

# Characterization of the SPECT 5-HT<sub>2A</sub> Receptor Ligand <sup>123</sup>I-R91150 in Healthy Volunteers: Part 2—Ketanserin Displacement

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As part of the radioiodinated 4-amino-*N*-1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidiny]5-iodo-2-methoxybenzamide (<sup>123</sup>I-R91150) characterization study, ketanserin challenges were performed on healthy volunteers with the aim of assessing the specificity of <sup>123</sup>I-R91150 binding to subtype 2A of the 5-hydroxytryptamine receptor (5-HT<sub>2A</sub>), the sensitivity of <sup>123</sup>I-R91150 SPECT in measuring ligand displacement, the relationship between ketanserin plasma concentrations and <sup>123</sup>I-R91150 displacement, and the suitability of the cerebellum as a reference region for quantification. **Methods:** Dynamic SPECT was performed on 6 healthy men (mean age  $\pm$  SD, 21  $\pm$  0.89 y) from the time of <sup>123</sup>I-R91150 injection until 470 min afterward. Ketanserin was administered intravenously at 210 min after injection at 3 doses: 0.1 mg/kg ( $n$  = 2), 0.05 mg/kg ( $n$  = 2), and 0.015 mg/kg ( $n$  = 2). Blood samples for measurement of ketanserin plasma concentrations were drawn. MRI was performed on all subjects and coregistered to the SPECT data for region-of-interest drawing on cortical regions and cerebellum. The simplified reference tissue model (SRTM) was considered the gold standard for quantification, and results were compared with those obtained with the tissue ratio method (TR). The percentage <sup>123</sup>I-R91150 displacement was calculated with both methods as the percentage difference between baseline and postketanserin scans. **Results:** Depending on the cerebral regions with the maximum ketanserin dose studied, SRTM and TR mean displacements were 57.1%–95.4% and 71.9%–101.2%, respectively, for the 0.1 mg/kg dose; 51.7%–91.4% and 56.7%–102.8%, respectively, for the 0.05 mg/kg dose; and 7.7%–54.5% and 13.8%–47.0%, respectively, for the lowest dose, 0.015 mg/kg. A good correlation was found between the 2 methods. No ketanserin-induced displacement was observed in the cerebellum time–activity curves, supporting the use of the cerebellum as a reference region. The relationship between displacement and ketanserin plasma concentration fit with a rectangular hyperbola, with a 5.6 ng/mL con-

centration associated with 50% of the maximum displacement (EC<sub>50</sub>). EC<sub>50</sub> values calculated using occupancies derived both with SRTM and with TR were in good agreement. **Conclusion:** <sup>123</sup>I-R91150 SPECT is sensitive enough to measure ketanserin dose-dependent displacement in cerebral regions rich in 5-HT<sub>2A</sub> receptors. These results support the selectivity of <sup>123</sup>I-R91150 for 5-HT<sub>2A</sub> receptors and its use as a SPECT ligand for measurements of drug-induced 5-HT<sub>2A</sub> receptor occupancy in humans.

**Key Words:** ketanserin; neurotransmission; SPECT; <sup>123</sup>I-R91150; 5-HT<sub>2A</sub>

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**B**rain kinetics, plasma behavior, and the accuracy of quantification methods have been reported in healthy volunteers for subtype 2A of the 5-hydroxytryptamine receptor (5-HT<sub>2A</sub>) SPECT ligand radioiodinated 4-amino-*N*-1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidiny]5-iodo-2-methoxybenzamide (<sup>123</sup>I-R91150) (1). The specificity of <sup>123</sup>I-R91150 binding to the 5-HT<sub>2A</sub> receptor site has previously been suggested by preclinical displacement studies. <sup>123</sup>I-R91150 displacement induced by the potent 5-HT<sub>2A</sub> antagonist ketanserin has been reported in both dogs (2) and baboons (3), with lack of effect of the selective dopamine D<sub>2</sub> receptor antagonist raclopride in the latter (3). However, experience with the effects of ketanserin on <sup>123</sup>I-R91150 binding in humans is limited. Preliminary results showed full <sup>123</sup>I-R91150 displacement in cortical brain regions, with no effect on cerebellar uptake in 3 healthy volunteers at a ketanserin dose of 0.1 mg/kg (4), which was previously found to be a saturating dose in primates (5).

The present study was part of the <sup>123</sup>I-R91150 characterization study (1). Ketanserin challenges at different doses in healthy volunteers were used to assess the specificity of <sup>123</sup>I-R91150 binding to 5-HT<sub>2A</sub> receptors, the sensitivity of the

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$^{123}\text{I}$ -R91150 SPECT technique in measuring ligand displacement, the relationship between ketanserin plasma concentrations and  $^{123}\text{I}$ -R91150 displacement, and the suitability of the cerebellum as a reference region for quantification.

## MATERIALS AND METHODS

### Subjects

Six men (mean age  $\pm$  SD,  $21 \pm 0.89$  y; range, 20–22 y) determined through the Structured Clinical Interview for DSM-IV to be healthy were enrolled in this study. None had a history of psychiatric treatment or significant medical events. None was taking psychotropic drugs or other relevant medication. Absence of illegal drug abuse was assessed by questioning and supported by urine drug screening (InstaCheck Drug Screen Test; Applied Biotech/Forefront Diagnostics). Subjects had to withdraw from caffeine and alcohol 24 h before the study day and were not allowed to take any drugs during the 7 d before the study day. All had normal findings on physical examination, 12-lead electrocardiography, clinical chemistry, and hematology. The study was approved by the local Ethics Committees and the Spanish Ministry of Health, and written informed consent was obtained from all subjects before inclusion in the study.

