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ARTICLE

The *Pyridinyl*-6 Position of WAY-100635 as a Site for Radiofluorination—Effect on 5-HT_{1A} Receptor Radioligand Behavior *In Vivo*

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PURPOSE: We aimed to evaluate radiofluorination at the *pyridinyl*-6 position of the selective 5-HT_{1A} receptor antagonist, WAY-100635 [*N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-pyridinyl)cyclohexanecarboxamide)], on 5-HT_{1A} receptor radioligand behavior *in vivo*.
PROCEDURES: The *pyridinyl*-6 [^{18}F]fluoro derivative of WAY-100635 ([^{18}F]6FPWAY) was obtained by direct nucleophilic substitution with [^{18}F]fluoride ion in a bromo precursor. After intravenous injection of [^{18}F]6FPWAY into Cynomolgus monkey, the uptake of radioactivity into brain regions was assessed with positron emission tomography (PET) and blood samples analyzed by high performance liquid chromatography (HPLC) for parent radioligand and radioactive metabolites. The experiment was repeated after pretreatment of the monkey with a dose of WAY-100635 that blocks brain 5-HT_{1A} receptors.
RESULTS: After intravenous injection of [^{18}F]6FPWAY into Cynomolgus monkey, the uptake of radioactivity into whole brain reached 4.33% of injected dose at 7.5 min. Uptake was highest in 5-HT_{1A} receptor-rich regions. Pretreatment with WAY-100635 reduced uptake in these regions to near the levels in receptor-devoid cerebellum. [^{18}F]6FPWAY was rapidly metabolized *in vivo*, as evidenced by the rapid appearance of radioactive metabolites in plasma.
CONCLUSION: [^{18}F]6FPWAY is selective and moderately useful for imaging brain 5-HT_{1A} receptors in vivo. The *pyridinyl*-6 position is resistant to defluorination and may be an attractive site for the ^{18}F-labeling of 6FPWAY analogs that resist hydrolysis. © 2004 Elsevier Inc. All rights reserved.

Key Words: 5-HT_{1A} receptor; Radioligand; Fluorine-18; PET; [^{18}F]6FPWAY; Brain; Monkey.

Introduction

The selective radioligand, [*carbonyl*-^{11}C]WAY-100635 (**1**),[1] is being used extensively with positron emission tomography (PET) in many centers to establish the role of central 5-HT_{1A} receptors in neuropsychiatric disorders and also for drug development (Figure 1).[2] The rapid clearance and metabolism of [*carbonyl*-^{11}C]WAY-100635[3] plus the short half-life of carbon-11 ($t_{1/2}$ = 20.3 minutes) result in low radioactivity in plasma samples, thus hampering the application of kinetic methods for quantitation of the radioligand in brain.[4–9] Radioligands labeled with longer-lived fluorine-18 ($t_{1/2}$ = 109.7 minutes) have an important advantage of enabling more accurate determination of the parent radioligand in plasma throughout the course of a PET scan. An ^{18}F-labeled radioligand also has potential to be distributed to satellite PET centers for multiple applications.

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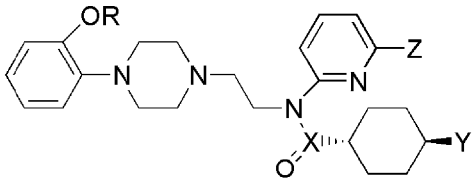


[*carbonyl*-^{11}C]WAY-100635 (**1**): X = ^{11}C, Y = H, Z = H, R = Me.

[^{18}F]*trans*-FCWAY (**2**): X = C, Y = ^{18}F, Z = H, R = Me.

WAY-100635 (**4**): X = C, Y = H, Z = H, R = Me.

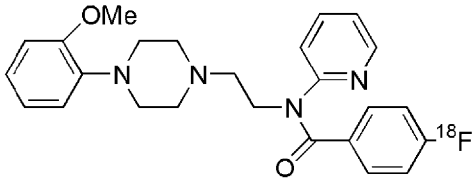
6FPWAY (**5**) X = C, Y = H, Z = F, R = Me.

[*carbonyl*-^{11}C]6FPWAY (**6**) X = ^{11}C, Y = H, Z = F, R = Me.

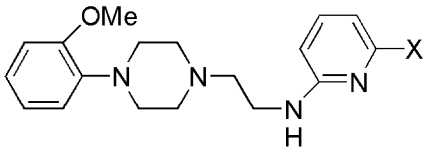
6BPWAY (**7**) X = C, Y = H, Z = Br, R = Me.

[^{18}F]6FPWAY (**9**) X = C, Y = H, Z = ^{18}F, R = Me.

[*O-methyl*-^{11}C]WAY-100635 (**10**): X = C, Y = H, Z = H, R = ^{11}CH$_3$.



[^{18}F]*p*-MPPF (**3**)



descyclohexylcarbonyl-6FPWAY (**8**): X = F

[^{18}F]*descyclohexylcarbonyl*-6FPWAY (**11**): X = ^{18}F

WAY-100634 (**12**): X = H

Figure 1. Structures of 5-HT$_{1A}$ receptor ligands and related compounds.

