in the rat brain are depicted (mean +/- SEM).

In addition to increasing monoaminergic activity, acute administration of vortioxetine increases extracellular acetylcholine (ACh) and histamine (HA) levels in the mPFC (Table 2) (Mork et al., 2013), which may contribute to the positive effects of vortioxetine on cognitive function (see Section 2.2.4). Vortioxetine's effect on these neurotransmitter systems is most likely mediated via 5-HT receptor modulation, as vortioxetine has low affinity for cholinergic and histaminergic receptors (Bang-Andersen et al., 2011; Sanchez unpublished results). Several of the 5-HT receptors targeted by vortioxetine, including the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_3 receptors, are involved in the release of ACh either directly via their presence on cholinergic neurons or via GABA interneurons, depending on the brain region (extensively reviewed by Fink and Gothert, 2007). The serotonergic regulation of cortical HA release is not well understood although stimulation of 5-HT_4 receptors increases extracellular HA (Johnson et al., 2012). Since vortioxetine increases the endogenous 5-HT tone beyond the level of an SSRI, this may hypothetically lead to increased stimulation of cortical 5-HT_4 receptors modulating HA release. However, more studies are needed to understand the receptor mechanisms underlying the effect of vortioxetine on cortical ACh and HA levels.

In summary, vortioxetine modulates several neurotransmitter systems in the brain including 5-HT, NE, DA, ACh and HA through complex mechanisms involving SERT inhibition and modulation of several 5-HT receptor subtypes; the contribution of the individual receptor activities to the net effect of vortioxetine differs between brain regions.

2.2.2. Synaptic transmission and neuroplasticity

Long-term potentiation (LTP) has been used to study long-lasting changes in synaptic function and is thought to play an important role in learning and memory (Malinow et al., 2000). In vitro electrophysiological recordings in hippocampal slices indicated that vortioxetine

enhances synaptic transmission. Thus, vortioxetine (but not the SSRI escitalopram) potentiated theta burst-induced LTP and counteracted 5-HT-induced spontaneous inhibitory post-synaptic currents (sIPSCs) in patch clamp studies of hippocampal pyramidal neurons (Table 2) (Dale et al., in press). In both studies, 5-HT_3 receptor antagonism played a key role. Since the majority of 5-HT_3 receptors are located on GABAergic interneurons (Morales and Bloom, 1997; Puig et al., 2004), vortioxetine might attenuate the inhibitory control of pyramidal neurons by GABAergic interneurons and thereby increase glutamatergic neurotransmission, LTP and neuroplasticity (Pehrson and Sanchez, 2013). Finally, while an acute dose of vortioxetine reduced LTP in vivo in non-stressed rats (similar to other 5-HT enhancing drugs), vortioxetine reversed the LTP reduction induced by an acute stressor consisting of exposure to an elevated platform (Haddjeri et al., 2012). A unique and distinctive feature of 5-HT_3 receptors vs. other monoamine receptors is their ionic nature, being able to strongly excite GABAergic interneurons after their physiological stimulation by endogenous 5-HT (Puig et al., 2004). Thus, 5-HT_3 receptor blockade by vortioxetine may strongly disinhibit pyramidal neuron activity, as seen in preliminary observations (Riga et al., 2013).

A drug-induced increase in hippocampal neurogenesis in the dentate gyrus in the adult brain has been associated with antidepressant effects (Banasr and Duman, 2007), although its functional impact on the etiology of depression and the mechanism through which it may reduce depression remain to be understood (Lucassen et al., 2010). Vortioxetine administered to rats under steady-state conditions via an osmotic minipump produced a significant increase in cell proliferation (measured as the number of 5-bromo-2-deoxeyurine (BrdU) positive cells) in the dentate gyrus after only 1 day of treatment, whereas the SSRI fluoxetine, tested under similar conditions, required >7 days to produce a similar effect (Haddjeri et al., 2012). In mice dosed once daily, vortioxetine significantly increased markers of cell proliferation and 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 higher dose of vortioxetine increased dendritic length and the number of dendrite intersections compared to vehicle controls, suggesting that vortioxetine accelerates the maturation of immature neurons (Guilloux et al., 2013). In the same study, fluoxetine administered once daily for 21 days significantly increased cell survival and stimulated maturation of immature granule cells, whereas fluoxetine administered for 14 days had no effect on dendritic length or number of dendritic intersections compared to vehicle controls.

In summary, vortioxetine differs from the SSRIs fluoxetine and escitalopram in promoting several measures of synaptic transmission and neuroplasticity. Vortioxetine significantly enhances excitatory synaptic transmission (measured as LTP and pyramidal neuron stimulation), and increases neuroplasticity (measured as increased cell proliferation and maturation) and dendritic branching to a larger degree than an SSRI. Thus, via its receptor activities, vortioxetine enhanced glutamatergic neurotransmission in key brain areas implicated in depression and cognitive function in rodents.

2.2.3. Effects of vortioxetine in behavioral models of antidepressant and anxiolytic activity

Vortioxetine has been studied extensively in behavioral models, as summarized in Table 2. After acute administration, vortioxetine is effective in most standard behavioral tests, including the forced swim test in mice and Flinders Sensitive Line rats, the rat social interaction test, the rat conditioned fear-induced vocalization test, and the mouse novelty-suppressed feeding test (NSF) and open-field test (OF) (Mork et al., 2012; Guilloux et al., 2013). Vortioxetine remains active in the mouse NSF, OF and FST (forced swim test) after dosing for 14 and 21 days (Guilloux et al., 2013) and is also active in SSRI-insensitive 12-month-old C57Black mice treated for 1 month (Li et al., 2013b). In contrast, vortioxetine was inactive in a rat chronic mild stress model of depression (Papp, personal communication). In this model repeated exposure to stressors reduces the intake of a palatable 1% sucrose solution and attenuates DA neurotransmission in the NAc (Willner, 1997; Papp, 2012). The model is thought to mimic aspects of anhedonia, a core symptom of depression, and antidepressants, regardless of their mechanism of action, are suggested to reverse the sucrose intake to control level through sensitization of DA D_2/D_3 receptors in this model (Willner, 1997). At this point vortioxetine's lack of effect in the model has not been investigated. However, since extensive literature suggests that the mesolimbic DA pathway plays a key role in mediating reinforcing effects of drugs as well as natural rewards such as sucrose, and since 5-HT_3 receptors are thought to be important mediators of serotonergic modulation of this pathway (e.g. review by Engleman et al., 2008), it might be speculated that vortioxetine's failure to reverse stress-induced reduction of sucrose drinking at least partly is associated with its 5-HT_3 receptor antagonism. In contrast to these preclinical observations, clinical studies with vortioxetine (see Section 3.3) show a significant effect of the drug on all items of the Montgomery–Åsberg Depression Rating Scale (MADRS), including anhedonia (*lassitude*) (Thase et al., 2013). Thus, the rat sucrose-drinking model of anhedonia failed to predict the clinical efficacy of vortioxetine on this symptom. This discrepancy likely reflects the limitations of the sucrose-drinking model as a simplistic approach to investigate vortioxetine's effect on a complex clinical symptom such as anhedonia, and alternative experimental approaches would be needed to fully address its effect on anhedonia.

