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Occupancy of Agonist Drugs at the 5-HT_{1A} Receptor

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Drugs acting on the 5-HT_{1A} receptor are used in the treatment of depression, generalized anxiety disorder, and schizophrenia. This study investigated 5-HT_{1A} receptor occupancy by the 5-HT_{1A} agonist drugs flesinoxan (a highly selective probe for the 5-HT_{1A} receptor) and ziprasidone (a novel atypical antipsychotic drug). Using a within-subject design, 14 healthy volunteers each received two positron emission tomography scans using the selective 5-HT_{1A} antagonist radiotracer [^{11}C]WAY-100635. One scan constituted a baseline, while the other followed either 1 mg flesinoxan or 40 mg ziprasidone orally. In addition, rats were pretreated with intravenous flesinoxan at doses ranging from 0.001 to 5 mg/kg then [^{11}C]WAY-100635 binding measured *ex vivo*. Cerebral cortical and hippocampal regions of interest, and cerebellar reference regions were sampled to estimate 5-HT_{1A} receptor occupancy (inferred from reductions in specific radioligand binding). In man, occupancy was not significant despite volunteers experiencing side effects consistent with central serotonergic activity. The mean cerebral cortex occupancy ($\pm$1 SD) for flesinoxan was 8.7% ($\pm$13%), and for ziprasidone 4.6% ($\pm$17%). However, in rats, flesinoxan achieved significant and dose-related occupancy (17–57%) at 0.25 mg/kg and above. We conclude that 5-HT_{1A} receptor agonists produce detectable occupancy only at higher doses that would produce unacceptable levels of side effects in man, although lower doses are sufficient to produce pharmacological effects. The development of agonist radiotracers may increase the sensitivity of detecting agonist binding, as 5-HT_{1A} antagonists bind equally to low- and high-affinity receptor states, while agonists bind preferentially to the high-affinity state.

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INTRODUCTION

The 5-HT_{1A} receptor is implicated in the pathophysiology of depression and schizophrenia and is a target for conventional and novel psychotropic drugs. It is an inhibitory G-protein coupled receptor that is located both presynaptically, as autoreceptors in the raphe nuclei, and postsynaptically, as heteroreceptors at highest density in the hippocampus and neocortex. In depression, reductions in 5-HT_{1A} receptor density have been found in a number of cortical regions using [^{11}C]WAY-100635 PET imaging (Drevets *et al*, 2000; Sargent *et al*, 2000), while in schizophrenia increases in receptor density have been found in prefrontal cortical regions using post-mortem techniques (reviewed in Bantick *et al*, 2001) although not with PET (Lombardo *et al*, 2002; Tauscher *et al*, 2002; Bantick *et al*, 2004).

Many psychiatric drugs modulate serotonergic transmission, and several specifically target the 5-HT_{1A} receptor (Table 1). The 5-HT_{1A} antagonist/weak partial agonist pindolol has been used, with mixed success, to augment and reduce the latency of response to selective serotonergic reuptake inhibitors in the treatment of depression (reviewed in Martinez *et al*, 2000). It facilitates increases of 5-HT in the cortical and limbic projection areas by blocking raphe autoreceptors that are otherwise stimulated by raised levels of 5-HT. The 5-HT_{1A} partial agonist buspirone is effective in the treatment of generalized anxiety disorder (Gale and Oakley-Browne, 2002). Studies in animal models suggest that it may act by stimulating raphe 5-HT_{1A} receptors (De Vry, 1995), so reducing serotonergic overactivity in the projection pathways, although the delay in action suggests that adaptive mechanisms are important. Among the antipsychotic drugs, clozapine and quetiapine have moderate affinity as 5-HT_{1A} partial agonists, while the novel drugs ziprasidone and aripiprazole are potent 5-HT_{1A} partial agonists (Jordan *et al*, 2002; Newman-Tancredi *et al*,

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Table 1 Properties of Compounds at the 5-HT$_{1A}$ Receptor Determined Using Transfected Chinese Hamster Ovary (CHO) Cells Stably Expressing Recombinant Human (h) 5-HT$_{1A}$ Receptors (Data from Newman-Tancredi *et al*, 1998a, b)

	Affinity (nM)[a]	Agonist efficacy: E_{max} (%)[b]
(+)Flesinoxan	0.54 ± 0.11	94.3 ± 10.4
(±)8-OH-DPAT	0.58 ± 0.03	75.7 ± 3.2
5-HT	0.61 ± 0.15	100
Ziprasidone	1.24 ± 0.06	54.9 ± 4.2
Ipsapirone	2.5 ± 0.5	49.0 ± 3.6
Tandospirone	5.9 ± 1.1	100.5 ± 0.7
(−)Pindolol	6.4 ± 1.2	20.3 ± 2.1
Buspirone	8.9 ± 1.2	65.4 ± 4.4
Clozapine	132 ± 30	53.3 ± 4.0
Quetiapine	250 ± 36	60.4 ± 10.9

[a]K_i values from competition experiments using [^{3}H]8-OH-DPAT. Results expressed as means ± SEM of at least three independent determinations. [b]Using a receptor-mediated G-protein activation method (stimulation of [^{35}S]GTPγS binding—a nonhydrolyzable GTP analog), and expressed relative to 5-HT (= 100%). This models activation of native hippocampal 5-HT$_{1A}$ receptors—agonist efficacies at presynaptic receptors are likely to be greater because of receptor reserve.

1998b). This is of interest because 5-HT$_{1A}$ agonists reduce neuroleptic-induced catalepsy and increase dopamine release in the frontal cortex in animals. In schizophrenia, they may therefore have efficacy against neuroleptic-induced extrapyramidal side effects, negative symptoms, and perhaps cognitive impairment (reviewed in Bantick *et al*, 2001; Millan, 2000; Sharma and Shapiro, 1996).

A drug's *in vitro* affinity for a receptor can be translated into adequate *in vivo* brain receptor occupancy if it has favorable absorption, metabolism, and passage across the blood–brain barrier. Using PET, brain receptor occupancy of clinical doses of many novel and conventional psychotropic drugs has been measured. Relationships can be described with the administered dose, plasma concentration, and clinical effects. The use of the highly selective 5-HT$_{1A}$ receptor radioligand, [*carbonyl*-^{11}C]WAY-100635, to measure occupancy of 5-HT$_{1A}$ receptors by 5-HT$_{1A}$ antagonists is well established (see Discussion). The occupancy achieved by clinical doses of 5-HT$_{1A}$ receptor *agonists* in man is unclear. This study investigated the 5-HT$_{1A}$ receptor occupancy of the agonist drugs flesinoxan and ziprasidone in man using [^{11}C]WAY-100635 PET scanning, and of flesinoxan in rats using *ex vivo* [^{11}C]WAY-100635 binding.

