Vilazodone: A 5-HT_{1A} Receptor Agonist/Serotonin Transporter Inhibitor for the Treatment of Affective Disorders

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Vilazodone (EMD 68843; 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride) is a combined serotonin specific reuptake inhibitor (SSRI) and 5-HT_{1A} receptor partial agonist currently under clinical evaluation for the treatment of major depression. This molecule was designed based on the premise that negative feedback circuitry, mediated via 5-HT₁ receptors, limits the acute SSRI-induced enhancements in serotonergic neurotransmission. If the hypothesis is correct, combination of SSRI with 5-HT_{1A} partial agonism should temporally enhance the neuroplastic adaptation and subsequently hasten therapeutic efficacy compared to current treatments. Preclinical *in vitro* evaluation has confirmed vilazodone's primary pharmacological profile both in clonal and native systems, that is, serotonin reuptake blockade and 5-HT_{1A} partial agonism. However, *in vivo* and in contrast to combination of 8-OH-DPAT and paroxetine, vilazodone selectively enhanced serotonergic output in the prefrontal cortex of rats. Behavioral evaluations, in the ultrasonic vocalization model of anxiety in rats, demonstrated anxiolytic efficacy. In the forced swim test (a putative model of depression), vilazodone also showed efficacy but at a single dose only. In man, vilazodone abolished REM sleep and demonstrated clinical antidepressant efficacy equivalent to an SSRI. Ongoing clinical evaluations will hopefully reveal whether the founding hypothesis was valid and if vilazodone will produce a more rapid onset of antidepressant efficacy.

Introduction

Since the initial observations, which linked 5-hydroxytryptamine (5-HT or serotonin) to psychiatric illness [1], evidence for a role of 5-HT in the pathology of a plethora of related disorders has grown. These include schizophrenia [2], obsessive-compulsive disorder [3,4], panic, and a number of related anxiety disorders [5,6] such as general anxiety (GAD) and social anxiety (SAD). However, it is the role of 5-HT in the pathogenesis of depression and the mechanism of action of antidepressants (AD) that has received the most attention. Although, reserpine-induced neurotransmitter depletion studies [7] first alluded to the role of 5-HT in the induction of depression, it was Coppen et al. [8,9] who first proposed that depressive illness can arise specifically from a decreased brain 5-HT function. However, the depletion

studies of Shopsin and Delgado et al. [10,11] caused an acceleration of our understanding of depression and of the interrelationship between this neurotransmitter and the disorder. Inhibition of tryptophan hydroxylase, by *p*-chlorophenylalanine (PCPA), produced a rapid return to a depressive state in patients who had previously responded to tricyclic ADs. Cessation of the inhibitor resulted in a rapid return to the nondepressed state [10]. Similarly, using a tryptophan-free diet in depressed patients resulted in a relapse to the depressive state for patients who had previously responded to serotonin specific reuptake inhibitor (SSRI) treatment. Again, this was rapidly reversed upon reintroduction of tryptophan to the diet [11]. Although these studies demonstrated the key role of 5-HT in the pathophysiology of the disease, perhaps the strongest support to the serotonin hypothesis of depression has been the development

of the SSRIs. The SSRI family is a chemically diverse class of compounds that all have serotonin transporter (SERT) blockade as a common characteristic and thus result in increases in extracellular levels of 5-HT. As a consequence, enhanced serotonergic neurotransmission is proposed as the unifying mechanism of action of modern day ADs such as monoamine oxidase inhibitors (MAOI), tricyclic ADs, serotonin, and noradrenaline reuptake inhibitors (SNRI) and, of course, the SSRIs [12].

Interestingly, long periods of treatment are required with all these types of drugs before any therapeutic efficacy is observed [13,14] even though compounds such as SSRIs elicit their reuptake blockade immediately. Svensson [15] was the first to demonstrate that this was due to negative feedback circuitry limiting the acute enhancement of serotonergic neurotransmission, an effect that was reduced following a period of chronic treatment. Subsequently, other techniques, such as *in vitro* autoradiographic [³⁵S]GTPγS binding, *in vivo* electrophysiological, neurochemical, and neuroendocrine studies, have suggested that prolonged treatment with SSRIs results in desensitisation of 5-HT_{1A} autoreceptors [16–20]. Furthermore, chronic SSRI treatment has also been demonstrated to result in desensitisation of terminal 5-HT_{1B} autoreceptors both *in vitro* and *in vivo* [21,22] suggesting that plasticity in both the 5-HT_{1A} and 5-HT_{1B} mediated autoregulatory function may be important for the clinical efficacy of therapeutics such as SSRIs.

