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Original Article

Initial in vivo PET imaging of 5-HT_{1A} receptors with 3-[¹⁸F]mefway

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Abstract: 4-*trans*-[¹⁸F]Mefway is a PET radiotracer with high affinity for 5-HT_{1A} receptors. Our preliminary work indicated the positional isomer, 3-[¹⁸F]mefway, would be suitable for PET imaging of 5-HT_{1A} receptors. We now compare the in vivo behaviour of 3-mefway with 4-mefway to evaluate 3-[¹⁸F]mefway as a potential 5-HT_{1A} PET radiotracer. Two male rhesus macaques were given bolus injections of both 3- and 4-*trans*-[¹⁸F]mefway in separate experiments. 90 minute dynamic PET scans were acquired. TACs were extracted in the mesial temporal lobe (MTL) and caudal anterior cingulate gyrus (cACg). The cerebellum (CB) was used as a reference region. In vivo behavior of the radiotracers in the CB was compared based upon the ratio of normalized PET uptake for 3- and 4-*trans*-[¹⁸F]mefway. Specific binding was compared by examining MTL/CB and cACg/CB ratios. The subject-averaged ratio of 3-[¹⁸F]mefway to 4-*trans*-[¹⁸F]mefway in the cerebellum was 0.96 for 60-90 minutes. MTL/CB reached plateaus of ~2.7 and ~6 by 40 minutes and 90 minutes for 3- and 4-*trans*-[¹⁸F]mefway, respectively. cACg/CB reached plateaus of ~2.5 and ~6 by 40 minutes and 70 minutes for 3- and 4-*trans*-[¹⁸F]mefway, respectively. The short pseudoequilibrium times and sufficient uptake of 3-[¹⁸F]mefway may be useful in studies requiring short scan times. Furthermore, the similar nondisplaceable clearance in the CB to 4-*trans*-[¹⁸F]mefway suggests the lower BP_{ND} of 3-[¹⁸F]mefway is due to a lower affinity. The lower affinity of 3-[¹⁸F]mefway may make it useful for measuring changes in endogenous 5-HT levels, however, this remains to be ascertained.

Keywords: 5-HT_{1A}, PET, serotonin, mefway

Introduction

N-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-N-(2-pyridyl)-N-(*trans*-4-[¹⁸F]-fluoromethylcyclohexane)carboxamide (4-*trans*-[¹⁸F]mefway) (shown in **Figure 1**) has demonstrated high affinity for the 5-hydroxytryptamine_{1A} (5-HT_{1A}) receptor with similar in vivo kinetics to the commonly used 5-HT_{1A} receptor antagonist [¹⁴C]WAY-100635 and high in vivo stability with no apparent bone uptake in rhesus due to defluorination [1, 2]. Preclinical studies examining the in vivo kinetics of 4-*cis*-[¹⁸F]mefway have shown that the isomeric state of [¹⁸F]FCH₃ on the 4 position of cyclohexane ring significantly altered the

affinity as binding potentials were 13 times lower for 4-*cis*-[¹⁸F]mefway [3]. 4-*Cis*- and 4-*trans*-[¹⁸F]mefway demonstrated near identical cerebellar time courses and plasma clearance, indicating that differences in binding were due to the lower affinity of 4-*cis*-[¹⁸F]mefway for 5-HT_{1A} receptors. Similar results have been observed for 4-*trans*-[¹⁸F]FCWAY and 4-methoxy WAY-100635 analogues that demonstrated 5-HT_{1A} binding had a large dependency on the *cis/trans* isomeric state [4, 5]. Additionally, it was shown that the labeling ¹⁸F at different positions on the cyclohexane ring dramatically altered the receptor-ligand binding affinity [4]. Our initial in vitro work indicated

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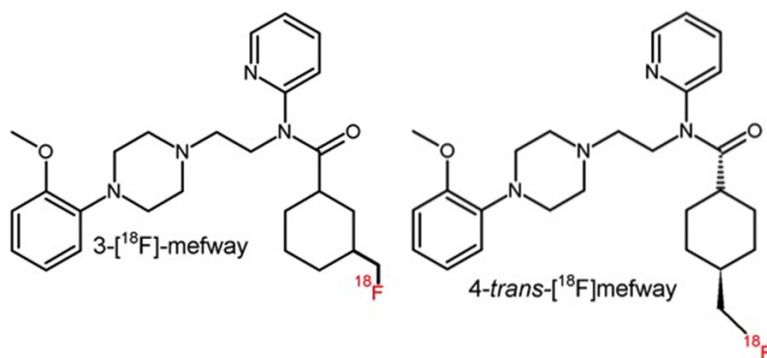


Figure 1. Molecular structure of 3-[¹⁸F]mefway and 4-trans-[¹⁸F]mefway.

