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Synthesis and biological evaluation of ^{18}F -Norfallypride in the rodent brain using PET imaging

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Norfallypride (*N*-[(2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-(3'-fluoropropyl)benzamide), an analog of fallypride, has been synthesized and evaluated as a potential PET imaging agent for dopamine receptors with increased subtype selectivity. In order to synthesize ^{18}F -Norfallypride, the substituted benzamide tosylate (*S*)-*N*-[(1-BOC-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-(3'-tosyloxypropyl)-benzamide) was radiolabeled with ^{18}F using Kryptofix and K_2CO_3 in acetonitrile and deprotected with trifluoroacetic acid to yield (*S*)- ^{18}F -Norfallypride in approx. 10% radiochemical yields. Norfallypride exhibited an IC_{50} of 0.63 μM for displacing ^{18}F -fallypride in rat brain slices. In vitro rat brain autoradiographic studies revealed weak binding of ^{18}F -norfallypride to striatal regions. PET imaging in rats showed low brain uptake of ^{18}F -norfallypride in the rat brain. Ex vivo brain PET analysis displayed binding of ^{18}F -norfallypride in several brain regions. With respect to the cerebellum, ex vivo PET ratios were: striatum > 3; hypothalamus > 2; hippocampus ~ 2; cerebellar nuclei > 2 while autoradiographic ratios were 14, 9, 4 and 6 respectively. ^{18}F -Norfallypride exhibited a unique binding profile to rat brain regions known to contain significant amounts of dopamine D3 and serotonin 5HT₃ receptors. Efforts are currently under way to increase brain permeability and fully characterize the binding of ^{18}F -norfallypride in vivo.

1. Introduction

Fallypride is a substituted benzamide that has been shown to be a good PET imaging agent for dopamine D2 and D3 receptors [1,2]. Dopamine D2-like receptors (D2, D3, D4 and their isoforms) have been found in several brain regions and are known to be involved in nigrostriatal, mesolimbic, mesolimbocortical, and other dopaminergic pathways [3]. These regions include the caudate, putamen, thalamus, hypothalamus, amygdala, nucleus accumbens, substantia nigra, frontal cortex, temporal cortex, including the hippocampus, colliculi (inferior and superior), pituitary, and others [1]. Several efforts have been under way to further differentiate D2-like receptors in vivo using selective PET radiotracers.

Substituted benzamides, which are generally antagonists, have been investigated in order to enhance receptor selectivity between dopamine D2 and D3 receptor subtypes. These

substituted benzamides have a high affinity for D2 and D3 subtypes and poor affinity for the D4 subtype [2,4]. Several other benzamide derivatives have been reported to have selectivity for D3 receptors over D2 [5].

Development of selective antagonists for the D3 receptors has been actively pursued. Both aminotetralin-based as well as dichloropiperazine-based compounds have been reported [6,7]. Additionally, tetrahydroisoquinoline-based compounds have been discovered which have been shown to exhibit high D3 selectivity. Initial attempts are beginning to be made in order to develop PET imaging agents of some of these derivatives.

Selectivity between D2 and D3 receptors has been achieved with agonists such as the 7-hydroxyaminotetralin analogs [8], naphthoxazine (^{11}C -PHNO, [9], references cited therein) and apomorphine analogs (^{11}C NPDA, [10] and ^{11}C -MNPA [11]). However, with antagonists, the ability to obtain selectivity has been difficult. Our molecular modeling studies of the D2 receptor indicated a high degree of homology between the D2 and D3 receptor subtypes which makes the development of selective D3 compounds challenging [12].

It is known from structure–activity relationship studies of substituted benzamides ([13] and references therein) that a small alkyl group ($\text{C}_2\text{--C}_4$) is preferred at the pyrrolidinyl nitrogen for optimal binding to the D2 receptor. The *N*-allyl group in the pyrrolidine nitrogen of ^{18}F -fallypride (Fig. 1, 1) fulfills this requirement. Previously we have prepared cyclopropylmethyl substituted benzamide derivatives in an effort to discriminate the receptor subtypes [14,15]. However, these derivatives did not profoundly affect the selectivity of binding or the in vivo binding properties. In order to assess the effects of the removal of this *N*-allyl group on the properties of ^{18}F -fallypride, we have prepared ^{18}F -norfallypride (Fig. 1, 2). Here we report the synthesis and radiosynthesis of ^{18}F -norfallypride and comparison of its in vitro and in vivo biological properties in the rat brain using PET with ^{18}F -fallypride.

