Neuropharmacology and analgesia

Electrophysiological evidence for rapid 5-HT$_{1A}$ autoreceptor inhibition by vilazodone, a 5-HT$_{1A}$ receptor partial agonist and 5-HT reuptake inhibitor

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ABSTRACT

This study examined the effect of vilazodone, a combined serotonin (5-HT) reuptake inhibitor and 5-HT$_{1A}$ receptor partial agonist, paroxetine and fluoxetine on the sensitivity of 5-HT$_{1A}$ autoreceptors of serotonergic dorsal raphe nucleus neurons in rats. These effects were assessed by determining the intravenous dose of ($\pm$)-8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) required to suppress the basal firing rate of these neurons by 50% (ID$_{50}$) in anesthetized rats using in vivo electrophysiology. 5-HT uptake inhibition was determined by the ability of the compounds to reverse ($\pm$)-p-chloroamphetamine (PCA)-induced rat hypothalamic 5-HT depletion ex vivo. Acute vilazodone administration (0.63 and 2.1 μmol/kg, s.c.), compared with vehicle, significantly increased (2–3-fold) the ID$_{50}$ of 8-OH-DPAT at 4 h, but not 24 h after administration. Subchronic administration (3 days) significantly increased the ID$_{50}$ value at 4 h (3–4-fold) and at 24 h ($\sim$2-fold). In contrast, paroxetine and fluoxetine at doses that were supramaximal for 5-HT uptake inhibition did not significantly alter the ID$_{50}$ value of 8-OH-DPAT after acute or subchronic administration. Vilazodone antagonized the action of PCA 3.5 h and 5 h after a single dose (ID$_{50}$ 1.49 and 0.46 μmol/kg, s.c., respectively), but was inactive 18 h post-administration, corroborating the electrophysiological results at 24 h following acute administration. The results are consistent with the concept of rapid and, following repeated treatment, prolonged inhibition of 5-HT$_{1A}$ autoreceptors by vilazodone. This effect could occur by either direct interaction with, or desensitization of, these receptors, an effect which cannot be ascribed to vilazodone's 5-HT reuptake inhibiting properties.

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1. Introduction

Vilazodone is a selective serotonin (5-HT) reuptake inhibitor (SSRI) and 5-HT$_{1A}$ receptor partial agonist with a novel chemical structure unrelated to conventional SSRIs (Bartoszyk et al., 1996; Heinrich et al., 2004). It exhibits anxiolytic-like actions in the ultrasonic vocalization test (Bartoszyk et al., 1997) and antidepressant-like effects in the forced swim test (Page et al., 2002), and has recently been approved for the treatment of major depressive disorder (Khan, 2009; Khan et al., 2011; Laughren et al., 2011; Reed et al., 2012; Rickels et al., 2009).

A significant body of evidence suggests that augmenting serotonergic transmission in the CNS, either by inhibition of 5-HT reuptake and/or by activating 5-HT$_{1A}$ receptors, may be effective approaches for the pharmacotherapy of depression and anxiety disorders (Heisler et al., 1998; Klemenhagen et al., 2006; Nutt, 2002). Although only one 5-HT$_{1A}$ agonist (buspirone) is approved for the treatment of anxiety, several compounds with 5-HT$_{1A}$ agonist/partial agonist activity have been shown to augment response to other antidepressants (Blier and Ward, 2003; Charney, 1998; Gammans et al., 1992; Parsey et al., 2006; Robinson et al., 1990; Rush et al., 2006).

Conversely, one widely-held theory states (for review, see Blier and Ward (2003)) that increase of 5-HT at terminal synapses

during acute SSRI-induced reuptake inhibition is immediately counteracted by neuronal negative feedback mechanisms mediated by 5-HT$_{1A}$ autoreceptors. Consequently, increased serotonergic transmission requires chronic SSRI treatment, which gradually desensitizes autoreceptors. The delay in the onset of the therapeutic effects of SSRIs, which occurs only after chronic treatment (e.g., 2–3 weeks), supports this view. The attenuation of 5-HT$_{1A}$ receptor-mediated feedback inhibition via compounds that decrease 5-HT$_{1A}$ receptor function is an approach that could potentially reduce the time to onset of antidepressant action by the SSRIs. Therefore, developing a molecule that inhibits the 5-HT transporter and attenuates the function of the 5-HT$_{1A}$ receptor offers a potential approach for decreasing the time of onset of antidepressant action. Vilazodone combines selective serotonin reuptake inhibition and 5-HT$_{1A}$ receptor partial agonism and may provide benefits in treating major depressive disorder.

