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Comparison of [^{18}F]altanserin and [^{18}F]deuteroaltanserin for PET imaging of serotonin$_{2A}$ receptors in baboon brain: pharmacological studies

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Abstract

The regional distribution in brain, distribution volumes, and pharmacological specificity of the PET 5-HT$_{2A}$ receptor radiotracer [^{18}F]deuteroaltanserin were evaluated and compared to those of its non-deuterated derivative [^{18}F]altanserin. Both radiotracers were administered to baboons by bolus plus constant infusion and PET images were acquired up to 8 h. The time-activity curves for both tracers stabilized between 4 and 6 h. The ratio of total and free parent to metabolites was not significantly different between radiotracers; nevertheless, total cortical R_T (equilibrium ratio of specific to nondisplaceable brain uptake) was significantly higher (34–78%) for [^{18}F]deuteroaltanserin than for [^{18}F]altanserin. In contrast, the binding potential (Bmax/K_D) was similar between radiotracers. [^{18}F]Deuteroaltanserin cortical activity was displaced by the 5-HT$_{2A}$ receptor antagonist SR 46349B but was not altered by changes in endogenous 5-HT induced by fenfluramine. These findings suggest that [^{18}F]deuteroaltanserin is essentially equivalent to [^{18}F]altanserin for 5-HT$_{2A}$ receptor imaging in the baboon.

Keywords: Serotonin; 5-HT$_{2A}$ receptor; PET; Primate brain

1. Introduction

From the time of the first *in vivo* human planar image of a radiolabeled hallucinogen which primarily displayed the whole body distribution of the serotonin 5-HT$_{2A}$ receptor, [35] considerable efforts have been applied towards the development of a selective radiotracer for PET and/or SPECT imaging of the brain 5-HT$_{2A}$ receptor. While several radiotracers have been developed to date, their utility for *in vivo* imaging has been limited due to high nonspecific binding or inadequate pharmacological selectivity over the dopamine D_2 and/or 5-HT$_{2c}$ receptor [4,7,38]. Recently, major advances towards selective imaging of brain 5-HT$_{2A}$ receptors occurred with the development of [^{18}F]altanserin, which demonstrated improved pharmacological specificity for binding to 5-HT$_{2A}$ receptors, with greater than 100- and 45-fold selectivity over D_2 and 5-HT$_{2C}$ receptors, respectively [1,2,13,16,41]. The value of [^{18}F]altanserin as a PET 5-HT$_{2A}$ radiotracer has been supported by its high specific brain uptake [3,15,34], relatively long half-life that permits equilibrium imaging using a bolus-plus-constant infusion paradigm [43], reasonable target to background ratios [3,15, 34,43], and reliable test-retest measures [37]. Despite these attributes, quantitative *in vivo* imaging of 5-HT$_{2A}$ receptors with [^{18}F]altanserin has been hindered by the generation of lipophilic radiometabolites which cross the blood-brain barrier. While not pharmacologically active, these metabolites increase nondisplaceable uptake and may hinder reliable quantitation [1,20,27,40].

Recently, a deuterated analog of [^{18}F]altanserin was synthesized in effort to decrease the rate of metabolism and production of radioactive metabolites (Fig. 1). Deuteration

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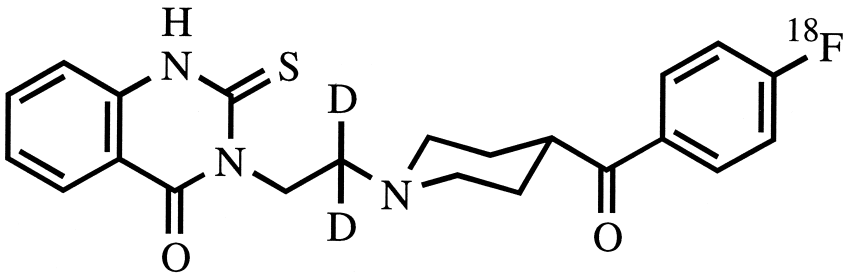

Fig. 1. Chemical structure of [18F]deuteroaltanserin. The 2′hydrogens in altanserin were substituted with deuteriums to generate deuteroaltanserin.

does not alter the pharmacological specificity or the neuroanatomical distribution of the radiotracer, however it may decrease the rate of metabolism if transient cleavage of the carbon-hydrogen bond is involved in the rate limiting or rate-contributing step in the metabolism of the parent radiotracer because the carbon-deuterium bond is more difficult to break than the carbon-hydrogen bond. In keeping, a preliminary study in humans demonstrated a 29% higher ratio of parent to labeled metabolites in plasma for [18F]deuteroaltanserin compared to [18F]altanserin suggesting that deuteration induced a modest but distinct reduction in the rate of metabolism of altanserin [42]. Despite a slower rate of metabolism in humans, administration of [18F]deuteroaltanserin by the bolus plus constant infusion paradigm permits equilibrium imaging of 5-HT_{2A} receptors [44] similar to [18F]altanserin [43]. In the present study, the regional distribution, metabolic stability, and pharmacological specificity of [18F]deuteroaltanserin brain uptake was examined and compared to [18F]altanserin in baboons using a constant infusion paradigm to achieve equilibrium receptor binding conditions.