Preclinical as well as clinical research show that an abrupt withdrawal of progesterone can produce a range of physical and affective symptoms including anxiety, irritability, anhedonia, social withdrawal and depression (Li et al., 2012). Vortioxetine and amitriptyline, unlike fluoxetine and duloxetine, produced antidepressant-like behaviors in the FST in female Long-Evans rats during an induced progesterone withdrawal state (Li et al., 2013a). Previous reports indicate that changes in GABAergic neurotransmission play a key role in producing the progesterone withdrawal state (Li et al., 2012). Flesinoxan (5-HT_{1A} receptor agonist) and ondansetron (5-HT_3 receptor antagonist) have antidepressant-like activity in the progesterone withdrawal model. Since *i*) both receptors can modulate the activity of GABAergic neurons, and *ii*) vortioxetine shows both activities, it can be hypothesized that vortioxetine exerts its antidepressant activity through these receptors and the subsequent modulation of GABAergic neurotransmission, as suggested by the above electrophysiological studies. Therefore, it appears that vortioxetine may mediate its antidepressant activity by interaction with targets that differ from those of SSRIs and SNRIs.

2.2.4. Effects of vortioxetine in behavioral models of cognitive function

Preclinical studies using receptor-selective compounds indicate that 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 and 5-HT_7 receptors can regulate cognitive functions. The enhancement of cholinergic and histaminergic neurotransmission by vortioxetine may contribute to its positive effect on cognitive function given the important roles of these neurotransmitter systems on cognition (Fink and Gothert, 2007; Haas and Panula, 2003). Furthermore, vortioxetine's receptor activities have the potential to modulate glutamate neurotransmission and thereby affect cognitive functions, either directly or indirectly via GABA interneurons (Pehrson and Sanchez, 2013).

Several lines of preclinical evidence indicate that vortioxetine can enhance cognitive function (Table 2). First, vortioxetine enhanced acquisition and retention of time-dependent contextual fear memory and time-dependent object recognition memory in Sprague Dawley male rats (Mork et al., 2013). Furthermore, vortioxetine (but not escitalopram or duloxetine) reversed memory impairment in rats depleted of 5-HT by treatment with the tryptophan hydroxylase inhibitor 4-chloro-DL-phenylalanine methyl ester HCl (PCPA) and

assessed in the novel object recognition and Y-maze spontaneous alternation tests of recognition and spatial memory, respectively (du Jardin et al., 2014; Jensen et al., 2014). The effect was sustained after chronic (23 days) treatment, as measured in the novel object recognition test (du Jardin et al., 2014). A selective 5-HT_{1A} receptor agonist (flesinoxan) and, rather surprisingly, a selective 5-HT_3 receptor antagonist (ondansetron), reversed memory impairments in the 5-HT depleted rats, indicating that these receptors play a role in the effect of vortioxetine on memory function (du Jardin et al., 2014). Administration of the 5-HT releasing agent, fenfluramine, to PCPA-treated rats resulted in a marked increase of extracellular 5-HT, suggesting that a biologically significant 5-HT pool remains available for synaptic signaling (Jensen et al., 2014). This may potentially explain the surprising effect of a 5-HT_3 receptor antagonist in the PCPA-treated rats. While 5-HT_{1A} receptor agonism restored memory function in both the novel object recognition and spontaneous alternation tasks, 5-HT_3 receptor antagonism only restored recognition memory. Finally, chronic (1 month) vortioxetine treatment in 12-month-old mice alleviated their visuospatial deficits in an object placement test, whereas fluoxetine was inactive (Li et al., 2013b). Vortioxetine was also effective in rat models assessing executive function. Thus, sub-chronic treatment (1 week) with the NMDA receptor antagonist phencyclidine (PCP), followed by a 1-week wash-out period, impaired the extra-dimensional shift in the attentional set shifting test (Goetghebeur and Dias, 2009). Vortioxetine reversed the PCP-induced deficits in the extra-dimensional part of the set-shifting test (Pehrson et al., 2013a). Furthermore, 5-HT depletion by treatment with PCPA or chronic intermittent cold stress impaired the intra-dimensional shift (reversal learning) of the set shifting test (Lapiz-Bluhm et al., 2009). Vortioxetine restored PCPA-induced reversal learning deficits after acute or 3 daily doses (Wallace et al., 2014). Similarly, chronic dietary administration of vortioxetine at doses corresponding to 50–90% SERT occupancy prevented reversal learning deficits in rats exposed to chronic intermittent cold stress (Wallace et al., 2014). While the neurobiological substrates for vortioxetine's cognition-enhancing properties remain to be studied in further detail, the in vitro electrophysiology studies of hippocampal slices, showing that vortioxetine facilitates LTP formation and pyramidal neuron firing, strongly support the notion that vortioxetine has the potential to regulate cognitive function in rodents (Dale et al., in press). Likewise, the enhanced activity of pyramidal neurons in mPFC evoked by vortioxetine (Riga et al., 2013) may contribute to the maintenance of persistent neuronal activity required for working (short-term) memory.

2.3. Preclinical studies with translational potential

2.3.1. Pharmacokinetics

The pharmacokinetics of vortioxetine was examined in rats after intravenous (i.v.), p.o. and s.c. dosing. Various pharmacokinetics parameters are presented in Table 3, as are PK data for studies in humans (discussed further in Section 3.1). Absolute oral bioavailability in the rat was approximately 10% compared with 75% in humans given vortioxetine. After single p.o. and s.c. dosing the times to maximal plasma drug levels in rats were approximately 2.5–3.9 and 1.6 h, respectively, while the elimination half-lives ($T_{1/2}$) of vortioxetine were approximately 3–4 (p.o.) and 7.9 h (s.c.), respectively. Plasma drug exposures were higher in female rats than in male rats after both single and multiple dosing. The $T_{1/2}$ in rats (2.9–3.9 h) was considerably shorter than in humans (57 h) (Areberg et al., 2012b). The in vitro protein binding of [^{14}C]vortioxetine in plasma from rats and humans was high (>99%).

2.3.2. Pharmacodynamics

Determining the relationship between vortioxetine doses and target occupancy offers a potential bridge between preclinical and clinical studies. Therefore, target occupancies were determined in rat studies using ex vivo autoradiography and in clinical studies using positron emission tomography (PET) imaging (see Section 3.2.1). Fig. 2 shows the dose–occupancy relations in rats 1 h after s.c. dosing of vortioxetine. The potency rank order ex vivo is the same as for the in vitro binding affinities in rats, i.e., 5-HT_3 > SERT > 5-HT_{1B} > 5-HT_{1A} ≈ 5-HT_7. The therapeutic dose range of 5 to 20 mg/day in humans corresponds to 50 to >80% SERT occupancy. Since 5-HT_{1A} and 5-HT_7 receptor affinities in rats are >10-fold less than in humans, it appears reasonable to conclude that all vortioxetine's targets are likely to be occupied at clinically relevant doses, primarily 5-HT_3 receptors and SERT at the lower doses and also 5-HT_{1B}, 5-HT_{1A} and 5-HT_7 receptors at the higher doses.

