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Neurochemical evaluation of the novel 5-HT_{1A} receptor partial agonist/serotonin reuptake inhibitor, vilazodone

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Abstract

Vilazodone has been reported to be an inhibitor of 5-hydoxytryptamine (5-HT) reuptake and a partial agonist at 5-HT_{1A} receptors. Using [^{35}S]GTPγS binding in rat hippocampal tissue, vilazodone was demonstrated to have an intrinsic activity comparable to the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Vilazodone (1–10 mg/kg p.o.) dose-dependently displaced in vivo [^{3}H]DASB (*N*,*N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine) binding from rat cortex and hippocampus, indicating that vilazodone occupies 5-HT transporters in vivo. Using in vivo microdialysis, vilazodone (10 mg/kg p.o.) was demonstrated to cause a 2-fold increase in extracellular 5-HT but no change in noradrenaline or dopamine levels in frontal cortex of freely moving rats. In contrast, administration of 8-OH-DPAT (0.3 mg/kg s.c.), either alone or in combination with a serotonin specific reuptake inhibitor (SSRI; paroxetine, 3 mg/kg p.o.), produced no increase in cortical 5-HT whilst increasing noradrenaline and dopamine 2 and 4 fold, respectively. A 2-fold increase in extracellular 5-HT levels (but no change in noradrenaline or dopamine levels) was observed after combination of the 5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide) (WAY-100635; 0.3 mg/kg s.c.) and paroxetine (3 mg/kg p.o.). In summary, vilazodone behaved as a high efficacy partial agonist at the rat hippocampal 5-HT_{1A} receptors in vitro and occupied 5-HT transporters in vivo. In vivo vilazodone induced a selective increase in extracellular levels of 5-HT in the rat frontal cortex. This profile was similar to that seen with a 5-HT_{1A} receptor antagonist plus an SSRI but in contrast to 8-OH-DPAT either alone or in combination with paroxetine.

Keywords: Serotonin (5-hydroxytryptamine, 5-HT); Microdialysis; Frontal cortex; Paroxetine; *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide) (WAY-100635); 5-HT_{1A} receptor; 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)

1. Introduction

Vilazodone is a combined serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) and 5-HT_{1A} receptor partial agonist (Sorbera et al., 2001) which exhibits anxiolytic-like behaviour in well established rodent models of anxiety, such as the rat ultrasonic vocalisation test (Bartoszyk et al., 1997) and antidepressant-like effects in the forced swim test (Page et al., 2002). Furthermore, vilazodone has been shown to increase extracellular 5-HT in the medial prefrontal cortex and ventral hippocampus of rats (Page et al., 2002).

It is widely accepted that acute increases in forebrain 5-HT levels, induced by administration of SSRIs, are restrained (Invernizzi et al., 1992; Malagie et al., 1995; Romero and Artigas, 1997) by an SSRI-induced reduction in serotonergic neurotransmission mediated by presynaptic 5-HT_{1A} autoreceptors (Blier and De Montigny, 1987; Gartside et al., 1995). Hence the effects of SSRIs can be augmented by co-administration of 5-HT_{1A} receptor antagonists such as WAY-100635 (Gartside et al., 1995; Hjorth et al., 1997; Sharp et al., 1997). However, partial agonists such

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as buspirone (Dawson and Nguyen, 1998) or agonists such as tandospirone (Yoshino et al., 2002) are not able to produce this augmentation. Pindolol is a partial 5-HT_{1A} receptor agonist with low intrinsic activity, yet its ability to augment SSRI-induced increases in 5-HT is equivocal. Some groups demonstrate a potentiation of fluoxetine or citalopram induced increases in 5-HT (Hjorth, 1996; Dawson and Nguyen, 2000), whereas others show no effect (Hughes and Sharp, 1998; Gartside et al., 1999). It has been suggested that the level of intrinsic activity of 5-HT_{1A} receptor partial agonists is critical in determining whether or not a drug of this class can potentiate the effect of an SSRI (Dawson and Nguyen, 1998).

As well as having no effect on 5-HT, 5-HT_{1A} receptor antagonists such as WAY-100635 also do not affect extracellular noradrenaline or dopamine levels (Hajos-Korcsok and Sharp, 1996; Gobert et al., 1997b). In contrast, 5-HT_{1A} receptor agonists and partial agonists such as (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), buspirone and MKC-242, have been shown to increase extracellular dopamine and noradrenaline in a number of brain regions in the rat (Done and Sharp, 1994; Suzuki et al., 1995; Chen and Reith, 1995; Gobert et al., 1998).

Thus in the present study, the neurochemical effects of vilazodone were compared to selective 5-HT_{1A} receptor ligands alone and in combination with the SSRI, paroxetine. The actions of vilazodone on 5-HT, dopamine and noradrenaline were compared to combinations of 5-HT_{1A} receptor agonist (8-OH-DPAT) plus paroxetine or 5-HT_{1A} receptor antagonist (WAY-100635) plus paroxetine. Extracellular levels of 5-HT, noradrenaline and dopamine in frontal cortex were measured using in vivo microdialysis in freely moving rats. In addition, vilazodone was assessed for its ability to occupy rat 5-HT transporter sites in vivo using displacement of [^{3}H]DASB (*N*,*N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine) binding (Wilson et al., 2000, 2002; Meyer et al., 2001). Furthermore, vilazodone was assessed in a native tissue GTPγS binding preparation as a functional measure of intrinsic agonist activity at postsynaptic 5-HT_{1A} receptors.

