Analyses of [18F]Altanserin Bolus Injection PET Data. II: Consideration of Radiolabeled Metabolites in Humans

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KEY WORDS positron emission tomography (PET); [18F]altanserin; serotonin receptor; 5-HT2A; imaging

ABSTRACT Imaging serotonin-2A (5-HT2A) neuroreceptors with positron emission tomography (PET) and [18F]altanserin has been the focus of a series of PET studies, as [18F]altanserin is one of the most selective 5-HT2A antagonist radiotracers. Previous animal studies showed that radiolabeled metabolites (radiometabolites) of [18F]altanserin crossed the blood–brain barrier (BBB) to localize nonspecifically in brain, consistent with a constant radioactivity “background.” In this work, we evaluated human bolus injection [18F]altanserin PET data with detailed consideration of the impact of BBB-permeable metabolites on the specific binding parameters. Data were quantified using either single (parent radiotracer), dual (parent radiotracer and radiometabolites), or no arterial input function(s) (cerebellum as reference tissue input function). A step-gradient high-performance liquid chromatography (HPLC) analysis provided distinct separation of [18F]altanserin and four radiolabeled components in plasma. After [18F]altanserin injection, the step-gradient data showed that the major BBB-permeable radiometabolites approached constant levels in plasma (>50 min), consistent with a constant metabolite “background.” The single-input Logan graphical results were highly correlated with the dual-input results and its bias was fairly constant across regions and subjects, as similarly observed for a nongraphical reference tissue method. The most comprehensive and quantitatively valid analysis for bolus [18F]altanserin PET data was the dual-input method that specifically accounted for BBB-permeable metabolites, although the Logan analysis was preferred because it provided a good compromise between validity, sensitivity, and reliability of implementation. Further study is needed to better understand how the cerebellar kinetics of [18F]altanserin and its radiometabolites impact the reference tissue measures. Synapse 41:11–21, 2001.

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INTRODUCTION Serotonin (5-HT) is a key neurotransmitter that has been implicated in several neuropsychiatric disorders. Selective serotonin reuptake inhibitors have been very effective in ameliorating depressive symptoms (see Frazer, 1997, for review) and the role of 5-HT in depression is of great research interest. Although there are at least 14 serotonin receptor subtypes (Peroutka, 1994), the 5-HT2A and 5-HT1A receptor systems have been most extensively studied in investigations of antidepressant drug action (see Meltzer et al., 1998a, for review). Accordingly, much effort has gone into the development and evaluation of 5-HT2A receptor antagonists for in vivo neuroimaging with positron emission tomography (PET).

Examples of PET radiotracers that have been used to assess 5-HT2A receptor binding include [11C]NMS and N1-([11C]-methyl)-2-Br-LSD (Wong et al., 1984, 1987), [11C]ketanserin (Baron et al., 1985), [18F]setoperone (Blin et al., 1988; Petit-Taboué et al., 1999), [18F]altanser-
serin (Lemaire et al., 1991), and [11C]MDL 100907 (Lundkvist et al., 1996; Mathis et al., 1996; Watabe et al., 2000). Several of these 5-HT2A radiotracers have been used to study neuropsychiatric disorders, including depression (Biver et al., 1997; Meyer et al., 1999; Meltzer et al., 1998a, 1999), and Alzheimer’s disease (Blin et al., 1993; Meltzer et al., 1999) and have revealed 5-HT2A binding alterations with age (Wong et al., 1984; Blin et al., 1993; Rosier et al., 1996; Meltzer et al., 1998b) and gender (Biver et al., 1996).

The majority of these studies were performed using [18F]altanserin because, relative to the other radiotracers, many of its properties are superior for PET imaging. These properties include its high affinity ($K_i = 0.51 \text{ nM}$) and selectivity (90- and 400-fold less potent at $\alpha_1$ and 5-HT2C, respectively) for 5-HT2A receptors (Smith et al., 1998; Leysen, 1989, 1990; Lemaire et al., 1991) and the existence of a method for its routine production (Lemaire et al., 1991). Previous bolus injection [18F]altanserin PET studies revealed a significant slow tissue component within the receptor-poor cerebellar kinetics (Biver et al., 1994; Sadzot et al., 1995; Smith et al., 1998; Mintun et al., 1996; Price et al., 1998). Studies in baboons showed that this component was partly the result of radiolabeled metabolites (radiometabolites) of [18F]altanserin that crossed the blood–brain barrier (BBB) and contributed to a background of nonspecific radioactivity that was fairly uniform across regions (Part I: Price et al., 2001). The regional uniformity of nonspecific binding is consistent with the relatively good performance of the previous quantitative analyses that involved single (parent only) and dual (parent and radiometabolites) input function(s) (Biver et al., 1994; Sadzot et al., 1995; Smith et al., 1998; Mintun et al., 1996; Price et al., 1998).