2. Materials and methods

2.1. General methods

All chemicals and solvents were of analytical or HPLC grade from Aldrich Chemical Co. and Fisher Scientific. Electrospray mass spectra were obtained on a Model 7250 mass spectrometer (Micromass LCT). Proton NMR spectra were recorded on a Bruker OMEGA 500 MHz spectrometer. Analytical thin layer chromatography (TLC) was carried out on silica coated plates (Baker-Flex, Phillipsburg, NJ). Chromatographic separations were carried out on preparative TLC (silica gel GF 20×20 cm 2000 μm thick; Alltech Assoc. Inc., Deerfield, IL) or silica gel flash column or semi-preparative reverse-phase columns using the Gilson high performance liquid chromatography (HPLC) systems. High specific activity ^{18}F -fluoride was produced in the MC-17 cyclotron using oxygen-18 enriched water (^{18}O to ^{18}F using p, n reaction). The high specific activity ^{18}F -fluoride was used in subsequent reactions which were carried out in automated radio-synthesis units (chemistry processing control unit (CPCU)). Fluorine-18 radioactivity was counted in a Capintec CRC-15R dose calibrator while low level counting was carried out in a Capintec Caprac-R well-counter. Radioactive thin layer chromatographs were obtained by scanning in a Bioscan system 200 Imaging scanner (Bioscan, Inc., Washington, DC). Rat brain slices were prepared at 10–40 μm thick using a Leica 1850 cryotome. Fluorine-18 autoradiographic studies were carried out by exposing tissue samples on storage phosphor screens (Perkin Elmer Multisensitive, Medium MS). The apposed phosphor screens were read and analyzed by OptiQuant acquisition and analysis program of the Cyclone Storage Phosphor System (Packard Instruments Co., Boston, MA). A preclinical Inveon dedicated PET scanner (Siemens Medical Solutions, Knoxville, TN) with a transaxial FWHM of 1.46 mm, and axial

FWHM of 1.15 mm [16] was used for the PET studies. Both in vivo and ex vivo PET images of rat brains were obtained and analyzed using ASIPro VM software. All animal studies were approved by the Institutional Animal Care and Use Committee of University of California-Irvine.

2.2. Synthesis of Norfallypride

2.2.1. (S)-N-[(1-BOC-2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-hydroxypropyl)benzamide 5

In order to synthesize norfallypride the reaction scheme described in Fig. 2 was followed. The 2,3-dimethoxy-5-(3'-hydroxypropyl) benzoic acid 3 (53 mg; 0.22 mmol) and *N*-[(1-BOC-2-pyrrolidiny)methyl]amine 4 (55 mg, 0.28 mmol) were dissolved in anhydrous acetonitrile (1 mL). To this was added triethylamine (0.12 mL) and subsequently BOP (124 mg, 0.28 mmol; benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate) was added and the reaction was stirred overnight. Solvents were removed in vacuo and the residue was taken up in dichloromethane and washed with water and saturated NaHCO₃. The organic portions were pooled together, dried (MgSO₄), filtered, evaporated, and purified by preparative TLC (silica gel; CH₂Cl₂:MeOH, 9 :1) to yield 5 (75 mg) as a yellow oil of (S)-*N*-[(1-BOC-2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-hydroxypropyl)benzamide. NMR (CDCl₃), 500 MHz) δ ppm: ¹H NMR δ 7.52 (br, 1H), 7.12-7.16 (d, 1H), 6.90 (d, 1H), 4.85 (br, 1H), 4.30 (t, 2H), 3.87 (s, 6H), 2.60-2.70 (9H), 3.70-1.45 (m, 14H). Mass spectra (m/z, %), 423 ([M + H]⁺, 70%).