There are currently two ^{18}F-labeled radioligands in use with PET for imaging human brain 5-HT$_{1A}$ receptors, namely [^{18}F]*trans*-(4-fluorocyclohexane carboxylic acid (2-(4-(2-methoxyphenyl)-piperazin-1-yl)ethyl)-pyridin-2-yl-amide) ([^{18}F]*trans*-FCWAY) (**2**)[10–12] and [^{18}F](4-fluorobenzoic acid (2-(4-(2-methoxyphenyl)-piperazin-1-yl)ethyl)-pyridin-2-yl-amide) ([^{18}F]*p*-MPPF) (**3**).[13–20] [^{18}F]*trans*-FCWAY can be obtained by a multistep radiosynthesis from cyclotron-produced no-carrier-added (NCA) [^{18}F]fluoride ion, but in low radiochemical yield.[10] Automation of this radiochemistry is also difficult. Nucleophilic displacement in a tosylate precursor with [^{18}F]fluoride ion provides a simpler single-step method to prepare [^{18}F]*trans*-FCWAY, but the radiochemical yield is also low.[21] [^{18}F]*trans*-FCWAY provides high receptor-specific signals in monkey and human brain.[11,12] However, there is rapid defluoridation giving rise to high skull uptake of radioactivity that may severely interfere with the ease of quantitation of brain scans.[11,12] [^{18}F]*p*-MPPF can be prepared simply in high radiochemical yield by treating a nitro precursor with NCA [^{18}F]fluoride ion.[14,20] However, evaluation in animals and humans has shown that this radioligand has low brain uptake and gives low receptor-specific signals.[13–20,22,23] It has been suggested that [^{18}F]*p*-MPPF binding to 5-HT$_{1A}$ receptors might be sensitive to endogenous 5-HT levels, as evidenced by studies in animals,[24,25] but recent work indicates that this is not so for biological circumstances likely to be encountered in human subjects.[26]

We have previously shown that an analogue of WAY-100635 (**4**), bearing a 6-fluoro substituent on the 2-pyridinyl ring (6FPWAY; **5**), displays high affinity for the 5-HT$_{1A}$ receptor *in vitro* (K_i = 0.31 nM, compared to 0.17 nM for WAY-100635) and is a full antagonist.[27] 6FPWAY has been labeled in the carbonyl group by ^{11}C-acylation of the corresponding secondary amine precursor and evaluated for binding to human brain slices

post mortem with autoradiography and to cynomolgus monkey brain *in vivo* with PET.[28] Autoradiography indicated that radioligand binding was highly selective for 5-HT_{1A} receptors. The lipophilicity of 6FPWAY, as indexed by calculated logP value (3.85), is somewhat higher than that of WAY-100635 (3.28),[2,28] but the ability of 6FPWAY to penetrate the blood–brain barrier is similar to that of WAY-100635.[28] In the monkey, after intravenous injection of [*carbonyl*-^{11}C]6FPWAY (**6**), there was a rapid high uptake of radioactivity into brain and high specific uptake of radioactivity in 5-HT_{1A} receptor-rich regions. We have recently shown that 6FPWAY can be readily labeled with NCA fluorine-18.[29] This finding encouraged us to evaluate the effect of radiofluorination of WAY-100635 in the *pyridinyl*-6 position on 5-HT_{1A} receptor radioligand behavior in vivo by experiments in monkey with PET and how this behavior might be influenced by peripheral metabolism.

Materials and Methods

Chemicals

6FPWAY [*N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)-ethyl))-*N*-(2-(fluoro-pyridinyl))cyclohexanecarboxamide, its 6-bromo analogue, 6BPWAY (**7**), and *descyclohexylcarbonyl*-6FPWAY (**8**) were synthesized as described previously.[27] 6BPWAY was sometimes re-purified before use by thin layer chromatography (TLC) (silica gel; CH_2Cl_2-MeOH, 98: 2 v/v). WAY-100635 was prepared as described previously.[30] All other chemicals were purchased and of analytical grade. Columns (μ-Bondapak-C18) for high performance liquid chromatography (HPLC) were purchased from Waters Associates.

Preparation of NCA [^{18}F]6FPWAY (9)

Aqueous NCA [^{18}F]fluoride ion (270 mCi; 9 GBq) was produced by the ^{18}O(p,n)^{18}F reaction on ^{18}O-enriched water using a Scanditronix MC16 cyclotron. The aqueous [^{18}F]fluoride ion was evaporated to dryness in the presence of Kryptofix® 2.2.2 (26 mg; 70 μmol) and potassium carbonate (4.6 mg; 33 μmol). Three cycles of addition and evaporation of acetonitrile (~1 mL) at 110°C ensured complete removal of water. 6BPWAY (5–11 mg; 10–22 μmol) in dry acetonitrile (1 mL) was added to the dry [^{18}F]fluoride ion and heated in a sealed glass vessel at 100°C for 20 minutes. [^{18}F]6FPWAY (retention time, 7.0 minutes) was isolated by HPLC on a μ-Bondapak-C18 column (300 × 7.8 mm o.d.; 10 μm particle size) eluted with acetonitrile-0.01M phosphoric acid (20: 80 v/v) at 2.0 mL/min for four minutes and then at 4.0 mL/min. [^{18}F]6FPWAY (~2% radiochemical decay-corrected yield; ~6 mCi) was formulated for intravenous injection by rotary evaporation of the collected product fraction to dryness, dissolution of the residue in sterile physiological phosphate buffer (pH = 7.4) and filtration through a sterile Millipore filter (0.22 μm) into a sterile vial.