Electroencephalography (EEG) is one of a number of techniques that can be useful in linking preclinical and clinical findings at the brain circuitry level Basar et al., 1999, 2000; Basar and Guntekin, 2008; Millan et al., 2012). A quantitative EEG (qEEG) analysis of the actively awake state in rats showed that vortioxetine dose-dependently increased arousal, as did flesinoxan, duloxetine, and ondansetron (but not escitalopram or the 5-HT_7 receptor antagonist SB-269970-A) (Table 2) (Leiser et al., in press). Furthermore, a quantitative spectral analysis showed that vortioxetine, unlike escitalopram or duloxetine, increased theta, alpha, and gamma power bands of the EEG (Leiser et al., in press). The combination of vortioxetine and a 5-HT_{1A} receptor

Table 3
Pharmacokinetic characteristics of vortioxetine in humans and rats.

Species	Human		Wistar rat; male:female		
Route of administration	Oral		p.o.		s.c.
Dosing regimen	Single	Multiple	Single	Multiple(b)	Single
Dose(s)	2.5, 5, 20, 60 mg/day (a)		2:2 mg/kg 6:6 mg/kg 20:20 mg/kg		2.9 mg/kg
T_{max} (hours)	8 [3–36]	7[4–10]	2:2	1:1.5	1.6
			1:2	1:4	
			2:2	1.5:2	
C_{max} (b) (ng/mL)	3.1	13	3.85:5.34	4.33:5.76	96.1
			20.7:42.1	13.4:29.2	
			83.9:161	111:186	
AUC (0-inf) (ng·h/mL	247	244	22.4:28.0	25.7:28.2	539.3
			116:155	87.7:173	
			564:1151	757:1419	
Elimination half-life ($T_{1/2}$)	57	57	3.0:2.9	2.9:2.9	7.9
			3.8:2.5	2.9:3.5	
			3.9:3.8	10:5.7	
Absolute oral bioavailability	75%		10%		

– = not examined; values in rats are for male:female rats dosed with vortioxetine.
(a) Doses pooled and dose normalized to 10 mg.
(b) After 7th dose. Value corrected for residual area attributed to drug remaining from previous dose.

agonist, flesinoxan, produced an even more pronounced effect on the power spectrum than vortioxetine alone. Furthermore, ondansetron and SB-269970 increased theta power significantly. These data suggest that vortioxetine increases frontal cortical activity through several of its receptor activities including 5-HT_{1A} receptor agonism and 5-HT_3 and 5-HT_7 receptor antagonism. In conclusion, the qEEG study in rats shows that vortioxetine has a pharmacodynamic profile very different from that of SSRIs and SNRIs; it increases arousal, which supports a potential to increase attention, and activates cortical networks involved in cognitive function.

2.4. Overall conclusion on preclinical findings

Vortioxetine's multimodal mechanism of action, combining 5-HT receptor modulation and SERT inhibition, results in a pharmacological profile that differs from that of SSRIs and SNRIs in various aspects. These include *i*) the direct and indirect modulation of multiple neurotransmitter systems (i.e., 5-HT, NE, DA, ACh, HA GABA and glutamate) via its interaction with several 5-HT targets, *ii*) effects in SSRI/SNRI-insensitive models of depression, and *iii*) the reversal of cognitive deficits in various animal models.

3. Clinical profile of vortioxetine and putative links to its pharmacological mechanism of action

3.1. Pharmacokinetics

The pharmacokinetics of single and multiple doses of vortioxetine was studied in 64 healthy men and 33 healthy women aged 18 to 53 years (Areberg et al., 2012b). As summarized in Table 3, vortioxetine has a bioavailability of 75% in humans, a mean T_{max} of 7–8 h and a mean elimination half-life ($T_{1/2}$) of 57 h upon oral administration. Assuming that it takes approximately 5 half-lives to reach steady-state, this would be reached within approximately 12 days. Vortioxetine shows linear and time-independent pharmacokinetics at doses of 2.5, 5, 20, 40, 60, and 75 mg.

Vortioxetine is extensively metabolized, primarily by oxidation and subsequent glucuronic acid conjugation (Uldam et al., 2011; Hvenegaard et al., 2012). The major metabolite is 3-methyl-4-(2-piperazine-1-yl-phenylsulfanyl)-benzoic acid (Lu AA34443) (Areberg et al., 2012b), which is considered pharmacologically inactive. Other metabolites are present in low concentrations in plasma or are not able to penetrate the blood–brain barrier and are therefore not considered pharmacologically relevant (Uldam et al., 2011; Hvenegaard et al., 2012). There were no differences between men and women in plasma levels for vortioxetine and Lu AA34443 after correction for weight; Lu AA34443 has a half-life similar to that of vortioxetine (Areberg et al., 2012b). The P450 enzymes responsible for the metabolism of vortioxetine include CYP2D6, CYP3A4/5, CYP2C9, CYP2C19, CYP2A6, CYP2C8, and CYP2B6 (Hvenegaard et al., 2012). The most important of these is CYP2D6. Vortioxetine shows no significant inhibition or induction of P450 enzymes, and is thus less prone to drug–drug interactions (Chen et al., 2013), although dosage adjustment may be required when vortioxetine is co-administered with bupropion (a CYP2D6 inhibitor and CYP2B6 substrate), or rifampicin (a CYP inducer) (Chen et al., 2013). This property of vortioxetine may be an advantage in comparison to other antidepressant drugs, such as paroxetine and duloxetine, which inhibit CYP2D6 (Hiemke and Hartter, 2000; Bourin et al., 2001; Spina et al., 2012).

3.2. Pharmacodynamics

3.2.1. Target occupancy studies

Positron emission tomography (PET) was used to examine the relation between dose/plasma concentration of vortioxetine and target occupancy in two clinical studies. In the first study, PET measurements were taken on day 1 (i.e., after a single dose) and on day 9 of multiple dosing of vortioxetine in 11 young healthy Caucasian subjects using the selective SERT ligand [^{11}C]-MADAM (N,N-dimethyl-2-(2-amino-4-methylphenylthio) benzylamine) (Lundberg et al., 2005). In the second study, PET measurements were taken after 13 days of multiple dosing of vortioxetine in 35 young healthy Caucasian or Japanese subjects with the selective SERT ligand [^{11}C]-DASB (3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile) (Houle et al., 2000). The two ligands have similar affinity for the SERT and vortioxetine's Emax and EC_{50} was not influenced by either PET ligand used. Study 1 investigated SERT occupancy at three dose levels of vortioxetine (2.5, 10 and 60 mg/day), whereas the doses in Study 2 were 2.5, 5 and 20 mg/day (Areberg et al., 2012a; Stenkrona et al., 2013). Occupancy was examined in the midbrain raphe nuclei, the area containing the highest SERT density in the human brain (Cortes et al., 1988). In both studies, consistent SERT occupancy levels in the midbrain raphe nuclei and EC_{50} values of vortioxetine ranging from 4.2 to 6.5 ng/mL were found (Areberg et al., 2012a; Stenkrona et al., 2013). The mean SERT occupancy in the raphe nuclei at steady-state conditions was ≈50% at 5 mg, 65% at 10 mg, and >80% at 20 mg of vortioxetine. Based on clinical efficacy studies (see Section 3.3), vortioxetine may exert antidepressant effects at SERT occupancies as low as 50%. In contrast, SSRIs and SNRIs may require SERT occupancy of at least 80% to exert their antidepressant effects (Meyer et al., 2004). This is consistent with the hypothesis that vortioxetine mediates its antidepressant effects through its activity at 5-HT receptors in addition to SERT inhibition (Section 1).