Taken together these observations have led to an improved understanding of the AD treatment-mediated neuroplastic changes in autoregulatory control of the serotonergic system. Assuming that an effective AD exerts its therapeutic activity as a direct consequence of its ability to increase serotonergic function, any mechanism that can do this should have clinical utility in the treatment of depression. This premise, together with our understanding of the underlying neurobiology, has led to a number of theories as to how the temporal onset and overall efficacy of current ADs can be enhanced. In this regard, much focus has gone into polypharmacy approaches, which combine blockade of the primary autoreceptors and the SERT (for review see [23] and references therein). These approaches acutely prevent the need for the temporal plasticity in the autoinhibitory processes and acutely augment 5-HT output. This has been demonstrated extensively in preclinical species (for review see [24]) and interestingly also supported by combination studies with pindolol in patients [25]. Perhaps a more novel approach has been to combine 5-HT_{1A} receptor agonism with SERT inhibition in an effort to hasten AD efficacy. SSRIs produce a desensitization of presynaptic 5-HT_{1A} autoreceptors, presumably via prolonged exposure to the SSRI-induced elevations in endogenous 5-

HT [17–19,26]. Thus, the rationale behind this combination approach is presumably to produce a more rapid and specific desensitization of the somatodendritic 5-HT_{1A} autoreceptors. This approach will also directly stimulate postsynaptic 5-HT_{1A} heteroreceptors, which could conceivably also be involved in the 5-HT mediated AD efficacy. In this regard, 5-HT_{1A} receptor agonists are efficacious in preclinical models thought to be predictive of AD activity, for example, forced swim test (FST; [27]) and may exert their effect indirectly via regulation of neurogenic processes [28,29] or altered expression of associated receptor subtypes, for example, 5-HT₂ [30,31]. Interestingly, early clinical studies with “immediate release” formulations of the selective 5-HT_{1A} receptor partial agonists buspirone, gepirone, and ipsapirone demonstrated limited efficacy and poor tolerability in depression and anxiety. Studies with “extended release (ER)” formulations have shown some subsequent improvement in treating the core symptoms of depression (see review [32]). However, dose-limiting side effects, such as dizziness, light-headedness, nausea, and vomiting [33], can be an issue with direct agonism of 5-HT_{1A} receptors. In contrast, 5-HT_{1A} receptor agonism may help to resolve some of the sexual dysfunction side effects associated with SSRI treatment [34,35]. However, a number of studies have demonstrated an adjunctive efficacy of buspirone when added to a variety of SSRIs, particularly in refractory patient populations (for review see [36,37]).

A number of molecules have been developed with the combined 5-HT_{1A} receptor agonist SERT blockade strategy in mind (for review see [23]). However, to these authors’ knowledge, the molecule that has progressed into the target clinical population, hence the focus of this review, is vilazodone.

Vilazodone

Vilazodone (EMD 68843; SB-659746A; 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride) is a dual 5-HT_{1A} receptor partial agonist and 5-HT reuptake inhibitor. Its synthesis was reported to have resulted from the combination of indole-butyl-amine and chromenonyl-piperazine structural elements into a single molecular entity [38]. Vilazodone (Fig. 1) was identified by modification of a series of indolealkyl-phenylpiperazines 5-HT_{1A} receptor agonists. Here it was found that, as with all azapirones, a four-carbon-saturated linker between the indole and piperazine rings, produced the optimal configuration for high 5-HT_{1A} receptor affinity. Introduction of electron-withdrawing groups in position 5 on the indole increased SERT affinity, with 5-fluoro and 5-cyano substituted indoles affording comparable

Figure 1 Chemical structure of vilazodone and its primary metabolite.

results. Vilazodone was synthesized by coupling ethyl 5-(piperazino)benzofuran-2-carboxylate and 3-(4-chlorobutyl)-1H-indole-5-carbonitrile. Several patents and publications describe the chemical synthesis, production of intermediates, and ultimately the identification of vilazodone and its active metabolites [38–40].