[^{18}F]6FPWAY Analysis

The radiochemical purity of [^{18}F]6FPWAY was determined on a sample of the final formulation with HPLC on a μ-Bondapak-C18 column (300 × 3.9 mm o.d.; particle size, 10 μm) eluted with acetonitrile-0.01M phosphoric acid (35: 65 v/v) at 3 mL/min with eluate monitored for radioactivity (Beckman β-flow detector) and absorbance at 270 nm. [^{18}F]6FPWAY (retention time, 6.9 minutes) was identified by coinjection with authentic 6FPWAY.

PET Experiments with [^{18}F]6FPWAY in Cynomolgus Monkey

The study was approved by the Animal Ethics Committee of Northern Stockholm. A male cynomolgus monkey (9.2 kg) was anaesthetized by repeated intramuscular injection of ketamine-xyalazine [Ketalar®; 1–2 mg/(kg.h)-(Rompun®; 1 mg/(kg.h)] and then positioned in a Siemens ECAT EXACT HR PET camera (resolution: 3.8 mm full width half maximum)[31] so that the transaxial imaging planes of the head were parallel to the cantomeatal line. An apparatus was used to secure a fixed position of the monkey head during the PET measurements.[32] Monkey body temperature was maintained with a thermostatically controlled heating pad. NCA [^{18}F]6FPWAY (0.757 mCi; 28 MBq) was injected into the left sural vein and regional cerebral radioactivity uptake was measured in 3-D mode for up to 60 minutes and corrected for physical decay. Data were displayed as 47 sections with a separation of 3.3 mm. Brain regions of interest (ROI) were drawn on the PET summation images, which represented radioactivity measured from nine minutes after injection to the end of scan. Brain ROI and the whole brain contour were defined *in situ* according to an atlas of cryosected cynomolgus monkey head.[32] Radioactivity was calculated from the sequence of time frames, corrected for physical decay and plotted versus time. In order to calculate the percentage of injected radioligand, the radioactivity concentration in the ROI for the whole brain was multiplied by the brain volume (estimated to be about 65 mL). The calculated value for radioactivity in the brain, as percentage of injected dose, was then divided by the radioactivity injected and multiplied by 100. In a second experiment, [^{18}F]6FPWAY (0.784 mCi; 29 MBq) was injected intravenously at 10 minutes after injection of WAY-100635 (0.5 mg/kg; i.v.) into the same monkey.

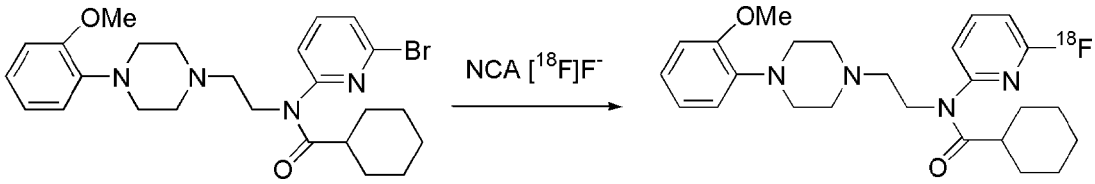


Figure 2. Radiosynthesis of NCA [^{18}F]6FPWAY from [^{18}F]fluoride ion and 6BPWAY.

Measurement of Unchanged [^{18}F]6FPWAY and Radioactive Metabolites in Monkey Plasma

The percentage of radioactivity in plasma as unchanged radioligand was determined by an HPLC method that has been shown to be effective for several other PET radioligands.[33,34] After intravenous injection of [^{18}F]6FPWAY into monkey, arterial blood samples were taken at 5, 25, 35, 45, and 55 minutes during the scan. Each blood sample was centrifuged at 2000 × g for one minute. The supernatant plasma (0.5 mL) was deproteinized with acetonitrile (0.7 mL) that had been prespiked with reference 6FPWAY. The radioactivity of this mixture was measured in a well counter and a portion (1 mL) analyzed by injection onto a gradient HPLC system, a μ-Bondapak-C18 column (300 × 7.8 mm o.d.; 10 μm particle size) eluted at 6.0 mL/min with acetonitrile-0.01M phosphoric acid.[33,34] More than 98% of the radioactivity in the blood sample was recovered in the deproteinized plasma taken for analysis.

Results

Preparation and Analysis of NCA [^{18}F]6FPWAY

The decay-corrected radiochemical yield for the incorporation of NCA [^{18}F]fluoride ion into isolated [^{18}F]6FPWAY (Figure 2) was about 2%. The separation by reverse phase HPLC gave [^{18}F]6FPWAY in greater than 99% radiochemical purity and free of the precursor, 6BPWAY. The overall preparation time was about 45 minutes. NCA [^{18}F]6FPWAY was obtained in useful amounts of radioactivity for monkey experiments (~6 mCi; 220 MBq).