### Synthesis of $^{123}\text{I}$ -R91150

The synthesis of  $^{123}\text{I}$ -R91150 was previously described (1,6). Briefly: 4-amino-*N*-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-2-methoxybenzamide (40  $\mu\text{g}$ ) was dissolved in glacial acetic acid (300  $\mu\text{L}$ ). The addition of  $\text{Na}^{123}\text{I}$  (370–740 MBq) and  $\text{H}_2\text{O}_2$  (30% solution, 100  $\mu\text{L}$  in 4 steps, 25  $\mu\text{L}$  each) yielded  $^{123}\text{I}$ -R91150. The reaction was stopped with 0.5 mL of a 0.5 mol/L solution of sodium sulfite in a 4.5 mol/L solution of NaOH, and the mixture was purified by high-performance liquid chromatography. The collected  $^{123}\text{I}$ -R91150 fraction was further diluted with an isotonic citrate/acetate buffer (end pH, 6.0–6.4), filtered through a Millex-FG filter (Millipore), stabilized with a trace of *o*-iodohippuric acid (1 mg/mL of diluent), and autoclaved. The specific activity of the autoclaved  $^{123}\text{I}$ -R91150 was approximately 300 TBq/mmol. Radiochemical purity at the time of administration (i.e., 24 h after synthesis) was higher than 96%.

### Subject Preparation and SPECT Procedure

SPECT was performed using a 3-head Prism 3000S camera (Philips) fitted with ultra-high-resolution fanbeam collimators. The preparation of each subject included administration of potassium perchlorate (8 mg/kg) up to 20 min before the  $^{123}\text{I}$ -R91150 injection to minimize exposure of the thyroid gland to radiation. Four multimodality SPECT/MRI markers (MM3003; IZI Medical Products Corp.), each filled with approximately 0.074 MBq of  $^{123}\text{I}$ , were firmly attached to the subject's head at the level of the mastoids and the corners of the eyes. A dynamic scan sequence of one 10-min, four 30-min, three 10-min, and three 40-min frames was acquired starting at 0, 11, 71, 120, 180, 210, 220, 230, 330, 400, and 470 min after injection. Ketanserin was administered at 210 min after injection in a slow intravenous injection (over 30 s) at 3 different doses—0.1, 0.05, and 0.015 mg/kg—each administered to a different subgroup of 2 subjects. The subjects were allowed to rest outside the SPECT camera during breaks between scans until the time of the ketanserin injection; then, for safety reasons, the subjects rested lying on the SPECT bed with continuous monitoring of electrocardiography, blood pressure, and heart rate until the end of the scanning session.

### Ketanserin Analysis

Blood samples for analysis of ketanserin levels were drawn at 180, 230, 270, 330, 400, and 470 min after the  $^{123}\text{I}$ -R91150 injection and were centrifuged for 10 min at 3,000 rpm and 25°C to separate the plasma. Plasma samples were analyzed for ketanserin using a validated analytic method based on protein precipitation, followed by high-performance liquid chromatography–mass spectrometry/mass spectrometry analysis. The limit of detection for ketanserin was 1 ng/mL, using a 300- $\mu\text{L}$  aliquot of human plasma with a higher limit of quantification, 100 ng/mL. Quality control samples, prepared at 3 different analyte concentrations and stored with the study samples, were analyzed with each batch of samples against separately prepared calibration standards. For the analysis to be acceptable, no more than one third of the quality control results were to deviate from the nominal concentration by more than 15%, and at least 50% of the results from each quality control concentration were within 15% of nominal.

### $^{123}\text{I}$ -R91150 Plasma Analysis

A total of 17 venous blood samples were collected manually from all subjects, at –1 (baseline), 0.25, 0.5, 1, 1.5, 2, 5, 10, 15, 71, 120, 180, 230, 270, 330, 400, and 470 min after injection. Plasma analysis of  $^{123}\text{I}$ -R91150 was previously described. Briefly: Samples 2–17 were centrifuged at 2,000g for 4 min, and plasma and residue fractions were counted in a  $\gamma$ -counter. For determination of the metabolite profile, 1 mL of pure acetonitrile was added to the plasma fraction, and after centrifugation (2,000g, 4 min), the supernatant was separated and evaporated at 40°C under a continuous flow of helium, and the residue was diluted in 100  $\mu\text{L}$  of mobile phase and injected into the high-performance liquid chromatography system. To the first blood sample (extracted before radiotracer injection), 100  $\mu\text{L}$  of  $^{123}\text{I}$ -R91150 reference solution ( $\sim 370$  kBq/mL) were added, and the resulting solution underwent the same experimental procedure as described for samples 2–17. All samples were processed within 60 min after they had been collected.

### Image Processing

Images were reconstructed using a filtered-backprojection algorithm with a Butterworth filter (exponent, 5.0; cutoff frequency, 0.4 cycle/pixel). Pixel sizes were between 2.45 and 2.51 mm on each slice and 3.56 mm in the axial direction. Attenuation correction was performed using the Chang algorithm and a manually fitted elliptic attenuation map on each slice ( $\mu = 0.1 \text{ cm}^{-1}$ ). SPECT and MRI scans were registered using in-house software implemented in C language. First, the corresponding external markers were manually identified on the MRI and SPECT scans. Then, rigid-body transformation (3 translations and 3 rotations) was estimated automatically by minimizing the sum of the squared distances between the corresponding marker positions (7).

Regions of interest (ROIs) were drawn manually and individually by the same investigator on the MR images of each subject. To control for ROI volumes across subjects, care was taken that approximately the same number of MRI slices was used in drawing the ROIs for each region across subjects. Moreover, anatomic landmarks were defined a priori in order to standardize the first (lower) and last (upper) brain slice within which each ROI was drawn. ROIs were drawn in the frontal, temporal, occipital, and parietal lobes and the cerebellum. The frontal lobe was divided into orbitofrontal, prefrontal, and superior frontal regions, and the occipital lobe was divided into calcarine and lateral regions.