The recent application of an equilibrium technique for [18F]altanserin PET (van Dyck et al., 2000) was promising because the specific binding measures ($V_3$) were consistent with 5-HT2A receptor binding, the postequilibrium $V_3$ measures were very stable, and a simple correction for the BBB-permeable metabolite radioactivity was performed ( subtraction of cerebellar radioactivity). However, in contrast to a 90 or 120 min bolus study, this equilibrium method required as much as 6 h of radiotracer infusion and acquisition of post-equilibrium PET data over a 4-h period (6.0–10.5 h), thereby limiting its routine use.

In the present work, we sought to evaluate analysis methods for human bolus injection [18F]altanserin PET data with detailed consideration of the impact of BBB-permeable metabolites on the specific binding parameters. This involved extensive characterization of the radiolabeled metabolites of [18F]altanserin and quantification of the contribution of these metabolites to the overall PET measure. This methodological evaluation considered correspondence of the PET binding parameters with the in vitro distribution of 5-HT2A receptors, as well as parameter dynamic range, variability, and sensitivity to specific binding differences and feasibility of methodological implementation.

**Materials and Methods**

**Radiochemistry**

[18F]Altanserin

Radiosynthesis of high specific activity (>1,500 Ci/mmol) [18F]altanserin was performed as previously described (Lemaire et al., 1991; Part I: Price et al., 2001). At high specific activity, the occupation of receptor sites by unlabeled tracer was assumed to be negligible.

**Imaging**

The [18F]altanserin PET data were acquired as part of other investigative protocols in our laboratory, as previously described (Smith et al., 1998; Meltzer et al., 1998b). Briefly, subjects underwent medical and psychiatric screening prior to PET imaging. Healthy control subjects were excluded based on history of or current psychiatric or neurologic illness. Data were also acquired in healthy female subjects that were recovered from an eating disorder for more than 1 year prior to the PET study (W. Kaye, unpublished data). Written informed consent was obtained as approved by the local Biomedical Institutional Review Board and Radioactive Drug Research Committee. Two human datasets were examined. For both sets, approximately 10 mCi of high specific activity [18F]altanserin was injected.

**Dataset one**

The first dataset was used to evaluate and compare the quantitative methods and consisted of data acquired in four healthy young subjects (one male, three female: 24.3 ± 5.0 years of age). Two of the females were recovered from an eating disorder for more than 1 year. The dynamic PET acquisition generally corresponded to multiple time frames: 6 × 20 sec, 2 × 30 sec, 2 × 1.5 min, 3 × 3 min, 1 × 5 min, 5 × 10 min, and 1 × 20 min (80 min midpoint for last frame). For one subject the data were collected over 121 min (last 100 min: 10 × 10 min). All data were analyzed over a 0–90 min period.

**Dataset two**

The second dataset was used to examine the extent to which differences in specific binding could be detected by using simplified methods (see Data Analyses). Dataset two corresponded to data acquired in healthy young (three male, six female: 18–29 years of age) and elderly (three male, six female: 61–76 years of age) subjects. Previously, these data showed an age-related decline in 5-HT2A receptor binding via conventional Logan analyses (Meltzer et al., 1998b). The observed age-related decline (50–60%) was regionally widespread and consistent with previous PET findings.
of decreased 5-HT$_{2A}$ receptor binding with age (Wong et al., 1984; Blin et al., 1993; Rosier et al., 1996). For dataset two, the dynamic PET time framing was over 91 min: 6 × 20 sec, 2 × 30 sec min, 1 × 1 min, 2 × 1.5 min, 3 × 3 min, 1 × 5 min, and 7 × 10 min.

**Plasma analyses**

The total radioactivity concentration in plasma ($C_{p\text{-}TOT}$) was determined at approximately 35 time points throughout the PET study from arterial blood samples. The contributions of $^{18}$F-altanserin and its radiometabolites (relative to $C_{p\text{-}TOT}$) were determined through high-performance liquid chromatography (HPLC) analyses of plasma obtained from arterial blood samples collected at 2, 10, 30, 60, and 90 min postinjection. The plasma input functions corresponded to fractional amounts of $C_{p\text{-}TOT}$ that were based on the HPLC proportions of $^{18}$F-altanserin and its radiolabeled metabolites, as previously described (Part I: Price et al., 2001).