2. Methods

2.1. Radiochemistry

[18F]Altanserin and [18F]deuteroaltanserin were prepared in a remote-control system as previously described [39,41]. For the studies herein, the overall radiochemical yields determined at the end of bombardment were 19.0 ± 5.2% and 20.8 ± 8.9%, with radiochemical purity of 95.7 ± 3.3% and 95.9 ± 2.5% for [18F]altanserin (n = 8; mean ± SD) and [18F]deuteroaltanserin (n = 14; mean ± SD), respectively. At the end of the synthesis, the specific activities were 233 ± 149 and 291 ± 177 GBq/μmol with corresponding synthesis times of 114 ± 10 min and 110 ± 7 min for [18F]altanserin (n = 8; mean ± SD) and [18F]deuteroaltanserin (n = 14; mean ± SD), respectively.

2.2. Animal protocol

All studies were approved by the Yale University Animal Care Committee. Three female ovarectimized baboons (*Papio anubis*) were studied. The animals were fasted for 18 to 24 h prior to each study, which occurred at a minimum of 2 week intervals for each animal. At approximately 2 h before the radiotracer injection, the animal was immobilized using ketamine (10 mg/kg i.m.) and then intubated for administration of 2.5% isoflurane. Glycopyrrolate (10 mg/kg i.m.), a long-acting peripheral anticholinergic drug that does not cross the blood-brain barrier [28], was coadministered with ketamine to decrease respiratory and digestive secretions. Body temperature was maintained between 35–36°C using a heated water blanket. Vital signs, including heart rate, respiratory rate, oxygen saturation and body temperature, were monitored every 30 min throughout each study. An intravenous perfusion line with 0.9% saline was used for the radiotracer injection. A second line with lactated Ringers solution was maintained at a rate of 11 mL/kg/h throughout the experiment and was used to obtain blood samples.

2.3. Plasma analysis

For three [18F]altanserin and seven [18F]deuteroaltanserin studies, blood samples were obtained at approximately 30, 60, 120, 180, 240, 300, 330, 360, 390, 420, 450 and 480 min post injection. For five [18F]deuteroaltanserin studies, plasma data were sampled at only 1 to 2 time points between 5 and 6 h to evaluate parent radiotracer levels for determination of plasma-dependent equilibrium outcome measures. Plasma analyses were conducted as described previously [43,44]. Mean count rates were decay corrected to the time of injection and normalized for the infusion rate. The final units of plasma activity are reported as (μBq/mL)/(Bq/h).

2.4. Imaging protocol

Baboons were placed with the head immobilized by a "bean bag" which hardens upon evacuation (Olympic Medical, Seattle, WA) within the gantry of a Posicam 6.5 camera (Positron Corp., Houston, TX) as described previously [43]. Frame mode acquisitions (2–20 min) were obtained every 30–60 min throughout the infusion period for initial studies with [18F]altanserin. Thereafter, PET acquisitions (2–10 min) were obtained every 15 min between 4 and 8 h of

infusion for both [^{18}F]altanserin and [^{18}F]deuteroaltanserin experiments.

To identify brain regions, MRI scans of 1.5 mm contiguous slices were obtained with a 1.5 Tesla GE Signa device. Axial images were acquired using a spoiled GRASS (gradient recall acquisition in the steady state) sequence with TR = 25 ms, TE = 5 ms, NEX = 2, matrix = 256 x 256, field of view = 16 cm.

2.5. Pharmacological displacement & challenge studies

Pharmacological doses of the 5-HT$_{2A}$ receptor antagonists, ketanserin [17,19] (Research Biochemicals International, MA) and SR 46349 B [30,31] (Sanofi, France), were administered intravenously after equilibrium had been established for at least 1 h (e.g. 5.5 to 6.5 h). Both drugs were dissolved initially in 1.1 mL of a 45% solution of hydroxypropyl-β-cyclodextrin (Encapsin HPB; Janssen Biotech N.V., Belgium) containing 10% alcohol. Upon dissolution, the compound was further diluted to 10 mL using 0.9% NaCl. In total**,** six displacement studies were done for [^{18}F]altanserin in two baboons, four using ketanserin at doses of 1.5–2 mg/kg and two using SR 46349B at a dose of 1.5 mg/kg. Three displacement studies were done for [^{18}F]deuteroaltanserin in three different baboons using SR 46349B at doses of 1.0–2.0 mg/kg. All displacing drugs were injected slowly over 30 s while vital signs were monitored.

To assess the potential for extracellular 5-HT to alter [^{18}F]deuteroaltanserin labeling, the 5-HT releaser S (+)-fenfluramine (Research Biochemicals International, MA) was dissolved in 0.9% NaCl and administered intravenously at doses of 1.0 and 1.5 mg/kg. Microdialysis studies have demonstrated that intravenous administration of S (+)-fenfluramine within this dose range induces 6 to 10-fold elevations in central 5-HT levels [33].