Flesinoxan is a high-affinity selective 5-HT$_{1A}$ receptor agonist. It is a substituted benzamide with over 40-fold selectivity for the 5-HT$_{1A}$ receptor over the next highest affinity receptor, the D$_3$ receptor (Solvay Pharmaceuticals, 1997). Flesinoxan is particularly useful as a pharmacological probe as it does not have pharmacologically active metabolites, unlike the azapirone 5-HT$_{1A}$ agonists such as buspirone which have the metabolite 1-PP (1-(2-pyrimidinyl)-piperazine), an α_2-adrenoreceptor antagonist. In rats, flesinoxan suppresses firing activity in dorsal raphe serotonergic neurons and dorsal hippocampus pyramidal neurons, and crosses the blood–brain barrier, albeit relatively slowly (Hadrava *et al*, 1995). In man, 1 mg intravenous flesinoxan produces a 5-HT$_{1A}$ agonist profile: it significantly elevates adrenocorticotropic hormone, cortisol, growth hormone, and prolactin while reducing temperature (Pitchot *et al*, 2002; Seletti *et al*, 1995). A 1 mg *oral* dose behaves similarly but does not elevate prolactin (de Koning and de Vries, 1995). Results from phase II studies for depression and generalized anxiety disorder have been encouraging: those from phase III studies are not yet available (Pitsikas, 1999).

Ziprasidone, a benzisothiazolylpiperazine, is a novel atypical antipsychotic drug with high affinity for the D$_2$ (K_i = 3.1 nM), D$_3$ (K_i = 7.2 nM), 5-HT$_{2A}$ (K_i = 0.39 nM), 5-HT$_{2C}$ (K_i = 0.72 nM) and 5-HT$_{1B/1D}$ (K_i = 2.0 nM) receptors as an antagonist, and for the 5-HT$_{1A}$ receptor (K_i = 2.5 nM) as an agonist (Schmidt *et al*, 2001; Seeger *et al*, 1995). It has moderate affinity for the α_1 and H$_1$ receptors. In rats, ziprasidone inhibits DRN firing (Sprouse *et al*, 1999) and increases prefrontal cortical dopamine release (Rollema *et al*, 2000). The former effect is prevented and the latter roughly halved by the 5-HT$_{1A}$ antagonist WAY-100635. In human PET studies, 40 mg oral ziprasidone occupies ~72% D$_2$ receptors using the D$_2$ antagonist radiotracer [^{11}C]raclopride (Bench *et al*, 1996) and ~95% 5-HT$_{2A}$ receptors using the 5-HT$_{2A}$ antagonist radioligand [^{18}F]setoperone (Fischman *et al*, 1996) 4–8 h post-dose. In patients with acute exacerbations of schizophrenia and schizoaffective disorder, randomized double-blind studies have shown ziprasidone to have efficacy superior to placebo (Daniel *et al*, 1999; Keck *et al*, 1998) and similar to haloperidol (Goff *et al*, 1998). In stable patients, it reduces relapse rate compared to placebo (Arato *et al*, 2002) and appears to have higher efficacy against negative symptoms than haloperidol (Hirsch *et al*, 2002). These studies have demonstrated a low incidence of movement disorders in patients treated with ziprasidone.

MATERIALS AND METHODS

Estimation of Flesinoxan Occupancy in Man Using [^{11}C]WAY-100635 PET Scanning

Six healthy male volunteers aged between 36 and 52 years (mean 47.2 years) were recruited by newspaper advertisement. Subjects were screened using questionnaires, an interview by a qualified psychiatrist and a physical examination. Exclusion factors were a General Health Questionnaire score above 5 (GHQ: 28 item self rated), Beck Depression Inventory score above 9 (BDI: 21 item self-rated), current or past psychiatric or neurological illness, current substance misuse, and any medication that could interfere with the study. The study was carried out in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local research ethics committee and permission to administer radioactivity was provided by the Administration of Radioactive Substances Advisory Committee (ARSAC). All volunteers gave written informed consent.

Each subject acted as his own control and received two PET scans on average 32 days apart (SD = 37 days) using

[^{11}C]WAY-100635 and an ECAT 953B PET scanner (CTI/Siemens, Knoxville, TN, USA). Subjects were asked to avoid alcohol for at least 24 h before each scan. Using a single-blind balanced order design, a tablet of either placebo (baseline scan) or 1 mg flesinoxan (flesinoxan scan) was administered 90 min before the start of the dynamic emission scan. Flesinoxan reaches maximum plasma concentration after 1–2 h, and single doses have a half-life of 5.5 h (Solvay Pharmaceuticals, 1997).

The ECAT 953B scanner has an axial field of view of 10.65 cm (Spinks *et al*, 1992). The mean spatial resolution is 5.8 mm (x) × 5.8 mm (y) × 5.9 mm (z) at full-width at half-maximum. The [*carbonyl*-^{11}C]WAY-100635 radioligand was prepared on site by carboxylation of Grignard Reagent (McCarron *et al*, 1996). Volunteers were positioned in the scanner such that a line running from the inferior edge of the orbit to the tragus was parallel to the planes of the detector rings. Head movement was minimized using a strap and padded head mold and could be detected using laser crosshairs and video camera monitoring. A 5 min scout transmission scan was performed to ensure complete inclusion of the cerebellar reference region in the field of view. A 10 min transmission scan was then performed in order to correct for tissue attenuation of 511 keV gamma photons. Subjects then received an average of 340 MBq [^{11}C]WAY-100635 (SD 51 MBq), with radiochemical purity exceeding 97%, intravenously through a cannula in the antecubital fossa over 20 s. The amounts of unlabeled WAY-100635 and precursor (WAY-100634) injected were measured using HPLC with on-line radioactivity and UV detection systems. A 21 frame emission scan was performed in 3D mode over 90 min 30 s with frames: 1 × 30 (background), 1 × 15, 1 × 5, 1 × 10, 1 × 30, 4 × 60, 7 × 300, and 5 × 600 s. Scans were scatter-corrected using a dual-energy window method and reconstructed using a reprojection algorithm. The top and bottom two planes of the 31 plane PET images were excluded from analysis as they are affected by higher levels of noise.

Venous blood samples were taken at a number of time points. The relative amount of parent [^{11}C]WAY-100635 remaining in the plasma was determined from plasma samples taken 1000, 2000, 3000, and 4000 s after the start of the dynamic emission scan, T, in order to check that flesinoxan did not affect the rate of [^{11}C]WAY-100635 metabolism. Flesinoxan, cortisol, and growth hormone (GH) levels were determined from samples taken just before flesinoxan administration (baseline) then at T−300, T + 2000, T + 4000, and T + 5400 s. The drug levels were measured in plasma using an HPLC method, and the neuroendocrine tests in serum using standard radioimmunoassay techniques. After the scan, patients were asked whether they had experienced any physical or psychological symptoms, and to rate severity as mild, moderate, or severe (given scores of 1, 2, and 3, respectively). Although flesinoxan is well tolerated, dizziness, and nausea occur more often than with placebo (Solvay Pharmaceuticals, 1997).

Estimation of Ziprasidone Occupancy in Man Using [^{11}C]WAY-100635 PET Scanning

Nine healthy volunteers were included in the study, aged between 31 and 57 years (mean age = 43 years). Subject recruitment and screening were as above, except that additional screening was performed using an electrocardiogram (exclusion if QT(ms)/RR(s)$^{0.5}$ > 440, or QT > 450 ms for males and > 470 ms for females) and plasma electrolytes (exclusion if $K^+ < 3.5$ mmol/l or $Mg^{2+} < 0.70$ mmol/l or corrected $Ca^{2+} < 2.15$ mmol/l), given the potential for ziprasidone to prolong the ECG QT interval (Glassman and Bigger, 2001).