2.2.2. (S)-N-[(1-BOC-2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-tosyloxypropyl)benzamide 6

The alcohol 5 (50 mg; 0.12 mmol) in CH₂Cl₂ (1 mL) was treated with triethylamine (20 μ L) and *p*-toluenesulfonyl chloride (23 mg, 0.12 mmol). The reaction vessel was stirred at room temperature for 18 to 20 h. The reaction mixture was washed with saturated NaHCO₃ and water. The pooled organic layers were dried (MgSO₄), filtered, concentrated, and purified by preparative TLC (silica gel; CH₂Cl₂: MeOH, 9 :1) to yield 6 (37 mg; 53% yield) as a colorless oil (S)-*N*-[(1-BOC-2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-tosyloxypropyl) benzamide: NMR (CDCl₃), 500 MHz) δ ppm: ¹H NMR δ 7.80 (br, 1H), 7.35 (dd, 4H, tosyl), 7.12-7.16 (d, 1H), 6.90 (d, 1H), 4.35 (br, 2H), 3.85-3.90 (d, 6H), 2.60-2.70 (9H), 3.70-1.45 (m, 14H). Mass spectra (m/z, %): 577 ([M + H]⁺, 30%).

2.2.3. (S)-N-[(2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-fluoropropyl)benzamide 7

The tosylate 6 (29 mg, 0.05 mmol) was dissolved in THF (1 mL). The solution was treated with tetrabutylammonium fluoride (TBAF), 1.0 M solution in THF (0.5 mL, 0.5 mmol) and heated at 60 °C for 2 h and cooled. The reaction mixture was concentrated to dryness in vacuo and the residue was then purified by flash column chromatography (silica gel DCM/MeOH, 95:5) providing (S)-*N*-[(1-BOC-2-pyrrolidiny)methyl]-2,3-dimethoxy-5-(3'-fluoropropyl)benzamide (95 mg, 60% yield) as a colorless oil (Mass spectra (m/z, %): 426 ([M + H]⁺, 100%). Deprotection of *N*-BOC fluorinated derivative was carried out by treatment with trifluoroacetic acid (TFA). The *N*-BOC derivative was dissolved in dichloromethane (2 mL) and the solution was cooled at 0 °C. The reaction solution was treated with TFA (0.3 mL) with stirring. The reaction solution was stirred at 0 °C for one hour followed by room temperature for 30 min. The reaction mixture was concentrated to dryness. The residue was neutralized with 1 N NaOH to pH 11 and the aqueous layer was extracted with dichloromethane, dried (MgSO₄), filtered, and concentrated to give crude norfallypride 7. Purification using preparative TLC provided pure product 7 (28 mg, 50%). NMR (CDCl₃), 500 MHz) δ ppm: ¹H NMR δ 8.35 (br, 1H), 7.55 (s, 1H), 6.85 (s, 1H), 4.45 (dt, 2H), 3.87 (s, 6H), 3.70-1.45 (m, 14H). MS, m/z 325 (100%, [M + H]⁺).

2.3. Radiosynthesis of ^{18}F -Norfallypride

The radiosynthesis of ^{18}F -norfallypride was conducted in the chemistry process control unit (CPCU). Hydrogen ^{18}F -fluoride in H_2^{18}O from the MC-17 cyclotron was passed through light QMA sep-pak (Waters Corp.), preconditioned with 3 mL of potassium carbonate, 140 mg/mL, followed by 3 mL of anhydrous acetonitrile. The trapped ^{18}F in QMA was eluted with 1 mL of Kryptofix 2.2.2 (Aldrich)/potassium carbonate solution (stock solution of 360 mg Kryptofix and 75 mg potassium carbonate in 24 mL acetonitrile and 1 mL water) and transferred to the reaction vessel in the CPCU. The SYNTH1 program was used for the synthesis that involved an initial drying step of the ^{18}F -fluoride, Kryptofix 2.2.2., and K_2CO_3 mixture at 120 °C for 10 min. The ^{18}F solution was further dried with acetonitrile (2 mL) at 120 °C for 7 min. Dried ^{18}F was treated with the precursor 8 (2 mg) in acetonitrile (0.5 mL). This solution was heated at 96 °C for 30 min and cooled.