2. Methods

2.1. Materials

WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl)cyclohexanecarboxamide) and paroxetine hydrochloride hemihydrate (3*S*-trans)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine hydrochloride hydrate (2:1)) were all synthesised by Medicinal Chemistry, GlaxoSmithKline (Harlow, UK). Vilazodone (EMD 68843; 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide hydrochloride) was synthesised by Medicinal Chemistry, Merck KGaA (Darmstadt, Germany). All drug salts were corrected for and drugs dosed at 1 ml/kg. [^{3}H]DASB (*N*,*N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine; in ethanol) was custom synthesised by Amersham Biosciences Inc. (Amersham, UK) and was administered in saline at approximately 8 μCi per 0.1 ml. Soluene (scintillation fluid) was obtained from Canberra Packard (Pangbourne, UK). Drugs involved in the surgical procedure were obtained from Vet Tech Solutions (Congleton, UK). All constituents of the mobile phase and artificial cerebrospinal fluid were analytical grade and obtained from BDH (Lutterworth, UK) or Fisher Chemicals (Loughborough, UK). Amine standards were obtained from Sigma (Poole, UK).

2.2. Animals

All animal studies were conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986 and conformed to GlaxoSmithKline ethical standards. Male Sprague Dawley rats (250–400 g, Charles River, UK), were group housed with free access to food and water under a 12 h light/dark cycle (lights on at 06:00 h).

2.3. [^{35}S]GTPγS binding studies in rat hippocampus

Hippocampal membranes were prepared according to Alper and Nelson (2000) with minor modifications. Rats were sacrificed by stunning and decapitation, their brains removed, hippocampi dissected and placed on dry ice. Tissue was homogenised in ice cold Tris buffer (50 mM Tris, pH 7.4). The homogenate was centrifuged at 4 °C, 39,800×*g* for 10 min. The supernatant was discarded and the pellet re-suspended in Tris buffer followed by incubation at 37 °C for 10 min. The suspension was then centrifuged again and the pellet re-suspended in Tris buffer. Following one final centrifugation the pellet was re-suspended in Tris buffer to yield a concentration of approximately 100 mg tissue protein/ml. The suspension was aliquoted and stored at −70 °C until needed. For the [^{35}S]GTPγS assay, membranes (50μg protein/well) were re-suspended in assay buffer (4 mM $MgCl_2$, 160 mM NaCl, 0.267 mM EGTA, 67 mM Tris and 3 μM SB-224289 (selective 5-HT_{1B} receptor antagonist, Selkirk et al., 1998), pH 7.4) and incubated with increasing concentration of test compound, 300 μM GDP and 0.1 nM [^{35}S]GTPγS at 37 °C for 20 min. The reaction was terminated by rapid filtration through Whatman GF/B filters, pre-soaked with distilled water, and washed with 4 mls ice cold Tris buffer. GTPγS (20 μM) defined the non-specific binding (NSB). Radioactivity was determined by liquid scintillation counting.

2.4. In vivo [^{3}H]DASB binding

Animals were administered vilazodone (1, 3 and 10 mg/kg p.o.), paroxetine (30 mg/kg p.o.) or vehicle and returned to their home cages. After the appropriate pretreatment time (2 h for vilazodone, 1 h for paroxetine; pre-determined from

in house efficacy studies and pharmacokinetic parameters) an i.v. bolus dose of 8 μCi [^{3}H]DASB was administered. Rats were sacrificed and hippocampus and cortex rapidly dissected from brain tissue under cooled conditions. Tissue weight was determined before being dissolved in 2 ml soluene. When the tissue had completely dissolved, 2 ml 0.5 M HCl and 15 ml scintillation fluid were added. Scintillation spectrometry was used to determine levels of radioactivity in each sample (dpm).

Levels of radioactivity (dpm) were corrected for tissue weight and % occupancy at 5-HT transporters was calculated according to Wadenberg et al. (2001) with the paroxetine (30 mg/kg) group used to define non-specific binding.

2.4.1. In vivo binding data analysis

Percentage occupancy of 5-HT transporters was calculated for each animal and presented as mean±S.E.M. for each treatment group.

2.5. Microdialysis surgical procedure

Rats were anaesthetised with a mixture of fentanyl (450 μg/kg i.p.) and medetomidine (0.4 mg/kg s.c.). Microdialysis guide cannulae (CMA 11, CMA microdialysis, UK) were implanted for sampling from the frontal cortex. Implantation coordinates (mm) were AP+3.2 ML±1.8 DV−0.4, (Paxinos and Watson, 1986). Guide cannulae were secured with dental cement and a tether anchor screw (Instech, Presearch, Hitchin, UK) attached. On completion of surgery anaesthesia was reversed using atipamezole (1 mg/kg s.c.) and nalbuphine (2 mg/kg s.c.). All rats were given the analgesic carprofen (7.5 mg/kg s.c.), the antibiotic amoxycillin (140 mg/kg s.c.) and saline (5 ml s.c.) and placed in an incubator until they regained their righting reflex. Following surgery the animals were housed individually, and received 5 days post-operative care. Animals were used in experiments 7 days after surgery.