In this study, in vivo extracellular electrophysiological recordings of spontaneously active 5-HT neurons in the dorsal raphe nucleus (DRN) in rats were used to evaluate the hypothesis that vilazodone produces a more rapid decrease in 5-HT$_{1A}$ autoreceptor sensitivity compared with conventional SSRIs (Page et al., 2002). The 5-HT$_{1A}$ receptor agonist ($\pm$)-8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) was used as a pharmacologic probe to assess changes in 5-HT$_{1A}$ autoreceptor sensitivity following administration of fluoxetine, paroxetine, or vilazodone either acutely or subchronically.

To establish whether 5-HT uptake inhibition influences the results in the electrophysiological studies, we determined the potency and duration of the action of vilazodone and conventional SSRIs in the ($\pm$)-p-chloro-amphetamine (PCA) model, a functional neurochemical ex vivo assay. PCA utilizes the 5-HT transporter to enter 5-HT neurons, causing a significant depletion of endogenous 5-HT (Berger et al., 1992). The systemic administration of SSRIs prior to PCA completely reverses 5-HT depletion and is therefore widely used to assess in vivo 5-HT reuptake inhibition (Fuller, 1980; Seyfried et al., 1989).

2. Material and methods

2.1. Materials

Vilazodone HCl was synthesized in the Medicinal Chemistry Department, Merck KGaA, Darmstadt. Fluoxetine HCl was a gift from Eli Lilly. Citalopram HBr was a gift from Lundbeck, and sertraline HCl and paroxetine HCl, 0.5H$_2$O were gifts from Pfizer and SmithKlineBeecham, respectively. 8-OH-DPAT HBr and R-(−)-fenfluramine were purchased from Research Biochemical (Natick, MA). PCA and chloral hydrate were obtained from Sigma-Aldrich (St Louis, MO). All other chemicals were at least of analytical grade, except the constituents of the mobile phase, which were of HPLC grade and were obtained from Merck KGaA.

2.2. Animals

For the electrophysiological experiments, male Sprague Dawley rats (Taconic Farms, Germantown, NY, 150–175 g upon arrival) were used, housed in the animal care facility at St. John's University, New York, which is an American Association for Laboratory Animal Care approved facility. For the neurochemical studies, male Wistar rats (IVA WiWu; 135–160 g, obtained from Ivanovas, Kisslegg, Germany) were used housed in the Department of CNS Research, Merck KGaA, Darmstadt, in accordance with the German guidelines for care and use of laboratory animals. Vivarium conditions were standardized as follows: temperature, 20–23 °C; relative humidity, 50–60%; 2–4 animals per cage; 12 h light/12 h dark cycle with lights turned on at 0600–0700 h; food

and water were available ad libitum. Rats were allowed at least 3–4 days acclimation in the vivarium prior to beginning of testing that occurred during the light cycle. Experimental protocols were approved by the Regierungspräsidium Darmstadt, Germany or by the St. John's University Institutional Animal Care and Use Committee (USA).

2.3. Electrophysiology: in vivo neuronal activity of 5-HT neurons in the DRN

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed on a heating pad (37 °C), and secured in a rat stereotaxic instrument. A lateral tail vein was catheterized by using a 26 gauge 3/8 in. needle. Additional doses of chloral hydrate were administered (50 mg/kg, i.v.) if the animals showed a nociceptive response to a pinching of the hind paw. The in vivo extracellular electrophysiological recording of dorsal raphe nucleus neurons was performed using micropipettes prepared as previously described (Aghajanian and Haigler, 1974). A small hole was drilled on the midline suture of the skull, 0.5–1.0 mm anterior to lambda. Slow stereotaxic descents were made (5.5–6.5 mm ventral to the skull surface), and a spontaneously active neuron was considered to be serotonergic if it met the following criteria: a slow, regular firing rate (0.5–2.5 Hz) and a positive action potential of 0.8–1.2 ms in duration. Animals were treated subcutaneously (1 ml/kg, s.c.) with 0.9% w/v of saline (vehicle), vilazodone (0.63 or 2.1 µmol/kg), paroxetine (13 µmol/kg), or fluoxetine (29 µmol/kg), administered once (acute group) or once daily for 3 days (subchronic group).