**Dataset one**

An extensive characterization of the radiolabeled metabolites of $^{18}$F-altanserin was performed using a step-gradient HPLC method. For the step-gradient analyses, a Waters C18 Symmetry column was eluted from 0–30 min with 15% acetonitrile / 85% buffer (0.294 M acetic acid / 0.0283 M ammonium acetate, pH 5.2) and from 31–60 min with an eluent consisting of 25% acetonitrile / 75% buffer. The ability of this method to resolve the more polar metabolic components and elute $^{18}$F-altanserin in less than 1 h permitted the determination of the kinetics of each major radiolabeled metabolite in plasma. For dataset one, two types of plasma input functions were determined: 1) unmetabolized parent $^{18}$F-altanserin ($C_{p\text{-}i}$) and 2) those radiolabeled metabolites in plasma that crossed the BBB ($C_{p\text{-}MET}$) (Smith et al., 1998).

**Dataset two**

An isocratic HPLC method was used to analyze $^{18}$F-altanserin in plasma, as previously described (Meltzer et al., 1998b). For dataset two, only $C_p$ was determined.

**Data analyses**

**Region-of-interest definition**

Regions-of-interest (ROIs) were hand-drawn on magnetic resonance (MR) images according to anatomic landmarks. The MR images were co-registered to “early” summed PET images (0–15 min postinjection, primarily reflective of blood flow) and resliced to match the PET data, based on the image alignment algorithm of Woods et al. (1993). PET time–activity data were generated for a set of ROIs that was chosen to sample a large range (high to low) of 5-HT$_{2A}$ receptor concentrations: lateral temporal association cortex (LTC), medial orbitofrontal cortex (OFC), anterior cingulate gyrus (ANC), prefrontal cortex (PFC), mesial temporal cortex (MTC; amygdala/hippocampus), basal ganglia (BSG; caudate and putamen), and cerebellum (CER) (Smith et al., 1998; Meltzer et al., 1998b). The ROIs were created using Imageool™ software (CTI PET Systems; Knoxville, TN). The regional PET time–activity data were generated using an internally developed Interactive Data Language (Research Systems, Boulder, CO) routine that utilized a phantom-based calibration factor to convert that PET data units to physiological units (µCi/ml).

**Compartmental analyses**

The compartmental models that were applied in this work are extensions of simpler nonspecific configurations that were applied to baboon data (Part I: Price et al., 2001). The more comprehensive configurations include specific receptor binding, as described in the equations below. The PET measurement was described in terms of the free, specifically bound, and nonspecifically bound concentrations of $^{18}$F-altanserin in brain ($C_F$, $C_S$, $C_{NS}$, respectively), its metabolites in brain ($C_{MET}$), and their respective input functions ($C_p$ and $C_{p\text{-}MET}$). These models do not account for the possible generation of radiometabolites in brain tissue itself (Gunn et al., 2000a); however, animal studies conducted in our laboratory have not revealed evidence of $^{18}$F-altanserin metabolism in brain (unpublished data).

For $^{18}$F-altanserin, bidirectional BBB transport was represented by $K_1$ (ml/min/ml) and $k_2$ (min$^{-1}$), association and dissociation of receptor binding by $k_3$ (min$^{-1}$) and $k_4$ (min$^{-1}$), and association and dissociation of nonspecific binding by $k_5$ (min$^{-1}$) and $k_6$ (min$^{-1}$). For the BBB-permeable radiometabolites, bidirectional BBB transport of radiometabolites was represented by $K_{1\text{-}MET}$ and $k_{2\text{-}MET}$. The equations included a vascular volume term ($VV$) that was the fractional amount of the total plasma radioactivity concentration ($C_{p\text{-}TOT}$) that contributed to the PET measurement.

\[
\frac{dC_p(t)}{dt} = K_1C_p(t) - (k_2 + k_3 + k_5)C_p(t) + k_2C_S(t)
\]

\[
+ k_3C_{NS}(t)
\]

(1)

\[
\frac{dC_S(t)}{dt} = k_3C_p(t) - k_4C_S(t)
\]

(2)

\[
\frac{dC_{NS}(t)}{dt} = k_5C_p(t) - k_6C_{NS}(t)
\]

(3)

\[
\frac{dC_{MET}(t)}{dt} = K_{1\text{-}MET}C_{p\text{-}MET}(t) - k_{2\text{-}MET}C_{MET}(t)
\]

(4)

The total model solution ($C_{MOD}$) was:

\[
C_{MOD} = C_F + C_S + C_{NS} + C_{MET} + VV(C_{p\text{-}TOT})
\]

