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Effect of vilazodone on 5-HT efflux and re-uptake in the guinea-pig dorsal raphe nucleus

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Abstract

The effect of vilazodone, a putative selective serotonin re-uptake inhibitor (SSRI) with 5-HT (5-hydroxytryptamine)$_{1A}$ receptor partial agonist activity, was investigated on 5-HT efflux and 5-HT re-uptake half life in the guinea-pig dorsal raphe nucleus, using in vitro fast cyclic voltammetry. The SSRI, fluoxetine, significantly increased 5-HT efflux. In contrast, vilazodone had no effect on 5-HT efflux at 100 nM but significantly decreased 5-HT efflux at 1 μM. Co-perfusion of 8-OH-DPAT (±8-hydroxy-2-(di-n-propylamino)tetralin) with fluoxetine significantly attenuated the fluoxetine-induced increase in 5-HT efflux. Co-perfusion of WAY 100635 with vilazodone did not attenuate the effect of vilazodone alone. In addition, the re-uptake half life for 5-HT was significantly increased by both fluoxetine and vilazodone. In conclusion, we have demonstrated that vilazodone (100 nM, 1 μM), in the guinea-pig dorsal raphe nucleus, blocks the serotonin transporter but does not display 5-HT$_{1A}$ receptor agonism.

Keywords: Vilazodone; EMD-68843; Voltammetry; 5-HT efflux; 5-HT re-uptake

1. Introduction

Antidepressant selective serotonin re-uptake inhibitors (SSRIs) given acutely block the serotonin (5-hydroxytryptamine, 5-HT) transporter, preventing the uptake of released 5-HT back into the neurone which results in an increase in local levels of 5-HT (Davidson and Stamford, 1995). However, the SSRI-induced increases of 5-HT in forebrain have been demonstrated to be limited by activation of 5-HT autoreceptors in cell body regions, such as the dorsal raphe nucleus (Roberts et al., 1999). Clinically, SSRIs do not exert their therapeutic action until after 2–3 weeks of drug administration (Asberg et al., 1986). This latency to therapeutic activity may be explained by the time needed for 5-HT autoreceptors (5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$) to desensitise and maximise the increase of 5-HT in forebrain regions (Blier and de Montigny, 1987). Therefore, combining SSRI activity with autoreceptor blockade may reduce the latency of action of antidepressants by elevating forebrain 5-HT levels after acute drug administration.

5-HT$_{1A}$ receptor antagonists, such as WAY 100635, have been shown to potentiate the effect of SSRIs on forebrain extracellular 5-HT (Hjorth et al., 1997). In addition, pindolol, a partial agonist at 5-HT$_{1A}$ receptors, has also been demonstrated to augment the effects of SSRIs — both preclinically (Hjorth and Auerbach, 1994; Dreshfield et al., 1996; Hjorth, 1996) and in patients (Artigas et al., 1994; Blier and Bergeron, 1995). However, these findings remain controversial due to the failure of others to replicate the studies.

Vilazodone is reported to be a 5-HT re-uptake inhibitor with partial agonist activity at 5-HT$_{1A}$ receptors (Sorbera et al., 2001). Vilazodone exhibits anxiolytic-like and antidepressant-like behaviour in well established rodent models (Bartoszyk et al., 1997; Treit et al., 2001; Page et al., 2002).

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Therefore, if vilazodone possesses 5-HT_{1A} receptor antagonism or partial agonism, together with its SSRI activity, it would potentially constitute a fast acting antidepressant.

Vilazodone has demonstrated partial agonist activity at the 5-HT_{1A} receptor in recombinant systems (Page et al., 2002) although data from in vivo models of 5-HT_{1A} receptor function were conflicting. Thus, vilazodone failed to show any 5-HT_{1A} receptor activity in the rat hypothermia model but displayed activities consistent with 5-HT_{1A} receptor agonist properties in the rat ultrasonic vocalisation test (Bartoszyk et al., 1997) and antagonist properties in the 5-HT syndrome model (Page et al., 2002). The conclusion from these studies was that further voltammetric and/or electrophysiological studies in native tissue were required to confirm the 5-HT_{1A} receptor functional activity of vilazodone.