Each subject acted as his or her own control and received two PET scans (baseline, and after ziprasidone, in variable order) on average 18 days apart (SD = 13 days) using [^{11}C]WAY100635 and an ECAT 966/EXACT3D PET scanner (CTI/Siemens, Knoxville, TN, USA). Subjects were asked to avoid alcohol for at least 24 h before each scan. For the ziprasidone scan, 40 mg ziprasidone was administered orally with a light meal (to enhance absorption) 4 h before the start of the dynamic emission scan. In the United States, the recommended starting dose of ziprasidone is 20 mg twice daily, while in Europe it is 40 mg twice daily (Gunasekara *et al*, 2002). The maximum dose is 80 mg twice daily in both regions. Maximum plasma concentration is reached after 4–5 h for a single dose of ziprasidone taken with food, and the half-life is 3.2–4.8 h (Miceli *et al*, 2000).

The ECAT 966 scanner has an axial field of view of 23.4 cm (Spinks *et al*, 2000). The mean spatial resolution is 5.1 mm (x) × 5.1 mm (y) × 5.9 mm (z) at full-width at half-maximum. The radioligand preparation and subject positioning were as above. A 5 min transmission scan was performed in order to correct for tissue attenuation. Subjects then received an average of 280 MBq [^{11}C]WAY-100635 (SD 16 MBq) with radiochemical purity exceeding 97% intravenously through a cannula in the antecubital fossa over 20 s. The amounts of unlabeled WAY-100635 and precursor injected were measured. The dynamic emission scan was obtained in list mode in 3D over 95 min. Post-acquisition rebinning was performed to produce a 23 frame dynamic image comprising 1 × variable length background frame, and 1 × 15, 3 × 5, 2 × 15, 4 × 60, 7 × 300, and 5 × 600 s frames (ie background + 90 min). Scans were scatter corrected using a model-based method then reconstructed using a reprojection algorithm. The top 15 and bottom 10 planes of the 95 plane PET images were excluded from analysis.

Venous blood samples were taken at a number of time points. The relative amount of parent [^{11}C]WAY-100635 remaining in the plasma was determined from plasma samples taken 1000, 1800, and 3000 s after the start of the dynamic emission scan, T. Ziprasidone levels were determined using an HPLC method from serum samples taken at T−300, T + 1800, T + 3600, and T + 5400 s during the ziprasidone scan.

PET Scan Analysis

This was performed by one investigator (RAB) blind to condition and was similar for both the flesinoxan and ziprasidone studies.

1. *Definition of regions*: The regions used were bilateral and comprised the cerebellar hemispheres (elliptical regions within each hemisphere), the entire cerebral cortex, the

frontal cortex (excluding the precentral gyri), the medial temporal cortex (hippocampi, amygdalae, and parahippocampal gyri), and the raphe. The cerebellar regions and cortical regions were contained within three-dimensional anatomical atlases drawn on the single subject MRI (in standard space). Using SPM99 software (Wellcome Department of Cognitive Neurology, University College, London), each atlas was normalized onto each patient's [^{11}C]WAY-100635 PET scan. The transformation matrix to achieve this was determined by normalizing an averaged [^{11}C]WAY-100635 scan template in standard space onto the patient's PET scan (see Rabiner *et al*, 2002b for full details). This automated template method enables the unbiased automatic placement of standard regions onto a quantitative PET image. The normalized regions were checked visually by superimposing them over an integral image of the subject's scan. In two individuals in the ziprasidone study, part of the cerebellum defined by the template fell in lower planes that were not used in the analysis. For these subjects, a manual cerebellar region was therefore used. This was a standard ellipse similar to the atlas region, positioned bilaterally on at least three consecutive planes (matched for both of a given subject's scans) using an integral image from the first 15 min of the scan in transverse orientation and Analyze version 4.0 software (Biomedical Imaging Resource, Mayo Foundation). The raphe regions are not well defined by MRI and were demarcated for each subject using an integral image from the last 75 min of the dynamic emission scan. Ellipses of standard size and orientation were manually positioned on four consecutive planes in sagittal orientation.

2. *Generation of time—activity curves using cerebellar hemisphere regions*: Each dynamic PET image was sampled using the cerebellar hemisphere regions, and time–activity curves (TACs) generated. Given the importance of the cerebellum as the reference region (see below), outliers were sought. Cerebellar activity values were converted into standard uptake values (decay-corrected activity/[injected dose of radioligand/subject weight]) and plotted against time, and any individual with a curve deviating by more than 2 SDs from the mean at 50% or more time points was excluded from the study.
3. *Generation of parametric images*: A simplified reference tissue model (SRTM) was used to determine binding potential (BP) and ratio of influx (R_I) in a voxel by voxel manner for each subject using a basis function method (Gunn *et al*, 1997, 1998). The cerebellar hemispheres are a suitable reference region as they are devoid of specific 5-HT$_{1A}$ receptor binding (Pazos *et al*, 1987; Pike *et al*, 1996). $BP = f_2\ B_{max}/K_D\ [1 + \Sigma\ (F_i/K_{Di})]$ where f_2 is the 'free fraction' of the unbound radioligand in tissue, B_{max} is the concentration of receptors, K_D is the equilibrium dissociation constant of the radioligand and F_i and K_{Di} are the free concentration and equilibrium dissociation constant of the competing drug. R_I (ratio of influx) is a measure of the relative delivery of radiotracer at a given site compared to the reference region. The f_2 and K_D values are assumed to remain constant between scans for a given subject.
4. *Region of interest (ROI) analysis*: The cortical and raphe ROIs were used to sample BP and R_I parametric maps from each subject. Percentage occupancy was calculated as follows: $\text{Occupancy} = ([BP_{baseline} - BP_{drug}]/BP_{baseline}) \times 100\%$.

Estimation of Flesinoxan Occupancy in Rats Using [^{11}C]WAY-100635 *Ex Vivo* Radioligand Binding

The work was carried out by licensed investigators according to the Home Office's 'Guidance in the Operation of Animals (Scientific Procedures) Act 1986.' Five adult male Sprague–Dawley rats were used in the control group and 14 in the experimental group. These latter animals were pretreated with intravenous flesinoxan at doses ranging from 0.001 to 5 mg/kg 5 min before the radioligand injection. [^{11}C]WAY 100635 was administered intravenously to each rat at a dose of ~10 MBq at experimental time zero in 0.2 ml via a previously catheterized tail vein. The rats were awake but lightly restrained in Bollman cages. The mean specific radioactivity at the time of injection was ~60 GBq/μmol which, using published data for *in vivo* saturation kinetics, can be estimated to result in a receptor occupancy of ~10% (Hume *et al*, 1998). At 60 min post-radioligand injection, rats were given intravenous Euthatal then the brains rapidly removed and the cerebellum, prefrontal with cingulate cortex and hippocampus dissected out. Blood was collected from five rats in the experimental group via a previously catheterized tail artery for measurement of plasma flesinoxan levels. Radioactivity was counted using a Wallac gamma-counter with automatic correction for radioactivity decay. Data were normalized for injected radioactivity (in counts per minute) and body weight, with 'uptake units' = (cpm/g tissue weight)/(injected cpm/g body weight).