(5)
Several conventional single-input models (no BBB-permeable metabolites, $C_p$ only) were examined (see Table I). These models were either 2-, 3-, or 4-compartments (2C, 3C, or 4C) and consisted of one vascular compartment and one ($C_p$, $F$, $S$, $C_{NS}$), two ($C_p$, $F$, $S$, $C_{NS}$, $S$), or three ($C_p$, $F$, $S$, $C_{NS}$) tissue compartments, respectively. In addition, a nonspecific 3C model ($3C_{NS}$) that consisted of one vascular compartment and two ($C_F$, $C_{NS}$) tissue compartments was applied to data in brain areas with low or negligible levels of specific binding. The $3C_{NS}$ model allowed assessment of the nonspecific tissue component ($DV_{NS}$) in receptor-poor regions, where $DV_{NS} = (K_1/k_2)(k_5/k_6)$.

For the dual-input models ([18F]altanserin and BBB-permeable metabolites, $C_p$ and $C_{p-MET}$), the configuration corresponded to a combination of a single-input

<table>
<thead>
<tr>
<th>Single input</th>
<th>2 Tissue compartments: $K_1$, $k_2$, $k_3$, $k_4$, $V_V$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3C</td>
<td>$3C_{DV} = K_1/(k_2(1 + k_3/k_4))$</td>
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<tr>
<td></td>
<td>$3C_{BP} = k_2/k_4 = (B_m/K_p)f_2$</td>
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<tr>
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<th>2 Tissue compartments: $K_1$, $k_2$, $k_5$, $k_6$, $V_V$</th>
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<td></td>
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<tr>
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*See Materials and Methods, Data analyses section.

**TABLE I. Compartmental modeling: nonspecific and specific components**

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model and a parallel metabolite model that allowed for BBB transport of radiolabeled metabolites. The metabolite model was a two-compartment model \(2C_{M_{ET}}\) that consisted of one vascular compartment and one tissue compartment \(C_{M_{ET}}\). Two types of dual-input models were examined; one model accounted for \([^{18}F]altanserin specific receptor binding \(3C + 2C_{M_{ET}}\), while the other did not \(2C + 2C_{M_{ET}}\). The primed term \(M_{ET}\) denotes the use of only BBB-permeable metabolites (in contrast to all measured radiometabolites: \(M_{ET}\), see Part I: Price et al., 2001).

Specific binding was assessed via the distribution volume (DV) and binding potential (BP); measures that are related to \(B_{max}\) (concentration of receptors) and to \(K_D\), the equilibrium dissociation rate constant (Mintun et al., 1984). In this work, DV values were determined for \([^{18}F]altanserin (DV) and BBB-permeable radiometabolites in tissue (DV_{M_{ET}})(see Table I). The BP was determined either as the \(k_3/k_2\) value or derived from the regional DV values. The BP that was defined as \(k_3/k_2\) was equivalent to \(B_{max}/K_D\) for a 4C model and to \((B_{max}/K_D) \times f_2\) for the 3C model (where \(f_2\) is the free fraction of radiotracer in brain) (Mintun et al., 1984; Koepp et al., 1994). The BP values obtained by the 3C and 4C models were denoted as 3C BP and 4C BP, respectively. The BP measure that was derived from the DV values (BP_{DV}) was determined using the DV ratio (DVR), which is the regional DV value normalized to the DV value of a reference region: BP_{DV} = DVR – 1 (Lammertsma et al., 1996). The BP_{DV} is equivalent to the 3C BP.

For the reference region, it was assumed that specific binding of the radiotracer was negligible (relative to receptor-rich regions) and that the concentrations of free and nonspecifically bound radiotracer were representative of other brain regions. For the present studies, the cerebellum was used as reference region (see Evaluation Criteria, below).

Curve fitting

Parameter estimation was performed as previously described (Part I: Price et al., 2001) and the various curve fitting strategies are listed in Table I. Parameters varied freely during the 2C and 3C model fits. Specific constraints were applied during the 4C model fits; the dual-input model fits were performed either with all parameters freely varying or with specific constraints (Mintun et al., 1996) (see Table I).

Simplified methods

Data were also analyzed using a single-input Logan graphical method (Logan et al., 1990, 1996) that utilizes linear regression analysis to obtain measures of DV (and BP_{DV}). For the graphical analyses, two types of input functions were used: 1) \([^{18}F]altanserin (C_P)\); or 2) cerebellar radioactivity \(C_{C_{ERR}}\). Implementation of the Logan method with \(C_P\) was denoted Logan_{PLAS}, while its implementation with \(C_{C_{ERR}}\) was denoted Logan_{CCER}. The Logan_{PLAS} slope provided a measure of the total radiotracer DV in tissue (including vascular volume), while the Logan_{CCER} slope provided a measure of DVR (Logan et al., 1990, 1996).

