

Short Communication

Assessing the Sensitivity of [¹¹C]P943, a Novel 5-HT_{1B} Radioligand, to Endogenous Serotonin Release

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ABSTRACT The main objective of the current study was to determine the sensitivity of the positron emission tomography (PET) radioligand [¹¹C]P943 to fenfluramine-induced changes in endogenous 5-HT in nonhuman primate brain. Fenfluramine-induced changes in 5-HT_{1B} occupancy were compared to those obtained by self-block with unlabeled P943. Two baboons and 1 rhesus monkey were given preblocking or displacing doses of fenfluramine (1–5 mg/kg) or preblocking doses of unlabeled P943 (0.2 mg/kg) and imaged with [¹¹C]P943 PET. Receptor occupancy by the low dose of fenfluramine (1 mg/kg) in the baboons was 25 and 29% and by the high dose of fenfluramine (5 mg/kg) in the rhesus macaque was 42%. Receptor occupancy by P943 (0.2 mg/kg) was 68 and 86% in the baboons. PET imaging of 5-HT_{1B} receptors with [¹¹C]P943 may be a useful approach for measuring changes in endogenous 5-HT in the living human brain. **Synapse 65:1113–1117, 2011.** © 2011 Wiley-Liss, Inc.

INTRODUCTION

The serotonergic system has been implicated in the neurochemical mechanisms underlying a variety of psychiatric disorders, especially depressive disorders. This is best highlighted by the widespread use of antidepressant drugs acting on the serotonin (5-HT) system and the association of a significant mood change with the procedure of tryptophan depletion, which results in lowering of central serotonin levels (Delgado et al., 1991). Serotonin and the 5-HT_{1B} receptor in particular are involved in a variety of physiological functions including food intake, sexual activity, and locomotion (Ruf and Bhagwagar, 2009; Sari, 2004) all of which may be disrupted in depressive disorders. Significant interest in the 5-HT_{1B} receptor stems from the fact that these receptors occupy a key position at the nerve terminals and are responsible for synaptic 5-HT release and neuronal firing (Sari, 2004). Given this key location and function, the 5-HT_{1B} receptor may be sensitive to endogenous levels of 5-HT as it provides a modulatory effect on the 5-HT system.

Using positron emission tomography (PET), it is possible to measure the availability of 5-HT receptors and potentially changes in synaptic 5-HT levels (Paterson et al., 2010). Quantification of changes in endogenous 5-HT has been attempted using a number of 5-HT receptor-related radiotracers including [¹⁸F]MPPF, [¹¹C]WAY100635, [¹¹C]DASB, and [¹¹C]MDL100907, but results have been inconclusive (Huang et al., 2005; Paterson et al., 2010). A recent study using [¹¹C]AZ10419369, a 5-HT_{1B} ligand, showed promising results in sensitivity to changes in endogenous 5-HT (Finnema et al., 2010). [¹¹C]P943 has recently been developed and applied to quantify

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5-HT_{1B} receptor availability in nonhuman primates (Nabulsi et al., 2010) and human subjects (Gallezot et al., 2010), and a reduction in ventral striatal 5-HT_{1B} availability was recently demonstrated in subjects with major depressive disorder (Murrrough et al., 2010). Our objective in the current study was to determine the sensitivity of [¹¹C]P943 to fenfluramine-induced changes in endogenous 5-HT in non-human primate brain.

MATERIALS AND METHODS

Two female baboons (B1, B2, *Papio anubis*, 9.8 and 10.4 kg) and one male rhesus monkey (M1, *Macaca mulatta*, 6.0 kg) served as subjects. The housing facility is fully accredited by the American Association for the Accreditation of Laboratory Animals (AAALAC), and all experiments were conducted in accordance with the Institutional Animal Care and Use Committee guidelines.

For the PET scans, fasted animals were initially anesthetized with ketamine (6–10 mg/kg, i.m.) and then maintained on 1.5–2.5% isoflurane. A 2-h time period is allowed between ketamine and injection of the radiotracer. Vital signs including respiration rate, blood pressure, heart rate, and temperature were monitored continuously and recorded every 15 min, and temperature was kept constant at 37°C with heated water blankets. An intravenous (i.v.) line was established in each leg, one for injection of fluids for hydration and injection of the radiotracer and the second for blood sampling. At equilibrium, the arterial:venous differences are expected to be small, and so venous sampling was assumed to be appropriate (Carson, 2000). [¹¹C]P943 was synthesized as previously described (Gallezot et al., 2010; Nabulsi et al., 2010) and administered as a bolus plus constant infusion, with a K_{bol} value (ratio of bolus dose to infusion rate) of 150 min. A study duration of 120 min for baboons and 150 min for rhesus macaque was used. Equilibrium (<5% change/h) was established by 60 min for all regions. A total of 9 PET scans were performed in the three animals.