## Quantification of $^{123}\text{I}$ -R91150 Displacement

The simplified reference tissue model (SRTM) (8) and the tissue ratio method (TR) were applied for quantification. SRTM was used as the gold standard for quantification, and the cerebellum time–activity curve was used to provide the input function to the kinetic model. A changing binding potential (BP) value at the moment of ketanserin injection was included in the model to fit the SRTM to both parts of the curve, that is, before and after ketanserin. BP before ketanserin ( $\text{BP}_{\text{pre}}$ ) and after ketanserin ( $\text{BP}_{\text{post}}$ ) were the SRTM outcome measurements. Radioligand displacement measured using SRTM was compared with the displacement calculated using TR at pseudoequilibrium. For the TR, specific uptake ratios (SUR) were calculated as  $([\text{C}_{\text{reg}} - \text{C}_{\text{cer}}]/\text{C}_{\text{cer}})$ , where  $\text{C}_{\text{reg}}$  and  $\text{C}_{\text{cer}}$  were the mean counts in the studied cortical region and in the cerebellum, respectively. For displacement measures, SUR values from 2 scans were used: the baseline scan frame at 180 min after injection ( $\text{SUR}_{180}$ ) and the scan frame starting at 400 min after injection, that is, 190 min after ketanserin administration ( $\text{SUR}_{400}$ ). This allowed pseudoequilibrium to be achieved again after ketanserin administration. Displacement was then calculated as the percentage change between the BP or SUR values before and after ketanserin:  $\% \text{Displ}_{\text{SRTM}} = 100 \times (\text{BP}_{\text{pre}} - \text{BP}_{\text{post}})/\text{BP}_{\text{pre}}$ , and  $\% \text{Displ}_{\text{TR}} = 100 \times (\text{SUR}_{180} - \text{SUR}_{400})/\text{SUR}_{180}$ .

The relationship between ketanserin plasma concentration and  $^{123}\text{I}$ -R91150 displacement in the different ROIs was modeled according to the Hill equation:

$$\text{Percentage displacement} = \frac{\text{Displ}_{\text{max}} \cdot \text{C}_p^\gamma}{\text{EC}_{50}^\gamma + \text{C}_p^\gamma},$$

where percentage displacement is 5-HT<sub>2A</sub> receptor displacement (i.e.,  $^{123}\text{I}$ -R91150 displacement),  $\text{Displ}_{\text{max}}$  is the theoretic maximum displacement for the particular ROI,  $\text{C}_p$  is the ketanserin plasma concentration (and is used as a functional surrogate of the ketanserin concentration at the synaptic level),  $\gamma$  is the slope factor, and  $\text{EC}_{50}$  is the concentration associated with 50% of the maximum displacement (a measure of drug potency in blocking 5-HT<sub>2A</sub> receptors). Because, in this study, we aimed at characterizing ketanserin displacement of our 5-HT<sub>2A</sub> receptor ligand, we decided neither to fix the slope factor to 1 (i.e., to assume that ketanserin interacts with a single 5-HT<sub>2A</sub> binding site in the brain) nor to fix  $\text{Displ}_{\text{max}}$  to 100% (i.e., to assume that  $^{123}\text{I}$ -R91150 can be completely displaced by ketanserin). Nonlinear mixed-effect modeling was used as implemented in NONMEM (version 5; Globo-Max). Because the scan obtained 400 min after injection was applied for displacement calculations using TR, the ketanserin plasma concentrations at 400 min were used to build up the relationship between displacement and plasma concentration for this method.

## RESULTS

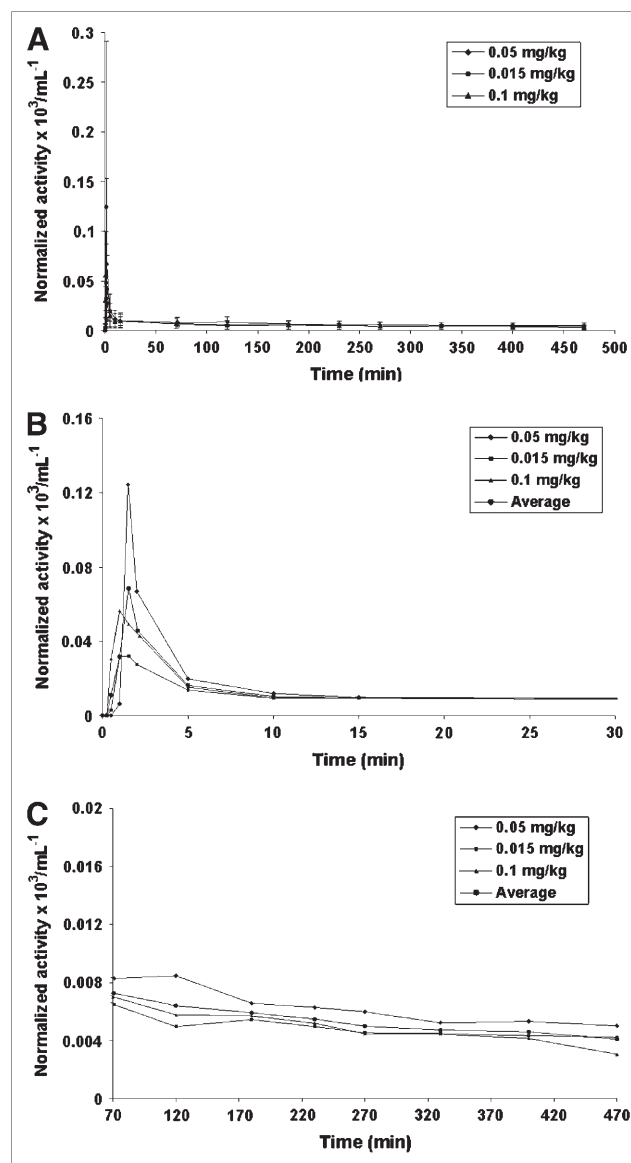
### Safety

Safety data recorded at the beginning and end of the scanning session, including heart rate, blood pressure, and QTc intervals, showed that these parameters remained within reference ranges during the entire scanning period. At baseline, the ranges were 100–133 (systolic)/54–69 (diastolic) mm Hg for blood pressure, 371–416 ms for QTc, and 55–96 bpm for heart rate. At the end of the experiment, the respective ranges were 99–140 (systolic)/51–65 (diastolic) mm Hg, 378–422 ms, and 52–80 bpm.