In the first study, no significant occupancy of 5-HT_{1A} receptors was found using [^{11}C]WAY 100635 as the radioligand after dosing with 30 mg vortioxetine (Stenkrona et al., 2013). This negative result could be explained by two factors. First, occupancy by an agonist may require displacement of an agonist radioligand. It is generally held that an agonist binds preferentially to the active state while an antagonist binds preferentially to the inactive state of the receptor in a two-state model (Ariens, 1954), and this may also be true in an extended model involving G proteins and effectors (Kobilka, 2004). An agonist might not be able to occupy the larger inactive state receptor population that is bound by an antagonist ligand, making the assay window too small to measure occupancy. In contrast, a partial agonist, especially a low-efficacy agonist, may be able to bind some or many of the inactive-state receptors. This may explain that successful PET studies using [^{11}C]WAY 100635 have been reported for the low-efficacy 5-HT_{1A} receptor partial agonist pindolol (Andree et al., 1999; Martinez et al., 2001), and the full 5-HT_{1A} receptor antagonist DU-125530 (Rabiner et al., 2002), but not for the agonist flesinoxan (Bantick et al., 2004). The second factor possible for the failure to find significant 5-HT_{1A} receptor occupancy is the endogenous 5-HT level. Serotonin has high affinity for the 5-HT_{1A} receptor (Newman-Tancredi et al., 1997) and may potentially impact the displacement of the 5-HT_{1A} receptor radioligand since vortioxetine substantially increases extracellular 5-HT levels in the brains of rats after single- and multiple-dose treatments (see Section 2.2.1).

3.2.2. Other pharmacodynamic studies

See Section 3.4 (driving performance (Theunissen et al., 2013), and Section 3.4.2 (sleep) (Wilson et al., 2013).

3.3. Clinical efficacy in MDD

3.3.1. Short-term studies

Table 4 summarizes the outcomes of 12 short-term (6, 8 or 12 weeks) MDD studies, 11 studies that evaluated the efficacy of vortioxetine 5, 10, 15, and 20 mg/day in randomized double-blind placebo-controlled studies, and one study using an active comparator. Seven of the placebo controlled studies were positive (vortioxetine was statistically superior to placebo on the pre-defined efficacy

Table 4
Overview of short-term (6–8 weeks) randomized, double-blind placebo controlled clinical studies in MDD patients. FAS: full analysis set, PBO: placebo, VOR: vortioxetine, VLF: venlafaxine, DLX: duloxetine, AGO: agomelatine MDE: major depressive episode.

Dose (mg) FAS (n)	Primary analysis	Inclusion criteria	Treatment period	Key findings	Clinical trial registry ID	Ref
PBO (105) VOR 5 mg (108) VOR 10 mg (100) VLF 225 mg (113)	MADRS (MMRM)	MADRS ≥30 MDE >3 and <12 months	6 weeks	Positive[1]: VOR 5 and 10 mg were superior to PBO on the pre-defined primary efficacy analysis.	NCT00839423	a
PBO (139) VOR 1 mg (139) VOR 5 mg (139) VOR 10 mg (139)	HAMD-24 (MMRM)	MADRS ≥26MDE >3 months	8 weeks	Positive[1]: VOR 10 mg was superior from PBO on the pre-defined primary efficacy analysis.	NCT00735709	b
PBO (149) VOR 2.5 mg (146) VOR 5 mg (153) DLX 60 mg (149)	HAMD-24 (ANCOVA, LOCF)	MADRS ≥22 MDE >3 months	8 weeks	Negative[3]: VOR 2.5 and 5 mg not significantly different from PBO on the pre-defined primary efficacy measure. DLX significantly different to PBO.	NCT00672620	c
PBO (286) VOR 5 mg (292)	HAMD-24 (ANCOVA, LOCF)	MADRS ≥30 MDE >3 months	6 weeks	Failed/negative[4]: VOR 5 mg not significantly different from PBO on the pre-defined primary efficacy measure.	NCT00672958	d
PBO (145) VOR 2.5 mg (155) VOR 5 mg (155) VOR 10 mg (151) DLX 60 mg (149)	MADRS(ANCOVA, LOCF)	MADRS ≥26 MDE >3 months	8 weeks	Failed[2] (supportive): VOR 5 and 10 mg not significantly different from PBO on the pre-defined primary efficacy analysis. DLX not significantly different to PBO.	NCT00635219	e
PBO (158) VOR 15 mg (149) VOR 20 mg (151) DLX 60 mg (146)	MADRS (MMRM)	MADRS ≥26 CGI-S ≥4 MDE >3 months recurrent	8 weeks	Positive[1]: VOR 15 and 20 mg were superior to PBO on the pre-defined primary efficacy analysis.	NCT01140906	f
PBO (153) VOR 15 mg (145) VOR 20 mg (147) DLX 60 mg (146)	MADRS (MMRM)	MADRS ≥26 CGI-S ≥4 MDE >3 months recurrent	8 weeks	Positive[1]: VOR 20 mg was superior from PBO on the pre-defined primary efficacy analysis.	NCT01153009	g
PBO (149) VOR 10 mg (143) VOR 15 mg (142)	MADRS (MMRM)	MADRS ≥26 &CGI-S ≥4 MDE >3 months recurrent	8 weeks	Failed/negative[4]: VOR 10 and 15 mg not significantly different from PBO on the pre-defined primary efficacy analysis.	NCT01179516	h
PBO (139) VOR 10 mg (124) VOR 20 mg (122)	MADRS (MMRM)	MADRS ≥26 CGI-S ≥4 MDE >3 months recurrent	8 weeks	Positive[1]: VOR 20 mg significantly different from PBO on the pre-defined primary efficacy analysis.	NCT01163266	i
PBO (145) VOR 5 (155) DLX 60 (148)	(HAMD-24 ANCOVA, LOCF)	≥65 years MADRS ≥26 & MMSE ≥24 MDE >4 weeks ≥1 prior MDE before 60 years	8 weeks	Positive[1]: VOR 5 mg significantly different from PBO on the pre-defined primary efficacy analysis.	NCT00811252	j
PBO (194) VOR 10 mg (193) VOR 20 mg (204)	MADRS (MMRM)[5]	MADRS ≥26 MDE >3 months recurrent	8 weeks	Positive[1]: VOR 10 and 20 mg significantly different from PBO on the pre-defined secondary efficacy analysis at Week 8.	NCT01422213	k
VOR 10-20 mg (252) AGO 25–50 mg (241)	MADRS (MMRM)	Inadequate response MADRS ≥22 MDE <12 months	12 weeks	Positive[1]: VOR 10–20 mg significantly different from AGO (25–50 mg) on the pre-defined primary efficacy analysis.	NCT01488071	l