Each baboon was studied four times with [¹¹C]P943 on two experimental days. On each day, a baseline measurement was followed by a preblock measurement with fenfluramine (1.0 mg/kg, i.v.) or unlabeled P943 (0.2 mg/kg, i.v.) given 5 min before the radiotracer injection. This resulted in 8 h total of anesthesia time, that is, 2-h set up, 2-h baseline scan, 2-h break, and 2-h preblock scan. The rhesus monkey was studied once with [¹¹C]P943 during which a displacement of a higher dose of fenfluramine (5 mg/kg, i.v.) was given over 5 min starting at 75 min after radiotracer injection. This resulted in 4.5 h total of anesthesia, that is, 2-h set up, and a 2.5-h scan. In the current study, experiments in baboons were con-

TABLE I. Experimental design

Animal	Drug	Dose (mg/kg)	No. scans/dy	Time of drug
B1	Fen	1.0	2	Preblock
B1	P943	0.2	2	Preblock
B2	Fen	1.0	2	Preblock
B2	P943	0.2	2	Preblock
M1	Fen	5.0	1	Displace

ducted with a low dose of fenfluramine (1 mg/kg) and, subsequently, a higher dose of fenfluramine (5 mg/kg) was used in an attempt to replicate a recent study in rhesus monkeys (Finnema et al., 2010; Table I). (±)-Fenfluramine was dissolved in saline, and the doses are expressed as relative to the free base.

A minimum of 14 days was allowed between experimental days. The baboon PET scans were performed on the HRRT PET camera (Siemens/CTI, Knoxville, TN), which produces 207 slices with a slice separation of 1.2 mm and has a reconstructed image resolution of ~3 mm. The rhesus PET scan was performed on the FOCUS 220, and a small animal PET scanner with a high resolution of ~1.5 mm at the center field of view. A transmission scan was acquired before radiotracer injection for attenuation correction. Dynamic images of radioactivity concentration were reconstructed with corrections for measured attenuation, normalization, random events, scatter, and dead-time. A magnetic resonance image of each animal was previously obtained to perform coregistration and guide the placement of regions of interest (ROIs). The ROIs were drawn manually on each animal's MR images and included occipital cortex, frontal cortex, cingulate cortex, globus pallidus, caudate, putamen, thalamus, insula, brainstem, and cerebellum (without the vermis).

The initial primary outcome measure examined was the equilibrium volume of distribution (V_T), where V_T is regional brain activity divided by metabolite-corrected plasma concentration. Parent fraction in plasma was determined by high-performance liquid chromatography as described previously (Gallezot et al., 2010). Occupancy plots (Cunningham et al., 2009; Esterlis et al., 2010) were constructed to determine the fraction of available 5-HT_{1B} receptor sites blocked by fenfluramine or unlabeled P943. Occupancy plots use the equation for the line [$y = rx + b$], where $y = [V_T(\text{baseline}) - V_T(\text{preblock or displacement})]$ and $x = V_T(\text{baseline})$, and linear regression of the fit to the brain regions provides receptor occupancy (r) values. This approach assumes uniform receptor occupancy across all brain regions but does not require the use of a reference region devoid of specific binding. Receptor occupancy was derived for each animal across all brain regions for each fenfluramine or unlabeled P943 preblocking (baboon) or fenfluramine displacement (rhesus macaque) scan compared to the

baseline scan. In the baseline and fenfluramine and unlabeled P943 preblocking scans, data obtained at equilibrium (60–90 min) were used. In the fenfluramine displacement study, data from 60 to 75 min were used for baseline, and values from 115 to 135 min were used for displacement.

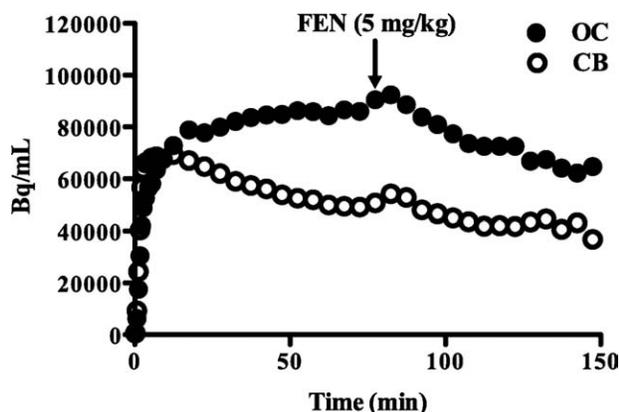


Fig. 1. Radioactivity (Bq/ml) in the occipital cortex (closed symbols) and cerebellum (open symbols) during an infusion of [¹¹C]P943 in rhesus monkey with a displacement of fenfluramine (5 mg/kg) given at 75 min. The "bump" in both curves following FEN is attributed to a transient increase in bioavailability of [¹¹C]P943 due to blockade throughout the body.