3-mefway had approximately 3-fold lower affinity than 4-mefway for the 5-HT_{1A} receptor [6].

Variability in binding affinities and in vivo kinetics in structurally related radioligands can be exploited to carefully match measurement sensitivity to a specific characteristic of a receptor system. For example, the high affinity 5-HT_{1A} radioligand 4-*trans*-[¹⁸F]-mefway possesses favorable in vivo behavior for measuring a high dynamic range of 5-HT_{1A} binding. For detecting alterations in endogenous neurotransmitter concentration at the receptor site, however, lower affinity radioligands may be preferable [7]. Specific to the serotonin system, a 5-HT_{1A} radioligand possessing properties of both fast kinetics and low affinity may allow measurement of acute time varying endogenous 5-HT changes. Thus far, detection of changes in endogenous 5-HT levels using 5-HT_{1A} antagonists and PET imaging have had limited success [8]. Previous studies using [¹⁴C]WAY-100635 have revealed no changes in binding due to manipulation of endogenous 5-HT [9-12]. Other ¹⁸F-labeled analogues of WAY-100635 have also revealed no significant alterations in binding due to changes in 5-HT levels [13]. A radiotracer capable of detecting changes in endogenous 5-HT levels would provide a valuable tool for measuring—in vivo—how drugs, gene, and environment influence 5-HT levels.

The goal of this work was to investigate the properties of a positional isomer of 4-*trans*-[¹⁸F]mefway, 3-[¹⁸F]mefway (**Figure 1**), to determine if it possesses characteristics necessary for PET imaging of the 5-HT_{1A} system. Our preliminary work demonstrated 3-[¹⁸F]mefway would have a suitable 5-HT_{1A} affinity for the use

in PET imaging [6]. Here, we provide initial in vivo imaging results of 3-[¹⁸F]mefway in the nonhuman primate.

Experimental procedure

Precursor and HPLC setup

The 4-*trans*-tosyl-mefway precursor for production of 4-*trans*-[¹⁸F]mefway (N-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-N-(2-pyridyl)-N-(4-*trans*-tosyloxymethyl)cyclohexane) was purchased from Huayi Isotopes Co. The 3-tosyl-mefway precursor was prepared using methods previously described for 4-*trans*-tosyl-mefway [1]. All other reagents for the chemical syntheses were purchased from commercial vendors and were used without further purification (Fisher Scientific, New Hampshire, USA and Sigma Aldrich, Missouri, USA). Purification of 3- and 4-*trans*-[¹⁸F]mefway was performed using reverse phase high performance liquid chromatography (HPLC). Mobile phase consisted of 50:50:0.1 (v/v/v) acetonitrile/water/triethylamine at a flow rate of 5 mL/minute through a Prodigy C18 10 μm 250 × 10 mm ODS Prep column (Phenomenex). Analytic HPLC was performed for quality assurance purposes and consisted of a mobile phase containing 45:55 (v/v) acetonitrile/0.1 M ammonium formate solution at a flow rate of 2.5 mL/minute. The analytic column was a Prodigy C18 5 μm 250 × 4.6 mm ODS3 100 Å column (Phenomenex). The masses of the final products were quantitatively assayed using a UV/Vis absorption detector (Waters model 2489) for estimation of specific activity based upon 4-mefway standard.

Radiochemical synthesis

The radiochemical synthesis of 3- and 4-*trans*-[¹⁸F]mefway was performed utilizing previously described methods [1, 3]. The ¹⁸F was produced by bombardment of protons on H₂¹⁸O using a 16 MeV PETtrace cyclotron. After removal of H₂¹⁸O, the [¹⁸F]F was dissolved in an aqueous solution of Kryptofix 222 and potassium carbonate using a chemistry process control unit. The solution was then dried using azeotropic distillation. Once dry, 1 mg of precursor (either 3-tosyl-mefway or 4-*trans*-tosyl-mef-