Radiosynthesis of ^{18}F -norfallypride took two steps (Fig. 3A). As described above, the first step was the nucleophilic displacement of the tosylate group in norfallypride tosylate 8 ((*S*)-*N*[(1-BOC-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-(3'-tosyloxypropyl)benzamide) by ^{18}F ion. The crude product *N*-BOC- ^{18}F -norfallypride was transferred out of CPCU using dichloromethane (5 mL). The dichloromethane containing *N*-BOC- ^{18}F -norfallypride was evaporated in vacuo for the deprotection step. Purity of an aliquot of this intermediate *N*-BOC- ^{18}F -norfallypride was performed by HPLC using an Alltech C_{18} column (10 μm , 250 $\times$ 10 mm) and UV detector (254 nm), mobile phase: 60% acetonitrile–40% 0.1% triethylamine, flow rate 2.5 mL/min, r.t. = 21 min (Fig. 3B).

The above *N*-BOC- ^{18}F -norfallypride 9 intermediate was taken in dichloromethane (1 mL) and trifluoroacetic acid (0.2 mL). The solution was heated at 80 °C for 30 min and evaporated to dryness. The residue was neutralized with 10% NaHCO_3 solution to pH 6–7. Semipreparative HPLC purification was performed using an Alltech C_{18} column (10 μm , 250 $\times$ 10 mm) and UV detector (254 nm), ^{18}F -Norfallypride was made in modest yields (10% decay corrected) in specific activities of $> 2 \text{ Ci}/\mu\text{mol}$. The collected fraction was taken to near dryness in vacuo. The final formulation was carried out using approx. 5 mL of saline (0.9% NaCl INJ) followed by filtration through a membrane filter (0.22 μm) into a sterile dose vial.

2.4. In vitro studies

Sprague–Dawley rats (250–300 g) were anesthetized and decapitated; the brain was rapidly removed and frozen in isopentane at -20°C . Horizontal slices were cut 10 μm thick using a Leica 1850 cryotome. Slices contained regions known to have dopamine receptors which include the striata, hippocampus, cortex and cerebellum as a reference region. Brain slices were preincubated in Tris buffer (50 mM Tris HCl, 2.5 mM CaCl_2 , 125 mM NaCl, 1 mM MgCl , 5 mM KCl, 0.1 mM sodium ascorbate, pH 7.4) at room temperature for 10 min. The slices were then incubated with 1–2 $\mu\text{Ci}/\text{mL}$ of ^{18}F -norfallypride or ^{18}F -fallypride at 37 °C for 1 h. Competition of ^{18}F -fallypride with various concentrations of norfallypride was carried out to assess binding affinity of norfallypride. Nonspecific binding was measured in the presence of 10 μM sulpiride. After incubation, slides were washed twice (each wash lasting one minute) with ice-cold buffer. Slides were then quickly dipped in cold deionized water, air dried, and exposed to a phosphor screen for 24 h. The autoradiographs were generated using the Phosphor Cyclone Imager. The amount of binding was evaluated in digital light units (DLU/mm^2) using OptiQuant acquisition and analysis program (Packard Instruments Co.).

2.5. MicroPET ^{18}F -Norfallypride studies

Male Sprague–Dawley rats (250–300 g) were fasted 24 h prior to time of scan. On the day of the study, rats were anesthetized using 4.0% isoflurane. The rat was then positioned on the scanner bed by placing it on a warm-water circulating heating pad and anesthesia applied using a nose-cone. A transmission scan of 20 min duration was subsequently acquired using the Co-57 source for attenuation correction. Preparation of dose injection was as follows: 0.7–1.0 mCi of ^{18}F -norfallypride was drawn into a 1 mL syringe with a 25 gauge needle and diluted with sterile saline to a final volume of 0.3 mL. The dose was injected intravenously into the tail vein of the rat. Isoflurane was reduced and maintained at 2.5% following injection. Scans were carried out for 120 min and acquired by the Inveon MicroPET in full list mode. List mode data were collected dynamically which were rebinned using a Fourier Rebinning algorithm. The images were reconstructed using Fourier rebinning and 2-dimensional filtered back-projection (2D FBP) method (ramp filter and cutoff at Nyquist frequency) with an image matrix of $128 \times 128 \times 159$, resulting in a pixel size of 0.77 mm and a slice thickness of 0.796 mm. Calibration was conducted to Bq/cc units using a Germanium-68 phantom which was scanned in the Inveon MicroPET and reconstructed under the same parameters as the subjects. Analyses of all data were carried out using Acquisition Sinogram Image Processing IDL's virtual machine (ASIPRO VM).