The data were also analyzed using a simplified compartmental modeling approach. Regional measures of BP were obtained using a reference tissue model (Blomqvist et al., 1989; Cunningham et al., 1991; Lammertsma et al., 1996; Lammertsma and Hume, 1996; Gunn et al., 1997). For the reference tissue model, the kinetics of the reference region \(R\) were used to simplify the compartmental modeling of the receptor-binding data and, thus, allow BP values to be obtained without arterial blood sampling. In the present work, a simplified reference tissue model (SRTM) as implemented by Gunn et al. (1997) was used:

\[
C_P(t) = \theta_1 C_P(t) + \theta_2 C_P(t) \otimes e^{-\lambda t} \tag{7}
\]

In this method, \(\theta_1 = R_1 = K_1/K_{R1}\) and \(\theta_2 ( \text{min}^{-1}) = k_2 - R_1 k_2/(1 + \text{BP})\) and \(\theta_3 ( \text{min}^{-1}) = k_3/(1 + \text{BP}) + \lambda\), where \(\lambda\) represents the radiotracer decay constant (\(\text{min}^{-1}\)) and \(R_1\) is proportional to the ratio of the cerebral blood flow values in the receptor-binding region to that in the reference tissue region. The values of \(\theta_3\) used in this work ranged from 0.017 min\(^{-1}\) to 0.156 min\(^{-1}\), based on the range of \(k_3\) values obtained by single-input compartmental modeling techniques. The SRTM BP is equivalent to the 3C BP and BP_{DV} (see Table I).

Evaluation criteria

The methods were evaluated primarily using dataset one and criteria that included physiological validity and methodological bias, correspondence of parameters with the in vitro distribution of 5-HT_{2A} receptors, as well as parameter dynamic range, intersubject variability, sensitivity to differences in receptor binding, and ease of methodological implementation. The percent coefficient of variation (CV\%) was used as a measure of variability (ISD/mean) * 100).

The correspondence of the regional binding parameters with the in vitro distribution of 5-HT_{2A} receptors was assessed through linear correlations that were determined between the \([^{18}F]altanserin binding measures and autoradiographic 5-HT_{2A} receptor concentration measures (fmol/mg protein) in postmortem human brain (Pazos et al., 1987). For each region, an average value was generated across the published postmortem tissue layer values and this average was used as the comparative autoradiographic measure. The PET ROIs (capital letters) were compared to postmortem human brain areas as follows: LTC = primary auditory cortex; OFC = gyrus rectus; ANC = anterogenual cingulate cortex; PFC = frontal cortex; MTC = amygdala and
RESULTS

Plasma analyses

Dataset one

Figure 1A (top) shows the average (±SD) relative contributions of [18F]altanserin and its radiolabeled metabolites in human plasma over time. The step-gradient method resolved five components: \([^{18}F]4\text{-FBP}\), \([^{18}F]4\text{-FBPmet}\), \([^{18}F]\text{ALTmet}\), \([^{18}F]\text{ALT-ol}\), and \([^{18}F]\text{altanserin}\) from least to most lipophilic (Mason et al., 1997; Lopresti et al., 1998; Smith et al., 1998). \([^{18}F]\text{ALTmet}\) and \([^{18}F]4\text{-FBPmet}\) correspond to unidentified metabolites of \([^{18}F]\text{altanserin}\) and \([^{18}F]4\text{-FBP}\), respectively (Mason et al., 1997; Lopresti et al., 1998). Only three of the four radiolabeled metabolites were present in concentrations that exceeded 3% of the plasma radioactivity at any time point in plasma following \([^{18}F]\text{altanserin}\) injection (\([^{18}F]4\text{-FBPmet}, [^{18}F]\text{ALTmet}, \text{and } [^{18}F]\text{ALT-ol}\).

Previous studies of rat brain radioactivity distributions following the injection of \([^{18}F]\text{ALTmet}\) isolated from baboon urine demonstrated that it did not enter brain tissue (Lopresti et al., 1998; Smith et al., 1998) and therefore indicated that only two metabolites (\([^{18}F]4\text{-FBPmet}\) and \([^{18}F]\text{ALT-ol}\)) were present in significant concentrations in the plasma of humans and were BBB-permeable. These findings are consistent with previous PET studies performed after the bolus injection of either \([^{18}F]4\text{-FBP}\) or \([^{18}F]\text{ALT-ol}\) in baboons (Part I: Price et al., 2001). These studies demonstrated nonspecific localization of brain radioactivity after either injection and substantial metabolic activity and large tissue:plasma radioactivity ratios after \([^{18}F]4\text{-FBP}\) injection (as a result of \([^{18}F]4\text{-FBPmet}\).