The identification of vilazodone's metabolites have been reported [41]. Using *in vitro* hepatocyte and microsomal preparations from rat, dog, monkey, and human liver microsomes, an unusual 6-hydroxy-5-cyano-indole compound was identified and demonstrated to be excreted as a glucuronidated salt form. The synthesis of the hydroxylated metabolite (Fig. 1) was reported by Heinrich and Böttcher [40] who also reported a reduction in the dual activity of this molecule (although no data were presented). Unfortunately, to these authors knowledge, no further information is available on the hydroxylated metabolite or any other minor metabolites, so it is diffi-

Table 2 Differences in [^3H]8-OH-DPAT and [^3H]WAY100635 derived binding affinities for 5-HT and vilazodone

	Human recombinant	Rat	Guinea pig	Mouse	Marmoset
5-HT	1.9	1.7	2.3	1.4	2.6
Vilazodone	2.3	1.7	2.0	1.6	1.8

cult to speculate on the likely contribution of the metabolites to vilazodone's *in vivo* and subsequent clinical profiles.

In Vitro Pharmacological Profile

The receptor binding profile of vilazodone was reported by Heinrich et al. [38]. Here vilazodone demonstrated an IC_{50} of 0.2 nM at the human 5-HT $_{1A}$ receptor and 0.5 nM for the SERT. Its closest cross affinity in these studies was to the dopamine D $_3$ receptor (IC_{50} of 71 nM) followed by the 5-HT $_4$ receptor (IC_{50} of 252 nM). Our own in house radioligand binding studies using the 5-HT $_{1A}$ receptor agonist [^3H]8-OH-DPAT have demonstrated that vilazodone displayed high affinity ($\text{pK}_i \geq 9.3$) for human recombinant and rat, guinea pig, mouse, and marmoset native tissue 5-HT $_{1A}$ receptors [42,43] (unpublished data in Table 1). In contrast, vilazodone displaced the antagonist radioligand, [^3H]WAY100635, binding (in the presence of Gpp(NH)p) with pK_i values up to 2 log units lower than those obtained using [^3H]8-OH-DPAT (Table 2). These data suggest that vilazodone preferentially binds to the high agonist affinity state of human 5-HT $_{1A}$ receptors, indicative of this molecule's partial agonist activity. It has been reported that the difference in affinity of a compound for 5-HT $_{1A}$ receptors, as measured using [^3H]8-OH-DPAT versus [^3H]WAY100635, is directly proportional to its intrinsic agonist activity [44]. Thus, given that the difference in affinity, as measured against [^3H]8-OH-DPAT cf. [^3H]WAY100635, was similar to that observed with the endogenous agonist 5-HT, these data suggest that vilazodone would act as a high efficacy partial agonist at 5-HT $_{1A}$ receptors. This hypothesis was supported in [^{35}S]GTP γ S binding studies

Table 1 Binding affinity (pK_i) of vilazodone for 5-HT $_{1A}$ receptors against [^3H]8-OH-DPAT (DPAT) and [^3H]WAY100635 (WAY)

Human recombinant		Rat		Guinea pig		Mouse		Marmoset	
DPAT	WAY	DPAT	WAY	DPAT	WAY	DPAT	WAY	DPAT	WAY
9.7 $\pm$ 0.2	7.4 $\pm$ 0.1	9.8 $\pm$ 0.1	8.1 $\pm$ 0.2	9.4 $\pm$ 0.3	7.4 $\pm$ 0.1	9.6 $\pm$ 0.1	8.0 $\pm$ 0.2	9.3 $\pm$ 0.3	7.5 $\pm$ 0.1

Data represent mean $\pm$ SEM in brackets of at least $n = 3$ experiments. Methodology as described by Watson et al. [74].