2.6. Ex vivo MicroPET

After completion of the in vivo MicroPET scans, rats were sacrificed and the brain was extracted for ex vivo MicroPET imaging. The whole brain was placed in a hexagonal polystyrene weighing boat (top edge side length 4.5 cm, bottom edge side length 3 cm) and covered with powdered dry ice. This boat was placed securely on the scanner bed and a transmission scan acquired. Subsequently a 60 min emission scan was acquired by the Inveon MicroPET scanner in full list mode. List mode was collected in a single frame and reconstruction of images was similar to the procedure previously described. Images were analyzed using ASIPRO VM software.

2.7. Ex vivo autoradiography

The brain after the MicroPET acquisition in ex vivo MicroPET analysis was removed from the dry ice and rapidly prepared for sectioning. Horizontal sections (40 μm thick) were cut using the Leica CM1850 cryotome, containing brain regions of the striatum, thalamus, hippocampus, cortex, cerebellum and other regions. The sections were air dried and exposed to phosphor films overnight. Films were read using the Cyclone Phosphor Imaging System. Region-of-interest (ROI) of same size was drawn and analyzed on brain regions using OptiQuant software and binding of ^{18}F -norfallypride measured in Digital Light Units/ mm^2 (DLU/ mm^2).

3. Results

3.1. Synthesis

Synthesis of norfallypride was carried out using modifications of procedures used for the synthesis of fallypride [17] by coupling of (*S*)-*N*-[(1-BOC-2-pyrrolidiny)methyl]amine instead of (*S*)-*N*-[(1-allyl-2-pyrrolidiny)methyl]amine with 2,3-dimethoxy-5-(3'-hydroxypropyl)benzoic acid as shown in Fig. 2. This reaction proceeded smoothly in modest yields. Tosylation of the alcohol 5 afforded the tosylate precursor molecule which served to incorporate the unlabeled fluorine in order to prepare norfallypride as well as fluorine-18 for preparing ^{18}F -norfallypride. Nucleophilic displacement of the tosylate 6 with tetrabutylammonium fluoride and subsequent acid deprotection of the N-BOC afforded norfallypride 7 in sufficient yields for biological studies.

3.2. Binding affinity

Since ^{18}F -fallypride labeled dopamine D2/D3 receptors in rat brain slices, competition binding with norfallypride was carried out. Norfallypride displaced ^{18}F -fallypride from the striata uniformly and did not show any regional differences between the nucleus accumbens areas versus the striatum (Fig. 4A). The displacement curve was smooth (Fig. 4B) and the binding affinity (IC_{50}) of norfallypride was found to be $0.63\ \mu\text{M}$ which suggests that norfallypride binds to dopamine receptor with weak affinity than is typically required for in vivo PET imaging.

3.3. Radiosynthesis

Unlike the radiosynthesis of ^{18}F -fallypride which required only one radiolabeling reaction, the radiosynthesis of ^{18}F -norfallypride required two chemical steps (Fig. 3A). The first step was incorporation of the fluorine-18 by radiolabeling the *N*-BOC norfallypride tosylate 8 under conditions that were similar for the ^{18}F -fallypride synthesis. This first step proceeded efficiently and radiolabeled with ^{18}F -Kryptofix- K_2CO_3 in CH_3CN at $96\ ^\circ\text{C}$ for thirty minutes. This *N*-BOC- ^{18}F -fallypride was purified and isolated (60% CH_3CN -0.1% Et_3N in water, flow rate 2.5 ml/min- C_{18} semiprep column; retention time 21 min; Fig. 3B). The pure ^{18}F - *N*-BOC-norfallypride 9 was subsequently deprotected with trifluoroacetic acid (10% in CH_2Cl_2) at $80\ ^\circ\text{C}$ to yield ^{18}F - norfallypride. ^{18}F -norfallypride was made in modest yields (10% decay corrected) in specific activities of $> 2\ \text{Ci}/\mu\text{mol}$.