Recently, Page et al. (2002) reported that acute vilazodone administration increased extracellular 5-HT in the medial prefrontal cortex and ventral hippocampus of rats. Confirming this, Dawson et al. (2004) reported that acute vilazodone treatment increased extracellular 5-HT in rat frontal cortex. In the present study, the acute effects of vilazodone on 5-HT efflux and re-uptake in the guinea-pig dorsal raphe nucleus, were compared to those of the SSRI, fluoxetine, the 5-HT_{1A} receptor agonist, 8-OH-DPAT (±8-hydroxy-2-(di-n-propylamino)tetralin), and the 5-HT_{1A} receptor antagonist, WAY 100635. This study will investigate whether vilazodone induces a comparable increase in 5-HT levels in the dorsal raphe nucleus as it has been reported to in the serotonergic terminal fields. In addition, we will assess whether vilazodone has any 5-HT_{1A} receptor agonist or partial agonist activity in the dorsal raphe nucleus. Together this information will help in elucidating the therapeutic potential of this compound in the treatment of depression.

2. Materials and methods

WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl) cyclohexanecarboxamide), 8-OH-DPAT, 5-CT (5-carboxamidotryptamine) and fluoxetine were purchased from Sigma. Vilazodone (EMD-68843; 5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl}-benzo furan-2-carboxamide hydrochloride) was synthesised by Medicinal Chemistry, Merck KGaA (Darmstadt, Germany). Compounds were dissolved in a dimethylsulphoxide (DMSO): polyethyleneglycol mixture (1:1 v/v) to give a stock solution of 1 mM. Subsequent dilutions were made into artificial cerebrospinal fluid (aCSF: NaCl 120 mM; KCl 2.5 mM; $NaHCO_3$ 26 mM; NaH_2PO_4 1.2 mM; $MgCl_2$ 1.3 mM; $CaCl_2$ 2.4 mM; glucose 10 mM) such that the final percent DMSO content during the experiments was less than or equal to 0.1%.

2.1. In vitro fast cyclic voltammetry

Male Dunkin Hartley guinea-pigs (200–250 g) were killed by terminal anaesthesia (isoflurane) and decapitated. The brain was rapidly removed and a 400 μm brain slice containing the dorsal raphe nucleus was prepared under ice-cold "slicing" buffer (Aghajanian and Rasmussen, 1989; KCl 2.5 mM; $NaHCO_3$ 26 mM; $MgCl_2$ 5 mM; NaH_2PO_4 1.2 mM; $CaCl_2$ 0.1 mM; sucrose 189 mM; glucose 10 mM). The slice was allowed to recover in oxygenated aCSF at room temperature for 60 min. The slice was then transferred to a brain slice chamber where it was perfused at 2.5 ml min^{-1} with oxygenated aCSF at 32 °C. A stimulating (bipolar tungsten, 100 μm tip diameter, 150 μm tip separation) and voltammetric (carbon fibre, 8 μm tip width, 100 μm tip length) electrode was placed in the ventral dorsal raphe nucleus at a depth of 100 μm. The voltammetric electrode tip was positioned between those of the stimulating electrode, just off linearity.

A triangular voltage waveform (−1.0 to +1.4 V, 1.5 cycles at a rate of 480 V s^{-1}) was applied to the carbon fibre microelectrode (CFe). The voltammetric scan was applied at a frequency of 2 Hz, the current sampled at 525 mV and the signal fed into a chart recorder. 5-HT efflux was evoked by electrical stimulation at 100 Hz for 20 pulses, 10 mA and 0.1 ms pulse width. The maximum signal peak was taken as a measure of efflux and the time taken to reduce this maximum signal to half its value was taken as a measure of re-uptake half life. Data were expressed as a percentage of control levels, prior to drug perfusion.