a:.Alvarez et al. (2012); b: Henigsberg et al. (2012); c: Mahableshwarkar et al. (2013b); d: Jain et al. (2013); e: Baldwin et al. (2012b); f: Boulenger et al. (2013); g: Mahableshwarkar et al. (2013c); h: Mahableshwarkar et al. (2013a); i: Jacobsen et al. (2013); j:Katona et al. (2012); k: McIntyre et al. (2014); l: Häggström et al. (2013).
[1]Positive: The primary efficacy analysis was statistically significant. [2]Failed: In the primary efficacy analysis, vortioxetine did not separate from placebo, nor did the active reference. [3]Negative: Vortioxetine did not but the active reference did separate from placebo on the primary efficacy analysis [4]Failed/negative: Vortioxetine did not separate from placebo on the primary efficacy analysis, and no active reference was included in the study. [5]Primary endpoint was cognitive dysfunction. Secondary endpoint was antidepressant efficacy assessed by MADRS (MMRM).

analysis) and vortioxetine was statistically significantly superior to the active comparator, agomelatine.

In the first (proof of concept) study, vortioxetine (5 and 10 mg/day) efficacy was assessed in MDD patients in a 6-week study (Alvarez et al., 2012). This randomized placebo-controlled study included patients with a baseline MADRS total score ≥30 and an active reference group treated with venlafaxine XR 225 mg/day. At week 6, both doses of vortioxetine were efficacious on the pre-defined primary efficacy analysis, with a statistically significantly higher mean change from baseline in the MADRS total score versus placebo of 5.9 (5 mg) and 5.7 (10 mg) points [$p < 0.0001$, last observation carried forward (LOCF)]. Henigsberg et al. conducted an 8-week randomized placebo-controlled study of vortioxetine 1, 5 and 10 mg/day in MDD patients with baseline MADRS score ≥26 (Henigsberg et al., 2012). The pre-defined primary outcome efficacy analysis was a reduction in the HAM-D24 total score versus placebo at week 8 (Table 4). Vortioxetine 10 mg/day showed a statistically significant reduction from baseline in HAM-D24 total score at week 8 compared to placebo ($p < 0.001$), with a mean difference from placebo of 4.9. In a study by Mahableshwarkar et al. (2013b), MDD patients were randomized to placebo, vortioxetine 2.5 or 5 mg/day, or the active reference duloxetine 60 mg/day for 8 weeks (Mahableshwarkar et al., 2013b). Neither dose of vortioxetine showed a statistically significant difference from placebo at week 8 on the HAM-D24 total score, in contrast to duloxetine (Table 4). This study therefore failed. Another randomized placebo-controlled 6-week study of patients with MDD showed no significant differences in change from baseline in HAM-D24 total score at week 6 between 5 mg/day vortioxetine and placebo (Jain et al, 2013). These results are difficult to interpret since no positive control was included. In an 8-week study conducted by Baldwin et al. (2012b), MDD patients with a baseline MADRS total score ≥26 were randomly assigned to 2.5, 5 or 10 mg/day vortioxetine, placebo, or 60 mg/day duloxetine (Baldwin et al., 2012b). There were no statistically significant differences from placebo for vortioxetine or duloxetine on the pre-defined primary efficacy analysis of mean change from baseline MADRS total score at week 8 (ANCOVA, LOCF). However, post-hoc analyses using a mixed model, repeated measures (MMRM) analysis showed that the primary endpoint and most secondary endpoints were supportive of efficacy for vortioxetine 5 and 10 mg/day and duloxetine.

Two similarly designed randomized placebo-controlled studies of vortioxetine 15 and 20 mg/day versus placebo were conducted in MDD patients with baseline scores of MADRS ≥26 and Clinical Global Impression-Severity (CGI-S) ≥4 (Boulenger et al., 2013; Mahableshwarkar et al., 2013c). Both studies included duloxetine

60 mg/day as an active reference. In one study (Boulenger et al., 2013), the pre-defined primary efficacy analysis of mean change from baseline in MADRS total score at Week 8 showed superiority versus placebo for both vortioxetine doses and mean treatment differences from placebo were −5.5 and −7.1 points for 15 and 20 mg/day, respectively. In the second study (Mahableshwarkar et al., 2013c) vortioxetine 20 mg/day was statistically significantly superior to placebo in reducing the MADRS total score at Week 8, with a mean difference from placebo of −2.8 points. Vortioxetine 15 mg/day was not statistically significantly superior to placebo on the pre-defined primary efficacy analysis.

In another randomized placebo-controlled study with vortioxetine 10 and 15 mg/day using the same inclusion criteria, neither vortioxetine dose was statistically significantly superior to placebo on the pre-defined primary efficacy analysis (Mahableshwarkar et al., 2013a), even though this study used centralized rating for the inclusion criteria and the same primary efficacy outcome measure. A randomized placebo-controlled study with vortioxetine 10 and 20 mg/day versus placebo in MDD patients showed that vortioxetine 20 mg/day was statistically significantly superior to placebo on the pre-defined primary efficacy analysis (mean change from baseline in MADRS total score at week 8) (Jacobsen et al., 2013).

In a randomized placebo-controlled study with vortioxetine 10 and 20 mg/day in which the primary outcome was cognitive function (McIntyre et al., 2014), both vortioxetine doses were statistically superior to placebo on the pre-defined secondary outcome using the MADRS at Week 8 (Table 4), with mean treatment differences from placebo of −4.7 and −6.7 points for 10 and 20 mg/day, respectively. Another study randomized MDD patients with an inadequate response to SSRI/SNRI monotherapy to flexible-dose treatment with vortioxetine (10–20 mg/day) versus agomelatine (25–50 mg/day) (Häggström et al., 2013). Vortioxetine was statistically superior to agomelatine on the pre-defined primary outcome using the MADRS at Week 8. In addition, a randomized, placebo-controlled, active-referenced study of vortioxetine 5 mg/day versus placebo was conducted in elderly patients (aged ≥65 years) with MDD (Section 3.3.3).