2.6. Image analysis

PET emission data were reconstructed with a Butterworth filter and corrected for attenuation using a theoretical μ (0.096 cm^{-1}) in an ellipse drawn around the edge of the baboon's skull. Images were co-registered to one another using the "realign" function in SPM 96 (Statistical Parametric Mapping 96 [10]; and were then reoriented approximately to the AC-PC line. For region of interest (ROI) analysis in the initial study, a summed PET image was co-registered to the MRI using surface registration (MEDx, Sensor Systems) [25]. Two-dimensional (2D) ROI templates were drawn on the co-registered MRI using a baboon brain atlas as a reference [29]. Thereafter, ROI analyses were performed using identical 2D ROI templates that were positioned by reference to the activity distribution on a summed PET image generated by addition of all images acquired in the study. The regional radioactive densities (cpm/pixel) were determined by forming three-dimensional (3D) volumes between two-dimensional ROIs placed on three adjacent slices (except for the cerebellum which included 2 adjacent slices) in MEDx. The final average 3D volumes (cm^3) were right and left frontal (R 3.6, L 3.6), temporoparietal (R 16.3, L 16.8), occipital (R 9.4, L 9.4) and temporal (R 4.2, L 4.2) cortices), and cerebellum (R 4.3, L 4.4). All regional cortical radioactive densities were averaged as a measure of total cerebral cortical uptake. The total cerebellar activity was reported as the average value of the right and left cerebellar hemispheres. Mean count densities (cpm/pixel) were decay corrected to the time of injection and normalized for the infusion rate. Thus, the final units of brain activity are (mBq/cm^3)/(Bq/h).

2.7. Outcome measures

Regional brain uptake was described by three outcome measures (V_3, V_3' and R_T) theoretically derived based upon a three-compartment equilibrium model as described previously [43,44]. For the baboon studies, V_3, V_3' and R_T are reported as the average between 4 and 6 h (except for 2 studies where displacement was initiated at 5.5 h) of infusion for [^{18}F]altanserin and 4.5 to 6.5 h of infusion for [^{18}F]deuteroaltanserin.

2.8. Statistical comparisons

The *a priori* hypothesis that the outcome measure R_T (but not V_3, V_3') would be higher for [^{18}F]deuteroaltanserin compared to that for [^{18}F]altanserin was assessed in baboons 1 and 2. A two-way analysis of variance (ANOVA) was performed in SPSS (SPSS Inc., Chicago, IL, USA) with radiotracer and baboon as independent variables. This analysis assessed the main effect of degree for a difference in the outcome measure between [^{18}F]altanserin and [^{18}F]deuteroaltanserin and the main effect of each baboon and the interaction of baboon by radiotracer.

3. Results

3.1. Neuroanatomical distribution

Visualization of the regional distributions of both radiotracers indicated that the neuroanatomical distribution of [^{18}F]deuteroaltanserin activity was identical to that of [^{18}F]altanserin. High uptake was found in cortical regions, including cingulate, frontal, parietal, insular, occipital, and temporal cortices. Both [^{18}F]altanserin and [^{18}F]deuteroaltanserin activity levels were virtually nondetectable in the striatum and cerebellum (data not shown).

3.2. Equilibrium imaging

The ability of [^{18}F]altanserin and [^{18}F]deuteroaltanserin to achieve stable levels of plasma and brain activity were

Table 1
Parameters for equilibrium modeling of [^{18}F]altanserin and [^{18}F]deuteroaltanserin

	[^{18}F]altanserin (n = 8)	[^{18}F]deuteroaltanserin (n = 14)
Bolus (MBq)	245.1 ± 43.9	180.2 ± 13.6
Infusion Rate (MBq/h)	68.5 ± 9.0	65.3 ± 3.9
B/I Ratio (h)	3.34 ± 0.11	2.92 ± 0.03

The data shown are the mean ± SD.

assessed using the bolus plus constant infusion paradigm (Table 1). As illustrated in the representative studies in Figure 2, plasma parent levels of [^{18}F]altanserin and [^{18}F]deuteroaltanserin stabilized by 3–4 h with bolus to infusion ratios of 3.8 and 3.0 respectively (Figure 2). The average levels of plasma total parent, free parent, deuteroaltanserinol and metabolites derived from 4-(p-fluorobenzoyl)piperidine [FBP] across all altanserin and deuteroaltanserin studies are shown in Table 2. Overall, the ratios of total parent to total metabolites and the ratio of free parent to total metabolites were not significantly different between [^{18}F]altanserin and [^{18}F]deuteroaltanserin for baboons 1 and 2.