Electrophysiological assessments were made at 4 h and 24 h after a single dose (acute group) or 4 h and 24 h after 3 days of dosing (subchronic group). In these experiments, a single spontaneously active 5-HT neuron in the DRN was isolated from each animal and a stable baseline was recorded for at least 3 min prior to administering 8-OH-DPAT. Subsequently, 8-OH-DPAT (1 dose per min) was given via the tail vein (doses of 3.8, 7.6, 14.2, 28.4, 56.8 or 113.6 nmol/kg dissolved in 0.9% saline with each injection being 0.05 ml) until the firing of the neuron was completely suppressed. Only one neuron was recorded from each animal. The mean dose of 8-OH-DPAT required to produce a 50% reduction in the firing rate of dorsal raphe 5-HT neurons (50% inhibitory dose [ID$_{50}$]) in the different drug groups was determined by using the curve fitting program of Prism Graph Pad 3.1 (GraphPad Software, Inc, La Jolla, CA). A decrease in 5-HT$_{1A}$ autoreceptor sensitivity was measured as a significant increase in the ID$_{50}$ of 8-OH-DPAT in drug-treated (compared with vehicle-treated) animals. At the completion of an experiment, the anatomic location of the 5-HT neurons isolated was determined by passing a −20 µA current through the recording electrode for 10 min, which resulted in the deposition of a discrete spot of fast green. Animals were then overdosed with chloral hydrate and perfused transcardially with a 10% formalin solution (pH 7.0, phosphate-buffered saline). The brains were removed and serial coronal sections were cut at 50 µm intervals, stained with cresyl violet, and counterstained with neutral red. Sections were scanned for a dye spot using a light microscope.

2.4. Neurochemical ex vivo determination of 5-HT reuptake inhibition

PCA (5 mg/kg, i.p.) was dissolved in saline and injected in a volume of 5 ml/kg body mass 3 h before euthanasia by decapitation. Vilazodone was dissolved in 100% dimethylsulfoxide and diluted with 1,2-propanediol to give a 1:1 (v/v) dilution, and the SSRIs were dissolved in pure 1,2-propanediol. The experimental drugs were administered (2 ml/kg, s.c) 3.5, 5, 8, or 18 h before decapitation. The dosing regimens used (µmol/kg) were: vilazodone: 0.21, 0.63, 2.1, 6.3, 21, 63, and 210; fluoxetine: 0.87, 2.9, 8.7,

and 29; citalopram: 0.25, 0.74, 2.5, 7.4, 25, 74, and 250; sertraline: 0.29, 0.88, 2.9, 8.8, 29, 88, and 290; and paroxetine: 0.080, 0.27, 0.80, 2.7, 8.0, 27, and 80. Four doses of each drug were tested at each of the 4 time points. Thus, in the interaction experiments, rats were pre-treated 3.5–18 h with vilazodone or the reference SSRIs and co-treated with PCA 3 h before euthanasia. Vehicle controls received vehicle and saline at the appropriate times, and the PCA controls were pre-treated with the drug solvent and received PCA 3 h before decapitation. In separate experiments, it was confirmed that the experimental drugs alone did not significantly alter hypothalamic 5-HT levels (results not shown). A typical experiment comprised 25 rats, *i.e.*, 3 vehicle controls (basal hypothalamic 5-HT levels, mean: 900–1000 ng/g hypothalamus fresh mass), 6 PCA-alone controls (depleted stores, mean: 400–500 ng/g) and 4 rats per each of the 4 co-treatment groups (mean: depending on uptake inhibition in the range of 400–1000 ng/g). Treatments, euthanasia, tissue dissection and workup were carried out according to a minute-by-minute time scheme. The interaction study with R-(−)-fenfluramine as the releasing agent was carried out in the same way as the PCA study. Vilazodone was administered s.c. into the neck 5.5 h before and R-(−)-fenfluramine, 10 mg/kg s.c. into the flank, 5 h before decapitation. Hypothalamus, frontal cortex and striatum from each rat were analyzed in this study.

Brain areas were dissected as described by Glowinski and Iversen (Glowinski and Iversen, 1966). Tissue samples were dissected out on ice and immediately processed for high-performance liquid chromatography (HPLC) analysis. 5-HT concentration was determined by an automated reverse-phase/ion pair, direct-injection HPLC method (Seyfried et al., 1986) within a 25 min run and electrochemical detection. N-Methyldopamine or N-ω-methylserotonin were used as internal standards, and the recovery rates were > 95%.