Therefore, with 8 positive short-term studies (Table 4), vortioxetine shows convincing clinical efficacy in patients suffering from moderate to severe MDD. Interestingly, the clinically effective doses range from 5 to 20 mg/day, which spans ~50 to >80% SERT occupancy. Given that effective doses of SSRIs typically correspond to ~80% SERT occupancy (Meyer et al., 2004), these results strongly support the hypothesis that the antidepressant effects of vortioxetine arise from both receptor modulation and inhibition of the SERT. Thus, it appears that the preclinical microdialysis observations that SERT occupancies as low as 40–50% significantly increased extracellular 5-HT levels translates into clinical efficacy in patients with MDD. Since vortioxetine's in vitro binding affinity for human 5-HT_3 receptors and SERT are of the same order of magnitude, it is plausible that both activities contribute to the clinical efficacy — even at the low end of the clinical dose range. Furthermore, the expression of 5-HT_3 receptors as heteroreceptors on GABA interneurons, and their robust ionic actions on neuronal activity, support the hypothesis that modulation of neurotransmitters systems beyond the serotonergic is involved, even at the low end of the clinical dose range. Vortioxetine's antidepressant efficacy in MDD patients with inadequate response to SSRI or SNRI treatment may potentially also support a different mechanism of action. Future mechanistic studies will hopefully address this in further detail.

3.3.2. Long-term studies

Evidence of the long-term efficacy of vortioxetine was shown in a placebo-controlled relapse-prevention study. Relapse after full remission in MDD is common after antidepressant treatment, especially in patients who experience residual symptoms such as sleep or appetite disturbances (Nierenberg et al., 2010). Relapse may occur if treatment is stopped as soon as symptomatic relief is obtained, but continued maintenance antidepressant treatment may reduce the risk of relapse (Geddes, 2003). To investigate the efficacy and tolerability of vortioxetine in the prevention of relapse in MDD patients in remission after short-term treatment, 639 patients were treated for 12 weeks with vortioxetine 5 or 10 mg/day in an open-label study, after which 396 patients in stable remission (MADRS ≤10 at both weeks 10 and 12) were randomly assigned to double-blind treatment with vortioxetine [at the dose (5 or 10 mg) that was fixed from Week 8 in the open-label period] or placebo (Boulenger et al., 2012). The study showed that the time to relapse (Cox proportional hazard model) was statistically significantly in favor of vortioxetine compared to placebo (hazard ratio of 2.01), with relapse rates of 13% with vortioxetine versus 26% in the placebo group.

Further support for the long-term effectiveness of vortioxetine came from two open-label studies: one by Baldwin et al. (2012a) that was an extension of a short-term study (Baldwin et al., 2012b) and a second (Alam et al., 2014) that was an extension of two short-term studies (Henigsberg et al., 2012; Mahableshwarkar et al., 2013b). In the Baldwin et al. (2012a) study, 535 MDD patients from the 8-week lead-in study, with a mean MADRS total score of 13.5, entered a long-term (52-week) safety study, mainly with vortioxetine 5 and 10 mg/day. At the end of the treatment period (week 52), the mean MADRS total score had improved by 8 points, with an observed cases (OC) response rate based on the start of the lead-in study of 94% (versus 63% in the lead-in study) and remission rate of 83% (versus 42% in the lead-in study). The corresponding LOCF rates were 71.2% for remission and 84.3% for response. Patients in remission (n = 226) at the start of this study had a relapse rate (MADRS ≥22) of 9.7%. In the second open-label extension study (Alam et al., 2014), 834 patients participated from the two lead-in studies, with a mean HAM-D24 total score of 17.6. At the end of treatment with vortioxetine 2.5, 5, or 10 mg/day (week 52), the mean HAM-D24 was 8.2 (OC, 9.7 LOCF). The HAM-D24 response rate based on the start of the lead-in study was 60.2% (OC) and 51.0% (LOCF) and the HAM-D17 remission rate (HAM-D17 ≤ 7) was 61.7% (OC) and 55.6% (LOCF). The results from these studies support the efficacy of vortioxetine in treating MDD and in decreasing the risk of recurrence of depressive episodes after remission is achieved.

3.3.3. Cognitive dysfunction in MDD

The efficacy, tolerability and safety of vortioxetine (5 mg/day) were investigated in elderly patients (aged ≥65 years, mean age 70.6 years) with recurrent MDD (mean baseline HAM-D24 score 29.0) and a mean Mini-Mental State Examination (MMSE) score of 28. This was an 8-week double-blind study with placebo control and duloxetine 60 mg/day as an active reference (Table 4) (Katona et al, 2012). Vortioxetine was superior to placebo on the pre-defined primary efficacy analysis of the HAM-D24 (ANCOVA, LOCF) at week 8, with a mean difference from placebo of −3.3 points (p = 0.0011).

In the same study, analyses of pre-defined secondary outcome measures showed that vortioxetine treatment also resulted in superior performance versus placebo in cognitive neuropsychological tests of executive function, attention, speed of processing, verbal learning and memory, as demonstrated by the Rey Auditory Verbal Learning Test (RAVLT) and the Digit Symbol Substitution Test (DSST) (Katona et al., 2012). The RAVLT and DSST are thought to assess aspects of cognition known to be impaired in patients with MDD (Rey, 1964; Wechsler, 1997). Both vortioxetine and duloxetine produced statistically significant improvement on the RAVLT compared to placebo, but only vortioxetine produced a statistically significant improvement versus placebo on the DSST (Katona et al., 2012). The fact that vortioxetine separated from placebo in both tests supports the hypothesis that it can improve cognitive dysfunction across a range of cognitive domains. In further support of this hypothesis, a post-hoc path analysis showed that more than two-thirds of the effect of vortioxetine on both the DSST and RAVLT was a direct treatment effect rather than an indirect effect through improvement in depressive symptom severity (Katona et al., 2012).

In a short-term MDD study with non-elderly adults, the pre-specified primary efficacy outcome measure was based on objective neuropsychological tests of cognitive function (McIntyre et al., 2014). The primary outcome measure was change from baseline to Week 8 in a composite Z-score comprising the DSST and RAVLT scores. Both doses of vortioxetine were significantly better than placebo, with mean treatment differences versus placebo of 0.36 (vortioxetine 10 mg, $p < 0.0001$) and 0.33 (vortioxetine 20 mg, $p < 0.0001$) on the composite cognition score. Path and subgroup analyses indicated that the beneficial effect of vortioxetine on cognitive performance was largely a direct treatment effect and not solely due to improvements in depressive symptoms. In addition to demonstrating efficacy on the composite cognition score, improvement with vortioxetine treatment was also noted on a range of secondary objective and subjective patient-reported measures of cognitive function. Significant improvement versus placebo was seen on all included measures of executive function, attention, and processing speed, as well as with learning and memory.

The positive effects of vortioxetine on cognitive dysfunction in MDD patients in these two studies are compatible with the substantial preclinical support for vortioxetine improving cognitive function (Sections 2.2.2 and 2.2.4). The cognitive enhancing effect of vortioxetine cannot be ascribed to its activity at any particular receptor as yet, but is probably mediated through a combination of activities at several receptors. More clinical studies, including comparative studies to SSRIs or SNRIs, are necessary to confirm these clinical observations.