2.5.1. Microdialysis sampling procedure

On the day prior to experiments animals were placed in microdialysis cages for overnight habituation. The following morning pins were removed from guide cannulae and replaced with microdialysis probes (concentric 2 mm cuprophane membrane probes (CMA 11), CMA microdialysis, UK). Probes were perfused with artificial cerebrospinal fluid containing (mM) NaCl 145, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2, Na_2HPO_4 2.0 (pH 7.4) at 1 μl min^{-1}.

After 2 h equilibration time, 4 basal microdialysates (30 min each) were collected. Animals were then dosed with test drugs either: (i) vilazodone (1 or 10 mg/kg p.o.) or vehicle (1% methyl cellulose, 1 ml/kg p.o.), or (ii) WAY-100635 (0.3 mg/kg s.c.), 8-OH-DPAT (0.3 mg/kg s.c.) or vehicle (0.9% NaCl, 1 ml/kg s.c.) followed 30 min later with paroxetine (3 mg/kg p.o.) or vehicle (1% methyl cellulose, 1 ml/kg p.o.). At the end of the experiment probes were removed and animals returned to their home cage for 7 days before re-use. Animals were re-used (up to 4 re-uses per study) in a randomised cross-over design. In all experiments microdialysates were collected for 3 h after final administration of drug. 5-HT, dopamine and noradrenaline content of microdialysates were measured using high performance liquid chromatography (HPLC) with electrochemical detection (see below).

2.5.2. Histology

After the final microdialysis experiment animals were euthanased and brains removed and stored in formal saline for verification of probe placement. Brains were sectioned (50 μm) using a cryostat. Brain slices were stained with cresyl violet to visualise sites of probe implantation. Data from animals with incorrect probe placement were discarded.

2.5.3. HPLC-electrochemical detection

Chromatographic separations were performed using a Capcell PAK, Strong Cation Exchange column (5 μm UG80, 1.5×150 mm; Shiseido, Japan). The mobile phase consisted of (mM) 13 Na_2HPO_4, 87 NaH_2PO_4, 0.1 EDTA, 5 NaCl and 20% methanol buffered to pH 6.0 and was delivered via a Jasco PU-980 HPLC pump (Jasco, Tokyo, Japan) at a flow rate of 0.2 ml/min at a temperature of 40 °C. 5-HT, noradrenaline and dopamine were detected via an electrochemical amperometric detector (Decade, Antec-Leyden, Netherlands) fitted with a 3 mm glassy carbon electrode (VT-03, Antec-Leyden, Netherlands) with a working electrode set at +500 mV vs. Ag/AgCl reference. The analogue data output was smoothed at 40 Hz before collection (LINK, Antec-Leyden, Netherlands). All data were acquired using Millennium^{32} software (Waters, Milford, MA, USA) for the PC. Samples (10 μl) were injected via a cooled (4 °C) Gilson model 234 autosampler (Gilson, Villiers-le-Bel, France) fitted with a six port rotary valve (Model 7125, Rheodyne, Berkley, CA, USA) with a 20 μl injection loop.

2.5.4. Microdialysis data analysis

The means of the microdialysate 5-HT, dopamine or noradrenaline concentrations of the four baseline samples were calculated and these values denoted as 100%. Levels of amines in microdialysates were expressed as a percentage of the preinjection baseline values. The transformed data were analysed by 2-way analyses of variance (ANOVA) with repeated measures followed by a post-hoc Fischer's test when significant effects were found.

3. Results

3.1. [^{35}S]-GTPγS binding to rat hippocampal membranes

The selective antagonists, WAY-100635 and SB-224289, were used to define the components of the 5-HT-induced

stimulation of [^{35}S]-GTPγS binding mediated via 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, respectively (Fig. 1A). In the presence of SB-224289 (3 μM) the response to 5-HT was assumed to be solely due to the activation of 5-HT$_{1A}$ receptors and this concentration of SB-224289 was included routinely in subsequent experiments to block the 5-HT$_{1B}$ receptor-mediated component of the 5-HT response. Under these experimental conditions 5-HT stimulated [^{35}S]-GTPγS binding to rat hippocampal membranes with a pEC$_{50}$ of 6.4±0.08 (Fig. 1B). In comparison, (±)8-OH-DPAT (pEC$_{50}$=7.2±0.11) produced a partial agonist response with an intrinsic activity of 0.45±0.02. The partial agonist buspirone showed a pEC$_{50}$ of 6.5±0.35 and an intrinsic activity of 0.19±0.02. In contrast, vilazodone was a potent 5-HT$_{1A}$ receptor partial agonist with a pEC$_{50}$ of 8.1±0.12 and an intrinsic activity of 0.61±0.05.

3.2. Effects of vilazodone on [^{3}H]DASB binding in rat cortical and hippocampal tissue

Vilazodone (1–10 mg/kg p.o.) displaced [^{3}H]DASB binding in hippocampus and cortex in a dose dependent manner (Fig. 2). In both brain regions vilazodone achieved 100% occupancy of 5-HT transporter sites (SERT) at 10 mg/kg and 50% occupancy in the range of 1–3 mg/kg (Fig. 2).