Statistics

Tests were performed using SPSS for Windows version 11.

For both PET studies, the following analyses were made. The injected dose, specific activity, radiochemical purity, and amount of unlabeled WAY-100635 and precursor injected were compared between conditions using independent *t*-tests. In order to confirm that the active drug did not affect the cerebellar time–activity curve (with the activity expressed as SUVs), baseline scan values were compared with active drug scan values using repeated measures analysis of variance (ANOVA). The relative amounts of parent [^{11}C]WAY-100635, and the drug and neuroendocrine levels were converted to areas under the curves (AUCs) using a trapezoid method, and those for growth hormone and cortisol were baseline corrected. The AUC results were then compared between conditions using two-tailed paired *t*-tests, or a nonparametric method if the sample deviated significantly from normality as assessed by the Lilliefors (Kolmogorov–Smirnov) test. Regional BP and R_I results were analyzed with repeated measures ANOVA. Greenhouse–Geisser correction was applied to ANOVAs when indicated by Mauchly's test of sphericity.

For the ziprasidone study, a relationship was sought between global cortical 5-HT$_{1A}$ receptor occupancy and ziprasidone level AUCs in the active drug group using Pearson's product moment correlation.

For the *ex vivo* radioligand binding rat study, ratios of ROI to cerebellar [^{11}C]WAY-100635 binding in uptake units (ROI/Cb) were calculated to reflect specific 5-HT$_{1A}$ receptor binding. The following equation was used for occupancy:

$$\text{Occupancy}\,\% = 100\% \times [(\text{ROI/Cb})_{\text{control}} - (\text{ROI/Cb})_{\text{flesinoxan}}]/(\text{ROI/Cb})_{\text{control}}$$

RESULTS

Estimation of Flesinoxan Occupancy in Man Using [^{11}C]WAY-100635 PET Scanning

No subjects were excluded on the basis of their cerebellar time–activity (SUV) curve. There were no between-treatment differences in the five radiochemical parameters (data not shown). Comparing the baseline and active drug scans, there was no between-treatment difference in the cerebellar time–activity (SUV) curves [$(F1, 5) = 0.17$; $p = 0.70$] and no treatment by time interaction, $F(19, 95) = 0.68$, $\varepsilon = 0.13$, $p = 0.55$]. There were no between-treatment differences in the AUCs for parent [^{11}C]WAY-100635, ($t = 0.33$, df = 3, $p = 0.98$), growth hormone (significant deviation from normality in one group with outlier present, thus Sign test used: $p = 0.38$) or cortisol ($t = -1.1$, df = 5, $p = 0.34$). Headache, sleepiness, and lightheadedness were reported by subjects and were more common in the flesinoxan group (group score 6) than the control group (group score 2). There was no between-treatment difference for either the binding potential (Table 2 and Figure 1), $F(1, 5) = 1.8$, $p = 0.24$, or the ratio of influx, $F(1, 5) = 0.34$, $p = 0.58$, and no region by treatment interaction, $F(3, 15) = 1.5$, $p = 0.26$ and $F(3, 15) = 2.0$, $p = 0.16$ respectively. The mean occupancy of flesinoxan at the 5-HT$_{1A}$ receptor (± 1 SD) was 8.7% ($\pm 13\%$) for the total cerebral cortex (Table 2).

Estimation of Ziprasidone Occupancy in Man Using [^{11}C]WAY-100635 PET Scanning

One subject was excluded from the study as his cerebellar time–activity curves fell more than 2 SDs above the mean for 60% (baseline scan) and 50% (ziprasidone scan) of the data points, leading to exceptionally low binding potential values. There were no between-treatment differences in the five radiochemical parameters (data not shown). Comparing the baseline and active drug conditions, there was no between-treatment difference in the cerebellar time–activity (SUV) curves, $F(1, 7) = 0.11$; $p = 0.75$, and no treatment by time interaction, $F(21, 147) = 1.5$, $\varepsilon = 0.095$, $p = 0.27$. There was no difference between conditions in the AUCs for parent [^{11}C]WAY-100635, ($t = -0.46$, df = 4, $p = 0.67$). All volunteers experienced mild or moderate somnolence with ziprasidone, and three of the eight experienced additional minor side effects. There was no between-treatment difference for either the binding potential (Table 3 and Figure 1), $F(1, 7) = 0.54$, $p = 0.49$, or the ratio of influx, $F(1, 7) = 0.44$, $p = 0.53$, and no region by treatment interaction, $F(3, 21) = 0.17$, $\varepsilon = 0.55$, $p = 0.81$ and $F(3, 21) = 0.11$, $\varepsilon = 0.41$, $p = 0.80$, respectively. The mean occupancy of ziprasidone at the 5-HT$_{1A}$ receptor (± 1 SD) was 4.6% ($\pm 17\%$) for the total cerebral cortex (Table 3). Ziprasidone levels achieved were similar to those of a single-dose pharmacokinetic study in healthy volunteers (Miceli *et al*, 2000). However, the ziprasidone level AUCs did not correlate with global cortical 5-HT$_{1A}$ receptor occupancy values ($r = -0.16$, $n = 8$, $p = 0.71$).

Estimation of Flesinoxan Occupancy in Rats Using [^{11}C]WAY-100635 *Ex Vivo* Radioligand Binding

A dose–occupancy curve was plotted with a log $_{10}$ dose axis (Figure 2). Consistent occupancy occurred at a dose of 0.25 mg/kg and above. A two-tailed independent *t*-test comparing experimental group animals that had received at least this dose of flesinoxan ($n = 6$) with the control group showed a highly significant difference for both the prefrontal/cingulate cortex ($t = 4.3$; df = 7; $p < 0.01$) and hippocampus ($t = 5.5$; df = 7; $p < 0.01$).

DISCUSSION

The PET studies in man did not identify significant occupancy of the 5-HT$_{1A}$ receptor by the 5-HT$_{1A}$ receptor agonists flesinoxan or ziprasidone at the doses used. However, in rats, at doses higher than those tolerable in man, significant occupancy was observed by flesinoxan using an *ex vivo* radioligand binding method.

The possibility that mechanisms other than 5-HT$_{1A}$ receptor occupancy by flesinoxan or ziprasidone could have produced confounding effects that may have masked 'true' occupancy was considered unlikely because the drugs did not modify the time–activity curves of the cerebellar reference region, or the proportion of parent [^{11}C]WAY-100635 remaining over time, or region of interest blood flow relative to the reference region (R_I).