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way) dissolved in 0.5 mL of anhydrous acetonitrile was added to the [¹⁸F]F. The reaction proceeded for 10 minutes at 95°C. The reaction was quenched with 3 mL of methanol and subsequently passed through a preconditioned alumina cartridge. The methanol was dried using a rotoevaporator, and the product mixture was then dissolved in 1.5 mL of mobile phase for injection onto preparative HPLC. The product eluted from the column at approximately 10 minutes and was subsequently diluted in 50 mL of H₂O and passed through a C18 Sep-Pak. The Sep-Pak was rinsed with 10 mL of 10% aqueous ethanol. The product was then eluted with 1 mL of ethanol (USP) followed by 9 mL of saline for injection through a 0.22 µm sterile filter (Millipore) into a sterile empty vial. Non-decay corrected radiochemical yields were ~1% for 3-mefway and ~10% for 4-mefway. Separate analytic HPLC measurements performed on the final products of 3- and 4-mefway revealed single radioactivity peaks in each and high specific activities of at end of synthesis (200 and 740 GBq/µmol for 3-[¹⁸F]mefway; 272 and 370 GBq/µmol for 4-*trans*-[¹⁸F]mefway in M1 and M2, respectively).

Subjects and scanning procedures

Two male *Macaca mulatta* (rhesus) (M1: 16.0 years, 10.8 kg; M2: 19.7 years, 9.4 kg) were scanned with both with 3- and 4-*trans*-[¹⁸F]mefway. An MR scan was performed on subject M1. The time between 3- and 4-*trans*-[¹⁸F]mefway PET scanning sessions were 3 and 1 months for M1 and M2, respectively. Prior to scanning procedures, subjects were administered atropine sulfate (0.27 mg im) to reduce secretions during the scan. For PET procedures, subjects were initially anesthetized using ketamine (10 mg/kg), and maintained with isoflurane (0.75-1.5%) for the duration of the scanning session. For MRI acquisition, M1 was anesthetized with 10 mg/kg ketamine, and 0.015 mg/kg dexmedetomidine prior to scan start. Heart rate, respiratory rate, SpO₂ levels, and body temperature were monitored throughout scanning procedures. PET radiotracer was administered in the saphenous vein and a catheter was placed in the femoral artery for acquisition of arterial samples in M1. The scans were acquired with either a Concorde microPET P4 scanner (M1) or a Siemens microPET Focus (M2). The microPET P4 has a field of view of 7.8 cm (axial) by 19 cm (transaxial) and an in-plane spatial resolution of 1.8 mm [14]. The microPET

Focus has a field of view of 7.6 cm (axial) by 19 cm (transaxial) and an in-plane spatial resolution of 1.3 mm [15]. A 518 second transmission scan was acquired using a ⁵⁷Co point source. Upon completion of the transmission scan, collection of 90 minutes of emission scan was initiated with the bolus injection of either 3-[¹⁸F]mefway (75 ± 8 MBq) or 4-*trans*-[¹⁸F]mefway (112 ± 11 MBq). Whole blood samples acquired at 10, 20, 30, 60, and 90 minutes were processed as previously described to obtain measurement of radiometabolites using thin layer chromatography [3]. MRI data was acquired on a 3.0 T X750 GE Discovery scanner with a 3-inch surface coil. Following MRI procedures, 0.15 mg/kg of atipamezole was administered. When the PET scanning session was completed, isoflurane administration was terminated. Upon completion of scanning procedures, subjects were returned to their cages and monitored until fully alert. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Data analysis

The raw list mode data from the microPET P4 and microPET Focus were binned into time frames of 4 × 1 minute, 3 × 2 minutes, and 16 × 5 minutes, applying appropriate corrections for scanner deadtime and random coincidence events. Sinograms were reconstructed using filtered back projection with a 0.5 cm⁻¹ ramp filter applying corrections for attenuation, radioactive decay, and scanner normalization. The final reconstructed microPET P4 images had voxel dimensions of 1.90 × 1.90 × 1.21 mm³ and a total matrix size of 128 × 128 × 63 voxels. The final reconstructed microPET Focus images had voxel dimensions of 0.95 × 0.95 × 0.80 mm³ and a total matrix size of 256 × 256 × 95 voxels. Time activity curves were extracted by drawing circular regions of interest (ROIs) in the high 5-HT_{1A} binding regions of the caudal anterior cingulate gyrus (cACg, 0.9 cm³) and mesial temporal lobe (MTL, 0.43 cm³) (which includes the hippocampus). The cerebellum (CB, 1.73 cm³) was used as a reference region as validated in our previous work [2].