Dataset one: tissue ratios

Figure 2 displays average tissue ratios for the four human subjects for which step-gradient analysis of the plasma was performed. Tissue ratios were calculated relative to the plasma concentrations of \([^{18}F]\text{altanserin}\) (\(C_p\)) and \([^{18}F]\text{altanserin } + \text{ MET}\) (\(C_p + C_p\text{-MET}\)) and relative to the cerebellar radioactivity (CER). Of note is the difference between the tissue ratios calculated relative to \(C_p\) and those calculated relative to \(C_p + C_p\text{-MET}\) or CER. The graph shows that tissue ratios generated using either \(C_p\) or \(C_p\text{-MET}\) or CER data resulted in earlier and more distinct plateaus relative to those determined using \(C_p\) only. The tissue ratios further indicated that the CER data may be a
good approximation to the “true” plasma concentration of all BBB-permeable compounds (i.e., $C_p^{1}C_p^{MET}$ 9) after 35 min. This point is exemplified in Figure 2 by the proximity of the CER/($C_p^{1}C_p^{MET}$) values (1.260.17) to a tissue ratio value of 1.

**Dataset one: compartmental and simplified analyses**

The average DV measures that were obtained across the four "step-gradient" subjects are shown in Figure 3 for four analysis methods and five ROIs. The $[^{18}F]$altanserin DV values that were obtained by the single-input methods (4C, 3C, and LoganPLAS slope) were larger in value than those obtained by the dual-input method (3C + 2C$^{MET}$). The estimation of the 4C DV relied upon the 3C cerebellar $K_i/k_2$ values and nonspecific parameters (i.e., $k_5$ and $k_6$); the latter measures (particularly $k_5$) were sometimes associated with large parameter errors (>50%). As a result of this occasional instability in the curve fits, the OFC and BSG 3C DV values in Figure 3 corresponded to only three of the four subjects. Generally, when the curve-fitting was stable the 3C or 4C model provided statistically better curve fits than the dual-input model, as observed also for baboon data (Price et al., 1998). Nevertheless, the curve fits did not differ greatly across methods (Fig. 4).

The single-input 3C$^{NS}$ model provided better curve fits to the CER data relative to the single-input 2C model (data not shown); a finding that was also observed in baboons (Part I: Price et al., 2001). Greater intersubject variability was associated with the cerebellar $3C^{NS}$ DV (2.6960.90) as compared to the LoganPLAS slope (1.3260.23) or the cerebellar radioactivity concentration. Tissue ratio values near 1 indicated that the cerebellar data may be a reasonable approximation to the plasma concentration of $[^{18}F]$altanserin + MET at later times. (MET: BBB-permeable radiolabeled metabolites).
PET measure included the contribution of BBB-permeable radiolabeled metabolites in the \[^{18F}\]altanserin DV measure (relative to the dual-input DV values). Despite the methodological differences, the \[^{18F}\]altanserin DV values for all methods were highly correlated with the 5-HT2A in vitro rank order (Pazos et al., 1987; LoganCER slope-1: 0.5 of dual-input value: 0.60; SRTM BP: 0.6 of dual-input value: 0.60; LoganPLAS slope: 0.09). As expected, the 3C BP was greater than the 4C DV r = 0.94; 3C DV r = 0.88; LoganPLAS slope r = 0.97; dual-input 3C BP r = 0.97).

The BP measures are shown in Table II. The relationship between the single-input BP values and the dual-input BP value in Table IIA did not vary greatly across ROIs: 4C BP \(BPDV\) (0.4 of dual-input value: 0.40 ± 0.02), LoganPLAS BPDV (0.7 of dual-input value: 0.69 ± 0.05), and SRTM BP (0.6 of dual-input value: 0.60 ± 0.06), with the exception of the LoganCER slope-1 (0.5 of dual-input value: 0.53 ± 0.09). As expected, the 3C + 2C\(_{MET}\) BPDV was nearly equivalent to the 3C + 2C\(_{MET}\) BP, while the 4C BPDV was much less than the 4C BP (Table IIB). The 4C BP was greater than the 3C + 2C\(_{MET}\) binding potential values and this difference was fairly stable across ROIs (1.2 of dual-input value: 1.2 ± 0.08). Differences between the BP measures were partially attributed to nonspecific binding effects. If the free fraction in tissue \(f_2\), was estimated as \(f_2 = \frac{1}{(1 + k_d/k_g)}\), then \(f_2 = 0.33 ± 0.13\), using the 3C\(_{NS}\) cerebral \(k_d/k_g\) values (data not shown). Correction of the 4C BP \(BPDV\) values by the \(f_2\) factor would nearly yield the 4C BP values in Table IIB (Koepp et al., 1994; Lamertsma et al., 1996). Based on this relationship, the 4C LTC BPDV \(\approx 0.33 \equiv 4C\) LTC BP and the 4C BSG BPDV \(\approx 0.33 \equiv 4C\) BSG BP (see Methods, Data analyses).