Antagonism of PCA-induced 5-HT depletion was calculated in the following way: $(X_{drug+PCA} - X_{PCA}) \times 100/(X_{controls} - X_{PCA})$, X being the means of each drug group. ID_{50} values and confidence intervals were calculated by using a nonlinear fit to the Hill equation (Mathematica 8, Wolfram Research Inc., Champaign, IL).

2.5. Statistical analysis

For the electrophysiological experiments, data were analyzed by using a one-way analysis of variance (ANOVA), and *post hoc* tests were conducted by using the Student–Newman–Keuls test. For the neurochemical experiments, ANOVA was performed followed by Dunnett's test for comparison of individual dose groups to the appropriate controls.

3. Results

3.1. 8-OH-DPAT-induced inhibition of dorsal raphe nucleus 5-HT neuron activity

Statistical analyses indicated that there was a significant effect of drug administration on the response of serotonergic dorsal raphe neurons to i.v. 8-OH-DPAT 4 h after administration ($F_{4,45} = 17.44$, $P < 0.0001$, one-way ANOVA). Subsequent *post hoc* tests revealed that 0.63 or 2.1 µmol/kg s.c. of vilazodone significantly increased the ID_{50} value of i.v. 8-OH-DPAT (2–3-fold) compared with animals treated with vehicle (Student–Newman–Keuls values of 17.4 and 48.7). In contrast, neither fluoxetine (29 µmol/kg) nor paroxetine (13 µmol/kg) significantly altered the ID_{50} value of i.v. 8-OH-DPAT for serotonergic neurons in the DRN compared with vehicle-treated animals. Fig. 1A summarizes the effects of the acute administration of vehicle, fluoxetine, paroxetine, and vilazodone on the (ID_{50}) values of i.v. 8-OH-DPAT.
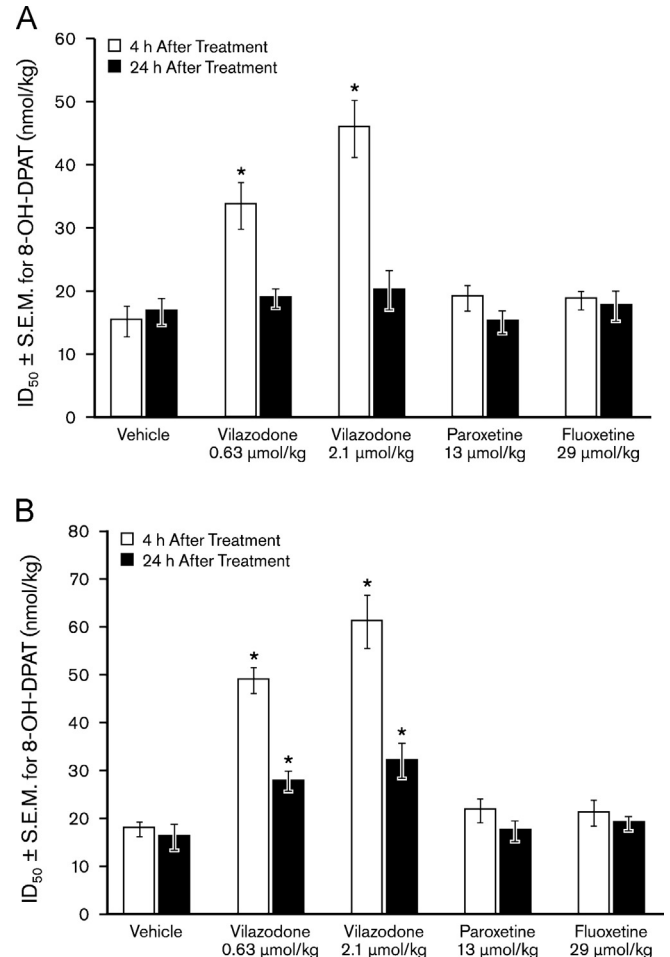

Fig. 1. Electrophysiological effects of vilazodone (0.63; 2.1 µmol/kg), paroxetine (13 µmol/kg), fluoxetine (29 µmol/kg), or vehicle on the inhibition of firing (ID_{50}) by intravenous 8-OH-DPAT at 4 h and 24 h after (A) acute or (B) subchronic administration. The firing rate of a single, spontaneously active dorsal neuron was measured in each animal. The values are the means ± S.E.M. of 10 rats. *$P < 0.01$ *versus* vehicle.