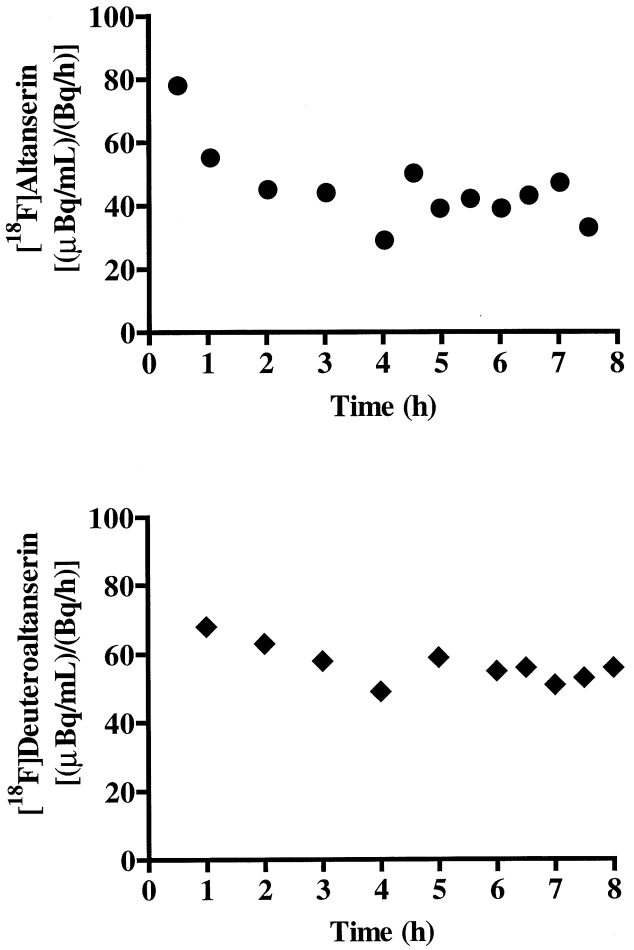

Fig. 2. Representative plasma time-activity curves for [^{18}F]altanserin and [^{18}F]deuteroaltanserin in baboon. The level of parent radiotracer was assessed in plasma samples taken at various time-points throughout the bolus plus constant infusion study. Top panel: [^{18}F]Altanserin was administered by intravenous bolus (170 MBq) plus constant infusion (41 MBq/h). [^{18}F]Altanserin activity in plasma stabilized by 3 h. Bottom panel: [^{18}F]Deuteroaltanserin was administered by intravenous bolus (237 MBq) plus constant infusion (78 MBq/h). [^{18}F]Altanserin activity in plasma stabilized by 4 h and remained constant for duration of infusion (8 h). The reported activity levels have been normalized to the infusion rate and thus are units of (μBq/mL) normalized to the radiotracer infusion rate (Bq/h).

[^{18}F]Altanserin activity in the cerebral cortex and cerebellum stabilized by 4–5 h and remained relatively constant for duration of infusion (8 h; Figure 3 top). Specific cortical uptake, defined as total cortical uptake–cerebellar uptake, changed on average 2.4 ± 3.7%/h (n =8) until the end of the infusion or the time displacement was induced (5.5–6 h). [^{18}F]Deuteroaltanserin activity in the cerebral cortex and cerebellum stabilized by 5–6 h and remained relatively constant for duration of infusion (8 h) (Figure 3 bottom). Specific cortical uptake changed on average–1.0 ± 3.9%/h (n = 14) for the duration of infusion or until the time displacement was induced (i.e., 6.5 h).

Regional brain uptake for both [^{18}F]altanserin and [^{18}F]deuteroaltanserin was assessed by three outcome measures derived between 4 and 6 h (except for two studies where displacement was initiated at 5.5 h) of infusion for [^{18}F]altanserin and 4.5 to 6.5 h of infusion for [^{18}F]deuteroaltanserin (Table 2). All three outcome measures were compared between [^{18}F]deuteroaltanserin and [^{18}F]altanserin for baboons 1 and 2 using a two-way ANOVA (analysis of variance). There was no significant two-way interaction between each outcome measure by baboon for all brain regions examined. The binding potential V_3 and also V_3' did not differ between [^{18}F]deuteroaltanserin and [^{18}F]altanserin uptake in frontal, occipital, temporoparietal, and total cortex (Table 3). In contrast, R_T was significantly higher for [^{18}F]deuteroaltanserin than [^{18}F]altanserin in the occipital (DF = 1,10; F = 12.758; p = 0.009) temporoparietal (DF = 1,10; F = 9.593; p = 0.017) and total cortex (DF = 1,10; F = 9.661; p = 0.017; Table 3).

3.3. Pharmacological displacement studies

Displacement studies were performed to assess the pharmacological specificity of the *in vivo* uptake of [^{18}F]altanserin and [^{18}F]deuteroaltanserin. After [^{18}F]altanserin activity stabilized in the cerebral cortex and cerebellum (5.5–6.5 h), ketanserin (1.5–2.0 mg/kg) was administered as an intravenous bolus. Cortical [^{18}F]altanserin activity decreased significantly within 30 min (data not shown). On average, across three displacement studies, a 73 ± 13% (n = 3) decrease in specific [^{18}F]altanserin activity was observed. [^{18}F]Altanserin activity levels in the cerebellar ROI were minimally displaced (6 ± 2%; n = 3) by the administration of ketanserin. Administration of 1.5 mg/kg SR46349B at 6 h resulted in 76% displacement of specific [^{18}F]altanserin cortical activity and 13% displacement of cerebellar activity from 30 min post-administration until the end of the infu-

Table 2
Plasma Parent & Metabolite Levels for [^{18}F]Deuteroaltanserin & [^{18}F]Altanserin