All BP values were similarly highly correlated with the 5-HT2A in vitro rank order (Pazos et al., 1987): 3C + 2C\(_{MET}\) BPDV: \(r = 0.94\); 4C BPDV: \(r = 0.94\); LoganPLAS BP: \(r = 0.94\); SRTM BP: \(r = 0.93\); LoganCER Slope-1: \(r = 0.93\). The dynamic range of the binding parameters was greater for the 4C BP and slightly less but comparable for the 3C + 2C\(_{MET}\) and LoganPLAS methods, particularly in receptor-rich regions. The regional dynamic range and intersubject variability was least for the LoganCER slope-1.

### Dataset two: evaluation of reference tissue methods

As a final step, we examined the applicability of the reference tissue methods to an additional \[^{18F}\]altanserin data set of 18 subjects: young: 18–29 years \((n = 9)\) and elderly: 61–76 years \((n = 9)\). Previously, we analyzed these data using the Logan PLAS method and the BP \(BPDV\) measures. The LoganPLAS BPDV yielded a large and widespread decline in 5-HT2A receptor binding with age that persisted after correction for cerebral atrophy effects (Meltzer et al., 1998b).

We compared the previous LoganPLAS BP \(BPDV\) measures to the reference tissue BP values by computing percent difference measures between the average young and elderly binding potential values (Table III). The LoganPLAS BPDV and the SRTM BP yielded similar age-related reductions in 5-HT2A receptor binding that were greater than the reductions measured using the LoganCER slope-1. For one of the elderly subjects, the SRTM BP measure for the basal ganglia was less than one (therefore, \(n = 8\) for BSG SRTM BP in Table III). Nevertheless, the SRTM method provided good dynamic range and sensitivity to changes in 5-HT2A receptor across regions, despite the nonspecific cerebellar component.
DISCUSSION

In this study, several methods were evaluated for the analysis of human bolus injection [18F]altanserin PET data. The most comprehensive and quantitatively valid analysis was the dual-input method that specifically accounted for BBB-permeable metabolites (MET'). This evaluation served to strengthen the validity of the LoganPLAS analyses for the assessment of [18F]altanserin binding changes in humans and further indicates that such binding changes can be measured using a reference tissue approach.

The LoganPLAS method was biased relative to the MET' method but yielded a comparable dynamic range of BP values, low intersubject variability (5–17%), sensitivity to 5-HT2A receptor binding differences (see Table III), and was previously shown to have an intra-subject variability of less than 10% (Smith et al., 1998). Investigation of the analyses that utilized the CER data, rather than the plasma input function, revealed mixed results. The analyses of the data that used the LoganPLAS curve, rather than the LoganPLAS method (relative to MET'). On the other hand, the LoganCER slope-1 demonstrated a limited dynamic range and poorer sensitivity to changes in 5-HT2A receptor binding.

Nonspecific binding component

The nonspecific component (DV_NS) measured by the single input function analyses was similar and consistent with the metabolite component (DV_MET') measured by the dual-input function metabolite analyses. In accordance, the step-gradient HPLC results showed that the major BBB radiometabolites reached constant levels during the PET study in a manner that was consistent with a constant “metabolite background” contribution to the nonspecific binding (Fig. 1B).

These findings are in agreement with the results of our evaluation of the [18F]altanserin nonspecific component in baboons (Part I: Price et al., 2001). These baboon studies showed that radiolabeled metabolites of [18F]altanserin crossed the BBB and contributed to a background of nonspecific radioactivity that appeared to be fairly uniform across regions. The notion of a constant background is further supported by the excellent correlation demonstrated between the LoganPLAS and 3C + 2C_MET BP values (r ~ 1.0). A constant metabolite background also is consistent with the suitability of the cerebellar metabolic subtraction correction that was recently applied in an equilibrium [18F]altanserin PET study (van Dyck et al., 2000).