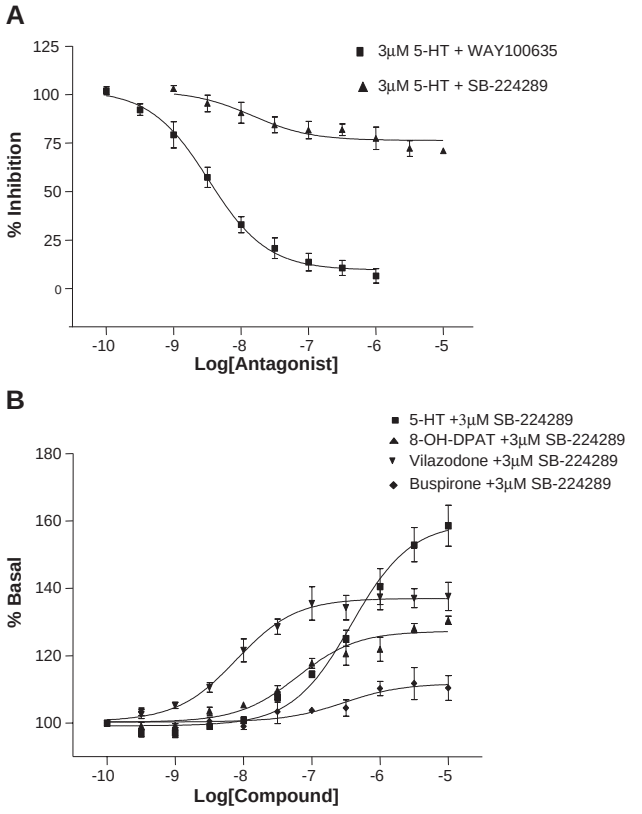

Fig. 1. (A) Effect of WAY-100635 and SB-224289 on 5-HT-induced stimulation of [^{35}S]GTPγS binding in rat hippocampal membranes (3 μM 5-HT), (B) agonist mediated [^{35}S]GTPγS binding in rat hippocampal membranes. Data expressed as mean±S.E.M. n=3–5 per group.

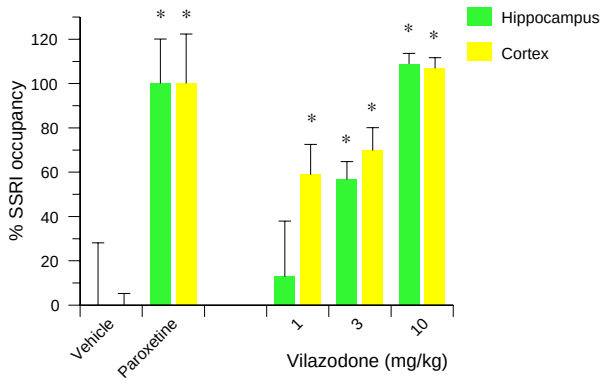

Fig. 2. The effect of vilazodone on [^{3}H]DASB binding in the hippocampus and cortex of male rats. Rats were dosed with vilazodone (1, 3 or 10 mg/kg, p.o.), paroxetine (30 mg/kg p.o.) or vehicle. Data are expressed as percent occupancy of 5-HT transporter sites (SERT) and are mean±S.E.M (n=4). *P<0.05 versus vehicle.

3.3. Effects of vilazodone on extracellular levels of 5-HT, noradrenaline and dopamine in rat frontal cortex

Basal microdialysate levels of 5-HT in the rat frontal cortex were 194.8±32.1 pM (n=22). Vilazodone (1 and 10 mg/kg p.o.) caused a dose dependent increase in extracellular 5-HT in rat frontal cortex (Fig. 3). The ANOVA revealed a significant effect of treatment ($F_{2,39}$ 11.9; P<0.0001) and a significant treatment×time interaction ($F_{12,234}$ 7.05; P<0.0001); post hoc analysis revealed significant increases at both 1 and 10 mg/kg (P=0.05 and P<0.0001, respectively). In the same animals basal levels of noradrenaline and dopamine were (pM) 315.6±45.7 (n=22) and 70.1±8.7 (n=23), respectively. Vilazodone (1 and 10 mg/kg p.o.) caused no change in extracellular levels of noradrenaline (main effect of treatment: $F_{2,26}$=2.90; P=0.07) or dopamine

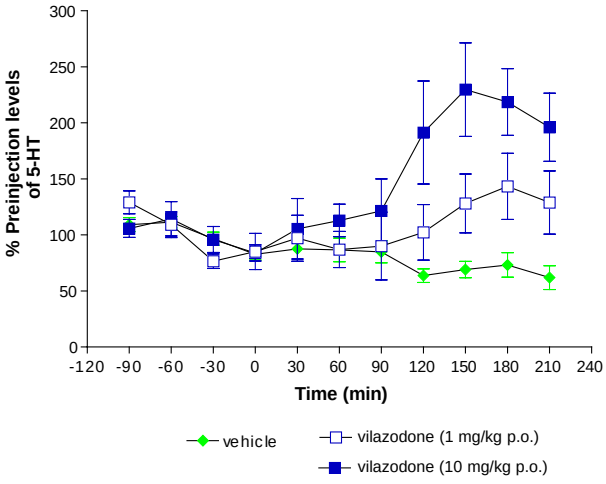

Fig. 3. Effect of vilazodone on extracellular 5-HT levels in rat frontal cortex. Rats were dosed with vilazodone (1 or 10 mg/kg p.o.) or vehicle at t=0. Data are expressed as a percentage of basal 5-HT and are mean±S.E.M. (n=6–9 per group).