2.2. Statistical analysis

Summary data were calculated as mean area under the curve (AUC) post drug perfusion. Statistical analysis was performed on the summary data using a one-way ANOVA (analysis of variance) followed by a post hoc *t*-test (Bonferroni). Significant effects were defined at the 5% level, i.e. $P<0.05$. To assess the number of animals needed in each group a power analysis was performed on the mean AUC. For the data reported in this study, group numbers of greater than 4 were not necessary.

3. Results

Control stimulation trains of 20 pulses at 100 Hz evoked the efflux of 55±4 nM 5-HT ($n=9$). The time taken for this efflux to reduce to half its maximum level ($T1/2$) was 0.83±0.05 s ($n=6$). Due to the nature of the fast cyclic voltammetry technique used in this study, it is necessary to confirm the origin of the signal being measured. Although the signal was recorded at the oxidation potential of 5-HT and displayed the characteristic faradaic current for 5-HT, additional controls were also employed. The 5-HT efflux was magnesium-sensitive, with 20 mM $MgCl_2$ significantly reducing 5-HT efflux to 35±3% of control ($n=5$). The ability of a high magnesium concentration to inhibit the evoked response confirmed that we were recording neurotransmitter release. The non-selective 5-HT receptor agonist, 5-CT (100 nM), also significantly inhibited 5-HT efflux to 48±6% of control ($n=3$) confirming that 5-HT release was being measured.

The 5-HT_{1A} receptor agonist, 8-OH-DPAT (100 nM), significantly inhibited 5-HT efflux to 41±2% of control ($n=6$; Fig. 1). In contrast, the 5-HT_{1A} receptor antagonist, WAY 100635 (100 nM), significantly increased 5-HT efflux to a maximum of 117±6% of control ($n=8$; Fig. 1). The 8-OH-DPAT-induced inhibition of efflux was significantly attenuated by WAY 100635 (Fig. 1).

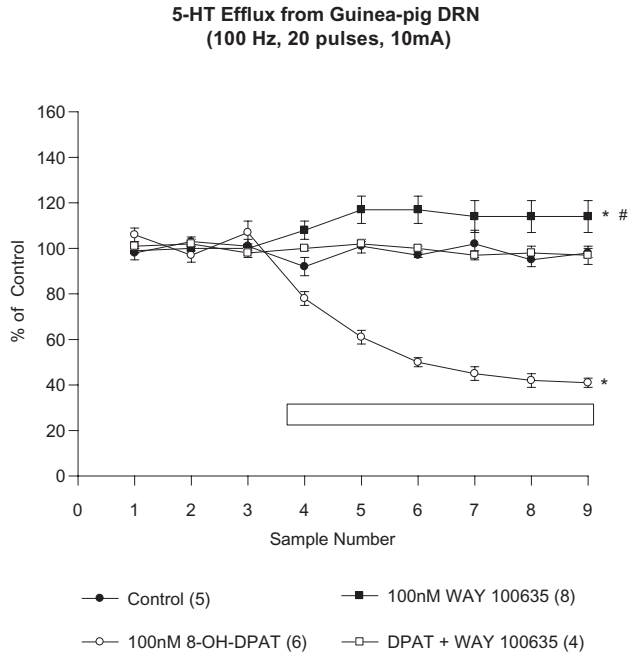

Fig. 1. The effect of 8-OH-DPAT (100 nM) and WAY 100635 (100 nM) on electrically-evoked 5-HT efflux from guinea-pig dorsal raphe nucleus slices. Bar denotes time of perfusion of drug(s). Figures in parentheses denote numbers per group. $^{*}P<0.05$ when comparing AUC with control and $^{\#}P<0.05$ when comparing to DPAT.