TABLE II. Percent occupancy for each animal between baseline and drug conditions

Animal	Fenfluramine (1–5 mg/kg)	P943 (0.2 mg/kg)
B1	25%	68%
B2	29%	86%
M1	42%	–

RESULTS

For the 9 PET scans, the mean (\pm SD) injected dose of [¹¹C]P943 was 249 ± 41 MBq, the mean specific radioactivity was 190.92 ± 75.85 MBq/nmol at time of injection, and the mean injected mass was 0.105 ± 0.046 μ g/kg. Under baseline conditions, [¹¹C]P943 uptake was high in globus pallidus, occipital cortex, thalamus and midbrain, intermediate in striatum, cingulate cortex, frontal cortex and brainstem, and low in the cerebellum.

Although the cerebellum has been proposed as an appropriate reference region for 5-HT_{1B} binding, there was a change in cerebellar [¹¹C]P943 uptake as measured with V_T between the baseline scans and fenfluramine (5% for B1; 21% for B2) or P943 (21% for B1; 23% for B2) preblock scans. Furthermore, in the rhesus infusion study, a fenfluramine-induced displacement of [¹¹C]P943 in the cerebellum (Fig. 1) was detected, and V_T was reduced, with a change of 29%. Hence we calculated receptor occupancy using the occupancy plots. Receptor occupancy by the low dose of fenfluramine (1 mg/kg) in the baboons was 25% and 29% and by the high dose of fenfluramine (5 mg/kg) in the rhesus macaque was 42% (Table II and Fig. 2). Receptor occupancy by P943 (0.2 mg/kg) was 68 and 86% in the baboons, respectively (Table II). The nondisplaceable volume of distribution (V_{ND}) can be estimated by the intercept of the occupancy plot. In the five studies (three with fenfluramine and two with unlabeled P943), V_{ND} was 9 ± 1 ml/cm³.

DISCUSSION

This study demonstrated significant occupancy of 5HT_{1B} receptors as measured with [¹¹C]P943 after

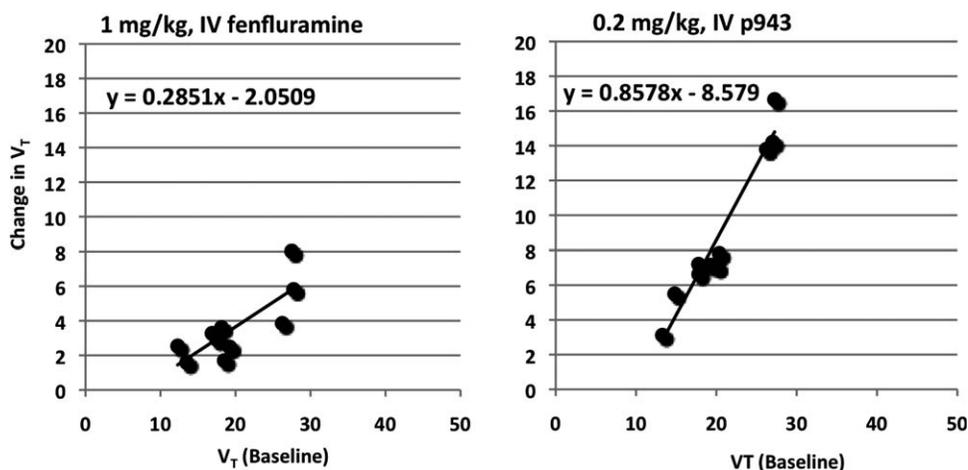


Fig. 2. Occupancy plots are shown for baboon B2 (left panel) for change in V_T between baseline and fenfluramine (1 mg/kg) scans and for baboon B2 (right panel) for change in V_T between baseline and P943 (0.2 mg/kg), both as a function of baseline V_T . Each data point represents a region-of-interest. The slope of these plots represents the 5-HT_{1B} receptor occupancy.