PET Study in Cynomolgus Monkey

The radioactivity in brain peaked at 4.33% of the total dose at 7.5 minutes after injection of NCA [^{18}F]6FPWAY into monkey and then steadily declined (Figure 3). This value was not greatly affected by pretreatment with WAY-100635 (Figure 3). Coronal, horizontal, and sagittal images acquired between 10 and 30 minutes revealed higher radioactivity uptake in many regions known to contain high densities of 5-HT$_{1A}$ receptors (e.g., insula, temporal, and frontal cortex) than in receptor-devoid cerebellum (Figure 4, Panels A–C) and no substantial radioactivity in skull. The time course for radioactivity uptake in cerebellum and certain 5-HT$_{1A}$ receptor-rich brain regions (cingulate gyrus, frontal cortex, hippocampus, insula, parietal cortex, and temporal cortex) are shown in Figure 5A. Cerebellum shows highest uptake of radioactivity at eight minutes, and thereafter

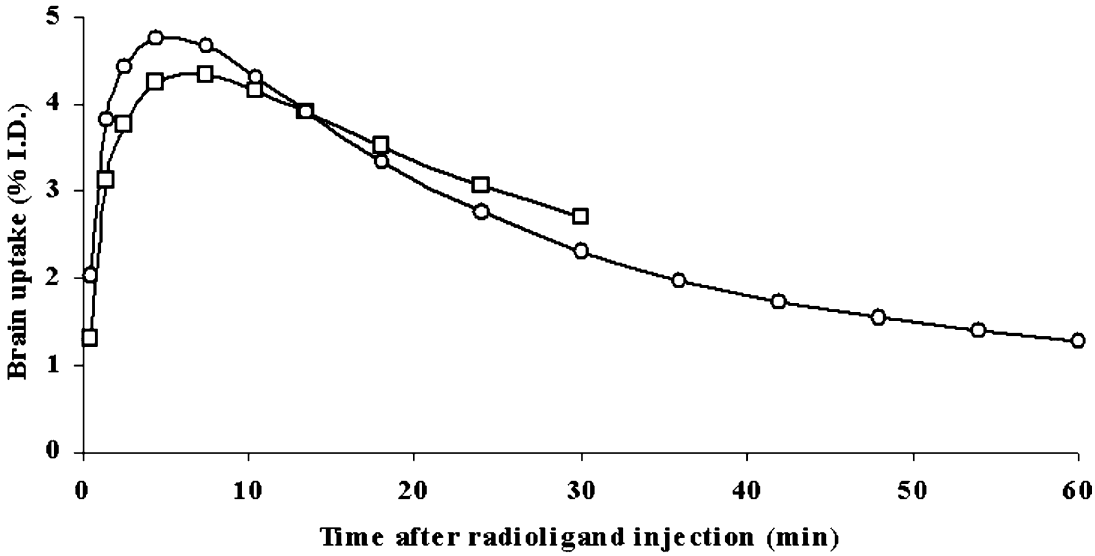


Figure 3. Brain uptake of radioactivity after intravenous injection of NCA [^{18}F]6FPWAY into cynomolgus monkey in the radioligand alone experiment (squares) and in the pretreatment experiment (circles).

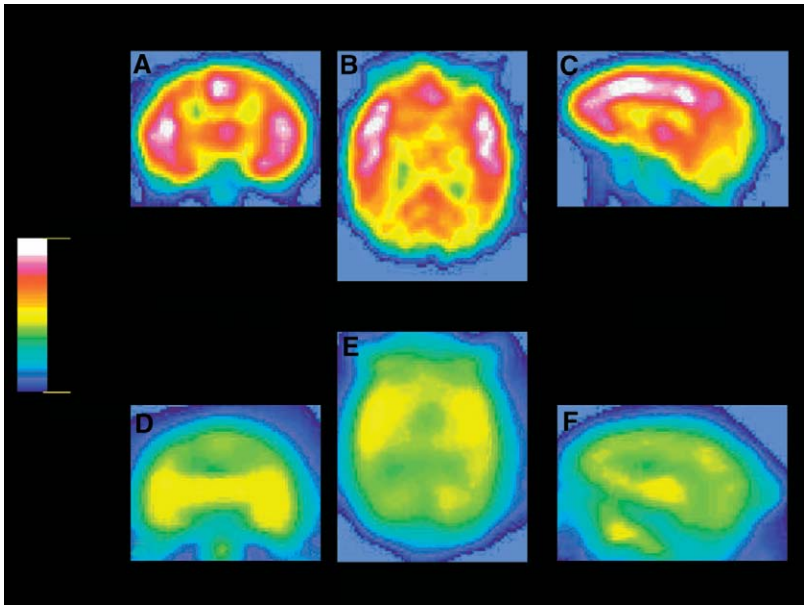


Figure 4. PET scans of cynomolgus monkey brain acquired between nine and 30 minutes after intravenous injection of NCA [18F]6FPWAY in a radioligand alone experiment (panels A–C) and in an experiment where the monkey was pretreated with WAY-100635 (0.5 mg/kg; i.v.) at 10 minutes before radioligand injection (panels D–F). A and D are coronal scans, B and E are horizontal scans taken at the level of the thalamus and striatum, and C and F are sagittal scans at the midline.

a fast clearance of radioactivity. The 5-HT_{1A} receptor-rich regions showed higher peak uptake of radioactivity and slower washout of radioactivity. The time course of the ratios of radioactivity concentration in the same regions compared to that in cerebellum at 30 minutes are shown in Figure 6. The highest ratio (~2) was found in insula at the end of data acquisition (30 min).* All the ratios were generally increasing during the course of the experiment. The ratios of radioactivity concentration compared to that in cerebellum at 30 minutes are shown for all sampled brain regions in Figure 7. The ratios for regions expected to have low densities of 5-HT_{1A} receptors (caudate nucleus, occipital cortex, pallidum, putamen, and thalamus) were low but above unity.