(main effect of treatment: $F_{2,26}$=0.04; P=0.959); data not shown.

3.4. Effect of acute administration of paroxetine in combination with 5-HT$_{1A}$ receptor ligands on extracellular 5-HT levels in rat frontal cortex

Similar to the previous set of experiments basal microdialysate levels of 5-HT were 178.9±28.0 pM (n=26). Evaluation of the effects of paroxetine in the presence and absence of 8-OH-DPAT revealed an overall significant effect of treatment ($F_{3,62}$ 4.7; P<0.005,) and a significant treatment×time interaction ($F_{18,372}$ 4.0; P<0.0001). Post hoc analysis showed that paroxetine (3 mg/kg p.o.) produced a small (maximum increase of 144±22% of baseline), but significant (P<0.0001) increase in extracellular 5-HT levels (Fig. 4). Whilst, 8-OH-DPAT (0.3 mg/kg s.c.) caused a significant (P=0.002) decrease in extracellular 5-HT, but a combination of 8-OH-DPAT with paroxetine had no overall effect on 5-HT levels when compared to vehicle treated animals (Fig. 4). Combination studies with paroxetine and the 5-HT$_{1A}$ receptor antagonist WAY-100635 (0.3 mg/kg s.c.) revealed a significant effect of treatment ($F_{3,75}$ 13.2; P<0.0001) and a treatment x time interaction ($F_{18,450}$ 8.5; P<0.0001). However, WAY-100635 alone had no effect on extracellular 5-HT, whilst pretreatment with WAY-100635 significantly potentiated (P< 0.0001) the effect of paroxetine producing a maximum increase of 270±35% of baseline (Fig. 5). Similarly, vilazodone (10 mg/kg p.o.) increased extracellular 5-HT levels to 230±42% of baseline (treatment $F_{1,14}$ 24.14; P=0.0002; Fig. 5). This increase was equivalent to that seen following the co-administration of WAY-100635 and paroxetine ($F_{1,26}$ 0.85; P=0.365; Fig. 5).

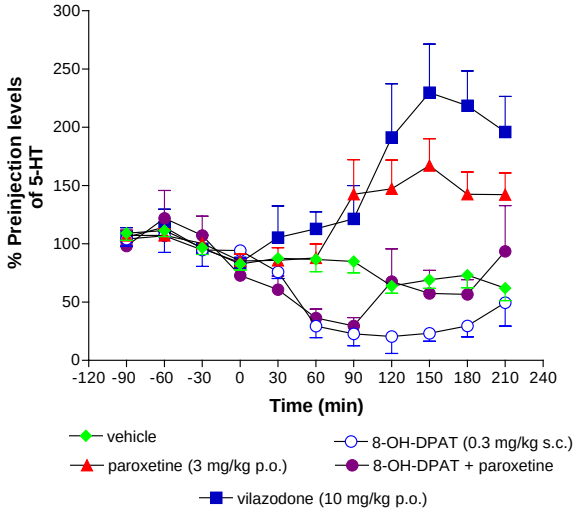

Fig. 4. Effects of vilazodone, paroxetine or paroxetine in combination with 8-OH-DPAT on 5-HT in rat frontal cortex. At t=−30 min rats were dosed with vehicle or 8-OH-DPAT (0.3 mg/kg s.c.). 30 min later, at t=0, rats were dosed with vehicle, paroxetine (3 mg/kg p.o.) or vilazodone (10 mg/kg p.o.). Data are mean±S.E.M. (n=6–8).

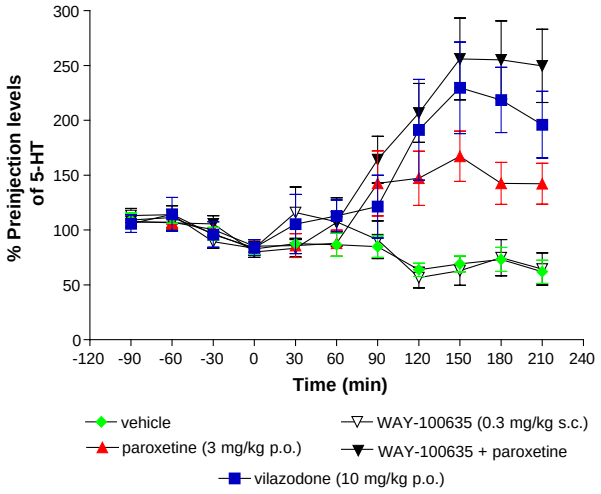

Fig. 5. Effects of vilazodone, paroxetine alone or paroxetine plus WAY-100635 on 5-HT in rat frontal cortex. At t=−30 min rats were dosed with vehicle or WAY-100635 (0.3 mg/kg s.c.). 30 min later, at t=0, rats were dosed with vehicle, paroxetine (3 mg/kg p.o.) or vilazodone (10 mg/kg p.o.). Data are mean±S.E.M. (n=6–8).