Fluoxetine significantly increased 5-HT efflux to 133±6% and 168±8% of control at 100 nM and 1 μM, respectively ($n=4$; Fig. 2). In contrast, vilazodone had no effect on 5-HT efflux at 100 nM but significantly decreased 5-HT efflux to 78±4% of control at 1 μM ($n=4$; Fig. 2). Co-perfusion of 8-OH-DPAT (100 nM) with fluoxetine (100 nM and 1 μM) significantly attenuated the fluoxetine-induced increase in 5-HT efflux (Fig. 3). Co-perfusion

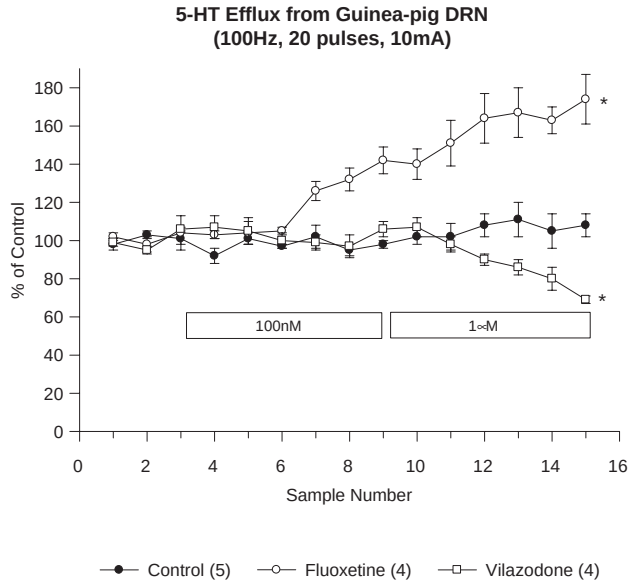

Fig. 2. The effect of fluoxetine (100 nM, 1 μM) and vilazodone (100 nM, 1 μM) on electrically-evoked 5-HT efflux from guinea-pig dorsal raphe nucleus slices. Figures in parentheses denote numbers per group. $^{*}P<0.05$ when comparing AUC with control.

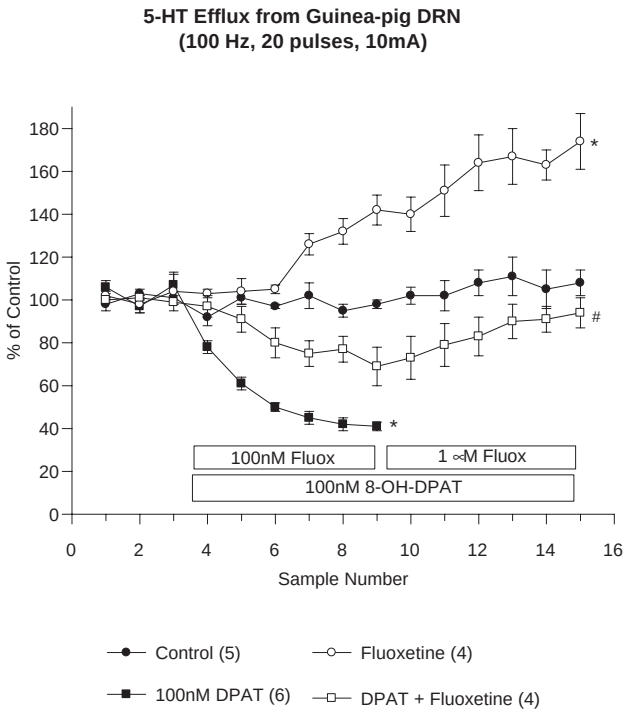

Fig. 3. The effect of 8-OH-DPAT (100 nM) and fluoxetine (100 nM, 1 μM) on electrically-evoked 5-HT efflux from guinea-pig dorsal raphe nucleus slices. Figures in parentheses denote numbers per group. $^{*}P<0.05$ when comparing AUC with control and $^{\#}P<0.05$ when comparing with Fluoxetine.

of WAY 100635 (100 nM) with vilazodone (100 nM and 1 μM) did not attenuate the effect of vilazodone alone (Fig. 4).