	[^{18}F]-Deuteroaltanserin			[^{18}F]-Altanserin	
	Baboon 1 (n = 7)	Baboon 2 (n = 1)	Baboon 3 (n = 3)	Baboon 1 (n = 1)	Baboon 2 (n = 2)
Total Parent (μBq/mL)/(Bq/h)	64.1 ± 23.6	39.9	51.3 ± 7.6	65.3	39.6 ± 0.6
Free Parent (μBq/mL)/(Bq/h)	1.0 ± 0.4	0.8	1.1 ± 0.2	1.1	0.8 ± 0.1
FBP** (μBq/mL)/(Bq/h)	264.2 ± 82.7	101.1	121.8 ± 11.0	183.2	98.2 ± 49.9
D/Altanserinol#$^{\#}$ (μBq/mL)/(Bq/h)	33.5 ± 3.1	12.2	25.0 ± 2.0	51.7	24.5 ± 11.5
f_1	1.7 ± 0.9%	1.7%	2.1 ± 0.9%	0.9%	2.3 ± 0.3%
Total parent/total metabolites	0.29 ± 0.20	0.35	0.35 ± 0.08	0.28	0.37
Free parent/total metabolites	0.005 ± 0.003	0.007	0.007 ± 0.002	0.004	0.008 ± 0.004

The mean ± SD is shown for plasma activity levels normalized to the infusion rate as described in the text.
** FBP fraction represents the major lipophilic metabolite fraction, probably derived from 4-(p-flurobenzoyl)piperidine as described in the text.
deuteroaltanserinol/altanserinol for deuteroaltanserin and altanserin respectively.

sion (Figure 4, top panel). After [^{18}F]deuteroaltanserin brain activity had stabilized (6.5 h), administration of SR 46349B (1.5–2.0 mg/kg), decreased cortical binding by 77 ± 5%; however, cerebellar activity levels decreased by 15 ± 6%. Because displacement with ketanserin and SR 46349B was not complete, it was not possible to reliably assess the uniformity of residual [^{18}F]altanserin and [^{18}F]deuteroaltanserin activity.

3.4. 5-HT challenge studies

To determine if changes in 5-HT levels would alter [^{18}F]deuteroaltanserin uptake, the 5-HT releaser S (+)-fenfluramine was administered under equilibrium binding conditions (Figure 5). S (+)-Fenfluramine was administered intravenously in three independent experiments, at doses of 1.0 (1 study) and 1.5 (2 studies) mg/kg in two different baboons. In all three studies, elevations in 5-HT induced by S (+)-fenfluramine failed to alter cortical [^{18}F]deuteroaltanserin uptake.

4. Discussion

In the present study, the PET 5-HT$_{2A}$ receptor radiotracer [^{18}F]deuteroaltanserin was evaluated and compared to its non-deuterated derivative [^{18}F]altanserin in nonhuman primates. Equilibrium imaging by administration of the radiotracer using the bolus plus constant infusion paradigm indicated that both radiotracers achieved steady-state between 4 and 6 h. Assessment of the metabolism of both radiotracers by comparison of the total parent and free parent to total metabolite ratios demonstrated no significant difference in the metabolism of the two radiotracers during equilibrium in baboons. The binding potential was not different between the two radiotracers, however [^{18}F]deuteroaltanserin demonstrated a higher ratio of specific to nondisplaceable uptake (R_T) compared to [^{18}F]altanserin. [^{18}F]Deuteroaltanserin cortical activity was displaced by 5-HT$_{2A}$ receptor antagonists but was not altered by changes in endogenous 5-HT induced by fenfluramine. Taken together, these findings indicate that [^{18}F]deuteroaltanserin is essentially equivalent to [^{18}F]altanserin for 5-HT$_{2A}$ receptor imaging in the baboon.

4.1. [^{18}F]deuteroaltanserin vs [^{18}F] altanserin uptake in baboon brain

Both radiotracers achieved steady-state in plasma by 3 h and apparent equilibrium receptor binding conditions in brain between 4 and 6 h using bolus to infusion ratios of 2.9 to 3.8. The ratios of total parent to total metabolite and free parent to total metabolite were not significantly different between [^{18}F]deuteroaltanserin and [^{18}F]altanserin for the two baboons studied. This finding was surprising given the evidence that deuteration should decrease the rate of dealkylation of the piperidine nitrogen and the generation of metabolites in the FBP fraction [40], and that [^{18}F]deuteroaltanserin was more slowly metabolized and its parent to total metabolite ratio was 29% higher compared to its non-deuterated derivative in humans [42]. The lack of a significant difference in the present study suggests possible species differences in metabolism that preclude a decrease in metabolic rate by deuteration in baboons in contrast to humans. In keeping with this premise there is clear evidence supporting differences in metabolism of [^{18}F]altanserin between rats and humans [40] and probably baboons [27]. Paradoxically, the significant increase of [^{18}F]deuteroaltan-