Figure 2 Effect of vilazodone on [35 S]GTP γ S binding to HEK cells expressing h5-HT $_{1A}$ receptors. Data represent mean $\pm$ SEM from 3 independent experiments, performed in duplicate. Methodology as described by Watson et al. [46].

in Sf9 cells expressing h5-HT $_{1A}$ receptors, whereby a single concentration of vilazodone (100nM) increased basal binding by approximately 70% of that produced by the full 5-HT $_{1A}$ receptor agonist, 8-OH-PIPAT [45]. However, given that only single concentrations were used in this study, accurate determination of intrinsic activity or functional potency at h5-HT $_{1A}$ receptors could not be achieved. More extensive studies in HEK cells expressing h5-HT $_{1A}$ receptors have since been performed (unpublished data). In these studies, vilazodone acted as a full agonist, as compared to 5-HT, with a pEC $_{50}$ of 9.0 (Fig. 2). In comparison, the 5-HT $_{1A/B/D}$ receptor partial agonist SB-272183 ((5-Chloro-2, 3-dihydro-6-[4-methylpiperazin-1-yl]-1[4-pyridin-4-yl]naphth-1-ylaminocarbonyl)-1H-indole), which has been reported to act as a 5-HT $_{1A}$ receptor antagonist at rodent and human native tissue 5-HT $_{1A}$ receptors [46], displayed an intrinsic activity of approximately 0.3 in the same study (Fig. 2). Interestingly, in [35 S]GTP γ S binding studies in rat hippocampal membranes (a functional preparation in which 5-HT $_{1A}$ receptors predominate; [47]), vilazodone acted as a potent 5-HT $_{1A}$ receptor partial agonist with a pEC $_{50}$ of 8.1 and an intrinsic activity of 0.61 [48]. In comparison, ($\pm$) 8-OH-DPAT (pEC $_{50}$ 7.2) produced a partial agonist response with an intrinsic activity of 0.45 and the partial agonist buspirone showed a pEC $_{50}$ = 6.5 ± 0.35 and an intrinsic activity of 0.19. Taken together, these data suggest that vilazodone would be a high efficacy 5-HT $_{1A}$ receptor partial agonist, *in vivo*. The apparent difference in intrinsic activities between recombinant and native tissue systems may be a consequence of varying degrees of receptor reserve. Indeed, receptor reserve has been reported to be present for native tissue somatodendritic 5-HT $_{1A}$ autoreceptors *in vivo* [49] but not native postsynaptic 5-HT $_{1A}$ receptors *in vitro* [50,51],

Figure 3 Inhibition of [3 H]5-HT uptake into LLC PK cells expressing human recombinant SERT by vilazodone. Methodology as described by Scott et al. [84].

which would explain why vilazodone demonstrated partial agonist properties in the hippocampus. Therefore, one possible conclusion is that vilazodone is a 5-HT $_{1A}$ receptor partial agonist with the potential to act as a full agonist in systems with high receptor reserve and/or improved receptor–G protein coupling efficiency. This concept is supported by the observation that vilazodone appeared to have much higher efficacy at pre- versus postsynaptic 5-HT $_{1A}$ receptors in some *in vivo* models [43] but is perhaps contradicted by some of the neurochemical observations ([48]; see below).

Combinations of biochemical and electrophysiological studies have demonstrated that vilazodone acts as a potent 5-HT reuptake inhibitor in rat and guinea pig cortex [42,52]. We have confirmed this high potency in LLC PK cells expressing human SERT, whereby vilazodone inhibited [3 H]5-HT uptake with a pIC $_{50}$ of 8.8 ± 0.05 (Fig. 3). This potency value is approximately 1 log unit greater than that for fluoxetine but similar to that for paroxetine (unpublished observations). These data, taken together with the observation that vilazodone is a potent 5-HT $_{1A}$ receptor partial agonist, puts this molecule in a novel class of compound that has the potential to rapidly desensitize 5-HT $_{1A}$ autoreceptors both directly, via 5-HT $_{1A}$ receptor agonism, and indirectly, via elevations in endogenous 5-HT through SERT inhibition.