There was also a significant effect of treatment on the ID_{50} value of i.v. 8-OH-DPAT for serotonergic neurons in the DRN 4 h after subchronic administration (one s.c. injection per day for 3 days; $F_{4,45} = 35.8$, $P < 0.0001$, one-way ANOVA) (Fig. 1B). Statistical analyses indicated that both 0.63 and 2.1 µmol/kg of vilazodone (Student–Newman–Keuls values of 45.4 and 87.3, respectively), but not paroxetine or fluoxetine, produced a significant 3–4-fold shift in the ID_{50} value of 8-OH-DPAT, compared with vehicle-treated animals.

In contrast, 24 h following acute drug administration, none of the compounds produced a significant change in the ID_{50} of 8-OH-DPAT ($F_{4,45} = 0.67$, $P = 0.617$) (Fig. 1A). However, there was an effect of treatment on the ID_{50} values of 8-OH-DPAT 24 h after subchronic administration ($F_{4,45} = 7.62$, $P < 0.001$, one-way ANOVA). The subchronic administration of 0.63 or 2.1 µmol/kg of vilazodone produced a significant increase in the ID_{50} of 8-OH-DPAT 24 h after the last injection (Student–Newman–Keuls values of 10.4 and 19.5). In contrast, the subchronic administration of paroxetine or fluoxetine (Fig. 1B) did not significantly alter the ID_{50} value of 8-OH-DPAT. Thus, vilazodone produced a long-lasting effect on 8-OH-DPAT-induced inhibition of neuronal DRN activity in anesthetized rats.

3.2. PCA and R-(−)-fenfluramine-induced 5-HT depletion in rat brain

Vilazodone dose-dependently inhibited PCA-induced 5-HT depletion in rat hypothalamus with an ID_{50} of 1.49 µmol/kg when

Table 1
Duration of action of vilazodone and various SSRIs to prevent PCA-induced rat hypothalamic 5-HT depletion.

Drug	ID_{50} (µmol/kg s.c.) (95% confidence intervals)			
	3.5 h	5 h	8 h	18 h post-application
Vilazodone	1.49 (1.30–1.65)	0.46 (0.25–0.73)	1.17 (1.09–1.28)	169 (100–∞)
Fluoxetine	6.88 (5.93–7.69)	4.80 (3.85–5.70)	7.63 (7.61–7.66)	22.7 (8.96–28.9)
Sertraline	1.81 (0.99–2.71)	1.37 (0.76–2.42)	2.42 (1.66–3.33)	9.51 (3.97–25.3)
Citalopram	1.90 (0.86–3.36)	2.64 (1.46–5.11)	7.97 (2.79–22.3)	57.7 (47.9–71.3)
Paroxetine	0.45 (0.37–0.53)	0.27 (0.21–0.32)	0.69 (0.45–1.17)	37.9 (13.6–72.3)

The values in the table represent the ID_{50} values (50% prevention of 5-HT depletion) and 95% confidence intervals (in parentheses) in µmol/kg s.c. Four doses of each drug were tested at each of the 4 time points. Doses were chosen to obtain dose response curves ranging from very low to at least 100% prevention of 5-HT depletion except for vilazodone, which only produced a 55% prevention at the highest dose of 210 µmol/kg 18 h post-application. Four rats were used for each drug plus PCA dose group, 6 rats for the PCA alone and 3 rats for the vehicle control group.
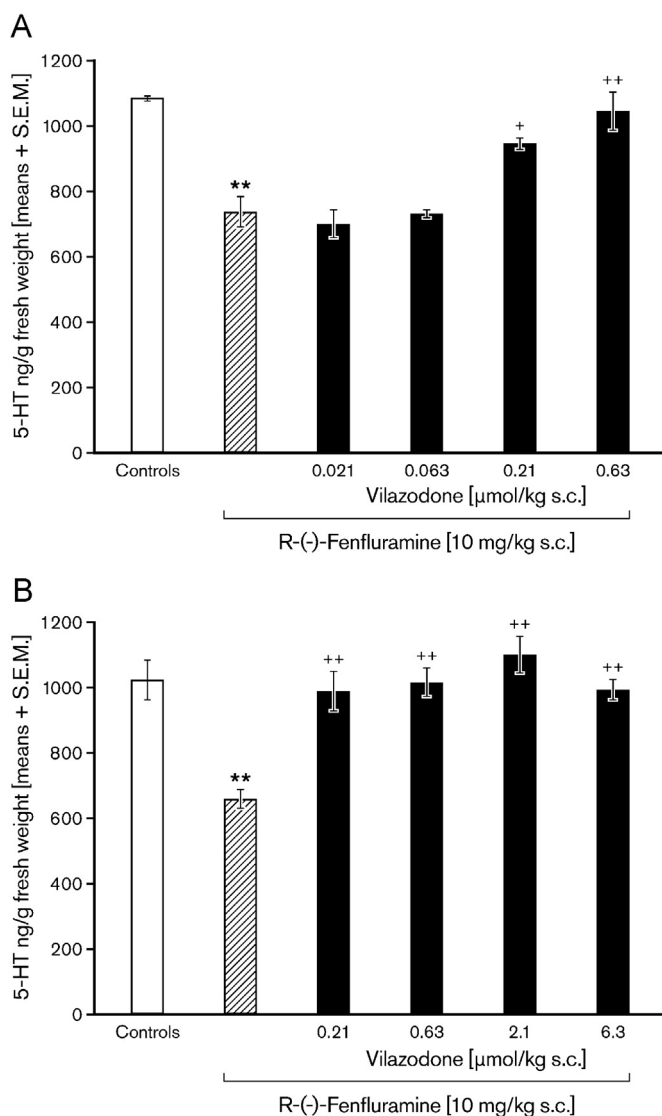