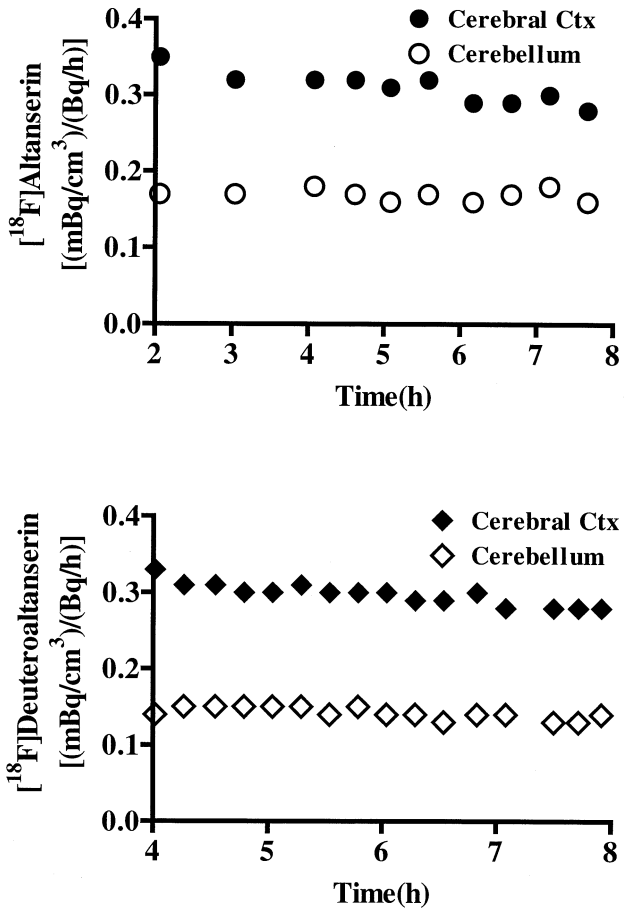

Fig. 3. Representative time-activity curves for equilibrium imaging studies of [^{18}F]altanserin and [^{18}F]deuteroaltanserin brain uptake in baboon. Top panel: [^{18}F]Altanserin was administered by intravenous bolus (170 MBq) plus constant infusion (41 MBq/h) and PET images were acquired for up to 8 h. [^{18}F]Altanserin activity in the cerebral cortex (solid circle) and cerebellum (open circle) stabilized by 4 h. Bottom panel: [^{18}F]Deuteroaltanserin was administered by intravenous bolus (237 MBq) plus constant infusion (78 MBq/h) and PET images were acquired between 4 and 8 h. [^{18}F]deuteroaltanserin activity in the cerebral cortex (solid diamond) and cerebellum (open diamond) stabilized by 5 h and remained constant for duration of infusion (8 h). The reported activity levels have been normalized to the infusion rate and thus are in brain activity (mBq/cm^3) normalized to the radiotracer infusion rate (Bq/h).

serin R_T (but not V_3 and V_3') in these two baboons *was* consistent with the findings in human subjects. If the metabolic rate was decreased by deuteration, it would be expected that R_T (but not V_3 and V_3') would be higher for [^{18}F]deuteroaltanserin, since it is determined in part by the nondisplaceable uptake, which may be altered by differential formation of lipophilic radiometabolites. While it is tempting to conclude that this finding supports the *a priori* hypothesis and the observations in human subjects [42,44], the limitations inherent to the present study including comparisons of R_T made from studies conducted at variable and lengthy time intervals and the unequal proportions within each statistical cell of the two-way ANOVA including two cells with a n = 1, as well as the lack of significant difference in the parent to metabolite ratio, weakens this conclusion.

4.2. Neuroanatomical specificity of [^{18}F]altanserin and [^{18}F]deuteroaltanserin brain uptake

The neuroanatomical distribution of [^{18}F]altanserin and [^{18}F]deuteroaltanserin uptake in brain was indistinguishable and expected since deuteration does not alter the pharmacological or neuroanatomical specificity of the radiotracer. Qualitative visualization of [^{18}F]altanserin and [^{18}F]deuteroaltanserin brain uptake indicated high uptake throughout the cerebral cortices with similar densities observed in the frontal, parietal, and temporal cortices. Virtually no uptake was visualized in the striatum and cerebellum. This neuroanatomical distribution of both radiotracers agrees with the distribution of 5-HT_{2A} receptors previously demonstrated in postmortem human brain [21,23].

4.3. Pharmacological specificity of [^{18}F]altanserin and [^{18}F]deuteroaltanserin brain uptake

The specificity of [^{18}F]altanserin and its deuterated analog, [^{18}F]deuteroaltanserin were confirmed by displacement studies using relatively selective 5-HT_{2A} receptor antagonists. The bolus plus constant infusion paradigm for equilibrium imaging is highly amenable to assessing the pharmacological specificity of a radiotracer. Once equilibrium has been achieved and sustained for a period of time, an intravenous bolus of a pharmacological dose of the receptor specific antagonist is administered and specific radiotracer uptake declines to baseline values within a brief time period. In the present study, after administration of high doses of the 5-HT_{2A} receptor antagonist ketanserin, total cortical [^{18}F]altanserin activities decreased to levels within $<$10% of cerebellar activity levels. Ketanserin, the archetypal 5-HT_{2A} receptor antagonist, exhibits high affinity for binding to the 5-HT_{2A} receptor [17], high selectivity compared to its affinities for other serotonin and dopamine receptors [13,19], but also has been shown to have high to moderate affinity for the α_1 adrenergic receptor [16] and to primarily label the vesicular monoamine transporter in striatal and midbrain regions [8,18]. Since altanserin and ketanserin both display high nanomolar affinity for binding to the α_1 adrenergic receptor (Ki values = 4.6 and 11.0 nM respectively [16] which imparts only a two- [13] to thirteen-fold (α_1/5-HT_{2A}) [16] and three- [5] to seventeen [16]-fold (α_1/5-HT_{2A}) selectivity, pharmacological specificity was assessed also using the 5-HT_{2A} receptor antagonist SR 46349B, which has significantly lower affinity for the α_1 receptor (3.4 μM; [30]). Both [^{18}F]altanserin and [^{18}F]deuteroaltanserin cortical uptakes were displaced by SR 46349 B. In addition, a low but notable amount of cerebellar activity (approximately 15%) was also displaced. While not a consistent finding [6,31], very low levels of 5-HT_{2A} receptors have been localized to cerebellum [9,21,24] and