administration of 1 and 5 mg/kg fenfluramine. Fenfluramine causes an increase in endogenous 5-HT levels by directly increasing 5-HT release and by inhibiting reuptake of 5-HT at the 5-HT transporter (Rothman and Baumann, 2002) and has been successfully used in previous studies to produce 5-HT-induced displacement of 5-HT_{1B} (Finnema et al., 2010) and 5-HT_{1A} (Hume et al., 2001; Zimmer et al., 2002) radioligands. A previous dual PET and microdialysis study in awake rhesus monkeys demonstrated 20-fold increases in 5-HT levels in the prefrontal cortex 15 min after administration of 5 mg/kg fenfluramine (Udo de Haes et al., 2006) illustrating that these doses of fenfluramine are sufficient to produce large increases in endogenous 5-HT. Finnema and colleagues (2010) demonstrated average regional decreases of 27 and 50% in [¹¹C]AZ10419369 binding, a 5-HT_{1B} radioligand, after 1 and 5 mg/kg fenfluramine, respectively, in anesthetized cynomolgus monkeys. This is highly consistent with our findings of 25–29% occupancy after 1 mg/kg and 42% occupancy after 5 mg/kg administration of fenfluramine.

Taken together, the most straightforward interpretation of these findings is that changes in 5-HT_{1B} ligand binding are due to fenfluramine-induced increases in endogenous 5-HT, consistent with the competition model (Laruelle, 2000). Alternatively, preclinical evidence suggests that 5-HT_{1B} receptors undergo rapid internalization upon exposure to 5-HT (Janoshazi et al., 2007), and if the radiotracer has a reduced affinity for internalized receptors as has been shown for dopamine D₂ receptors (Guo et al., 2010), this would result in reduced 5-HT_{1B} availability that is not reflective of 5-HT occupancy of the receptor. The extent to which receptor internalization contributes to the measurement of changes in synaptic 5-HT will have to be further examined.

A limitation of this radioligand is that there was a fenfluramine- and P943-induced change in cerebellar 5-HT_{1B} receptor binding. Changes in cerebellar binding were not reported in the previous study using a similar 5-HT_{1B} compound (Finnema et al., 2010) and were not examined in a previous study using the same compound (Nabulsi et al., 2010), because metabolite-corrected input function data were not available. It is generally accepted that the cerebellum is a good reference region for 5-HT_{1B} radioligands as it is thought to be devoid of 5-HT_{1B} receptors. Specifically, in a human autoradiography study, no 5HT_{1B} receptors were detected in the cerebellum (Bonaventure et al., 1997); however, rodent studies have reported moderate 5-HT_{1B} receptor-like immunoreactivity in the deep nuclei of the cerebellum (Sari et al., 1999), and 5-HT_{1B} receptor mRNA has been identified in the Purkinje cell layer of the cerebellum (Bruinvels et al., 1994; Maroteaux et al., 1992). Thus, in this study, we used the occupancy plot, which uses V_T values, instead of binding potential values, which depend on

the use of reference regions. The potential changes in cerebellar binding with this compound will need to be examined in human subjects using occupancy studies before fluctuations in endogenous 5-HT can be reliably measured in humans with reference region methods.

The occupancy plot used in this study has two major assumptions. First, it is assumed that the non-displaceable volume of distribution (V_{ND}) is uniform across brain regions, an assumption commonly applied in receptor imaging studies. Second, the occupancy plot assumes that the fractional receptor occupancy is uniform across brain regions. This latter assumption is reasonable when using an exogenous drug, where brain concentration is assumed to be driven by plasma concentration. However, in this study, it is not at all clear that fenfluramine will produce equal concentrations of 5HT across brain regions. A previous microdialysis study suggests differential fenfluramine-induced 5-HT release between brain regions (Hume et al., 2001). Thus, potential variation in 5-HT level across brain regions may be a contributing factor to the variability found in the occupancy plots (Fig. 2), where each data point represents a different brain region.

In general, the two 5-HT_{1B} receptor ligands, [¹¹C]P943 and [¹¹C]AZ10419369, demonstrate greater sensitivity to changes in synaptic 5-HT levels than ligands selective for the 5-HT_{1A} (Jagoda et al., 2006; Maeda et al., 2001; Udo de Haes et al., 2002) or 5-HT_{2A} (Meyer et al., 1999; Staley et al., 2001) receptors [and see Paterson et al. (2010) for review]. Reasons for the differences are not clear but may be related to differences in receptor subtype affinities, distribution, localization, or clearance rates of the ligands from the tissue to plasma. Also of note, the studies using 5HT_{1B} ligands to date have used anesthetized nonhuman primates, and so the results will have to be replicated in awake animals or human subjects. It may be hypothesized that if anesthesia is dampening neuronal circuits, a larger response may be evoked in conscious human or nonhuman primates. In summary, PET imaging of 5-HT_{1B} receptors with [¹¹C]P943 may be a useful approach for measuring changes in endogenous 5-HT in the living human brain.

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