In Vivo Pharmacological Profile

Neurochemical Effects of Vilazodone

The effects of vilazodone have been evaluated in two separate *in vivo* microdialysis studies [45,48]. Page et al. [45] demonstrated that vilazodone produced increases in extracellular levels of 5-HT in both the frontal cortex (FC) and ventral hippocampus (vHipp) of rats. Maximum

increases were observed at 3 mg/kg (ip) and reached 527% and 558% of preinjection baseline values in the FC and vHipp, respectively. Interestingly, the SSRI and fluoxetine in the same assay produced maximum increases of only 165% and 273% of preinjection baseline, respectively. Subsequent administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (1 mg/kg ip), at a suitable time point (i.e., once maximal increases had been attained) resulted in only a partial and transient decrease in the vilazodone-induced increase in extracellular 5-HT in both brain regions. Whilst the same challenge to the fluoxetine-treated subjects produced a similar decrease to that seen with vilazodone in the vHipp, a full reversal back to baseline was observed in the FC. The authors suggested that the relatively large acute increase in 5-HT induced by vilazodone was likely due to the combined inhibition of both the SERT and partial agonist/"interference" at the level of 5-HT_{1A} autoreceptor, as confirmed by the partial effect of 8-OH-DPAT in reversing this increase. A similar conclusion was reached by Hughes et al. [48]. A dose related acute increase in extracellular 5-HT was also observed in the FC of rats reaching a maximum of 230% of preinjection baseline at 10 mg/kg (po) of vilazodone. These doses were selected according to the relative *ex vivo* SERT occupancy, as measured using the tritiated form of the [¹¹C] PET radioligand [53,54], that is, [³H]DASB. These data revealed that vilazodone (1–10 mg/kg po) occupied rat hippocampal and cortical SERT in a dose-dependent manner [48]. In addition, these studies concurrently compared the findings with vilazodone to those induced by paroxetine when coadministered with either WAY100635 or 8-OH-DPAT. In this case, the vilazodone-induced effects on extracellular 5-HT were similar to those induced by the WAY100635/paroxetine combination. Furthermore, it was shown that 8-OH-DPAT ± paroxetine produced marked increases in FC dopamine and noradrenaline (a known pre- and postsynaptic 5-HT_{1A} receptor agonist-induced effect, respectively; [55–57]) while vilazodone was without effect on either transmitter. Together, these data suggested that *in vivo* vilazodone showed no evidence of pre- or postsynaptic 5-HT_{1A} receptor activation but may, as suggested by Page et al. [45], be actually blocking 5-HT_{1A} mediated effects. In support of this, Roberts et al. [52] also demonstrated that vilazodone behaved as an SSRI in the guinea pig dorsal raphe, using voltametric measures of 5-HT, but displayed no evidence of direct agonism at 5-HT_{1A} receptors. These neurochemical findings are somewhat surprising, in light of vilazodone's *in vitro* profile (discussed above) that suggested a relatively high intrinsic activity at both human cloned and rat native 5-HT_{1A} receptors. Thus it would appear that vilazodone's *in vivo* neurochemical profile is somewhat different to that which may have

been predicted from its primary *in vitro* pharmacological profile.

Pharmacodynamic Effects of Vilazodone

Two studies have examined the pharmacodynamic effects of vilazodone, specifically 5-HT_{1A} receptor mediated behavioral/physiological changes. Administration of 5-HT_{1A} receptor agonists produce a characteristic behavioral syndrome that includes changes in posture, hind limb reduction, head weaving, tremors, forepaw treading, and straub tail. Using these measures, Page et al. [45] examined the effects of vilazodone, at doses that were shown to enhance cortical 5-HT output, and compared them to 8-OH-DPAT. Vilazodone (3 mg/kg ip) produced none of these characteristic 5-HT syndrome behaviors, whilst 8-OH-DPAT produced all of the symptoms. Interestingly, pretreatment with vilazodone dose-dependent manner blocked the 8-OH-DPAT-evoked behaviors, whilst in contrast, pretreatment with fluoxetine did not alter the 8-OH-DPAT-induced syndrome.

5-HT_{1A} receptor agonists have been shown to decrease body temperature in the rat and this has been reported to be a postsynaptic receptor mediated event [58–60]. Using this measure, Bartoszyk et al. [43] examined the effects of vilazodone and compared to those of fluoxetine, WAY100635, and 8-OH-DPAT. Neither fluoxetine (100 mg/kg po), WAY100635 (1 mg/kg sc), nor vilazodone (55 mg/kg po) produced any change in rat body temperature. In contrast, 8-OH-DPAT (0.55 mg/kg sc) produced a significant 2–3°C decrease; an effect that was blocked by WAY100635 but not by vilazodone.