Table 3
Outcome measures for equilibrium modeling of [^{18}F]deuteroaltanserin and [^{18}F]altanserin

	[^{18}F]-Deuteroaltanserin			[^{18}F]-Altanserin	
	Baboon 1	Baboon 2	Baboon 3	Baboon 1	Baboon 2
R_T	(n = 7)	(n = 1)	(n = 3)	(n = 1)	(n = 2)
Frontal cortex	0.97 ± 0.23	1.07	1.23 ± 0.20	0.62	0.86 ± 0.05
Occipital cortex**	1.13 ± 0.20	1.48	1.17 ± 0.20	0.62	0.91 ± 0.07
Temporo-parietal cortex**	0.97 ± 0.19	1.26	1.14 ± 0.19	0.51	0.85 ± 0.02
Cerebral cortex**	1.05 ± 0.19	1.33	1.18 ± 0.19	0.59	0.88 ± 0.04
V_3' (mL/cm^3)	(n = 7)	(n = 1)	(n = 3)	(n = 1)	(n = 2)
Frontal cortex	2.48 ± 1.01	3.02	3.44 ± 0.56	2.90	3.51 ± 0.50
Occipital cortex	2.81 ± 0.93	4.01	3.39 ± 1.02	2.37	3.47 ± 0.22
Temporo-parietal cortex	2.43 ± 0.84	3.43	3.18 ± 0.66	2.87	3.71 ± 0.02
Cerebral cortex	2.63 ± 0.91	3.62	3.35 ± 0.81	2.75	3.56 ± 0.13
V_3 (mL/cm^3)	(n = 7)	(n = 1)	(n = 3)	(n = 1)	(n = 2)
Frontal cortex	155.2 ± 62.9	143.9	163.8 ± 26.9	181.1	167.4 ± 23.8
Occipital cortex	175.9 ± 57.9	190.9	161.6 ± 48.5	179.2	176.9 ± 1.0
Temporo-parietal cortex	151.9 ± 52.7	163.3	151.5 ± 31.5	148.1	165.0 ± 10.2
Cerebral cortex	164.7 ± 57.0	172.3	159.6 ± 38.5	171.9	169.5 ± 6.2

The data shown are the mean ± SD. R_T = [(cortical ROI-CB)/CB]; V_3' = [(cortical ROI-CB)/total plasma parent]. V_3 = [(Cortical ROI-CB)/total plasma parent** mean f_1].

** Two-way ANOVA for baboons 1 & 2, deuteroaltanserin vs altanserin $p < 0.02$.

thus may be detected by [^{18}F]altanserin and [^{18}F]deuteroaltanserin. Alternatively, the displacement in cerebellum may be due to a low level contamination of the 5-HT$_{2A}$ receptor signal by other receptors, such as the 5-HT$_{2c}$ receptor for which both altanserin and SR 46349B (but not ketanserin) display moderate affinities. Furthermore, the slightly enhanced displacement by SR 46349B (compared to ketanserin) in both cortical and cerebellar regions may reflect trace labeling of another receptor. Regardless of the source of displacement, the low but measurable level of displacement of cerebellar activity by 5-HT$_{2A}$ receptor antagonists raises concerns about the suitability of the cerebellum as a background region for assessments of 5-HT$_{2A}$ receptor number using [^{18}F]deuteroaltanserin and [^{18}F]altanserin.

4.4. Sensitivity of cortical [^{18}F]deuteroaltanserin labeling to endogenous 5-HT

Sensitivity of brain radiotracer uptake to endogenous neurotransmitters has been studied previously by the induction of an acute elevation in neurotransmitter levels by administration of a drug that stimulates release, and depletion of endogenous neurotransmitter by administration of a key enzyme inhibitor that inhibits the rate–limiting synthetic enzyme [14]. S (+)-fenfluramine, which elevates extrasynaptic 5-HT levels by inhibition of 5-HT reuptake and release of cytoplasmic 5-HT through reversal of the 5-HT transporter [11,22] may be used to assess the sensitivity of 5-HT$_{2A}$ receptor radiotracers to endogenous 5-HT. In the present study, intravenous administration of S (+)-fenfluramine at doses known to rapidly stimulate a 5–10 fold elevation of extracellular 5-HT levels, did not alter [^{18}F]deuteroaltanserin binding. The lack of an effect is most likely not due to inadequate distribution of S (+)-fenfluramine to the cerebral cortex or prohibition by general anesthesia, since fenfluramine distribution is uniform across brain regions [12] and elevates 5-HT even in the presence of anesthetics [36]. The lack of an effect may be a consequence of the low affinity of endogenous 5-HT for binding to the antagonist site on 5-HT$_{2A}$ receptors [13,26] or of receptor internalization [32], which would allow antagonist binding but preclude access to agonist.