In the second PET experiment, in which WAY-100635 was administered before the radioligand, there was noticeably much less radioactivity uptake in brain 5-HT_{1A} receptor-rich regions as assessed by coronal, horizontal and sagittal PET scans acquired over the period between 10 and 30 minutes after radioligand injection (Figure 4, panels D–F). Acquired kinetic data showed that cerebellum had a very similar uptake and washout of radioactivity to that in the first experiment (Figure 5B). Uptake of radioactivity in 5-HT_{1A} receptor-rich regions (cingulate gyrus, frontal cortex, hippocampus, insula, parietal cortex, and temporal cortex) was also similar to that in the first experiment, but washout of radioactivity was faster, such that at 60 minutes the levels of radioactivity in these regions were almost reduced to that in cerebellum. The ratios of radioactivity in these receptor-rich regions to cerebellum at 30 minutes after radioligand injection were much lower than in the first experiment (Figure 7). Pretreatment with WAY-100635 resulted in slightly reduced ratios for other brain regions. The ratio of whole brain radioactivity concentration to cerebellum radioactivity concentration was also substantially reduced at 30 minutes.

Analysis of Parent Radioligand and Radioactive Metabolites in Plasma

The recovery of radioactivity from blood for analysis was high (>98%). Two main peaks of radioactive metabolites were observed on reverse phase HPLC (Figure 8) and these both eluted before the parent radioligand. One radioactive metabolite (Figure 8, peak B) comigrated with *descyclohexylcarbonyl*-6FPWAY and this represented 19% of radioactivity in plasma at 55 minutes. The percentage of radioactivity in monkey plasma represented by unchanged [18F]6FPWAY decreased rapidly to 19% at 55 minutes (Figure 9).

Discussion

[18F]6FPWAY was produced in satisfactory isolated yield for evaluation in monkey by nucleophilic substitution

*Due to unrecoverable technical failure, data are not available beyond the initial 30 minutes of scanning. However, data from the first 30 minutes of scanning are sufficient for the evaluation of the radioligand and for drawing the main conclusions from the study.

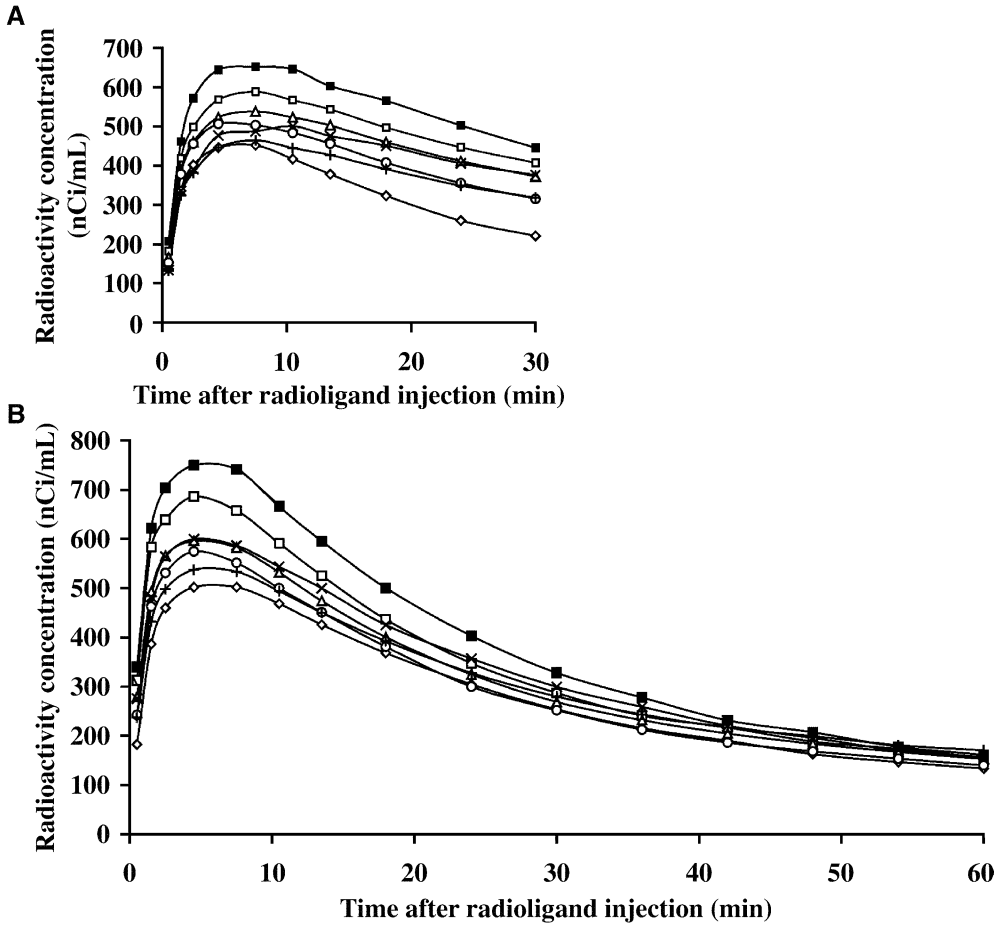


Figure 5. Time-radioactivity (decay-corrected) curves for cerebellum (diamonds) and 5-HT_{1A}-receptor-rich brain regions [cingulate gyrus (open squares); frontal cortex (triangles); hippocampus (x); insula (filled squares); parietal cortex (circles); temporal cortex (+)] after intravenous injection of NCA [^{18}F]6FPWAY in (A) baseline (radioligand alone study) and (B) pretreatment study (WAY-100635 given 10 minutes before radioligand). Data are nonnormalized for injected dose ([0.757 mCi (28 MBq) in A and 0.784 mCi (29 MBq) in B).

in the corresponding bromo precursor (6BPWAY) with NCA [^{18}F]fluoride ion (Figure 2). The decay-corrected isolated radiochemical yield was low, but not optimized in this study. Our separate experimental study [29] has shown that high radiochemical yields (15%–25% nondecay corrected) of [^{18}F]6FPWAY can be obtained reliably by the substitution of either a bromo or a nitro substituent in the 6-position of the pyridine ring of WAY-100635 with NCA [^{18}F]fluoride ion under optimized conditions.