Cerebellar data as approximation of plasma input function

The apparent stability of the nonspecific binding component and the results of the step-gradient HPLC plasma analyses strongly indicated that the cerebellar data was a reasonable approximation to the “true” plasma input (Cp + C_p_MET) at later times. The CER/Cp value approached 2 and did not exhibit a distinct plateau during the time frame of the study (Fig. 2). In contrast, when the CER data were normalized to C_p + C_p_MET, these data exhibited a distinct plateau as early as 20 min after injection (Fig. 2). These results prompted the investigation of reference-tissue based methods.

Study limitations: sources of bias

Of the methods evaluated, the dual-input metabolite model was the most valid because it accounted for BBB passage of BBB-permeable radiolabeled metabolites. Therefore, we assessed methodological bias relative to the MET’ results. The dual-input method (and all compartmental methods) accounted for vascular volume, unlike the Logan and SRTM implementations, for which the vascular contribution can be an additional source of bias. Relative to the MET’ model, the single-input methods yielded different absolute values (Fig. 3, Table II). These differences were partly attributed to the influence(s) of nonspecific binding on the binding parameters. We acknowledge that longer scan times (>90 min) may yield more stable 3C_NS nonspecific parameter estimates (Smith et al., 1998).

Low levels of cerebellar 5-HT2A receptor binding may also contribute to bias, particularly for the reference tissue methods. For the SRTM, bias also can be introduced if a reduced order model is used (i.e., one rather than two tissue compartments). The SRTM assumes one tissue compartment for the cerebellar data and it is this assumption that is proposed as the most important source of bias contributing to an underestimation of the BP values (Lammertsma et al., 1996; Parsey et al., 2000; Gunn et al., 2000b).

As a consequence of using a reduced or simplified model, a sensitivity of the SRTM BP to regional cerebral blood flow can be introduced. In dataset one, the Rf values ranged from 0.99–0.86 in the basal ganglia and some cortical regions but were lower in the LTC (0.81 ± 0.05) and MTC (0.72 ± 0.04) regions. These values are similar to those previously reported for other radiotracers (Gunn et al., 1997) but indicate greater bias for the LTC and MTC regions. Two tissue compartments were needed to describe the cerebellar kinetics when the plasma input function corresponded to [18F]altanserin only. However, when the plasma input function corresponded to C_p + C_p_MET, one tissue compartment provided a reasonable description of the [18F]altanserin cerebellar data (data not shown). Therefore, the [18F]altanserin cerebellar kinetics may be approximated fairly well by a single tissue compartment.

Method of choice

Of the analysis methods, the MET’ method was the most comprehensive and valid because it specifically...
accounted for BBB-permeable radiometabolites based on step-gradient HPLC analysis of plasma and a dual-input compartmental model for data analysis. The LoganPLAS method accounted for parent radiotracer based on an isocratic HPLC analysis and involved a simple linear regression for data analysis. Despite these simplifications, the LoganPLAS method was found to be valid, since it was highly correlated with the \(3C + 2C_{MET}\). BP \((r \sim 1.0)\) with a fairly constant bias across regions and subjects \((0.69 \pm 0.05\) of MET BP\). In addition, the LoganPLAS method yielded broad dynamic range of BPDV values, low intersubject variability \((5–17\% \text{ across regions})\), and sensitivity to in vivo 5-HT\textsubscript{2A} binding differences, and was reliably implemented on a routine basis.

The SRTM and LoganCER methods were the least complicated analyses to implement because neither required arterial blood sampling or HPLC analysis of plasma samples. Similar to the LoganPLAS method, the SRTM BP measures were highly correlated and moderately biased relative to the MET BP measures, with good dynamic range, intersubject variability, and sensitivity to in vivo 5-HT\textsubscript{2A} binding differences. The LoganCER method was most practical because it utilized linear regression rather than least-squares curve-fitting. Unfortunately, the LoganCER slope-I value exhibited a greater bias than the SRTM BP \(\text{(relative to MET)}\). This bias in the LoganCER slope-I value was more variable across regions, possibly indicating that the general implementation of the graphical method was not optimal in this case (Logan et al., 1996; Ichise et al., 1999). The future application of reference tissue methods will require a better understanding of how the cerebellar kinetics of \([^{18}\text{F}]\)altanserin and its radiolabeled metabolites impact the specific binding measures.

**CONCLUSIONS**

We conclude that the most comprehensive and valid method for the analysis of human bolus injection \([^{18}\text{F}]\)altanserin PET data is a dual-input function compartmental model that specifically accounts for BBB-permeable metabolites of \([^{18}\text{F}]\)altanserin. Overall, we found the LoganPLAS method to provide the best compromise among validity, bias, dynamic range, intersubject variability, sensitivity to in vivo 5-HT\textsubscript{2A} binding differences, and ease and reliability of implementation.

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