3.4. In vitro binding

Weak binding of ^{18}F -norfallypride in the striatal regions was observed in rat brain slices (Fig. 5A). Ratio of striata to cerebellum was approx. 1.5 to 2. This is significantly lower compared to the striata to cerebellum ration using ^{18}F -fallypride (> 50 , for ^{18}F -fallypride in Fig. 5B). Sulpiride reduced the binding of both ^{18}F -norfallypride and ^{18}F -fallypride. Some binding of ^{18}F -norfallypride was also observed in the cerebellar midline which was displaced by sulpiride. The small amount of specific binding of ^{18}F -norfallypride in the rat brain was not always consistent and suggested the weak affinity of ^{18}F -norfallypride to dopaminergic sites.

3.5. MicroPET ^{18}F -Norfallypride studies

Brain uptake of ^{18}F -norfallypride was low (Fig.6A–C). Uptake of ^{18}F -norfallypride in the brain reached initial value up to 1600 Bq/cc in the striata which gradually went down to 200 to 400 Bq/cc in 2 h (Fig. 6D). Activity levels in the cerebellum were lower (approx. 50 to 100 Bq/cc). Because of the low activity in vivo other brain regions were difficult to visualize. MicroPET images were normalized to the standard space described by the stereotaxic coordinates via co-registration to an MRI rat template as previously described in our ^{18}F -fallypride rodent studies [18]. The volumes of interest (VOIs) for the different brain regions were spheres approx 2 to 3 mm in diameter. The striatum (Fig. 6B) retained greater levels of activity at the end of the scans compared to the cerebellum, providing striatum to cerebellum ratios of approx > 3 .

3.6. Ex Vivo ^{18}F -Norfallypride studies

After the in vivo scan the brain was excised from the rat and an ex vivo scan revealed several brain areas that had retention of ^{18}F -norfallypride (Fig. 6 E–G). Apart from the striatum, dorsal and ventral, Fig. 6E, hippocampus (Fig. 6F), hypothalamus and cerebellar nuclei (Fig. 6G) were visualized.

Fig. 7A shows ex vivo rat brain PET image of ^{18}F -norfallypride showing binding in numerous brain regions. With respect to the cerebellum, ex vivo PET ratios were: striatum =

3.3; hypothalamus = 2.2; hippocampus = 1.8; cerebellar nuclei = 2.4. Distribution of ^{18}F -norfallypride in vivo was similar to that confirmed in brain slices. Using ex vivo autoradiography, sections of the brain in Fig. 7A were obtained and brain regions were identified by scanning the brain sections (Fig. 7C, D, F, H). Localization of ^{18}F -norfallypride was confirmed in several brain regions and ratios with respect to cerebellum were: striatum = 14; cerebellar nuclei = 6; hypothalamus = 9; dorsal hippocampus = 4.7; ventral hippocampus = 4.0.

4. Discussion

Norfallypride was successfully synthesized using previously reported methods for fallypride synthesis [17]. Radiolabeling to produce ^{18}F -norfallypride was longer, because it involved two reaction steps. The overall radiochemical yield after purification was somewhat lower than radiochemical yields obtained for ^{18}F -fallypride but the specific activity of ^{18}F -norfallypride was similar to that obtained with ^{18}F -fallypride. ^{18}F -Norfallypride was found to be stable for biological studies.

Affinity of norfallypride was weak compared to fallypride for dopaminergic sites and supports the need for the *N*-allyl substituent present in fallypride for optimal binding [13]. Regional selectivity for displacement of ^{18}F -fallypride in rat brain slices was not evident and therefore its selectivity for the D3 receptor subtype, which is more prevalent in the nucleus accumbens, was difficult to ascertain from the in vitro ^{18}F -fallypride displacement study. Binding of ^{18}F -norfallypride in the striatum was low and at times inconsistent and was in keeping with the low to moderate affinity of norfallypride for dopamine receptor sites.

In vivo binding uptake of ^{18}F -norfallypride was low and an extensive amount of activity was unable to penetrate the blood brain barrier. The secondary nitrogen of norfallypride appears to dramatically affect the ability of the radiotracer to permeate the blood brain barrier (BBB) when compared to fallypride in the rat brain [18]. However, the small amount of activity detected in the in vivo brain suggested preferred retention in certain parts of the brain (such as the striatum) compared to the cerebellum.