The re-uptake half life for 5-HT was significantly increased by fluoxetine to maxima of 151±8% and 219±27% of control at 100

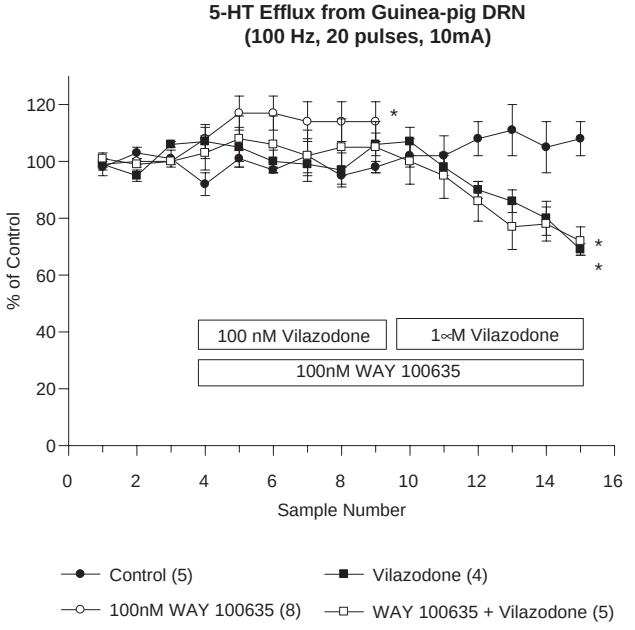

Fig. 4. The effect of WAY 100635 (100 nM) and vilazodone (100 nM, 1 μM) on electrically-evoked 5-HT efflux from guinea-pig dorsal raphe nucleus slices. Figures in parentheses denote numbers per group. $^{*}P<0.05$ when comparing AUC with control.

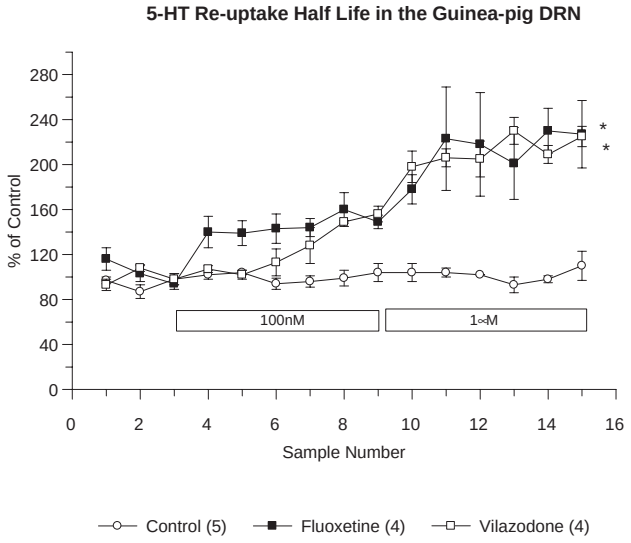

Fig. 5. The effect of fluoxetine (100 nM, 1 μM) and vilazodone (100 nM, 1 μM) on the re-uptake half life of 5-HT following electrical stimulation in guinea-pig dorsal raphe nucleus slices. Figures in parentheses denote numbers per group. *$P<0.05$ when comparing AUC with control.

nM and 1 μM, respectively ($n=4$; Fig. 5). Similarly, vilazodone significantly increased the re-uptake half life to maxima of 168±3% ($n=4$) and 217±8% of control ($n=4$) at 100 nM and 1 μM, respectively. The effects of fluoxetine and vilazodone were not significantly different.

4. Discussion

WAY 100635 and 8-OH-DPAT were used as reference compounds and demonstrated the expected activities of a 5-HT_{1A} receptor antagonist and agonist, respectively, in the guinea-pig dorsal raphe nucleus. Blockade of 5-HT_{1A} receptors with WAY 100635 had a small but significant effect on 5-HT efflux. This is in concordance with literature data (Hopwood and Stamford, 2001; Roberts et al., 2001) and indicates that there is a small amount of endogenous 5-HT tone present. In contrast, 5-HT_{1A} receptor agonists elicit large and significant decreases in 5-HT efflux, which can be fully attenuated with WAY 100635, as demonstrated in this present study.