3.5. Effect of acute administration of vilazodone or paroxetine in combination with 5-HT$_{1A}$ receptor ligands on extracellular noradrenaline levels in rat frontal cortex

Basal levels of noradrenaline (pM) were 343.6±38.6 (n=38). Evaluation of the effects of paroxetine in the presence and absence of 8-OH-DPAT on extracellular noradrenaline levels revealed an overall significant effect

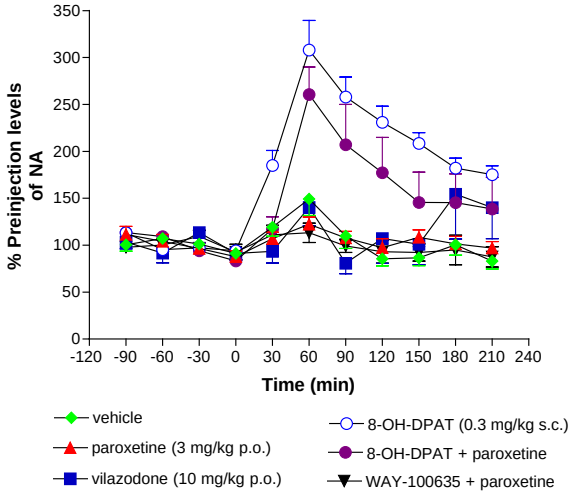

Fig. 6. Effects of vilazodone or paroxetine alone or in combination with WAY-100635 or 8-OH-DPAT on extracellular noradrenaline levels in rat frontal cortex. At t=−30 min rats were dosed with vehicle or 8-OH-DPAT (0.3 mg/kg s.c.). 30 min later, at t=0, rats were dosed with vehicle, paroxetine (3 mg/kg p.o.) or vilazodone (10 mg/kg p.o.). Data are mean±S.E.M. (n=6–8).

of treatment ($F_{3,45}$ 17.4; $P<0.0001$) and a significant treatment x time interaction ($F_{18,270}$ 7.1; $P<0.0001$). Acute administration of paroxetine (3 mg/kg p.o.) produced no change in extracellular levels of noradrenaline. However, and in contrast, 8-OH-DPAT, both in the presence and absence of paroxetine, produced a significant ($P<0.0001$) increase in extracellular noradrenaline reaching maximum values of 260±30% and 308±35% of baseline, respectively (Fig. 6). By comparison, combination studies with paroxetine and WAY-100635 (0.3 mg/kg s.c.) revealed no significant effects (Fig. 6) of treatment ($F_{3,35}$ 0.5; $P=0.67$). Similarly, vilazodone (10 mg/kg p.o.) did not affect extracellular noradrenaline levels ($F_{1,22}$ 0.204; $P=0.654$; Fig. 6).

3.6. Effect of acute administration of vilazodone or paroxetine in combination with 5-HT$_{1A}$ receptor ligands on extracellular dopamine levels in rat frontal cortex

Basal levels of dopamine (pM) were 64.5±7.3 ($n=32$). In concurrence with the noradrenaline data, paroxetine in the presence and absence of 8-OH-DPAT produced an overall significant effect of treatment ($F_{3,35}$ 27.1; $P<0.0001$) and a significant treatment×time interaction ($F_{18,210}$ 16.1; $P<0.0001$). 8-OH-DPAT (0.3 mg/kg s.c.) alone induced a significant ($P<0.0001$) increase in dopamine to a maximum value 540±87% of baseline (Fig. 7). Whilst, administration of paroxetine with 8-OH-DPAT also produced a significant ($P<0.0001$) increase in extracellular dopamine (maximum value of 723±74%) which was not significantly different from 8-OH-DPAT alone ($F_{1,13}$ 0.81; $P=0.384$; Fig. 7). By comparison, combination studies with paroxetine and WAY-100635 (0.3 mg/kg s.c.) revealed no significant effects of treatment ($F_{3,39}$ 2.1; $P=0.23$). Similarly, vilazodone (10 mg/kg p.o.) did not affect extracellular dopamine levels ($F_{1,20}$ 0.1; $P=0.9$; Fig. 7).

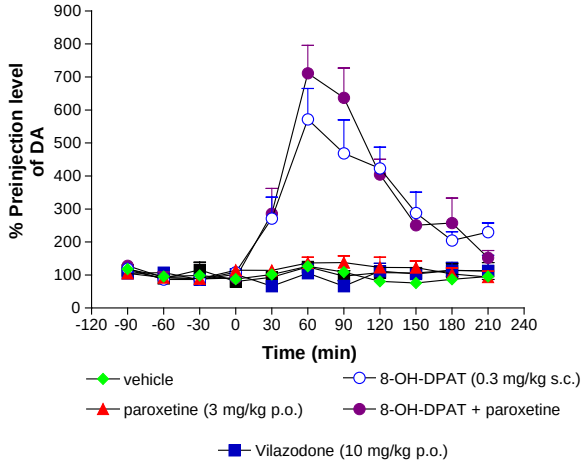

Fig. 7. Effects of vilazodone or paroxetine alone or in combination with WAY-100635 or 8-OH-DPAT on extracellular dopamine levels in rat frontal cortex. At $t=-30$ min rats were dosed with vehicle, WAY-100635 (0.3 mg/kg s.c.) or 8-OH-DPAT (0.3 mg/kg s.c.). 30 min later, at $t=0$, rats were dosed with vehicle, paroxetine (3 mg/kg p.o.) or vilazodone (10 mg/kg p.o.). Data are mean±S.E.M. ($n=6$–8).