A PET experiment with [^{18}F]6FPWAY in monkey showed that brain uptake of radioactivity was rapid and was followed by a slower clearance of radioactivity (Figure 3). The maximal uptake, 4.33% of the injected dose at 7.5 minutes after radioligand injection, is somewhat lower than that for [*carbonyl*-^{11}C]6FPWAY (8% of the total dose at 10 minutes),[28] but similar to the values reported for [*O-methyl*-^{11}C]WAY-100635 (**10**)[35,36] and [*carbonyl*-^{11}C]WAY-100635.[3] PET scans revealed a distribution of radioactivity composed mainly of the sum of selective binding to brain 5-HT_{1A} receptors plus a level of other binding (Figure 4, panels A–C). Time-radioactivity curves showed higher uptake of radioactivity in several selected 5-HT_{1A} receptor-rich regions than in cerebellum (Figure 5, panel A), indicating a significant degree of 5-HT_{1A} receptor binding. In these regions, the ratio of radioactivity concentration to that in receptor-poor cerebellum reached modest but increasing values (1.4–2.0) at 30 minutes during the PET scan (Figure 6). However, these ratios are somewhat lower than those attained in similar PET experiments with [*carbonyl*-^{11}C]6FPWAY where, for example, ratios peaked at about 3 in temporal and frontal cortex at about 20 minutes after injection[28] and with [^{18}F]*p*-MPPF (hippocampus to cerebellum ratio 3.1 at 30 minutes after injection.[20] The ratios for [^{18}F]6FPWAY are also much lower than values for [^{18}F]FCWAY in monkey (e.g., 8.7 for frontal cortex to cerebellum at 90–120 minutes).[12] In this study the small raphé nuclei, which are rich in 5-HT_{1A} receptors, were not successfully

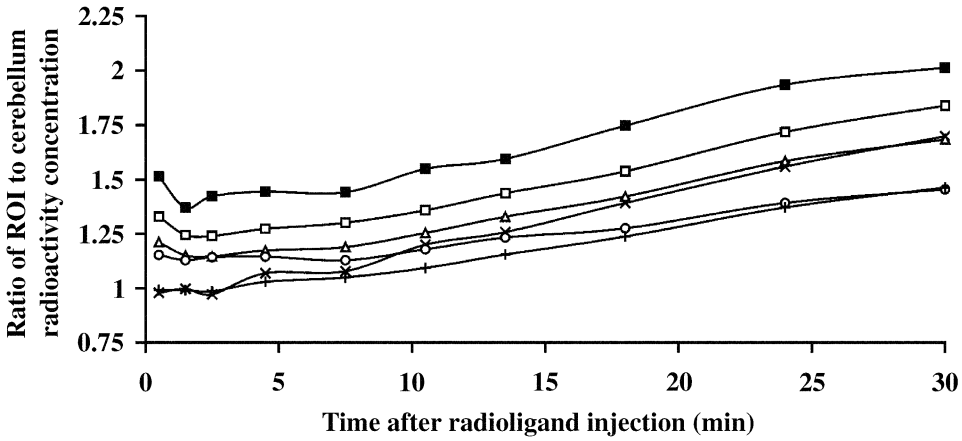


Figure 6. Time course of ratios of ROI radioactivity to cerebellar radioactivity concentration after intravenous injection of NCA [^{18}F]6FPWAY into cynomolgus monkey [cingulate gyrus (open squares); frontal cortex (triangles); hippocampus (x); insula (filled squares); parietal cortex (circles); temporal cortex (+)].

identified for measurements. Receptor-poor regions including, amygdala, brainstem, caudate nucleus, occipital cortex, pallidum, putamen, and thalamus, gave low ratios above unity (Figure 7).

WAY-100635 is highly selective for binding to 5-HT_{1A} receptors[35] and has been shown previously to block the binding of radioligands to 5-HT_{1A} receptors completely in monkey brain at a dose of 0.5 mg/kg i.v.[36] In the second experiment, in which the cynomolgus monkey was predosed with WAY-100635 (0.5 mg/kg i.v.) before [^{18}F]6FPWAY injection, PET scans of the brain acquired between 10 and 30 minutes showed much lower radioactivity than in the experiment where radioligand was given alone. This radioactivity was also more uniformly distributed in the brain. The acquired kinetic data showed there was rapid and high uptake of radioactivity again into brain followed by slow clearance (Figure 3). The uptake and clearance of radioactivity in cerebellum were very similar to that in the first experiment (compare Figure 5A and B), consistent with an absence of significant 5-HT_{1A} receptor binding of radioligand in this region. The early pattern of radioactivity uptake into 5-HT_{1A} receptor-rich brain regions was similar to that in the first experiment (Figure 5B). However, the clearance of radioactivity from these regions was faster, such that by 60 minute radioactivity levels nearly matched the low level in cerebellum (Figure 5B). Hence, the scan and kinetic data show the 5-HT_{1A} receptor-selectivity of a high proportion of bound radioactivity in the experiment in which [^{18}F]6FPWAY was injected alone.