Comparison of nondisplaceable uptake in the CB was performed by examining the ratio of normalized PET uptake (PET concentration × subject mass/injected activity) for 3- and 4-*trans*-[¹⁸F]mefway in each subject separately. Specific binding for 3- and 4-*trans*-[¹⁸F]mefway

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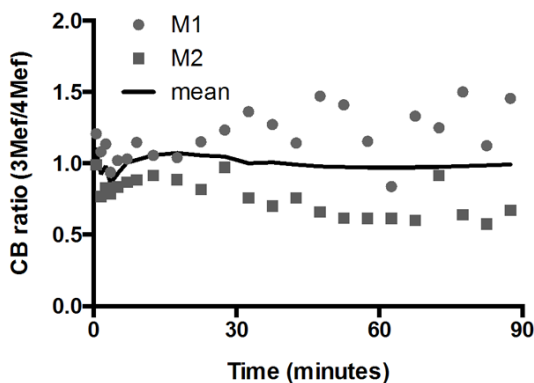


Figure 2. Plot of normalized 3-[¹⁸F]mefway CB/4-trans-[¹⁸F]mefway CB for M1 (circles) and M2 (squares) along with the average (solid line) for the comparison of kinetics in the CB. For calculation of the average, the individual CB uptake curves were modeled as a two-exponential function.

was analyzed by comparing the subject and radiotracer specific ratio of binding in either MTL or cACg to CB.

Results

In vivo metabolism

TLC analysis performed on the ethyl acetate extracted plasma samples drawn at 10, 20, 30, 60, and 90 minutes of M1 revealed one peak eluting with the standard, 3-[¹⁸F]mefway.

Comparison of clearance in the cerebellum

To allow a comparison of nondisplaceable uptake (i.e. regions with negligible specific binding), ratio plots of normalized 3-[¹⁸F]mefway to 4-*trans*-[¹⁸F]mefway uptake in the CB are shown in **Figure 2**. The clearance of 3- and 4-*trans*-[¹⁸F]mefway showed almost identical behavior in this region with negligible specific binding, as the ratio for times of 60-90 minutes averaged 0.96 for the two subjects.

Comparison of uptake in specific binding regions

A comparison of brain uptake (normalized to CB) between 3- and 4-*trans*-[¹⁸F]mefway is shown in **Figure 3**. Higher binding was observed with 4-*trans*-[¹⁸F]mefway in the MTL and cACg. To enhance visualization of the 3-[¹⁸F]mefway binding, thresholding level was decreased and is shown in the right panel of **Figure 3**. 3-[¹⁸F]mefway images were visually assessed for

defluorination and showed no evidence of bone uptake. MTL and cACg to CB ratio plots are shown in **Figure 4**. 3-[¹⁸F]mefway reaches a plateau of about 2.7 by approximately 40 minutes in the MTL and 2.5 by 40 minutes in the cACg. 4-*trans*-[¹⁸F]mefway reached a plateau at approximately 70 minutes in the cACg and 90 minutes in the MTL.

Discussion

Our previous work with 4-*trans*-[¹⁸F]mefway validated its utility as a PET radioligand for studying the 5-HT_{1A} system [2]. The closely matched *in vivo* behavior and affinity for the 5-HT_{1A} receptor of 4-*trans*-[¹⁸F]mefway to the commonly used [¹¹C]WAY-100635 make it an excellent ¹⁸F-labeled alternative, benefiting from a simpler radiosynthesis and a longer lived radiolabel. However, 4-*trans*-[¹⁸F]mefway and [¹¹C]WAY-100635 require intermediate scan times (~90 minutes) to reach a pseudo-equilibrium. Additionally, it is believed that the high affinity of [¹¹C]WAY-100635 for the 5-HT_{1A} receptor may be the source of an insensitivity to detecting endogenous competition with 5-HT evoked by challenges that alter synaptic neurotransmitter concentration [8]. This technique has most successfully been applied to the study of endogenous dopamine release using positron emission tomography, but has not yet been adequately extended to other neurotransmitter systems. Lower affinity radioligands can offer increased sensitivity to detecting changes in endogenous neurotransmitter competition [7]. The goal of this work was to compare the *in vivo* behavior of 3-[¹⁸F]mefway with 4-*trans*-[¹⁸F]mefway and examine the relative affinities for 5-HT_{1A} binding. These studies therefore assess if 3-[¹⁸F]mefway may be a suitable radiotracer for measuring 5-HT changes at the receptor site, however, 5-HT depletion and competition studies will be needed to determine the extent of these effects on binding.