## $^{123}\text{I}$ -R91150 Plasma Analysis

The average activities of unmetabolized parent compound in plasma from venous samples at each ketanserin dose are presented in Figures 1A (entire study), 1B (0–30 min), and 1C (70–470 min). Figures 1B and 1C also present the average values for the 6 subjects. As can be seen in Figure 1B, the 3 curves reached a plateau after a rapid distribution phase. Activity peaked at 1.5, 1, and 1 min after injection of 0.05, 0.015, and 0.1 mg, respectively, of ketanserin per kilogram. The maximum normalized activities were 0.123, 0.032, and  $0.056 \times 10^3 \text{ mL}^{-1}$ , respectively.

Attention to the percentage of unmetabolized parent compound in plasma over time at each of the tested



**FIGURE 1.** Average values for activity corresponding to unmetabolized parent compound in plasma as function of time for 0.05, 0.015, and 0.1 mg of ketanserin per kilogram for entire study (A), first 30 min after injection (B), and 70–470 min after injection (C). All values are decay corrected to injection time and normalized with injected activity. Values in A include SD.

ketanserin doses revealed that this fraction dropped from initial values of  $96\% \pm 2\%$ ,  $95\% \pm 3\%$ , and  $94\% \pm 3\%$  to final values of  $71\% \pm 5\%$ ,  $81\% \pm 10\%$ , and  $69\% \pm 3\%$  for 0.05, 0.015, and 0.1 mg, respectively, of ketanserin per kilogram. As previously found (1), only one species more hydrophilic than the parent compound (lower retention time) could be detected in all chromatograms. Coinjection of the samples with free  $^{123}\text{I}$ -iodine showed the same retention time as for the first peak ( $\sim 1.5$  min).

The fraction of activity in plasma over time (referred to as total blood activity, average value for 2 venous blood samples) showed a quick decrease ( $<10$  min), reaching a plateau of around 80% at 15 min after injection for all ketanserin doses.

#### Ketanserin-Induced Displacement of $^{123}\text{I}$ -R91150

Table 1 shows the individual BP and SUR values used for displacement calculations, as well as the percentage

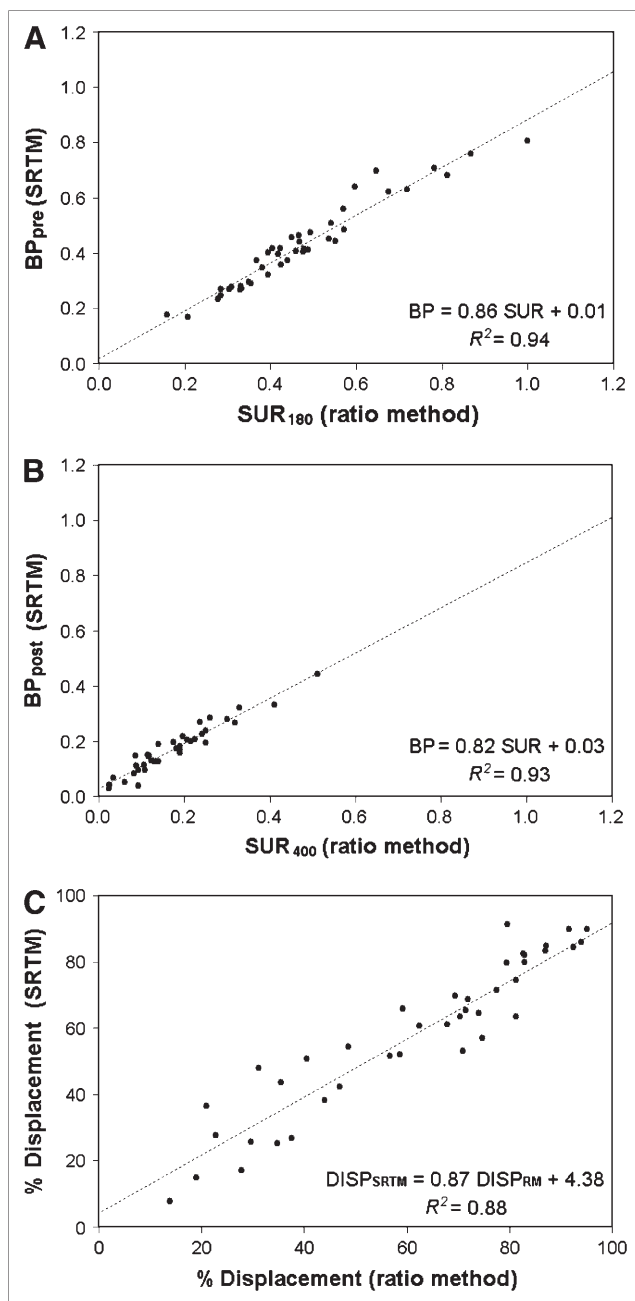
**TABLE 1**  
BP Values Calculated from SRTM, and SUR Calculated from TR, with Corresponding Percentages of  $^{123}\text{I}$ -R91150 Displacement for Each Region in Each Subject at the 3 Ketanserin Doses