Compared to the alternative plasma input function method (PIFM), the SRTM underestimates BP values, particularly in regions of higher receptor density (Oikonen *et al*, 2000; Parsey *et al*, 2000; Slifstein *et al*, 2000). It may thus be less sensitive—a concern when low levels of occupancy are being investigated. However, the SRTM avoids arterial cannulation and it is highly reproducible in all cortical areas (Gunn *et al*, 2000, 1998): test–retest variability is $\sim$12% (Rabiner *et al*, 2002b). Furthermore, BP values are little affected by changes in regional blood flow over the physiological range (Gunn *et al*, 2000). The SRTM *does* reliably detect 5-HT$_{1A}$ receptor occupancy by antagonist drugs (Andree *et al*, 2003; Rabiner *et al*, 2000a, 2002c), and reduced 5-HT$_{1A}$ receptor density in depression (Drevets *et al*, 2000; Sargent *et al*, 2000) similarly to corresponding studies using the PIFM (Mann *et al*, 2002; Martinez *et al*, 2001).

Previous PET studies have established that ziprasidone penetrates the brain at the dose used in this study: high levels of occupancy have been observed at both the D_2 receptor (Bench *et al*, 1996) and the 5-HT$_{2A}$ receptor (Fischman *et al*, 1996) as discussed in the introduction. Flesinoxan penetrates the brain in rats, albeit much less efficiently than the prototypical 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT (Hadrava *et al*, 1995). This relatively poor penetration appears to arise because flesinoxan is a substrate for P-glycoprotein,

Table 2 Flesinoxan PET Study Subject Description, Drug/Neuroendocrine Levels and [^{11}C]WAY-100635 Binding Results. SD = standard deviation

				Drug/neuroendocrine levels expressed as AUCs								
					Over 180 min and baseline corrected		Binding potential values				Percentage occupancy at 5-HT$_{1A}$ receptor	
Subject	Age	Scan treatment (+scan order)[a]	Side effects[b]	Flesinoxan (min ng/ml) over 95 min[c]	Growth hormone (min miu/l)	Cortisol (min μg/100 ml)	Total cerebral cortex (postsynaptic)	Frontal cortex region	Medial temporal cortex	Raphe (presynaptic)	Total cerebral cortex (postsynaptic)	Raphe (presynaptic)
1	51	P (2nd)	H+	—	0	78	4.3	3.8	6.2	2.8	25.3	9.1
		F (1st)	H+ S+	485	0	−388	3.2	3.0	4.8	2.6		
2	52	P (1st)		—	548	29	4.6	4.4	5.8	3.6	2.7	9.9
		F (2nd)		833	64	655	4.5	4.3	5.7	3.3		
3	47	P (2nd)		—	1722	−1258	4.7	4.3	7.1	3.8	24.7	28.3
		F (1st)	L+	347	545	−1146	3.5	3.2	5.3	2.7		
4	45	P (1st)		—	0	−241	4.2	4.0	5.8	3.4	5.3	−2.9
		F (2nd)		762	364	706	4.0	3.8	5.8	3.5		
5	52	P (1st)	S+	—	−39	−352	3.6	3.2	5.2	2.7	−7.6	−17.9
		F (2nd)	S++	N/A	−406	−480	3.9	3.7	5.9	3.2		
6	36	P (2nd)		—	39	−1324	4.2	3.8	6.0	3.0	2.0	−0.2
		F (1st)	L+	N/A	−22	−1081	4.1	3.9	5.8	3.0		
Mean	47	P	0.3	—	567	−348	4.3	3.9	6.0	3.2	8.7	4.4
		F	1.0	607	243	−43	3.9	3.6	5.5	3.1		
SD	6.2	P	0.5	—	812	622	0.4	0.4	0.6	0.4	13.3	15.5
		F	0.9	229	256	891	0.4	0.5	0.4	0.4		

[a]P = placebo; F = flesinoxan.
[b]H = headache, S = somnolence, L = lightheadedness; + = mild, ++ = moderate, +++ = severe. Scores are used for mean and SD rows.
[c]Note flesinoxan is approximately 90% bound to human plasma proteins *in vitro* (Solvay Pharmaceuticals, 1997).

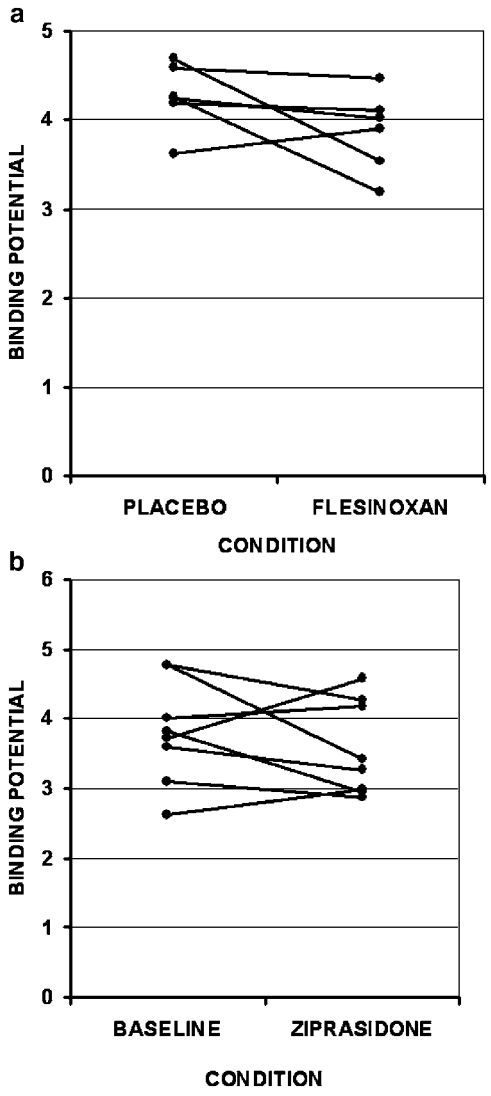

Figure 1 Binding potential value changes after flesinoxan (1a: $n=6$) and ziprasidone (1b: $n=8$).

a multidrug transporter, which actively effluxes it from the brain at the blood–brain barrier (van der Sandt *et al*, 2001). In man, the evidence that flesinoxan crosses the blood–brain barrier is indirect: it causes elevation of ACTH, cortisol, and growth hormone while reducing temperature by mechanisms that are believed to be centrally mediated (de Koning and de Vries, 1995; Pitchot *et al*, 2002; Seletti *et al*, 1995). Furthermore, a single 0.5 mg dose produces similar EEG activity to 20 mg buspirone (Solvay Pharmaceuticals, 1997). We did not find the significant increases in GH (actually reduced) and cortisol previously described with 1 mg oral flesinoxan (de Koning and de Vries, 1995). This is presumably because of our smaller sample size.

In man, the observed cortical occupancy effect for 1 mg flesinoxan was 8.7%$\pm$13% (1 SD) while that for 40 mg ziprasidone was 4.6%$\pm$17% (1 SD). A much larger study would be required to demonstrate a statistically significant occupancy effect given the low effect sizes. Unlike some studies with pindolol (Martinez *et al*, 2001; Rabiner *et al*, 2000a), we did not observe a differential occupancy of somatodendritic and postsynaptic 5-HT$_{1A}$ receptors. Increasing plasma levels of drug did not produce increasing occupancy for either flesinoxan (the two subjects with higher plasma concentrations of flesinoxan paradoxically had lower occupancies than the two subjects with lower concentrations) or ziprasidone.