Both 3- and 4-[¹⁸F]mefway exhibited near identical nondisplaceable uptake in the cerebellum as shown in **Figure 2**. However, major differences between specific 5-HT_{1A} binding were found for these positional isomers. Based upon the closely matched nondisplaceable behavior of 3- and 4-*trans*-[¹⁸F]mefway, it can be concluded that lower specific 5-HT_{1A} binding for 3-[¹⁸F]mefway is due to lower affinity and not reduced tissue delivery.

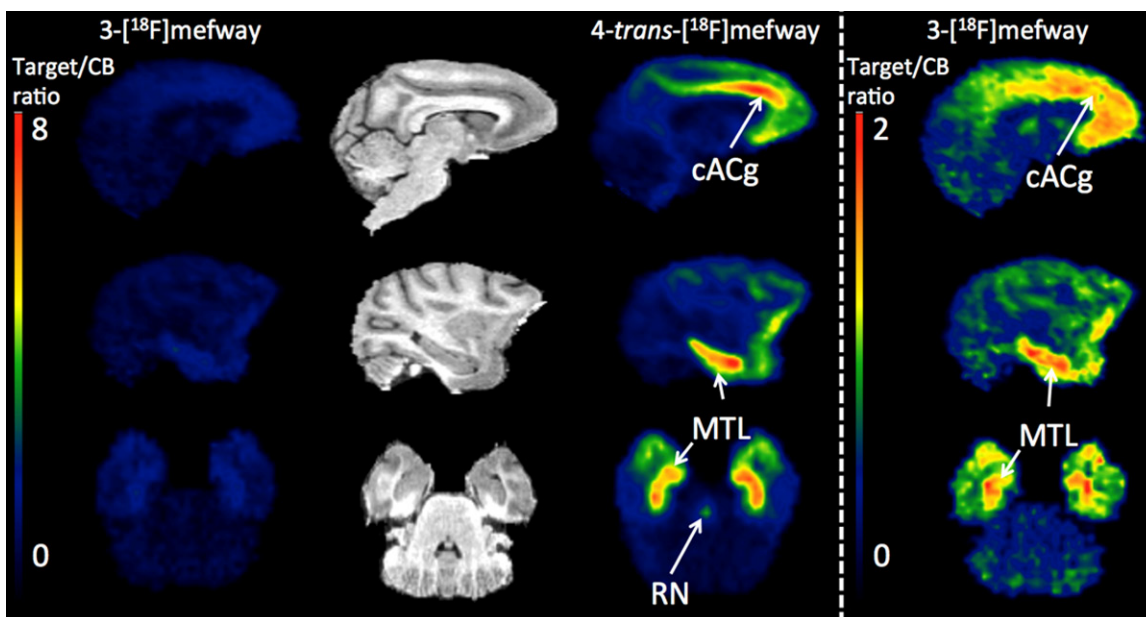


Figure 3. Brain uptake (normalized to CB) of 3-[¹⁸F]mefway and 4-trans-[¹⁸F]mefway at a similar threshold (left) along with corresponding T1 MRIs. (Right) 3-[¹⁸F]mefway with the threshold adjusted to enhance visualization of binding.

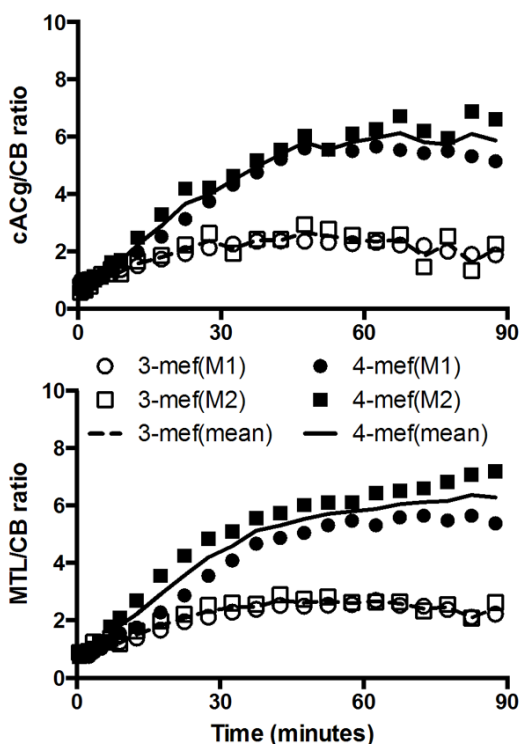


Figure 4. MTL (bottom) and cACg (top) to cerebellum ratio plots for 3-mefway (open symbol) and 4-mefway (solid symbol) for M1 (circle) and M2 (square) along with within radioligand average (lines). Ratios were calculated using subject and radiotracer specific CB uptake modeled by a decaying two-exponential function to minimize noise effects.