ROI	Subject	BP <sub>pre</sub>	BP <sub>post</sub>	SUR <sub>180</sub>	SUR <sub>400</sub>	%Displ <sub>SRTM</sub>	%Displ <sub>TR</sub>
0.1 mg/kg							
Orbitofrontal	1	0.44	0.19	0.55	0.14	57.1	74.8
	2	0.81	0.29	1.00	0.26	64.6	74.1
Prefrontal	1	0.41	0.04	0.49	0.02	89.9	95.1
	2	0.68	0.11	0.81	0.11	83.3	87.0
Superior frontal	1	0.29	0.01	0.35	0.00	95.4	101.2
	2	0.63	0.10	0.72	0.09	84.9	87.2
Calcarine occipital	1	0.40	0.13	0.48	0.13	68.7	71.9
	2	0.71	0.13	0.78	0.13	82.2	82.9
Lateral occipital	1	0.42	0.08	0.48	0.08	79.8	82.9
	2	0.49	0.07	0.57	0.03	86.0	94.0
Temporal	1	0.44	0.11	0.47	0.09	74.6	81.3
	2	0.76	0.22	0.87	0.20	71.5	77.5
Parietal	1	0.27	0.03	0.28	0.02	89.8	91.7
	2	0.62	0.13	0.67	0.14	79.6	79.5
0.05 mg/kg							
Orbitofrontal	3	0.37	0.18	0.44	0.19	51.7	56.7
	4	0.56	0.27	0.57	0.24	52.0	58.6
Prefrontal	3	0.29	0.05	0.35	0.06	82.5	82.6
	4	0.51	0.20	0.54	0.17	61.1	67.8
Superior frontal	3	0.27	0.04	0.33	0.02	84.5	92.5
	4	0.40	0.15	0.39	0.12	63.5	70.4
Calcarine occipital	3	0.41	0.15	0.46	0.09	63.5	81.4
	4	0.42	0.13	0.42	0.13	69.8	69.4
Lateral occipital	3	0.25	0.10	0.28	0.11	60.7	62.5
	4	0.46	0.04	0.45	0.09	91.4	79.6
Temporal	3	0.32	0.15	0.39	0.11	53.2	70.9
	4	0.46	0.16	0.47	0.19	65.8	59.2
Parietal	3	0.17	0.04	0.21	-0.01	76.5	102.8
	4	0.42	0.14	0.40	0.12	65.5	71.5
0.015 mg/kg							
Orbitofrontal	5	0.35	0.32	0.38	0.33	7.7	13.8
	6	0.70	0.44	0.65	0.51	36.4	20.9
Prefrontal	5	0.27	0.23	0.33	0.24	17.0	27.8
	6	0.48	0.27	0.49	0.32	43.6	35.4
Superior frontal	5	0.23	0.17	0.28	0.18	25.3	34.8
	6	0.36	0.21	0.42	0.23	42.4	47.0
Calcarine occipital	5	0.28	0.20	0.33	0.21	26.8	37.6
	6	0.45	0.28	0.54	0.30	38.2	44.0
Lateral occipital	5	0.18	0.13	0.16	0.12	27.6	22.8
	6	0.37	0.17	0.37	0.19	54.5	48.5
Temporal	5	0.28	0.24	0.31	0.25	14.8	19.0
	6	0.64	0.33	0.60	0.41	48.0	31.1
Parietal	5	0.27	0.20	0.30	0.21	25.8	29.6
	6	0.40	0.20	0.42	0.25	50.8	40.5

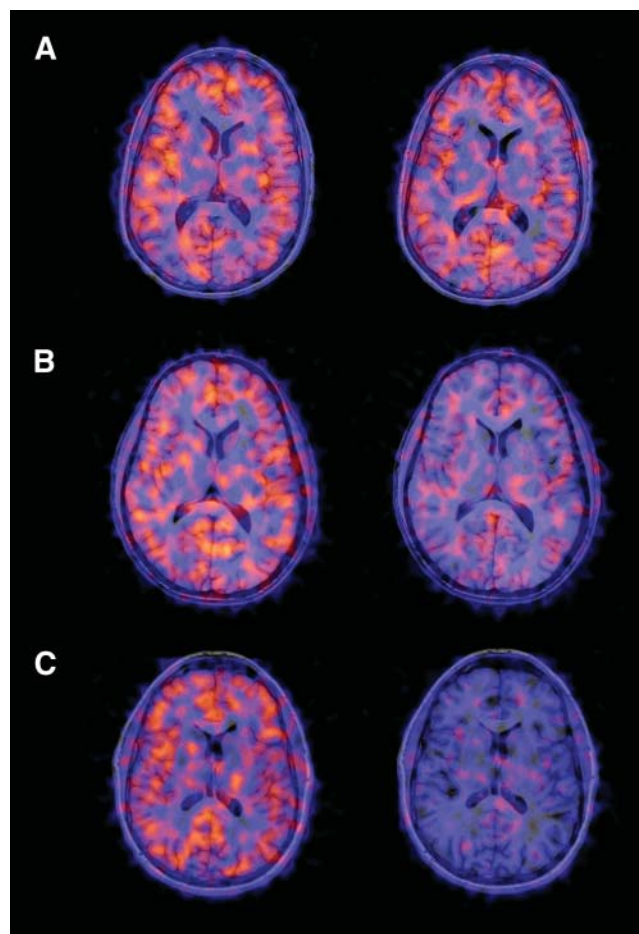


displacement calculated using SRTM ( $\%Displ_{SRTM}$ ) and TR ( $\%Displ_{TR}$ ) for each cerebral region at each ketanserin dose studied. SRTM and TR results correlated well, with  $R^2$  values of 0.94 ( $BP_{pre}$  vs.  $SUR_{180}$ ), 0.93 ( $BP_{post}$  vs.  $SUR_{400}$ ), and 0.88 ( $\%Displ_{SRTM}$  vs.  $\%Displ_{TR}$ ) (Fig. 2). Depending on the cerebral regions with the maximum ketanserin dose studied, SRTM/TR mean displacement ranges were 57.1%–95.4%/71.9%–101.2% for the 0.1 mg/kg dose, 51.7%–91.4%/56.7%–102.8% for the 0.05 mg/kg dose, and 7.7%–54.5%/13.8%–47.0% for the lowest dose, 0.015 mg/kg. The gradual dose-dependent displacement was also seen in all cortical regions (Fig. 3). Independently of the