The SSRI, fluoxetine, increased 5-HT efflux and prolonged the re-uptake half life of 5-HT in a concentration-dependent manner. This is in agreement with Davidson and Stamford (1995) and is a result of serotonin transporter (SERT) blockade slowing the re-uptake of 5-HT into the neurone and hence increasing synaptic 5-HT levels. In contrast, vilazodone did not increase 5-HT efflux. Instead, the SERT activity of vilazodone was confirmed by the demonstration that this compound increased the re-uptake half life of 5-HT in a concentration-dependent manner and to a level that was comparable to that seen with fluoxetine. The inability of vilazodone to increase 5-HT efflux may be explained if the SSRI-induced increases were attenuated by direct or indirect activity at another receptor, such as 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1D} autoreceptors.

There are many reports in the literature where increases in 5-HT release can be modulated via activation of 5-HT autoreceptors, such as 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors (Roberts et al., 1999; Stamford et al., 2000; Hopwood and Stamford, 2001; Roberts et al., 2001; Roberts and Price, 2001). Data in this study demonstrate that a 5-HT_{1A} receptor agonist attenuated the fluoxetine-induced increase in 5-HT efflux. Literature reports have shown that vilazodone has high affinity at 5-HT_{1A} receptors (Bartoszyk et al., 1997). However, there are conflicting reports on whether functionally it acts as a 5-HT_{1A} receptor agonist or antagonist. Thus, Page et al. (2002) demonstrated that 100 nM vilazodone stimulated [^{35}S]-GTPγS binding in human cloned 5-HT_{1A} receptors, indicating that it was a 5-HT_{1A} receptor partial agonist with intrinsic activity of 0.7. In contrast, the same group failed to show a vilazodone-induced 5-HT syndrome in vivo and in fact, vilazodone attenuated an 8-OH-DPAT-induced 5-HT syndrome, indicating that it had 5-HT_{1A} receptor antagonist activity (Page et al., 2002).

Recent investigations in our group have confirmed that vilazodone is a 5-HT_{1A} receptor agonist in cloned h5-HT_{1A} receptors (unpublished observations). In addition, vilazodone has been shown to have partial agonist activity in rat hippocampus stimulating [^{35}S]-GTPγS binding (unpublished observations). However, Dawson et al. (2004) failed to demonstrate any 5-HT_{1A} receptor agonist or partial agonist activity in vivo. In agreement with this, the inability of WAY 100635 to attenuate the vilazodone effect on 5-HT efflux in the present study suggested that it did not exhibit 5-HT_{1A} receptor agonism in guinea-pig dorsal raphe nucleus.

Although we have shown that the SSRI-induced increase in 5-HT efflux can be attenuated with co-perfusion of a 5-HT_{1A} receptor agonist, the inability of vilazodone to increase 5-HT efflux did not result from activation of 5-HT_{1A} receptors. As vilazodone does not have appreciable affinity at the other 5-HT autoreceptors, its failure to increase 5-HT efflux may be via an indirect agonist action at 5-$HT_{1B/1D}$ autoreceptors. That is, the increase in 5-HT elicited through 5-HT re-uptake blockade may activate 5-HT_{1B} or 5-HT_{1D} receptors to decrease 5-HT release.

In conclusion, we have demonstrated that vilazodone (100 nM, 1 μM), in the guinea-pig dorsal raphe nucleus, blocks the serotonin transporter but is not a 5-HT_{1A} receptor partial agonist. The failure of vilazodone to increase 5-HT efflux in the dorsal raphe nucleus will potentially ensure maximal synaptic levels of 5-HT in forebrain regions, by negating activation of 5-HT autoreceptors in the cell body, which would have attenuated SSRI-induced increases of terminal 5-HT release in forebrain regions.

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