The radioligand 4-trans-[¹⁸F]mefway demonstrates a large dynamic range in receptor specific binding, capable of detecting 5-HT_{1A} receptors in lower density regions. 4-Trans-[¹⁸F]Mefway requires approximately 90 minutes to reach the target to cerebellum plateau in high 5-HT_{1A} receptor density regions such as the hippocampus (included in the MTL). Although 90 minutes is a reasonable duration for PET experiments, shorter scanning durations offer several benefits. These include the possibility of several experiments from a single radiotracer batch, reduced scanning burden and discomfort on physically compromised subject populations and lower experimental associated expenses. Our results show 3-[¹⁸F]mefway reaches pseudoequilibrium within approximately 40 minutes in the high receptor density regions of the MTL and cACg. The faster equilibration times and sufficient uptake of 3-[¹⁸F]mefway make it suitable for use in experiments requiring shorter scanning durations.

Previous studies examining kinetic properties of 3-cis-, 3-trans-, 4-cis-, and 4-trans-[¹⁸F]FCWAY demonstrated that the isomeric state and placement of ¹⁸F-label on the cyclohexane ring had significant effects on 5-HT_{1A} receptor specific binding [4]. This work revealed that 4-trans-[¹⁸F]FCWAY yielded the highest hippocampus to cerebellum ratios, which is congru-

ent to our findings with 4-*trans*-[¹⁸F]mefway. Furthermore, these results support our preliminary in vitro analysis indicating 3-mefway has a lower affinity for 5-HT_{1A} receptors [6]. The reason for the large difference in 5-HT_{1A} specific binding of the positional isomers is unclear, however, it may be due to greater steric interactions of the 3-[¹⁸F]mefway isomer in the cyclohexyl ring binding pocket within the 5-HT_{1A} binding site compared to the 4-*trans*-[¹⁸F]mefway isomer. Ultimately, this will decrease the association rate and increase the dissociation rate leading to a lower affinity for 5-HT_{1A} receptors.

The use of two different microPET systems may have introduced additional intersubject variability into our results. Although both high-resolution scanners were designed for nonhuman primate imaging, there was an improvement in spatial resolution (1.3 mm versus 1.8 mm) and detection sensitivity with the newer generation scanner. The same system was used for the paired scan of each subject (i.e. 3- and 4-*trans*-mefway) to minimize intrasubject variability. However, we did not scan the same subject on both scanners and cannot offer insight into potential intersubject differences introduced by the PET scanners.

The radiochemical synthesis of 3-[¹⁸F]mefway produced a much lower radiochemical yield when compared with 4-*trans*-[¹⁸F]mefway. It is possible that the tosylate leaving group at the 3-position of the 3-[¹⁸F]mefway precursor is more sterically hindered when compared to the 4-position. However, this remains to be ascertained. Our previous work with *cis*- and 4-*trans*-[¹⁸F]mefway indicated distinct in vivo properties for the two conformers [3]. Presence of such conformer effects in the case of 3-[¹⁸F]mefway remains to be assessed before a full determination can be made about the usefulness of 3-[¹⁸F]mefway.

Conclusion

The in vivo uptake of 3-[¹⁸F]mefway was compared with 4-*trans*-[¹⁸F]mefway in the nonhuman primate to preliminarily investigate its use as a 5-HT_{1A} receptor antagonist. The shorter equilibration times and sufficiently high binding of 3-[¹⁸F]mefway make it a potential 5-HT_{1A} PET radiotracer for studies requiring a shorter scan time. Furthermore, the lower affinity of 3-[¹⁸F]mefway, as indicated by these results, may make it useful for measuring changes in endog-

enous 5-HT levels. 5-HT depletion and competition studies will be needed to determine the extent of 3-[¹⁸F]mefway's susceptibility to measuring changes in 5-HT levels.

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Disclosure of conflict of interest

None.

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