Figure 7 compares the ratios of radioactivity concentration in all selected brain regions to that in cerebellum

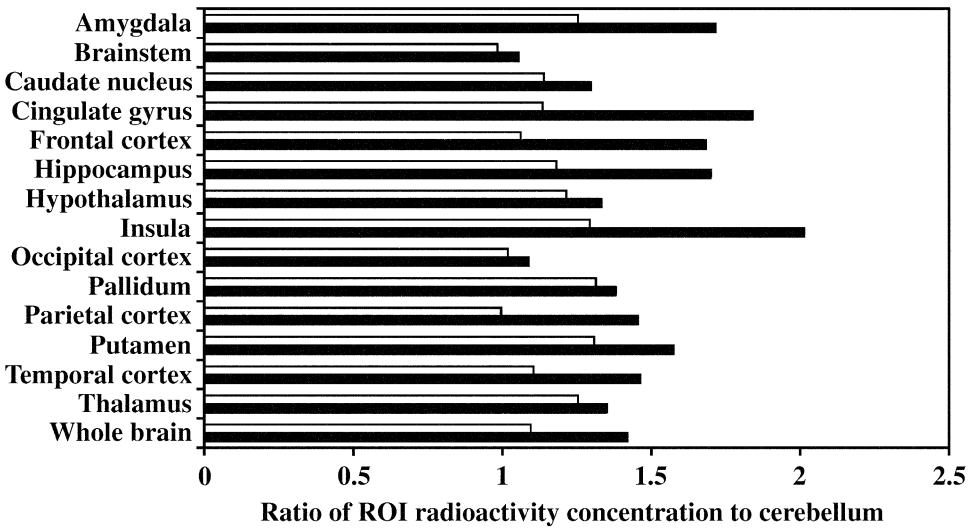


Figure 7. Ratios of radioactivity concentration in brain regions of interest to cerebellar radioactivity concentration at 30 minutes after intravenous injection of a cynomolgus monkey with NCA [^{18}F]6FPWAY in (A) the baseline experiment (filled bars) and (B) the pretreatment experiment (open bars).

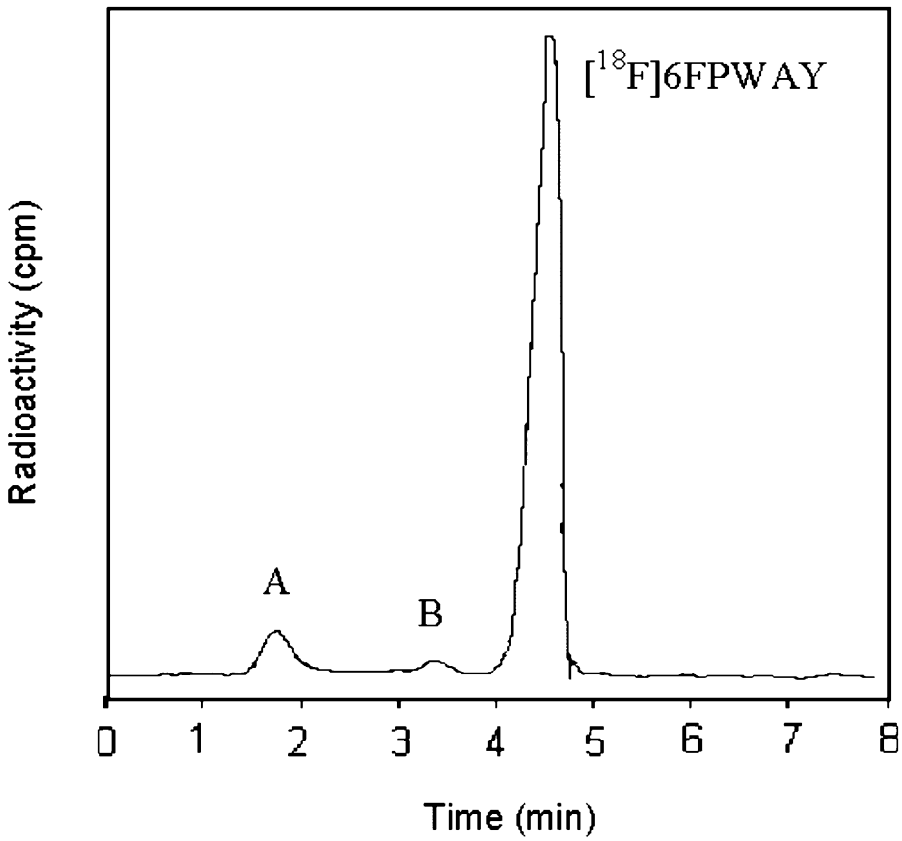


Figure 8. Radio-chromatogram from the reverse phase HPLC analysis of plasma at five minutes after intravenous injection of NCA [^{18}F]6FPWAY into cynomolgus monkey, showing the formation of radioactive metabolites. (A) polar radioactive metabolites; (B) [^{18}F]descyclohexylcarbonyl-6FPWAY.

at 30 minutes for both PET experiments. In the pretreatment experiment compared to the radioligand alone experiment, these ratios in nearly all 5-HT$_{1A}$ receptor-rich regions were lower. However, they were not reduced to unity. The small ratios in many receptor-poor regions also remained slightly above unity in both experiments (Figure 7). This may indicate that the binding of radioactivity after [^{18}F]6FPWAY administration is not highly selective for 5-HT$_{1A}$ receptors, but may also involve some binding to other receptors.