Fig. 2. Antagonism by vilazodone of R-(–)-fenfluramine-induced rat hypothalamic 5-HT depletion. Vilazodone (A) 0.021–0.63 µmol/kg s.c., and (B) 0.21–6.3 µmol/kg s. c., was administered into the neck 5.5 h before euthanasia. R-(–)-fenfluramine administration (10 mg/kg s.c. into the flank) was administered 5 h before euthanasia. Number of rats were: 3 (controls), 6 (R-(–)-fenfluramine) and 4 (vilazodone plus R-(–)-fenfluramine). **P < 0.01 versus controls. +P < 0.05; ++P < 0.01 versus R-(–)-fenfluramine alone group (t-Dunnett).

administered 3.5 h prior to euthanasia. The complete time-course of this effect is shown in Table 1. Although vilazodone produced significant inhibition of PCA-induced 5-HT depletion 3.5 h, 5 h,

and 8 h after administration, its potency was more than 100-fold lower when evaluated 18 h after administration ($ID_{50}=169$ µmol/kg). The duration of action of citalopram and paroxetine were similar to that of vilazodone, whereas fluoxetine and sertraline had longer-acting effects, with relatively low ID_{50} values at 18 h post-administration.

In agreement with the PCA results, R-(–)-fenfluramine-induced hypothalamic 5-HT depletion was inhibited with an ID_{50} of 0.18 µmol/kg s.c. (Fig. 2A); the ID_{100} was ~0.63 µmol/kg s.c. Increasing this dose 10-fold did not change the results (Fig. 2B). Similar values were obtained for frontal cortex and striatum (0.23 and 0.19 µmol/kg s.c., respectively; results not shown). In a separate study (in the absence of fenfluramine), subchronic administration of supramaximal doses of vilazodone alone (up to 21 µmol/kg, s.c., daily for 3 days) did not deplete tissue 5-HT in the rat striatum and frontal cortex 3 h or 24 h after the last application (results not shown).

4. Discussion

The present electrophysiological results show, for the first time, direct in vivo interactions of vilazodone with the 5-HT$_{1A}$ receptors on DRN neurons, and support conclusions of earlier in vivo studies, using indirect behavioral and neurochemical models (Bartoszyk et al., 1996, 1997) and microdialysis studies (Hughes et al., 2005; Page et al., 2002; Roberts et al., 2005). Importantly, the results indicate that the reduction of sensitivity of 5-HT$_{1A}$ autoreceptors on rat dorsal raphe neurons in the current study is unrelated to the inhibition of 5-HT uptake, since the conventional uptake inhibitors fluoxetine and paroxetine did not significantly alter the ID_{50} for 8-OH-DPAT, a finding consistent with that of Czachura and Rasmussen (2000).

It can be estimated that the electrophysiological model used here is quite sensitive, approximately in the same range as microdialysis studies, where threshold doses of vilazodone for increasing rat hippocampal 5-HT output were also 0.63 µmol/kg (Page et al., 2002). In the same dose range, vilazodone markedly inhibited 5-HT uptake in vivo, as reflected in the PCA model (Table 1). Also, in vitro, both the affinity for the 5-HT$_{1A}$ receptor and the inhibition of 5-HT uptake using native rat brain membranes, are in the same range with IC$_{50}$ values of 0.5 and 0.2 nM, respectively (Bartoszyk et al., 1996).