Taken together both studies suggest that vilazodone is not behaving as a classic 5-HT_{1A} receptor agonist in these models. Bartoszyk et al. [43] has suggested that vilazodone can behave as preferential presynaptic 5-HT_{1A} receptor agonist and this may, at least in part, provide reasoning for these conflicting datasets. However, the observations of Hughes et al. [48] contradict this hypothesis since there were no decreases in 5-HT output or concurrent increases in extracellular dopamine levels in their studies, both of which are reputed to be presynaptic 5-HT_{1A} receptor-mediated events [56,61].

Efficacy in Models of Anxiety

Three separate studies have evaluated the efficacy of vilazodone in various rodent models thought to be predictive of anxiolytic activity. In the rat ultrasonic vocalizations test, stress-induced vocalizations were inhibited by vilazodone (55 mg/kg po) at 120 and 210 min post dose. In comparison, 8-OH-DPAT (0.55 mg/kg sc) produced a similar, but shorter duration, activity that was

reversed by WAY 100635. In contrast, the SSRI fluoxetine (100 mg/kg po) was without effect unless combined with 8-OH-DPAT. Together these data suggest that the vilazodone-mediated efficacy in this model was via its 5-HT_{1A} receptor agonist activity. In two further rat models of anxiety, that is, the elevated plus maze and the shock-probe burying tests [62–64], vilazodone demonstrated dose related efficacy (10–40 mg/kg ip) in the shock probe test but, interestingly, was without effect in the elevated plus maze. The positive control diazepam produced efficacy in both paradigms [65]. Finally, vilazodone was examined in a predator-induced stress paradigm, brought about by unprotected exposure to a domestic cat. Predator stress increased anxiety-like behaviors in the elevated plus maze and an increased response to an acoustic startle [66]. Vilazodone (20–40 mg/kg ip), administered acutely or prophylactically (1 week prior to behavioral testing), attenuated stress-induced potentiated startle but had no effect on stress potentiated anxiety response in the elevated plus maze. Interestingly, a lower dose of 10 mg/kg of vilazodone had the opposite effect in the startle response, indicating a somewhat unexplained bidirectional effect, and all doses produced a potentiation of the startle-induced stress response possibly suggestive of an anxiogenic-like response. Finally, the data from these studies also showed that vilazodone had neither sedation nor stimulatory effects but did produce context-dependent efficacy.

Efficacy in Models of Antidepressant Activity

The FST is widely used to assess the potential of molecules to exhibit an antidepressant profile [67,68] since all major classes of antidepressant drugs [69], including tricyclic antidepressants (TCA), SNRI and SSRI, monoamine oxidase inhibitors, and atypical ADs [70–74], are effective in this stress based model. Page et al. [45] demonstrated that vilazodone produced efficacy (i.e., a reduction in immobility time) in both the rat and mouse versions of this model at a single dose (1 mg/kg ip), whilst higher doses (3 and 10 mg/kg) were without effect. The magnitude of this efficacy was approximately similar to that of fluoxetine [45]. Efficacy in this model is generally considered to be mediated by 5-HT [73] and blocked by receptor antagonists and genetic deletions of 5-HT_{1A} receptors [75,76]. Thus vilazodone-mediated efficacy may be via direct 5-HT_{1A} receptor agonism or alternatively indirect through elevations in endogenous 5-HT.

Perhaps the major driving force behind this mechanistic combination of these two activities is a hastened desensitization of 5-HT_{1A} receptors leading to an enhanced onset of therapeutic activity. Therefore some evaluation of the onset of preclinical efficacy in models thought

to mimic this, such as rodent social interaction [77] or schedule induced polydipsia [78], would be useful but unfortunately has not been reported to date.