The HPLC analysis of monkey plasma indicates that the rate at which [^{18}F]6FPWAY is metabolized (Figure 9) is somewhat faster than that of [*O-methyl*-^{11}C]WAY-100635,[37,38] indicating that the fluorine atom in the 6-position of the pyridine ring has a small accelerating effect on metabolism. All the major radioactive metabolites are more mobile than the parent radioligand on reverse phase HPLC (Figure 8). The detection of [^{18}F]-*descyclohexanecarbonyl*-6FPWAY (**11**) in plasma shows that amide scission is the major route of metabolism for [^{18}F]6FPWAY, as for [^{11}C]WAY-100635 (**12**)[3,38] and many other analogs.[39–42] By analogy with the des-fluoro compound, (WAY-100634; **9**), which is known to cross the blood–brain barrier easily in monkey[38] and human,[40] [^{18}F] *descyclohexylecarbonyl*-6FPWAY is also likely to cross the blood-brain barrier so elevating nonspecific binding and reducing PET signal contrast. This radioactive metabolite is structurally close to WAY-100634 and would also therefore be expected to bind to 5-HT$_{1A}$ and α_1-adrenoceptors with quite high affinity [I.A. Cliffe & A. Fletcher, personal communication]. Therefore, the lower PET signals obtained with [^{18}F]6FPWAY compared to [*carbonyl*-^{11}C]FPWAY and their lower receptor selectivity, are almost certainly due to the rapid metabolism of [^{18}F]6FPWAY to give brain-penetrant [^{18}F] *descyclohexylcarbonyl*-6FPWAY, which appears as an increasing proportion of the radioactivity in plasma (Figure 9). The contrast in radioligand behavior between [*carbonyl*-^{11}C]6FPWAY and [^{18}F]FPWAY parallels that between [*carbonyl*-^{11}C]WAY-100635 and [*O*-methyl-^{11}C]WAY-100635 and reaffirms the importance of label position with respect to achieving optimal PET signal in radioligands based on WAY-100635; labeling in the ligand on the *N*-side of the amide bond leads to brain-penetrant radioactive metabolites and lower signal (Figure 1).

HPLC analysis of plasma after [^{18}F]6FPWAY administration to monkey shows that very polar radioactive metabolites account for an increasing proportion of

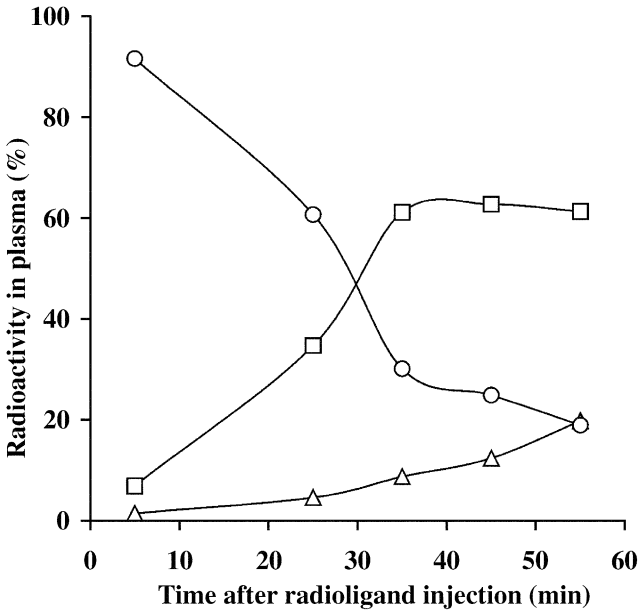


Figure 9. Percentage of radioactivity in cynomolgus monkey plasma represented by [^{18}F]6FPWAY (○), [^{18}F]descyclohexylcarbonyl-6FPWAY (triangles) and polar metabolites (squares) for the first 55 minutes after radioligand injection (baseline experiment).

the radioactivity, reaching 61% of the radioactivity in plasma at 55 minutes (Figure 8). However, all the PET scans of monkey brain obtained with this radioligand (Figure 4) show little or no skull uptake of radioactivity, indicating that rapid defluoridation does not occur. This supports the use of the 6-position of a pyridinyl ring as a metabolically resistant site for the introduction of fluorine-18 into PET radiopharmaceuticals.

Conclusion

The high affinity 5-HT$_{1A}$ receptor antagonist, NCA [^{18}F]6FPWAY, was produced ready for i.v. injection in useful isolated radiochemical yield by single-step radiochemistry. [^{18}F]6FPWAY is moderately effective as an in vivo radioligand in monkey. However, this radioligand is rapidly metabolized, probably primarily by amide scission resulting in a radioactive metabolite, putatively [^{18}F]*descyclohexylcarbonyl*-6FPWAY, that is expected to enter brain. The results reaffirm that labeling with a positron-emitter on the nitrogen side of the amide bond in WAY-100635 is unfavorable to desirable radioligand behavior. A fluoro substituent in the 6-position of the pyridinyl ring appears resistant to rapid defluoridation and this may be a significant advantage for PET imaging. Therefore, nucleophilic substitution of a good leaving group in the *pyridinyl*-6 position of WAY-100635 is a useful approach to labeling compounds of this type with fluorine-18 and would be valuable for labeling WAY-100635 analogs as prospective PET radioligands if coupled with a strategy to block amide hydrolysis *in vivo*.

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