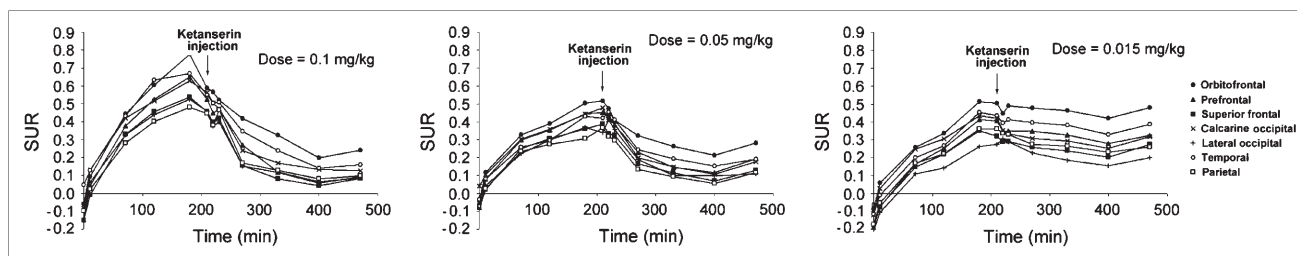
dose or degree of displacement, the behavior of each cerebral region was the same. The highest displacements were measured in the superior frontal, prefrontal, and lateral occipital regions, and the lowest displacements were measured in the orbitofrontal region. The effects of ketanserin on the time course of SUR values are shown in Figure 4. All subjects showed increasing cortical uptake up to the first pseudoequilibrium time point (180 min after injection), as reported previously (1). In all cases,  $^{123}I$ -R91150 cortical uptake immediately decreased after ketanserin injection at 210 min, showing the largest decrease within the first 30 min. From 210 to 240 min, when the cortical time–activity curve decreased the most, a small decrease in the slope of the cerebellum time–activity curve was observed but was transient and recovered quickly. Thus, at 270 min after injection, the slope of the cerebellum time–activity curve recovered to a steady decline indicative of tracer washout, with no indication of tracer displacement (Fig. 5).



**FIGURE 2.** Scatterplots of SRTM vs. TR measurements:  $BP_{pre}$  vs.  $SUR_{180}$  (A),  $BP_{post}$  vs.  $SUR_{400}$  (B), and  $\%Displ_{SRTM}$  vs.  $\%Displ_{TR}$  (C).



**FIGURE 3.** Coregistered MRI/SPECT images from 3 representative healthy volunteers, each receiving a different ketanserin dose. Baseline scans at 180 min after injection are on left; postketanserin scans at 400 min after injection are on right. Ketanserin doses are 0.015 mg/kg (A), 0.05 mg/kg (B), and 0.1 mg/kg (C). Gradual dose-dependent displacement is seen. (Reprinted from (9 [Fig. 19]) with the kind permission of Springer Science and Business Media.)



**FIGURE 4.** Mean SUR values over time for all cerebral regions studied from each pair of subjects on same ketanserin dose.

### Ketanserin Plasma Concentration and Relationship with $^{123}\text{I}$ -R91150 Displacement

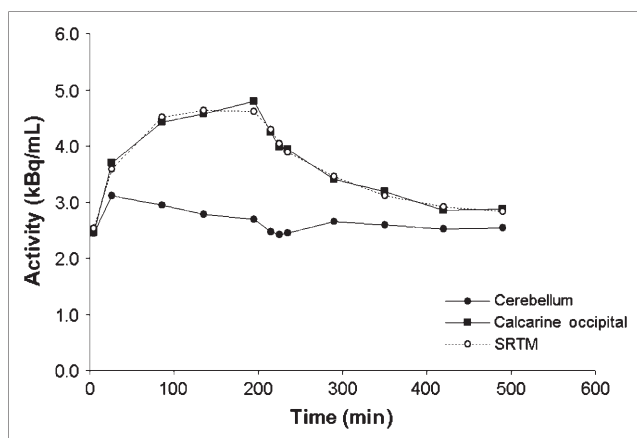
Mean ketanserin plasma levels at the times used for displacement calculations with TR (400–440 min after injection of  $^{123}\text{I}$ -R91150) were  $18.9 \pm 0.35$  ng/mL for the 0.1 mg/kg dose,  $9.00 \pm 0.27$  ng/mL for the 0.05 mg/kg dose, and  $3.60 \pm 1.10$  ng/mL for the 0.015 mg/kg dose. Given the limited time over which ketanserin plasma levels were observed, no formal pharmacokinetic analysis was run.

The Hill equation fitted the displacement data adequately in all regions both when the displacement was calculated with SRTM and when the displacement was calculated with TR (Fig. 6). The model parameters calculated for each region (Table 2) showed that  $\text{Displ}_{\text{max}}$  was close or equal to 100% in all regions, indicating that ketanserin can completely displace  $^{123}\text{I}$ -R91150.  $\text{EC}_{50}$  ranged from 4.2 to 7.1 ng/mL using SRTM data (population mean, 5.6 ng/mL) and from 3.4 to 6.5 ng/mL using TR data (population mean, 5.0 ng/mL). Estimates of the Hill slope factor were closer to 1 with SRTM data (population mean, 1.3) than with TR data (population mean, 1.8).

### DISCUSSION

This study reports for, what is to our knowledge, the first time the effect of different intravenous doses of ketanserin

on cerebral uptake of  $^{123}\text{I}$ -R91150 in humans. Ketanserin is a potent 5-HT<sub>2A</sub> receptor antagonist (inhibition constant, 0.38 nmol/L), with less affinity for 5-HT<sub>2C</sub> receptors (inhibition constant, 14.5 nmol/L) (10). Ketanserin has also an affinity for  $\alpha$ 1-adrenergic receptors and histamine  $^1\text{H}$  receptors (11). Ketanserin lowers blood pressure in patients with hypertension, likely because of its  $\alpha$ 1-adrenergic antagonism. The compound has been shown to be tolerable, and severe side effects after treatment have not been reported (12). The clinical oral dose for hypertension is 20–80 mg/day. The maximum ketanserin doses administered in this study were 7.47 and 7.72 mg (subjects 1 and 2, respectively). An intravenous dose of 10 mg has been reported effective in the treatment of postanesthetic shivering in humans (13). The present study showed that large 5-HT<sub>2A</sub> receptor occupancies were achieved even with subtherapeutic doses of ketanserin. In the preliminary SPECT study using the 0.1 mg/kg dose as a challenge for  $^{123}\text{I}$ -R91150 in humans, no changes were observed in blood pressure, temperature, or pulse up to 12 h after administration (4). Similarly, no safety issues were found in the present study with any of the doses tested. Blood pressure, heart rate, and the electrocardiogram, including QTc interval, were maintained within reference ranges for the entire scanning period.