5. Summary

The present study demonstrates that the deuterated derivative of the 5-HT$_{2A}$ PET radiotracer [^{18}F]altanserin, ([^{18}F]deuteroaltanserin) exhibits high brain uptake with a neuroanatomical signature characteristic of 5-HT$_{2A}$ receptor distribution and exhibits reasonable pharmacological specificity *in vivo*. Elevations in extrasynaptic 5-HT induced by S (+)-fenfluramine failed to alter cortical uptake indicating that [^{18}F]deuteroaltanserin binding is insensitive to endogenous 5-HT levels. Overall, the properties of [^{18}F]deuteroaltanserin were comparable to those observed for its more established non-deuterated derivative [^{18}F]altanserin, with the exception of the outcome measure R_T which was modestly higher than that observed for [^{18}F]altanserin. Collectively, these findings suggest that [^{18}F]deuteroaltanserin is suitable for, and may be superior to [^{18}F]altanserin for PET imaging of the 5-HT$_{2A}$ receptor.

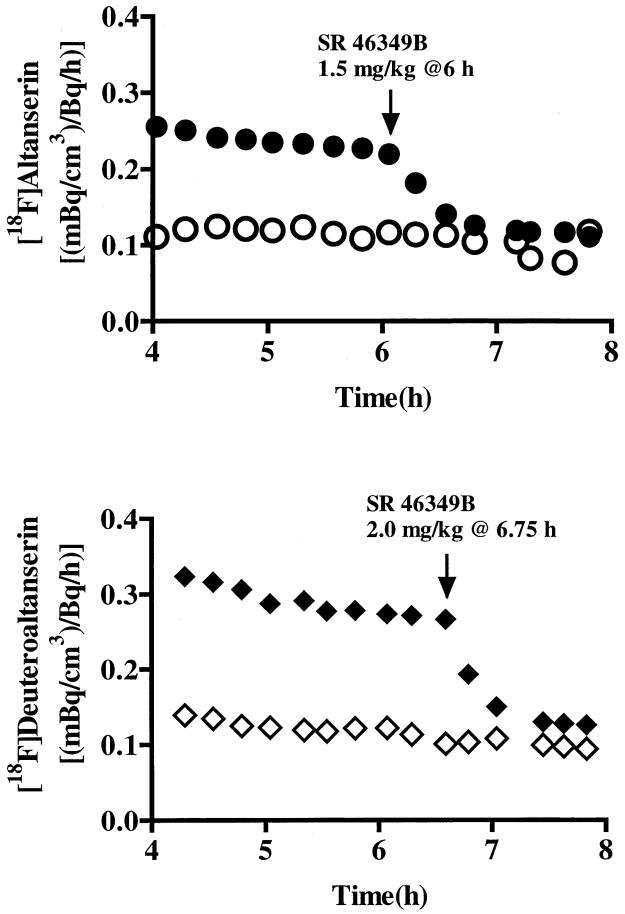

Fig. 4. Representative pharmacological displacement studies for [^{18}F]altanserin and [^{18}F]deuteroaltanserin. Top panel: [^{18}F]Altanserin was administered by intravenous bolus (229 MBq) plus constant infusion (78 MBq/h), and PET images were acquired for 8 h. SR46349B (1.5 mg/kg) was administered by intravenous bolus after 6 h of infusion and scanning was continued for 2 h. Bottom panel: [^{18}F]Deuteroaltanserin was administered by intravenous bolus (207 MBq) plus constant infusion (74 MBq/h) and PET images were acquired between 4 to 8 h. SR46349B (2.0 mg/kg) was administered by intravenous bolus after 6.75 h of radiotracer infusion and scanning was continued for 1.5 h.

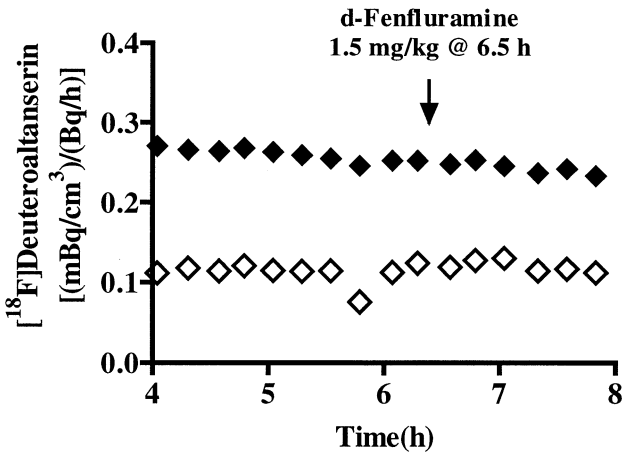

Fig. 5. Representative illustration of the effects of S (+)-fenfluramine on [^{18}F]deuteroaltanserin equilibrium binding. [^{18}F]Deuteroaltanserin was administered by intravenous bolus (181 MBq) plus constant infusion (63 MBq/h) and PET images were acquired between 4 to 8 h. The 5-HT releaser S (+)-fenfluramine (1.5 mg/kg) was administered by intravenous bolus after 6.5 h of radiotracer infusion.

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