Preclinical Perspective

The *in vitro* profile of vilazodone, both from our own in house studies and literature data, supports this molecule's activity at both the 5-HT_{1A} receptor and SERT in both clonal and native tissue preparations. It is also clear that *in vitro* vilazodone behaves as a relatively high intrinsic activity partial agonist with potency and agonist activity equal to/or greater than that of the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT. However, it is in its *in vivo* profile that there appears to be some interesting differential effects. Its neurochemical profile, as highlighted by two separate studies described previously [45,48], demonstrated an enhancement of 5-HT output in rodents. In contrast, 8-OH-DPAT + SERT blockade produced no such elevation in terminal 5-HT, but enhanced dopamine and noradrenaline output; again an effect that was not observed with vilazodone. Previous studies examining the effects of 5-HT_{1A} receptor antagonists versus partial agonists demonstrated that even molecules with lower levels of intrinsic activity than vilazodone, for example, buspirone (reported here to have pEC₅₀ of 6.5 and an intrinsic activity of 0.3), are unable to augment the SSRI induced effects [79]. Furthermore, vilazodone's acute neurochemical profile is in contrast to the observations with a further 5-HT_{1A} receptor agonist/SERT inhibitor, VN2222, which acutely reduced 5-HT output in the rat cortex and suppressed the firing rate of dorsal raphe neurons upon systemic administration [80]. Unfortunately, no dopamine or noradrenaline data were presented for this compound and we also do not have the equivalent native tissue intrinsic activity data for comparison. Behaviorally, vilazodone also appeared to produce a mixture of anxiolytic and anxiogenic-like effects in the various anxiety models [66] and an inverted U-like dose relationship in the FST [45].

A possible explanation for these conflicting observations may lay in vilazodone's partial agonist properties. The degree of agonism versus antagonism exerted *in vivo* by a partial agonist depends, in part, on the concentration of endogenous agonist, in this case 5-HT. In the absence of 5-HT, a partial agonist would behave as an agonist, activating target receptors, whilst in the presence of a preferential agonist (i.e., 5-HT) partial agonism may become antagonism. In the case of vilazodone, the extracellular levels of 5-HT are generally going to be higher due to the SERT activity of this molecule. It is therefore feasible that the functional activity of vilazodone at 5-HT_{1A}

receptors will vary based on not only the tonic serotonergic output of a given projection system but also the number and degree to which vilazodone inhibits the terminal SERTs. This added complexity maybe contributing to the inconsistent dose-related effects of vilazodone in behavioral assays and may explain why vilazodone's neurochemical profile is more akin to an antagonist + SERT rather than an agonist. However, what is not clear is why vilazodone's *in vivo* profile is so different to that of 8-OH-DPAT + paroxetine despite similar *in vitro* affinity/efficacy at 5-HT_{1A} receptors. So there may be complexities to vilazodone's preclinical *in vivo* activity, which cannot be fully interpreted at present.

It is not clear what impact this may have on clinical dose selection/estimations and the dose-response in humans. Furthermore, if the pathophysiological output of 5-HT differs in the various anxiety conditions and depression the therapeutic utility of vilazodone may change. Given this molecule has entered the clinical arena (discussed below) it will be interesting to see if any of these hypothesizes are supported or disproved by the disease efficacy data.

Clinical Perspective

Vilazodone has undergone significant clinical evaluation to date. In a double-blind randomized placebo-controlled crossover study in 10 healthy male subjects, the effect of 20 mg vilazodone was assessed on sleep electroencephalography [81]. A reduction in rapid eye movement and changes in slow wave sleep and wakefulness confirmed central activation, and specific observations could be the consequence of vilazodone's SERT inhibition and 5-HT_{1A} receptor agonist properties, respectively. A PET study was performed using the antagonist radiotracer [¹¹C] WAY100635 in six healthy male subjects, in an attempt to estimate central 5-HT_{1A} receptor occupancy following a single dose of either 20 mg (n = 2) or 40 mg (n = 4) of vilazodone. These studies suggested that vilazodone preferentially occupied pre- versus postsynaptic 5-HT_{1A} receptors at the 40 mg dose, where a plasma exposure of >50 ng/mL was achieved, albeit in only two subjects [82]. Variable degrees of occupancy was observed at plasma exposures < 50 ng/mL. Preferential occupancy for 5-HT_{1A} autoreceptors could be due to several factors including, but not limited to, varying levels of high and low agonist affinity states in the two regions. Given that vilazodone is a partial agonist at native tissue 5-HT_{1A} receptors and, from the radioligand binding studies previously mentioned in this review, the compound does seem to show some discrimination between high and low agonist affinity states. As such, the preferential