3.3.4. Clinical efficacy: overall conclusions

Vortioxetine is an effective antidepressant after short-term as well as long-term treatment. Both clinical and preclinical studies indicate that the multimodal mechanism of action, with both inhibition of the SERT and direct modulation of 5-HT receptors, differentiates vortioxetine from currently used SSRI and SNRI antidepressants. Vortioxetine is clinically effective at SERT occupancies as low as 50%, which supports the hypothesis that receptor modulation is involved in its antidepressant activity. Furthermore, assessments of vortioxetine's effects on cognitive function indicate that it may have beneficial effects by alleviating cognitive dysfunction in addition to depressive symptoms. Future preclinical and clinical studies will extend these observations and shed light on their underlying biological substrates.

3.4. Tolerability and safety

In clinical studies vortioxetine was well tolerated in healthy subjects and in adult and elderly patients with MDD. In a study in 24 healthy subjects, vortioxetine 10 mg/day and mirtazapine 30 mg/day were evaluated for possible effects on driving, cognitive, and psychomotor performance in a double-blind, placebo-controlled, three-way cross-over design (Theunissen et al., 2013). Mirtazapine produced inferior driving performance at day 2 but not day 16 of treatment compared to placebo and impaired cognitive and psychomotor performance on day 2, whereas vortioxetine showed no difference from placebo.

In a pooled analysis of 10 randomized, double-blind, placebo-controlled, short-term (6–8 weeks) trials of vortioxetine, most treatment-emergent adverse events (TEAEs) reported by MDD patients treated with therapeutic doses of vortioxetine (5–20 mg/day) were of mild or moderate intensity (89.3% for vortioxetine versus 90.5% for placebo) (Baldwin et al., 2013). The rate of withdrawal due to TEAEs was 4.5 to 8.4% compared to placebo (3.5%), venlafaxine (14.2%) and duloxetine (8.8%). TEAEs with an incidence ≥5% for vortioxetine 5–20 mg/day and at least twice the incidence of placebo were nausea (20.9 to 31.2%) versus placebo (8.6%), venlafaxine (33.6%) and duloxetine (34.1%) and vomiting (2.9 to 6.5%) compared to placebo (1.2%), venlafaxine (3.5%) and duloxetine (4.1%). Nausea showed a clear dose effect and most patients who had nausea during treatment with vortioxetine reported nausea during the first weeks of dosing. Nausea was most often transient, with a median duration of 10 to 16 days, for doses from 5 to 20 mg/day (Baldwin et al., 2013). These data suggest an association of nausea with vortioxetine use. Nausea is a well-known adverse effect of SERT inhibitors and of selective 5-HT_{1A} receptor agonists (Heiser and Wilcox, 1998). Hence, vilazodone, which displays both activities, requires dose titration for up to 2 weeks in order to reach the daily target dose of 40 mg due to high rates of gastrointestinal adverse effects such as nausea and vomiting (Wang et al., 2013). Despite the fact that vortioxetine also inhibits SERT and stimulates 5-HT_{1A} receptors, its additional antagonism of 5-HT_3 receptors may partly counteract gastrointestinal adverse effects due to these activities, since 5-HT_3 receptor antagonists display clear antiemetic properties and have been used to counteract SSRI-induced nausea (McManis and Talley, 1997).

As reported in the review of vortioxetine conducted by the European Medicines Agency, the incidence of serious adverse events was generally low (<3%). Six patients (from a total of 4972 patients treated with vortioxetine) died during the clinical trials: 2 patients died from cancer, one patient from suicide, one patient from morphine overdose and 2 patients from accidents (CHMP, 2013). Furthermore, no clinically meaningful differences between vortioxetine and placebo were found for clinical laboratory values, vital signs, physical examination findings, or electrocardiograms.

3.4.1. Sexual dysfunction

The incidence of treatment-emergent sexual dysfunction (TESD), judged by the investigators as decreased libido, abnormal orgasm, delayed ejaculation, erectile dysfunction, anorgasmia, loss of libido, ejaculation disorder, disturbance in sexual arousal, orgasmic sensation decreased, sexual dysfunction, and ejaculation failure, was not significantly different from placebo (CHMP, 2013). This contrasts with most existing antidepressants, which are known to cause this side effect during the course of antidepressant therapy (Serretti and Chiesa, 2009; Baldwin and Foong, 2013). Duloxetine is also known to have sexual dysfunction as a side effect, but seems to have a lower incidence than most other antidepressants (Serretti and Chiesa, 2009). Duloxetine was included as the active reference in 5 of the 10 clinical studies involving vortioxetine, and the incidence of treatment-emergent sexual dysfunction in patients treated with vortioxetine ranged from 1.6 to 2.6% versus 4.5% with duloxetine (Baldwin et al., 2013). Using the Arizona Sexual Experience Scale (ASEX) to assess the incidence of TESD, there was no clear dose–response relationship during treatment with vortioxetine over the therapeutic dose range (5 mg to 20 mg). The overall incidence of TESD during treatment with vortioxetine was higher than placebo (20%) for vortioxetine 15 and 20 mg (33 and 34%) for women and higher than placebo (14%) for vortioxetine 20 mg/day (29%) for men (FDA, 2013). The underlying mechanism for the relatively low incidence of sexual dysfunction remains to be studied in more detail. Generally speaking, an increased 5-HT level (e.g., after SSRI treatment) will inhibit sexual function (Olivier et al., 2011). Preclinical studies indicate that 5-HT_{1A} receptor agonists under conditions of elevated 5-HT levels facilitate sexual performance in male rats, whereas the role in female rats under similar conditions remains to be studied (Snoeren et al., 2014a, 2014b). Clinically this mechanism may potentially limit sexual dysfunction. Preclinical research indicates that other 5-HT receptor subtypes may be involved in regulation of sexual function; however these mechanisms are less studied (Olivier et al., 2011). The biological substrate underlying vortioxetine's relatively limited interference with sexual function remains to be studied in further detail.

3.4.2. Sleep disruption

In clinical trials with vortioxetine the incidence of sleep-related TEAEs (insomnia, initial insomnia, middle insomnia, hyposomnia, sleep disorder, dyssomnia, poor quality sleep, terminal insomnia) was low and not dose related, with an incidence of 2.0 to 5.1% compared

to 4.4% with placebo (Baldwin et al., 2013). This differs from SSRIs and SNRIs, which have an incidence of sleep-related TEAEs significantly higher than placebo (Brecht et al., 2007; Stein and Lopez, 2011). Serotonin is a key regulator of the sleep/wake cycle, with the serotonergic system being active during awake time and inactive during sleep (Adrien, 2002; Datta and Maclean, 2007). Since preclinical studies indicate that vortioxetine increases brain 5-HT levels to a greater extent than SERT inhibitors, vortioxetine's placebo-like level of sleep-related TEAEs is intriguing and remains to be investigated.