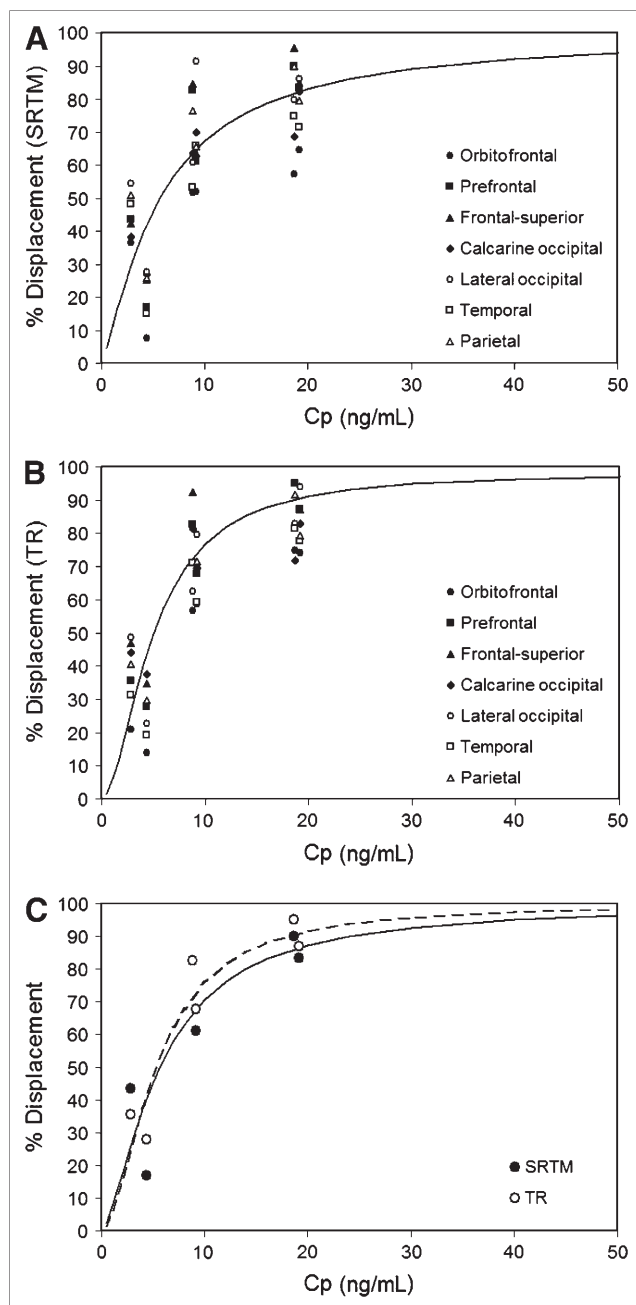


**FIGURE 5.** Time-activity curves of cerebellum and occipital calcarine region, with corresponding SRTM fit, from subject receiving highest ketanserin dose, 0.1 mg/kg. Slight and transient decrease in cerebellum uptake is seen from 210 to 240 min, when largest decrease in cortical time-activity curve occurred. Cerebellum slope recovered completely by 270 min.

### $^{123}\text{I}$ -R91150 Plasma Analysis

The activity corresponding to unmetabolized parent compound versus time (average value for 9 venous blood samples) was previously reported (1). As shown in the present work, during the basal state (before ketanserin administration), results similar to those previously reported (1) were found. Taking into account average values ( $n = 6$ ), we can state that a peak is found at 1.5 min after injection and that a quick decrease in activity ending in a plateau is found at less than 15 min after injection. The activity value at maximum (1.5 min) was  $0.068 \times 10^3 \text{ mL}^{-1}$ , which differs from that previously reported,  $0.035 \times 10^3 \text{ mL}^{-1}$  (1). Variability between subjects at short times has proven to be important (Fig. 1A, in which the SD for each group is shown).

One aim of analyzing plasma metabolites in the present work was to find out whether displacement of radioligand  $^{123}\text{I}$ -R91150 by ketanserin could be detected as an increase in the concentration of free parent compound in plasma. As



**FIGURE 6.** Relationship between ketanserin plasma concentration and  $^{123}\text{I}$ -R91150 displacement using SRTM, for all cortical regions (A); using TR, for all cortical regions (B); and comparing model fit for both SRTM and TR in representative region (prefrontal cortex) (C).

Figure 1 shows, no conclusions could be drawn on this point. With only 2 subjects included in each group, differences between subjects of the same group were often greater than differences between subjects of different groups. The expected increase in the concentration of free parent compound in plasma after administration of ketanserin (210 min after injection) is not detected as an increase in the amount of radioactivity (corresponding to unmetabolized parent compound) in plasma.