occupancy of vilazodone for presynaptic 5-HT_{1A} autoreceptors may be the consequence of a more dense population of the high agonist affinity in the presynaptic cf. postsynaptic regions. Interestingly, the 5-HT_{1A} receptor partial agonist, pindolol, was also assessed in this study and showed preferential occupancy for 5-HT_{1A} autoreceptors at the 10 mg dose. The recent development of a 5-HT_{1A} receptor agonist PET ligand, [¹¹C]MPT [83], may allow for a more accurate prediction of receptor occupancy by compounds with 5-HT_{1A} receptor agonist properties.

Several phase II trials have been conducted by the compound's originator, Merck KGaA (Darmstadt, Germany), and GSK (Brentford, Germany), who licensed it in 2001. Specifically, vilazodone was administered to a total of 369 healthy subjects and 1163 depressed patients but failed to demonstrate significant efficacy against placebo. Interestingly, in the Phase II trials that included an active comparator, the active comparator also failed to demonstrate efficacy. Given that the latter trials did not result in satisfactory data to support progression to phase III, GSK withdrew from the alliance in 2003 (Scrip, 23rd April 2003). In 2004, Clinical Data (who acquired Genaissance Pharmaceuticals in 2005; Newton, MA, USA) obtained an exclusive license on the asset and have recently demonstrated statistical significance efficacy against placebo in a phase III trial in depression. The randomized, double-blind, placebo-controlled, 10-site trial, which consisted of 410 adult patients with major depressive disorder, achieved the primary endpoint of a significant change from baseline at 8 weeks in the Montgomery-Asberg depression rating scale total score compared to placebo ($P = 0.001$). In addition, key secondary endpoints were met, including a significant mean change from baseline on the Hamilton depression 17 rating scale ($P = 0.022$). Furthermore, potential candidate biomarkers to predict for therapeutic efficacy were identified a number of which are reported to be key genes in the 5-HT pathway. Vilazodone was well tolerated, with the most commonly observed adverse events being diarrhea, nausea, somnolence, and dizziness. A new drug application filing is targeted for 2009 (Bear Stearns Healthcare conference; Newton, MA, USA (Business Wire), September 2007). Since the premise behind the combination of these two activities was a hastened onset to therapeutic efficacy, we await with interest the release of further data to prove or disprove the founding hypothesis behind this polypharmacy approach.

Summary and Conclusion

Current antidepressant therapies are associated with a delay to therapeutic onset and treatment resistance in some

patients. The postulated reason for the delay in onset is the need for temporal neuroplastic changes in the control of serotonergic neurotransmission, and more specifically changes in 5-HT₁ autoreceptor function. Vilazodone is a combined 5-HT reuptake inhibitor and 5-HT_{1A} receptor partial agonist, which has been developed to presumably produce a more rapid and specific desensitization of the somatodendritic 5-HT_{1A} autoreceptors and also directly stimulate postsynaptic 5-HT_{1A} heteroreceptors, thus hastening onset and perhaps enhancing therapeutic efficacy. *In vitro* data have clearly demonstrated that vilazodone is indeed a high efficacy partial agonist at human and rodent 5-HT_{1A} receptors, and occupies and functionally blocks central SERT sites. There are, however, some discrepancies in the reported *in vivo* data as to whether vilazodone behaves as an agonist at pre- and/or postsynaptic 5-HT_{1A} receptors. That said, vilazodone appears to enhance forebrain serotonergic output and is efficacious in preclinical models of anxiety and depression. Unfortunately, no preclinical data on onset of anxiolytic activity are currently available.

Recent clinical observations have suggested some evidence of efficacy in depression. However, we await with interest further clinical information on vilazodone's efficacy and whether these mechanisms do actually culminate in an enhanced onset of therapeutic activity compared to current therapeutics.

Conflict of Interest

The authors declare no conflict of interest.

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