Similar to other in vivo studies, the partial agonist vilazodone reduces the sensitivity of the 5-HT$_{1A}$ receptors in the in vivo electrophysiological model and thus is distinct from the 5-HT$_{1A}$ agonist 8-OH-DPAT. Thus, it has been shown that vilazodone does not induce the so-called 5-HT syndrome in rats, but instead antagonizes the actions of 8-OH-DPAT in this model (Page et al., 2002). In contrast, in various in vitro models, vilazodone exhibits

the profile of a potent, high efficacy 5-HT$_{1A}$ receptor partial agonist (Dawson and Watson, 2009; Hughes et al., 2005; Page et al., 2002). Depending on the *in vitro* assay system utilized, the intrinsic activity of vilazodone ranged from 0.61 to 100 and pEC$_{50}$ values from 8.1 to 9.0 (for review, see Dawson and Watson (2009)). Based on the *in vitro* data, one would expect to also have observed 5-HT$_{1A}$ agonistic activity *in vivo*. Therefore, it has been speculated that metabolites of vilazodone might explain the *in vitro/in vivo* discrepancies. However, in pharmacokinetic studies using [^{14}C]-labeled vilazodone, more than 90% of the radioactivity in rat brain consisted of unchanged vilazodone (Forest Laboratories, data on file). Furthermore, one of the primary active metabolites, 6-hydroxy-vilazodone (Hewitt et al., 2001), is > 10 times less potent than vilazodone in inhibiting 5-HT uptake (IC$_{50}$=9 nM) and at 5-HT$_{1A}$ receptors (IC$_{50}$=8.4 nM) (H. Boettcher, personal communication). Therefore, it is unlikely that active metabolites of vilazodone play a role in its *in vivo* CNS effects.

A more likely explanation for the different activities seen *in vitro versus in vivo* is due to possible differences in receptor reserves found *in vitro* in membrane preparations relative to *in vivo* environments, *i.e.*, the percentage of the receptor population necessary to elicit a full agonist response. It is conceivable that vilazodone, being a 5-HT$_{1A}$ receptor partial agonist, has a markedly lower receptor reserve *in vivo* than 8-OH-DPAT, but due to its higher affinity, displaces full agonists from binding sites and decreases the receptor occupancy of 8-OH-DPAT. Receptor reserve has been shown to be variable in different recombinant and various native tissue *in vitro* systems, which adds a complication to interpreting these data (Dawson and Watson, 2009). Apparently, *in vitro* agonist binding assays measuring the effect on [^{35}S]-GTP-γ-S binding might have limited predictive value for *in vivo* experiments.

Measurement of 5-HT$_{1A}$ agonist activity of vilazodone *in vivo* can be challenging due to its potent inhibition of serotonin reuptake, which may potentially confound any direct assay of 5-HT$_{1A}$ agonist activity. Currently, it is not possible to estimate the *in vivo* intrinsic activity of vilazodone at the 5-HT$_{1A}$ receptor as receptor inactivation studies similar to other 5-HT$_{1A}$ partial agonists (Cox et al., 1993; Meller and Bohmaker, 1994; Meller et al., 1990) are lacking.

It has been suggested that rapid desensitization of 5-HT$_{1A}$ autoreceptors may explain vilazodone's *in vivo* profile, notably the earlier microdialysis findings that suggested vilazodone increased serotonergic output in the prefrontal cortex of rats. (Dawson and Watson, 2009; Hughes et al., 2005; Page et al., 2002). Indeed, the desensitization of these autoreceptors is believed to be a critical rate-limiting step in the process of augmenting 5-HT neurotransmission by antidepressant drugs (Blier and Ward, 2003). The results presented herein do not exclude this interpretation: following a 24 h washout period, there was still a 1.7-fold and a 2-fold increase in the ID$_{50}$ values of 8-OH-DPAT when vilazodone was given subchronically at doses of 0.63 and 2.1 μmol/kg s.c. per day, respectively, whereas paroxetine and fluoxetine were inactive. This lack of desensitizing activity of paroxetine and fluoxetine is also unrelated to their duration of action as the ratio of the ID$_{50}$ values at 18 h and 3.5 h is 3.3 for fluoxetine and 84 for paroxetine (Table 1), reflecting fluoxetine's long duration of action as compared with that of paroxetine and vilazodone (ratio 114). In order to obtain desensitizing effects comparable to vilazodone, fluoxetine has to be given for much longer periods. Indeed, chronic treatment with 29 μmol/kg s.c. per day for 21 days results in a 3-fold increase in the ID$_{50}$ of 8-OH-DPAT whereas vilazodone, 21 or 63 μmol/kg s.c., also given for 21 days, produces an 8–10-fold increase, which is apparently the maximal effect of vilazodone in this model (Ashby, unpublished results). Similarly, Hughes et al. (2007) in microdialysis studies found that chronic paroxetine (26 μmol/kg p.o., daily for 21 days)