Vortioxetine is active at 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 and 5-HT_7 receptors, all of which are involved in sleep stage regulation (Adrien, 2002; Monti and Jantos, 2008; Hedlund, 2009). Thus, 5-HT_{1A} receptor stimulation promotes wakefulness and 5-HT_{1A} and 5-HT_{1B} receptor stimulation and 5-HT_7 receptor antagonism reduce rapid eye movement (REM) sleep (Adrien, 2002; Bonaventure et al., 2007, 2012). In line with this, a recent polysomnographic study of vortioxetine in healthy subjects showed REM-suppressing properties (Wilson et al., 2013). In rats, stimulation of 5-HT_3 receptors in the dorsal raphe nucleus has been reported to reduce REM sleep (Monti and Jantos, 2008). It remains to be studied whether a 5-HT_3 receptor antagonist could have the opposite effect on REM sleep. Interestingly, vortioxetine at a given SERT occupancy seemed to affect REM sleep to a lesser degree than paroxetine in healthy subjects, which may support the hypothesis that 5-HT_3 receptor antagonism contributes to its overall effect on sleep stages (Wilson et al., 2013). A study in rats indicated that a 5-HT_7 receptor antagonist can counteract SSRI-induced micro-arousals (i.e., non-REM or REM sleep interrupted by a 10-s epoch of wakefulness), which is thought to mimic clinically observed SSRI-induced sleep fragmentation (Bonaventure et al., 2007). Hence, it may be hypothesized that vortioxetine's 5-HT_7 receptor antagonism could have a beneficial effect against sleep fragmentation. In conclusion, 5-HT and several 5-HT receptors have a prominent and complex role in sleep/wake regulation. Understanding the impact of vortioxetine's 5-HT_3 and 5-HT_7 receptor antagonistic properties on its benign effect on sleep is particularly interesting for future studies.

3.4.3. Discontinuation symptoms

Discontinuation symptoms were assessed in three 8-week placebo-controlled studies in MDD (Mahableshwarkar et al., 2013c; Jacobsen et al., 2013; Boulenger et al., 2013). All three studies used the Discontinuation Emergent Signs and Symptoms (DESS) checklist (Rosenbaum et al., 1998). The mean DESS total score was 1.55 and 1.58 for vortioxetine (10–20 mg/day) and 0.96 and 1.19 for placebo in the first and second week following abrupt discontinuation (Baldwin et al., 2013). The mean DESS total score for duloxetine was 1.33 when duloxetine was down-tapered from 60 mg/day to 30 mg/day and 2.85 when it was abruptly discontinued (Baldwin et al., 2013). The DESS scores were low and similar in nature to the TEAEs and do not indicate a dependence liability. Vortioxetine's low level of discontinuation symptoms is likely to be at least partly related to its relatively long elimination half-life (57 h; Table 3). Comparisons across SSRIs suggest that a short elimination half-life increases the incidence of discontinuation symptoms (Rosenbaum et al., 1998).

3.4.4. Long-term safety

In an open-label long-term safety study involving 535 MDD patients followed for 52 weeks, it was found that vortioxetine (2.5, 5, and 10 mg/day) met general safety requirements, as evaluated by vital signs, weight, ECG parameters, and clinical laboratory results (Baldwin et al., 2012a). The mean weight gain was 1.1 kg from the start of the lead-in study and no patients withdrew from the study due to weight increase. The long-term adverse event profile of vortioxetine was similar to that observed during acute 8-week treatment and no new safety findings were noted in long-term use. It was noted that sexual dysfunction associated with long-term treatment of vortioxetine was low overall; only 6 patients (1.1%) reported sexual dysfunction-related TEAEs and none withdrew due to this TEAE. This long-term safety study for vortioxetine was as large as that for escitalopram (n = 590) (Wade et al., 2006) and larger than that for duloxetine (n = 177) (Dunner et al., 2008). The completion rate of 61% with vortioxetine was lower than with escitalopram (74%) but similar to the rate with duloxetine (58%). The rate of withdrawal due to TEAEs in this study (7.9%) was comparable or lower than with escitalopram (8.8%) or duloxetine (11.9%). The results were similar to those from a second large open-label extension study, with 834 patients (Alam et al., 2014). The limitation of both studies was the lack of placebo control.

3.4.5. Tolerability and safety — conclusions

Overall, vortioxetine was well tolerated in short-term as well as long-term clinical studies, with a low level of discontinuation symptoms likely due to its relatively long half-life. The incidence of withdrawals related to adverse effects was close to placebo level. The adverse event with the highest incidence was nausea, which was usually mild to moderate and caused few patients to withdraw from treatment. It may be hypothesized that vortioxetine's potent 5-HT_3 receptor antagonism suppresses the gastrointestinal adverse effects produced by the general increase of 5-HT tone and the direct stimulation of 5-HT_{1A} receptors – at least to some extent. Rates of sexual dysfunction, a typical adverse effect caused by increased 5-HT, were low with vortioxetine in both short-term and long-term studies, possibly related to its 5-HT_{1A} receptor agonistic properties. The occurrence of sleep disruption was low in vortioxetine-treated patients; understanding how 5-HT_3 and 5-HT_7 receptor antagonism contribute to this would be particularly interesting.

4. Overall conclusions

The antidepressant vortioxetine has a novel mechanism of action that combines direct 5-HT receptor modulation and SERT inhibition. The result of this target profile is that vortioxetine interrupts one or more negative feedback mechanisms that control neuronal activity in key areas of the brain involved in major depression, in particular the dorsal and median raphe nuclei and the prefrontal cortex (Lanzenberger et al., 2012; Hahn et al., 2014). This leads to a distinct preclinical profile compared to currently used SSRIs and SNRIs. In particular, enhanced synaptic plasticity and cognitive function may be ascribed to vortioxetine's modulation of one or more 5-HT receptors. The blockade of ionotropic 5-HT_3 receptors appears to play a prominent role in its mechanism of action. In a comprehensive short- and long-term clinical program in MDD patients, vortioxetine showed convincing and dose-dependent efficacy and was generally well tolerated. The comparatively low incidence of sexual dysfunction and sleep disruption may be ascribed to vortioxetine's receptor modulation. In line with the cognition-enhancing profile in preclinical studies, vortioxetine was superior to placebo on various cognitive function measures in MDD patients. The efficacy of vortioxetine in MDD patients with an inadequate response to SSRI or SNRI monotherapy also potentially supports a different mechanism of action. In conclusion, the multimodal mechanism of action of vortioxetine indicates a differentiated antidepressant profile compared to currently used antidepressants. Its future use in clinical practice will likely substantiate these differences and perhaps reveal other novel attributes of the compound.

Conflict of interest

FA is the Primary Investigator of a grant from Lundbeck on the mechanism of action of vortioxetine. He also declares having received lecture and consultancy fees from Lundbeck. CS is a full time employee at H Lundbeck A/S. KEA is a full time employee of Takeda Development Center of the Americas.

Role of the Funding Source

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