**TABLE 2**

Model Parameters Calculated for Each Region Using Both SRTM and TR

ROI	SRTM			TR		
	Displ <sub>max</sub>	EC <sub>50</sub>	$\gamma$	Displ <sub>max</sub>	EC <sub>50</sub>	$\gamma$
Orbitofrontal	82	7.1	1.2	82	6.5	2.2
Prefrontal	100	5.6	1.5	100	5.3	1.8
Superior frontal	100	5.1	1.6	100	4.2	1.6
Calcarine occipital	92	5.1	1.3	86	3.4	1.5
Lateral occipital	100	4.2	1.1	100	5.1	1.4
Temporal	100	6.9	1.0	92	6.0	1.7
Parietal	100	4.8	1.2	93	4.2	2.2
Population means	99.8	5.6	1.3	98.6	5.0	1.8

#### Ketanserin-Induced Displacement of $^{123}\text{I}$ -R91150

This study found a ketanserin dose-dependent displacement of  $^{123}\text{I}$ -R91150, supporting the specificity of  $^{123}\text{I}$ -R91150 binding to 5-HT<sub>2A</sub> receptor. A ketanserin dose of 0.1 mg/kg was previously reported to be a saturation dose, that is, a dose inducing more than 90% displacement, using  $^{18}\text{F}$ -altanserin in monkeys (5). The present study showed that this dose induced a  $^{123}\text{I}$ -R91150 displacement in the human brain that varied depending on the cortical region studied, ranging from 57.1% to 95.4% using SRTM values or from 71.9% to 101.2% using TR values. This finding agrees with the preliminary results of Travis et al. (4), who reported fast and intense displacement of  $^{123}\text{I}$ -R91150 to levels similar to those in the cerebellum in 3 healthy volunteers. The present study showed, in addition, intermediate (SRTM, 51.7%–91.4%/TR, 56.7%–102.8%) and low (SRTM, 7.7%–54.5%/TR, 13.8%–47.0%)  $^{123}\text{I}$ -R91150 displacements induced by, respectively, the subtherapeutic intermediate (0.05 mg/kg) and lowest (0.015 mg/kg) ketanserin doses tested, indicating that the SPECT technique is sensitive in detecting drug-induced occupancy of the 5-HT<sub>2A</sub> receptor. SRTM was used as the gold standard quantification method because the TR method may introduce errors in the determination of displacement. A good correlation was found between displacement measures using BP values (SRTM) and displacement measures using SUR values (TR). This finding supports the use of a single acquisition at pseudoequilibrium after bolus injection of  $^{123}\text{I}$ -R91150 and quantification using TR for measurement of drug-induced occupancy of the 5-HT<sub>2A</sub> receptor. The simplicity of this technique makes it suitable for most SPECT centers and increases patient compliance.

In previous SPECT studies with  $^{123}\text{I}$ -R91150 (2–4,14), and PET studies of the 5-HT<sub>2A</sub> receptor (15–17), the cerebellum was used as a reference region for quantification. In this study, a fast and transient decrease in the cerebellum time-activity curve was observed from 210 to 240 min after injection, that is, during the first 30 min after ketanserin administration, when the largest decrease in specific cortical binding occurred. This finding was also reported in a bolus/infusion study using  $^{18}\text{F}$ -altanserin (15), from which the cerebellum was concluded to be a valid representation

of free and nonspecifically bound ligand and brain radio-labeled metabolites. This conclusion was based on, first, the fact that although a small amount of 5-HT<sub>2A</sub> receptor is detectable by autoradiography, it may be difficult to detect by means of PET/SPECT and ketanserin displacement because of the poor statistical quality of the imaging data and spatial resolution of the images and, second, evidence that blocking the 5-HT<sub>2A</sub> receptor with unlabeled MDL 100,907 before a PET study did not eliminate the need for a 2-tissue-compartment model in the cerebellum (18). An acute and transient effect of ketanserin on cerebellar regional cerebral blood flow might also be considered. However, such an effect seems unlikely given that no blood pressure decrease was detected in any subject studied, and taking into account the marked decrease in systemic blood pressure needed to significantly decrease regional cerebral blood flow, because of the cerebral autoregulation mechanisms. The low <sup>123</sup>I-R91150 uptake in cerebellum and lack of displacement after ketanserin administration found in this study further support the suitability of using this region as a reference.

#### Ketanserin Plasma Concentration and Relationship with <sup>123</sup>I-R91150 Displacement

The ketanserin pharmacokinetics profile after intravenous injection increased linearly with increasing dose. The subtherapeutic dose range explored for ketanserin and the short pharmacokinetics collection time (less than 5 h) did not allow for a direct comparison between reported ketanserin pharmacokinetics parameters (19) and pharmacokinetics data from the present study. However, the ketanserin plasma levels found 4 h after injection at the highest dose (approximately 7.5 mg) were in line with the average concentration measured at the same time point after a 10-mg intravenous injection in healthy subjects (19). The Hill equation used for describing the concentration-displacement relationship in the different ROIs described the data well, both using displacement calculated according to SRTM and using displacement calculated according to TR, with little difference in ketanserin potency and maximal displacement among different brain regions. In particular, a 15%–20% variability in EC<sub>50</sub> was observed among different regions, indicating a fairly homogeneous displacement mechanism in brain areas expressing 5-HT<sub>2A</sub> receptors. Also, the EC<sub>50</sub> values calculated using SRTM- and TR-derived displacements agreed well. Differences were less than 20%, with the exception of the calcarine occipital and frontal superior regions. Overall, the major differences between the model parameters calculated using SRTM and those calculated using TR were in hill slope factor ( $\gamma$ ) estimates, supporting the fact that the concentration-displacement relationship conforms to a rectangular hyperbola (i.e., interaction with a single binding site) only for SRTM-derived data.

#### CONCLUSION

<sup>123</sup>I-R91150 is a SPECT radioligand specific for the 5-HT<sub>2A</sub> receptor in the human brain. The findings of the

present study demonstrate that <sup>123</sup>I-R91150 SPECT is sensitive enough to measure ketanserin-induced displacement in a dose-dependent manner and support the use of the cerebellum as a reference region for quantification. The relationship between displacement and ketanserin plasma concentration was well defined, with an EC<sub>50</sub> of 5.6 ng/mL based on SRTM displacement and of 5.0 ng/mL based on TR.

Use of the simplified method of acquiring a single scan at pseudoequilibrium after bolus injection of <sup>123</sup>I-R91150 and TR produced results comparable to those of using scanning over time and SRTM. Therefore, TR after a single acquisition at pseudoequilibrium can be used to measure drug-induced occupancy of the 5-HT<sub>2A</sub> receptor with <sup>123</sup>I-R91150 SPECT.

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