was required for a modest 1.5-fold increase in cortical 5-HT. The results in the PCA model, where vilazodone was inactive 18 h post-administration (Table 1), underlines the view that rapid 5-HT$_{1A}$ autoreceptor desensitization may be responsible for the sustained effects of vilazodone in the electrophysiological model.

Since the plasma half-life of vilazodone in rats is relatively short and was estimated in rat pharmacokinetic studies following a single injection (1 mg/kg i.v.) to be 1.4 h (Forest Laboratories, data on file), one can speculate that virtually no vilazodone should be present at 24 h post-administration. However, pharmacokinetic studies using s.c. administration are not available and rats were treated for three days u.i.d. in the electrophysiological studies, so we cannot exclude that some accumulation of vilazodone might have occurred.

It is difficult to determine if desensitization also plays a role in the experiments with the 4 h washout period. Notwithstanding the above caveats, if one considers the results of the *in vitro* agonist assays, vilazodone's agonist potency is at least one order of magnitude greater than that of 5-HT or 8-OH-DPAT (Dawson and Watson, 2009; Hughes et al., 2005), which would support the desensitization hypothesis. Additionally, the affinity of vilazodone for the 5-HT$_{1A}$ receptor is at least one order of magnitude higher than 5-HT and 8-OH-DPAT, both of which compete for the 5-HT$_{1A}$ binding site, lending further support for desensitization. Intriguingly, the onset of the purported vilazodone-induced desensitization must be very fast, since 40 min after an acute injection of 6.3 μmol/kg i.p. vilazodone, a maximally 7-fold increase in 5-HT levels occurs in frontal cortex dialysates (van Amsterdam, personal communication), which is in agreement with findings of Page et al. (2002). Alternatively, rapid desensitization could occur *via* receptor internalization (Riad et al., 2001) or by attenuation of receptor coupling to the G protein, although these assumptions are less parsimonious.

To exclude false positive results, especially in the previous microdialysis studies, it is imperative to rule out the possibility that vilazodone administration results in 5-HT release. This is unlikely as the subchronic treatment with supramaximal doses (up to 21 μmol/kg s.c. daily for 3 days) did not deplete tissue 5-HT levels in either the cortex or striatum (brain areas often chosen for 5-HT microdialysis studies) 3 h and 24 h after the last administration (results not shown). Furthermore, vilazodone completely antagonized the 5-HT depleting actions of the prototypical 5-HT releaser R-(−)-fenfluramine in rat hypothalamus (Fig. 2A), even at doses 10-fold higher than the ID$_{100}$ in this model (Fig. 2B).

Currently, no clinical studies have been conducted to specifically determine if vilazodone produces an early onset of antidepressant action in patients with major depressive disorders. In both Phase III clinical trials the antidepressant efficacy primary endpoint was statistically significant in favor of vilazodone compared with placebo (Khan et al., 2011; Rickels et al., 2009). The difference *versus* placebo was statistically significant at Week 1 (the earliest time point measured) in one trial, but not the other. Therefore, the hypothesis that vilazodone produces a rapid onset of antidepressant action in patients remains to be tested.

5. Conclusion

In summary, the present electrophysiological experiments indicate that vilazodone reduces the sensitivity of 5-HT$_{1A}$ autoreceptors in the DRN more rapidly than the two SSRIs fluoxetine and paroxetine. This effect could occur by either direct interaction with, or rapid desensitization of these receptors, and cannot be ascribed to vilazodone's 5-HT reuptake-inhibiting properties. Further clinical studies would be needed to determine whether

vilazodone produces an early onset of efficacy in patients with depression.

Authorship contributions

Participated in research design: Ashby, Seyfried, Bartoszyk.
Conducted experiments: Ashby, Seyfried.
Wrote the original reports, including analysis and interpretation of results: Ashby, Seyfried.
Wrote or contributed to the writing of the manuscript: Ashby, Seyfried, Pierz, Bartoszyk, Kehne Renda, Athanasiou.
Contributed new reagents or analytic tools: n/a.

Disclosure statement

Role of the funding source

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