4. Discussion

Vilazodone (EMD-68843) is reputed to be a combined 5-HT reuptake inhibitor and 5-HT$_{1A}$ receptor partial agonist (Bartoszyk et al., 1997; Sorbera et al., 2001). Previous data (Wilson et al., 2000, 2002) has demonstrated that the radiotracer, [^{11}C]DASB, binds selectively to the 5-HT transporter across a range of brain regions in the rat. Thus demonstrating the utility of this radioligand for the evaluation of occupancy of transporter sites within the rodent brain. The results of the [^{3}H]DASB in vivo binding in the present study indicate that vilazodone occupies rat hippocampal and cortical 5-HT transporters in a dose dependent manner and thus support previous findings (Bartoszyk et al., 1997). Although in vivo binding to assess 5-HT$_{1A}$ receptor occupancy was not undertaken in the resent study, vilazodone has equal affinity for both receptor and transporter sites (Bartoszyk et al., 1997; Sorbera et al., 2001). Therefore, one could speculate that 5-HT$_{1A}$ receptor occupancy is also likely to be high. Furthermore, the ability of vilazodone to promote [^{35}S]GTPγS binding in the rat hippocampus, a native tissue system lacking 5-HT$_{1A}$ receptor reserve (Alper and Nelson, 2000), demonstrates the 5-HT$_{1A}$ receptor partial agonism of this compound. However, the level of intrinsic activity of vilazodone (~61% vs. 100% for a full agonist) in this in vitro functional assay is comparable to that exhibited by 8-OH-DPAT (~45%) and is significantly less than buspirone (~19%). This level of intrinsic activity is comparable to that shown by Page et al. (2002) in a human clonal system and demonstrates vilazodone to be a high efficacy partial agonist at 5-HT$_{1A}$ receptors.

Based on these findings we have investigated the neurochemical effects of vilazodone both alone and as a comparison to the SSRI, paroxetine in the presence and absence of 8-OH-DPAT. Vilazodone showed full occupancy of hippocampal and cortical 5-HT transporter sites at 10 mg/kg. Positron emission tomography (PET) studies using [^{11}C]DASB have reported that clinically efficacious doses of SSRIs occupy approximately 80% of 5-HT transporter sites (Meyer et al., 2001). If clinical efficacy is directly related to increases in extracellular 5-HT (resulting from 5-HT transporter occupancy) 10 mg/kg vilazodone would be an optimal dose and has thus been used as the maximal dose in the present studies. Vilazodone produced an acute increase in 5-HT levels in frontal cortex of Sprague Dawley rats at 10 mg/kg. This increase in the dorsal lateral cortex is similar to that observed by Page et al. (2002) in the medial prefrontal cortex. By comparison, acute administration of the SSRI, paroxetine produced a much smaller increase in extracellular 5-HT when given alone. This paroxetine-induced increase was significantly augmented by the

5-HT_{1A} receptor antagonist, WAY-100635 but attenuated by the 5-HT_{1A} receptor agonist, 8-OH-DPAT. A comparison of magnitude of changes in extracellular 5-HT suggested that the vilazodone response was equivalent to the that produced by WAY-100635 with paroxetine.

In vivo electrophysiology and microdialysis studies, have demonstrated that agonist activity at both pre- and postsynaptic (via a "long feedback loop") 5-HT_{1A} receptors caused a reduction in serotonergic cell firing and subsequent decrease in extracellular 5-HT levels (Hajos et al., 1999; Casanovas et al., 1999). Furthermore, acute administration of an SSRI also results in a reduction in dorsal raphe cell firing and a reduction/limitation in terminal 5-HT output (Gartside et al., 1995; Blier et al., 1998). The effects of pre- and postsynaptic 5-HT_{1A} receptor agonists on dorsal raphe 5-HT cell firing and extracellular 5-HT in cortical regions, suggest that the mechanism of 5-HT_{1A} receptor agonism with 5-HT reuptake blockade would not acutely increase cortical 5-HT. Only a partial agonist with low intrinsic activity would be expected not to decrease serotonergic activity and consequently augment an SSRI-induced increase in 5-HT (Dawson and Nguyen, 1998). The effects of combining partial agonists with different levels of intrinsic activity with SSRIs on extracellular 5-HT have been reported. Buspirone, a partial agonist with low intrinsic activity does not potentiate the effect of SSRIs on cortical 5-HT (Hjorth, 1996; Gobert et al., 1997a; Dawson and Nguyen, 1998). Pindolol, a partial agonist with low intrinsic activity at 5-HT_{1A} receptors (but also with possibly confounding affinity for 5-HT_{1B} receptors) in the native tissue preparations (unpublished observations) has been shown by some groups to potentiate the effects of SSRIs on 5-HT (Romero et al., 1996; Hjorth, 1996; Dawson and Nguyen, 2000), whereas other groups have not observed an effect of this compound (Hughes and Sharp, 1998; Gartside et al., 1999). Based on the levels of intrinsic activity that vilazodone exhibited in the 5-HT_{1A} receptor hippocampal preparations (i.e. comparable to 8-OH-DPAT) and the fact that combination of 8-OH-DPAT with paroxetine produced no increase in cortical 5-HT, it would seem unlikely that the increase in 5-HT caused by vilazodone was due to partial agonist activity at 5-HT_{1A} autoreceptors (+5-HT reuptake blockade). One possible caveat to this conclusion is that there is a discord between intrinsic activity measured in an in vitro hippocampal preparation, which possess no appreciable receptor reserve (Alper and Nelson, 2000), and that exerted in vivo at dorsal raphe neurones. The level of intrinsic activity which a molecule exerts will depend on receptor reserve and concentrations of endogenous agonist, i.e. in a high receptor reserve environment with low endogenous agonist an antagonist can appear to be a partial agonist and conversely if conditions are reversed an agonist can appear to be an antagonist. Page et al. (2002) report that the vilazodone-induced increase in the medial prefrontal cortex 5-HT and suggested this was due to the partial agonism of vilazodone. Furthermore, they demonstrated that this increase was not attenuated by 8-OH-DPAT, again suggesting that vilazodone was able to block the 5-HT_{1A} receptor. Interestingly, another compound with 5-HT reuptake inhibition and 5-HT_{1A} receptor agonist activity, VN2222, produces no acute increase in striatal 5-HT (Romero et al., 2003). As the striatum is innervated by the dorsal raphe the effects of 5-HT_{1A} receptor/SSRI compounds would be expected to be comparable to those seen in the frontal cortex. The effects of VN2222 are more comparable to those of the paroxetine+8-OH-DPAT effects observed in the present study, whilst the effects seen with vilazodone are more comparable to those of WAY-100635+paroxetine.