In contrast, in rats, flesinoxan achieved consistent occupancy at doses of 0.25 mg/kg and above, corresponding to a plasma level of 27 ng/ml and above, and occupancy increased in a dose related fashion thereafter. Receptor saturation was not attained over the intravenous dose range, which was limited by the solubility of flesinoxan. We have replicated these *ex vivo* findings *in vivo* using small animal tomography (Hirani *et al*, unpublished observations). Flesinoxan produced comparable levels of 5-HT$_{1A}$ receptor occupancy in rats using [^{11}C]WAY-100635 with quad-HIDAC, a multi-wire detection system based on high-density avalanche chamber technology (Figure 3).

The human flesinoxan results suggest that, although the dosage used in the PET study is sufficient to produce pharmacological effects in the form of a higher incidence of side effects and, in larger studies, neuroendocrine responses (de Koning and de Vries, 1995), it is not sufficient to produce measurable occupancy. The rat *ex vivo* binding study and *in vivo* HIDAC results indicate that flesinoxan will produce significant occupancy at the 5-HT$_{1A}$ receptor when administered at a higher dosage. Despite the problems of making comparisons between species, it seems reasonable to conclude that the human oral dose of 0.012 mg/kg with plasma levels averaging 6.4 ng/ml during the scan is markedly less than the rat intravenous dose of 0.25 mg/kg which resulted in a plasma level of 27 ng/ml 60 min after injection. In man, a higher dose of flesinoxan could only be administered in the context of a gradually incrementing dose regimen in order to check tolerability for each volunteer. Subchronic dosing can, however, lead to adaptive changes in receptor populations that confounds the measurement of occupancy.

PET studies have readily demonstrated occupancy of 5-HT$_{1A}$ receptor *antagonists* in man. All but the lowest doses of the antagonist/weak partial agonist pindolol occupy cortical 5-HT$_{1A}$ receptors in studies using both [^{11}C]WAY-100635 (Andree *et al*, 1999; Martinez *et al*, 2001; Rabiner *et al*, 2000a) and [^{18}F]-MPPF (Passchier *et al*, 2001). Pindolol's dose range is limited by its action at β-adrenergic receptors. Studies using more selective novel 5-HT$_{1A}$ receptor antagonists have achieved higher levels of occupancy. A 40 mg dose of DU 125530 occupies 65% of postsynaptic 5-HT$_{1A}$ receptors (Rabiner *et al*, 2002c), while 10 mg of NAD-299 (robalzotan) occupies 71% of neocortical receptors (Andree *et al*, 2003).

However, it has not been possible to demonstrate occupancy by 5-HT$_{1A}$ receptor exogenous *agonists* (despite the tested drugs generally possessing affinities comparable to the antagonists mentioned above), or by the endogenous agonist serotonin in man using PET. The 5-HT$_{1A}$ agonist and putative antipsychotic drug EMD 128 130 has been studied using [^{11}C]WAY-100635 and [^{11}C]raclopride PET (Rabiner *et al*, 2002a). Significant occupancy at the D$_2$ receptor (mean 40%) was not paralleled by significant occupancy at the 5-HT$_{1A}$ receptor, despite EMD 128 130 having a higher affinity for the 5-HT$_{1A}$ receptor (K_i 1 nM at cloned human receptors) than for the D$_2$ receptor

Table 3 Ziprasidone PET study subject description, drug levels and [^{11}C]WAY-100635 binding results. SD = standard deviation

						Binding potential values				Percentage occupancy at 5-HT$_{1A}$ receptor	
Subject	**Age**	**Sex**	**Scan type (+scan order)[a]**	**Side effects[b]**	**Ziprasidone levels: AUCs over 95 min (min ng/ml)[c]**	**Total cerebral cortex (postsynaptic)**	**Frontal cortex region**	**Medial temporal cortex**	**Raphe (presynaptic)**	**Total cerebral cortex (postsynaptic)**	**Raphe (presynaptic)**
1	31	m	B (1st)	—	—	2.6	2.5	3.2	1.8	−13.7	−7.9
			Z (2nd)	S++	3489	3.0	2.9	3.7	2.0		
2	57	m	B (1st)	—	—	4.8	4.5	6.5	3.4	10.5	10.3
			Z (2nd)	S+	4101	4.3	4.1	6.3	3.1		
3	50	f	B (1st)	—	—	4.0	3.8	5.5	3.1	−4.2	1.5
			Z (2nd)	S++ D+	1361	4.2	4.0	5.9	3.0		
4	42	m	B (1st)	—	—	3.8	3.4	5.6	2.9	22.7	26.8
			Z (2nd)	S++	5272	2.9	2.7	4.0	2.1		
5	41	f	B (2nd)	—	—	3.1	3.0	4.2	2.4	7.3	26.2
			Z (1st)	S++	6439	2.9	2.8	3.9	1.7		
6	33	m	B (1st)	—	—	4.8	4.6	6.4	3.1	28.1	34.7
			Z (2nd)	S+ L+	3643	3.4	3.2	4.6	2.0		
7	55	f	B (1st)	—	—	3.6	3.4	4.3	2.2	9.3	26.9
			Z (2nd)	S++	696	3.3	3.1	4.3	1.6		
8	38	m	B (2nd)	—	—	3.7	3.6	4.3	2.6	−23.1	−55.6
			Z (1st)	S++ N+ D+	6957	4.6	4.3	6.2	4.1		
Mean	43					3.8	3.6	5.0	2.7	4.6	7.9
					3995	3.6	3.4	4.9	2.5		
SD	9.7					0.7	0.7	1.2	0.5	17.4	29.5
					2223	0.7	0.6	1.1	0.8		

[a]B = baseline, Z = ziprasidone.
[b]H = headache, S = somnolence, L = lightheadedness, D = dry mouth, N = nausea; + = mild, ++ = moderate, +++ = severe.
[c]Note ziprasidone is >99% protein bound (Tandon, 2000).

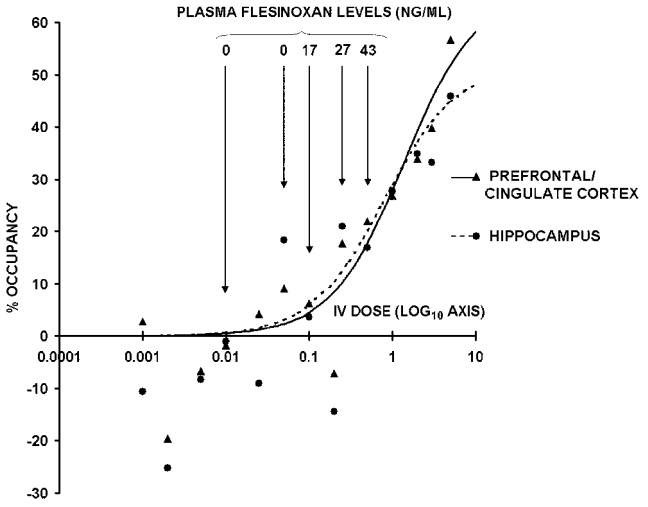

Figure 2 Dose–occupancy curve from rats pretreated with intravenous flesinoxan at doses ranging from 0.001 to 5 mg/kg for two regions of interest, with flesinoxan plasma levels shown. The curves were fitted using a single site binding model, using the Michaelis–Menten relationship, as described in Hume *et al* (1997). ED_{50} levels were estimated as 1.4 and 0.8 mg/kg for the prefrontal/cingulate cortex and hippocampus, respectively. The nondisplaceable component of binding was in the order of 50% at the maximal dose used.

(K_i 20 nM), and subjects experiencing side effects consistent with central 5-HT$_{1A}$ receptor agonism. As with ziprasidone, the drug's *in vitro* affinity for the 5-HT$_{1A}$ receptor (agonism) relative to the D_2 receptor (antagonism) was *not* predictive of relative *in vivo* occupancies. In the case of ziprasidone, the radioligands used to determine the *in vitro* affinities are documented (Seeger *et al*, 1995). The discrepancy may arise because ziprasidone's 5-HT$_{1A}$ receptor occupancy was measured with an antagonist, [^{11}C]WAY-100635, but the affinity was measured with an agonist, [^{3}H]8-OH-DPAT, while for the D_2 (and 5-HT$_{2A}$) receptor, both occupancy *and* affinity were measured using antagonists. A 60 mg dose of tandospirone did not affect [^{11}C]WAY-100635 binding in any brain region despite significantly reducing body temperature and increasing plasma growth hormone levels, while a 30 mg dose resulted in a nonsignificant *increase* in binding (Nakayama *et al*, 2002). Similarly, in two small studies, buspirone did not display an occupancy effect at either a 10 mg dose using the radiotracer [^{18}F]MPPF (Passchier *et al*, 2001) or a 20 mg dose using [^{11}C]WAY-100635 (Rabiner *et al*, 2000b). Finally, a between-subject [^{11}C]WAY-100635 PET study in patients with schizophrenia did not demonstrate occupancy of clozapine, an antipsychotic drug with 5-HT$_{1A}$ receptor partial agonist properties, at the 5-HT$_{1A}$ receptor (Bantick *et al*, 2004). Studies using tryptophan depletion (with a tryptophan-free drink) or infusion to, respectively, reduce or increase central 5-HT levels have not altered radioligand binding to the 5-HT$_{1A}$ receptor using either [^{11}C]WAY-100635 (Rabiner *et al*, 2002b) or [^{18}F]MPPF (Udo de Haes *et al*, 2002). These two studies suggest that the binding of these 5-HT$_{1A}$ receptor radioligands is relatively insensitive to changes in the level of endogenous 5-HT in man. The possibility that *exogenous* agonist occupancy has not been observed in man because it has been masked by a simultaneous reduction in the concentration of the *endogenous* agonist serotonin (as a result of stimulation of 5-HT$_{1A}$ autoreceptors in the raphe) is thus unlikely.

These negative PET results in man contrast with positive studies in animals using exogenous 5-HT$_{1A}$ receptor agonists, and mixed findings with endogenous serotonin manipulations. Several studies have been performed in monkeys, using single animals apart from the larger study of Tsukada *et al* (2001). Preadministration of a high dose of 8-OH-DPAT blocks [^{11}C]WAY-100635 specific uptake in the frontal cortex substantially (Farde *et al*, 1997; Mathis *et al*, 1994). Administered during a PET scan, 8-OH-DPAT displaces [^{11}C]WAY-100635 (Mathis *et al*, 1994; Tsukada *et al*, 2001) and [^{18}F]MPPF (Shiue *et al*, 1997). In *ex vivo* studies in rats, 8-OH-DPAT blocks the binding of [*O*-methyl-^{3}H]WAY-100635 (Hume *et al*, 1994), [^{11}C]WAY-100635 (Mathis *et al*, 1994) and [^{18}F]MPPF (Shiue *et al*, 1997). Buspirone and ipsapirone at doses of 2 mg/kg blocked $\sim$50% of [*O*-methyl-^{3}H]WAY-100635 specific binding in all regions examined in an *ex vivo* study in rats, while 5 mg/kg buspirone occupied 61% of frontal cortex 5-HT$_{1A}$ receptor in an [^{11}C]WAY-100635 PET study using a monkey (Farde *et al*, 1997). Studies in rats using 10 mg/kg fenfluramine to increase endogenous 5-HT levels have had mixed results, despite elevating 5-HT levels by 9–15 times in the hippocampus (Hume *et al*, 2001; Maeda *et al*, 2001; Zimmer *et al*, 2002). Using [^{11}C]WAY-100635 PET, Hume *et al* (2001) found a 10–20% reduction in specific binding in the hippocampus but not the prefrontal cortex (where the elevation of 5-HT was less), while Mathis *et al* (1995) found a 20% reduction in both hippocampal and cortical regions using an 8 mg/kg dose and an *ex vivo* dissection method. Using a β^+ probe, Zimmer *et al* (2002) identified displacement of [^{18}F]MPPF after fenfluramine in the hippocampus. In contrast, neither Maeda *et al* (2001) using 10 mg/kg fenfluramine in rats with [^{11}C]WAY-100635 PET, nor Rice *et al* (2001) using 3 mg/kg fenfluramine in mice with an *ex vivo* [^{3}H]WAY-100635 binding method found any effect on the hippocampus or frontal cortex, while Parsey *et al* (1999) found paradoxical *increases* in [^{11}C]WAY-100635 binding in baboons (using both 2.5 mg/kg fenfluramine and 1.5 mg/kg amphetamine). In studies in which 5-HT levels have been *depleted*, neither Maeda *et al* (2001) using reserpine in rats, nor Rice *et al* (2001) using *p*-chlorophenylalanine in mice, identified any changes in radiolabeled WAY-100635 binding. However, Zimmer *et al* (2003b), using a β^+ probe in rats, have recently reported a significant increase in [^{18}F]MPPF specific binding in the hippocampus after 5-HT depletion using *p*-ethynylphenylalanine (*p*-EPA).