Two possible alternative explanations for the observed increases in extracellular 5-HT are: (i) that vilazodone has 5-HT releasing properties however, this seems unlikely given that it produces no depletion of tissue levels of 5-HT, whilst fenfluramine produces marked effects (Page et al., 2002); (ii) vilazodone produces a rapid desensitization of presynaptic 5-HT_{1A} receptors. Both chronic SSRI treatment (for review, see Blier et al., 1998) and administration of 5-HT_{1A} receptor agonists and partial agonists has been shown to cause desensitisation of 5-HT_{1A} autoreceptors in the dorsal raphe (Blier and De Montigny, 1987; Dong et al., 1997; Le Poul et al., 1999; Okazawa et al., 1999; Dawson et al., 2000). However, the time course of such an effect is not clear. Many reports indicate that chronic treatment (3–14 days) is required (Blier and De Montigny, 1987; Le Poul et al., 1999; Dawson et al., 2002), whereas one group suggest that desensitisation can occur as soon as 15 min after administration of a 5-HT_{1A} receptor agonist (Riad et al., 2001). In deed this would have needed to occurred rapidly since an increase in 5-HT was observed within the first 30 min (i.e. first post injection microdialysate). Although this seems an unlikely mechanism further work using in vivo electrophysiological techniques would need to be performed to prove or disprove this hypothesis.

The effects of vilazodone on extracellular dopamine and noradrenaline also do not indicate agonist activity at 5-HT_{1A} receptors. In keeping with published literature, 8-OH-DPAT caused marked increases in dopamine and noradrenaline (Done and Sharp, 1994; Chen and Reith, 1995). Co-administration of paroxetine did not alter these responses. The partial agonist, buspirone, also increases cortical dopamine and noradrenaline (Gobert et al., 1999). In contrast, vilazodone had no effect on dopamine or noradrenaline indicating that this compound does not exhibit the predicted effects of 5-HT_{1A} receptor agonism or partial agonism. The increases in dopamine caused by 5-HT_{1A} receptor agonists such as 8-OH-DPAT, or buspirone have been shown to be due to stimulation of presynaptic 5-HT_{1A} receptors (Chen and Reith, 1995). In contrast, it is thought that the concomitant increase in

noradrenaline is due to stimulation of postsynaptic 5-HT_{1A} receptors (Suzuki et al., 1995; Chen and Reith, 1995). Thus these data would further suggest that vilazodone does not functionally activate either pre- or postsynaptic 5-HT_{1A} receptors.

In summary, the neurochemical profile of vilazodone is consistent with an SSRI with 5-HT_{1A} receptor antagonist or partial agonist properties. This confirms and extends the findings of Page et al. (2002). Reports on this compound suggest different activities depending on the model used. Vilazodone is effective in the forced swim test suggesting agonist activity at postsynaptic 5-HT_{1A} receptors (Page et al., 2002), whereas the same authors show that vilazodone blocks 8-OH-DPAT-induced 5-HT syndrome indicating it is either an antagonist, or a partial agonist. Furthermore, vilazodone has no effect on body temperature in the rat and does not affect 8-OH-DPAT-induced changes which indicate no effect at postsynaptic 5-HT_{1A} receptors (Bartoszyk et al., 1997). Clearly there are discrepancies between the in vivo and some disparity between in vitro and in vivo activity of this compound which require further investigation. However from these data it would appear that vilazodone has a neurochemical profile more akin to that of an antagonist or low efficacy partial agonist at 5-HT_{1A} receptors. Moreover, acute increases in extracellular 5-HT along with reported efficacy in behavioural models of anxiety/depression (Bartoszyk et al., 1997) indicate that this molecule may have therapeutic utility in the treatment of anxiety and depression.

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