The results of our study and the others described above suggest that antagonist, but not agonist, occupancy can be detected in man using 5-HT$_{1A}$ receptor antagonist radiotracers. However, occupancy effects can be demonstrated in animals with exogenous and possibly endogenous agonists. The discrepancy between the human and animal studies probably arises because the doses of agonists and the extent of 5-HT manipulations possible in man are restricted by tolerability. Although substantial *blockade* of 5-HT$_{1A}$ receptors appears to be well tolerated (Rabiner *et al*, 2002c), agonism produces pharmacological effects at low levels of occupancy, implying a high sensitivity to agonists. Such findings are consistent with a low level of tonic

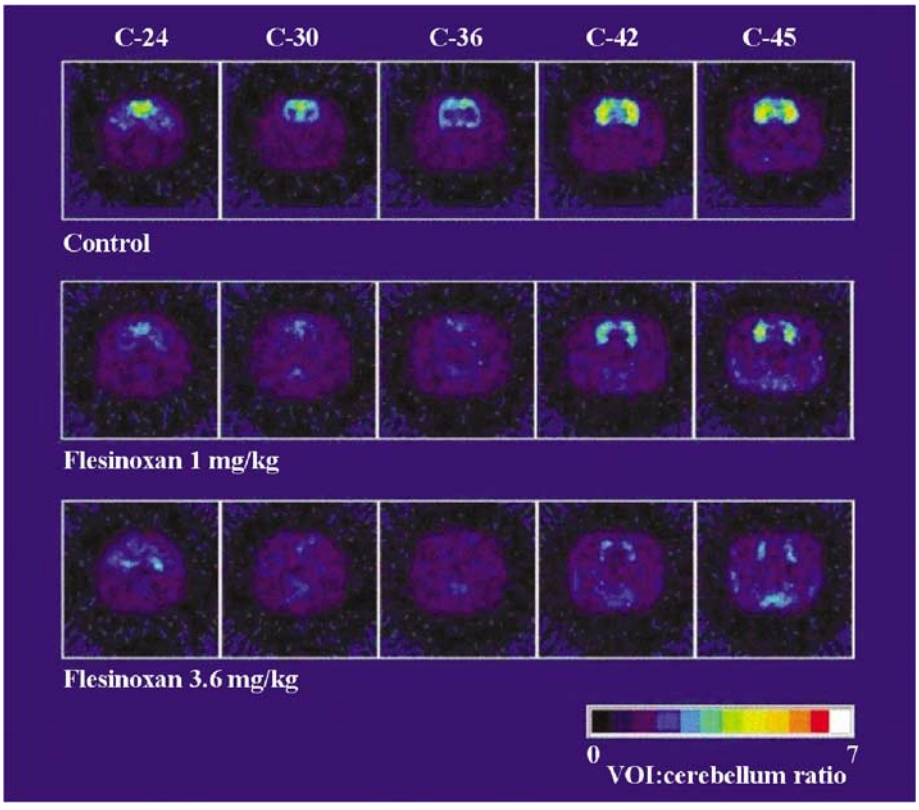

Figure 3 Effect of flesinoxan (1 and 3.6 mg/kg i.v.) on [^{11}C]WAY-100635 binding measured in rat brain using the novel small animal PET scanner, quad-HIDAC (Oxford Positron Systems, Weston-on-the-Green, UK: see Jeavons *et al*, 1999). These volume of interest (VOI) to cerebellum 'normalized' images (representing total: nonspecific binding) show data from three rats acquired over the 20–60 min period after [^{11}C]WAY-100635 injection. The upper set of images is from a control rat, the middle from a rat administered 1 mg/kg flesinoxan and the lower from a rat administered 3.6 mg/kg intravenous flesinoxan 5 min before radioligand injection. Five coronal slices (each 0.5 mm thick) are shown for each scan (numbered C-24 to C-45), corresponding to brain atlas slices Bregma +4.2 mm to Bregma −6.3 mm (Paxinos and Watson, 1996). The frontal cortex (C-24) and hippocampus (C-42 and C-45) are visible as areas of high binding, and the striatum (C-30 and C-36) as 'cold spots' (low binding). Detailed methodology is contained within Hume *et al* (2001). Briefly, each isoflurane anesthetized rat was positioned in the scanner and injected with [^{11}C]WAY-100635 via a previously catheterized tail vein. Scan data were acquired over 60 min in list mode, starting at the time of radioligand injection. Sinogram bins were reconstructed in 0.5 mm cubic voxels with a Hamming filter at 0.6 cut-off, using conventional filtered back-projection. From each image volume, data were sampled from a 40 min frame starting 20 min after radioligand injection. During this time, [^{11}C]WAY-100635 binding has been shown to reach a pseudo-equilibrium (Hirani *et al*, 2000). Data were transferred into Analyze software and an in-house ROI template was projected onto each image volume (Hume *et al*, 2001). Frontal cortex (452 voxels), hippocampus (264 voxels), and cerebellum (576 voxels) regions were sampled. ROI: cerebellum ratios were used to calculate occupancy as before. For the 1 and 3.6 mg/kg doses of flesinoxan, frontal cortex occupancies were 31 and 47% and hippocampal occupancies were 23 and 46%, respectively.

stimulation of postsynaptic neocortical 5-HT$_{1A}$ receptors (Hajos *et al*, 2001).

A limitation of antagonist radiotracers is that they may underestimate 5-HT$_{1A}$ receptor occupancy by agonist drugs. The 5-HT$_{1A}$ receptor can exist in high-affinity (coupled with G-protein) and low-affinity (free receptor) states. Full *antagonists* such as WAY-100635, bind homogeneously to both. However *agonists* such as flesinoxan and 8-OH-DPAT bind with high affinity only to G-protein-coupled receptors (Gozlan *et al*, 1995; Mongeau *et al*, 1992). In support of this, the ratio of [^{3}H]WAY-100635: [^{3}H]8-OH-DPAT receptor binding site densities in human neocortex is 1.7–2.7 in man (Burnet *et al*, 1997 using quantitative autoradiography). In rats, the low : high-affinity receptor ratio has been studied most frequently in hippocampal tissue where it averages ~1.5 (Chamberlain *et al*, 1993; Fletcher *et al*, 1996; Gozlan *et al*, 1995; Khawaja, 1995; Mongeau *et al*, 1992). The ratio *in vivo* is not known. The use of an agonist PET radiotracer could enable the determination of drug occupancy at just the high-affinity receptors—a more meaningful clinical measure. Although an agonist PET radioligand is not yet available for the 5-HT$_{1A}$ receptor, one has recently become available for the D$_2$ receptor (also G-protein linked), and this appears more sensitive to changes in dopamine levels than the antagonist D$_2$ radiotracer [^{11}C]raclopride (Cumming *et al*, 2002). A number of 5-HT$_{1A}$ receptor agonists have been investigated as potential PET radiotracers. The failure of these candidate compounds (Mathis *et al*, 1997; Passchier and van Waarde, 2001; Pike *et al*, 2001; Zimmer *et al*, 2003a), despite evidence of penetration into the brain for several, coupled with the human 5-HT$_{1A}$ agonist occupancy results above, may suggest that *in vivo*, only a small proportion of 5-HT$_{1A}$ receptors are configured in the high-affinity state. Alternatively, agonist binding to high-affinity state receptors may rapidly convert them into the low-affinity state, as the ternary complex of agonist, receptor and G protein breaks down, yielding active G-protein subunits that can alter effector activity (McLoughlin and Strange, 2000; Strange, 1999).

In summary, we did not observe significant occupancy by flesinoxan or ziprasidone at the 5-HT$_{1A}$ receptor in man using PET, but did observe significant and dose-related occupancy using higher doses of flesinoxan in rats with an *ex vivo* technique. We conclude that 5-HT$_{1A}$ receptor agonists display significant occupancy only at higher doses that would produce unacceptable levels of side effects in man, although lower doses are sufficient to produce pharmacological effects. In order to image 5-HT$_{1A}$ agonist occupancy in man, 5-HT$_{1A}$ agonist radiotracers may need to be developed.

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