Whole brain ex vivo PET imaging allowed a better assessment of the localization of ^{18}F -norfallypride in the different brain regions. It became evident that striatal regions, hippocampus, hypothalamus and cerebellar nuclei were some of the brain regions which showed preferential localization and retention of ^{18}F -norfallypride. Although all of these brain regions are known to contain dopamine receptors, both D2 and D3, their abundance varies between each of the regions.

A comparison of ^{18}F -fallypride and ^{18}F -norfallypride measurements of whole brain ex vivo PET was made to ascertain general differences in their distribution pattern (Fig. 8). Binding of ^{18}F -fallypride is strong in the striatal regions and several brain regions such as the colliculi, substantia nigra, brain stem, and others [2]. On the other hand ^{18}F -norfallypride regional localization was different and was more in the dorsal striatum, tapering to the ventral striatum. Significant binding was seen in the hippocampus, hypothalamus and cerebellar nuclei. These findings suggest that by removing the *N*-allyl group from fallypride, the binding pattern of ^{18}F -norfallypride has been significantly altered. The dopaminergic nature of in vivo ^{18}F -norfallypride binding remains to be confirmed. The dopamine D3 receptor selective agent 7-OH-DPAT displaces the striatal binding of ^{18}F -fallypride [19], its effects on ^{18}F -norfallypride were inconclusive.

Although substituted benzamides have been evaluated as dopamine receptor agents [13] another class of substituted benzamides such as zacopride has been studied as potential imaging agents for serotonin 5HT3 receptors [20]. Comparison of the structure of

norfallypride with zacopride (Fig. 9) suggests structural similarities between norfallypride and zacopride. Critical structural features such as the amide linkage, the critical nitrogen (highlighted by arrows in Fig. 9) and the substitution pattern in the aromatic ring suggest that it may be relevant to examine the serotonin 5HT₃ receptor as a potential target for ¹⁸F-norfallypride. Additionally, a possible explanation for low levels of binding during in vivo experiments may also be attributed to the distribution and low density of 5HT₃ receptors in the brain regions [21]. These findings suggest that an amendment to the primary structure of ¹⁸F-norfallypride may be necessary in order to increase its brain permeability which will assist in the in vivo characterization of binding in the brain and its regional selectivity difference compared to fallypride [22].

5. Conclusions

¹⁸F-Norfallypride exhibited a unique binding profile to rat brain regions known to contain significant amounts of dopamine D₃ receptors and serotonin 5HT₃ receptors. Efforts are currently under way to increase brain permeability of ¹⁸F-norfallypride and fully characterize the binding of ¹⁸F-norfallypride to the receptor subtype in vivo.

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1. ^{18}F -Fallypride

2. ^{18}F -Norfallypride

Fig. 1. Comparison of the chemical structures of 1. ^{18}F -fallypride and 2. ^{18}F -norfallypride. Note that norfallypride does not contain the 3-carbon N -allyl group. Other features of the two molecules are identical.

Fig. 2.

Synthesis scheme of Norfallypride 7 (BOP: Benzotriazol-1-yloxy-tris (dimethylamino) phosphonium hexafluorophosphate; Et₃N: Triethylamine; TsCl: p-Toluenesulfonyl chloride; Bu₄NF: Tetrabutylammonium fluoride; KF: Potassium fluoride; TFA: Trifluoroacetic acid; CH₃CN: Acetonitrile; CH₂Cl₂: Dichloromethane; THF: Tetrahydrofuran).

Fig. 3. (A). Radiolabeling of Norallypride Tosylate with ^{18}F -Kryptofix- K_2CO_3 in CH_3CN . (B). HPLC purification of ^{18}F -N-Boc-Norallypride (60% CH_3CN -0.1% Et_3N in water, flow rate 2.5 ml/min-C18 semiprep; retention time 21 min; RA = radioactivity; UV = ultraviolet absorbance at 254 nm).

Fig. 4. (A). In vitro competition of norfallypride (10^{-9} to 10^{-4} M) with ^{18}F -fallypride bound in the rat brain slices. (B). Displacement curve of ^{18}F -fallypride using norfallypride in rat brain slices.

Fig. 5.

(A). In vitro binding of ^{18}F -norfallypride showing binding in the striata (ST) and little binding in the cerebellum (CB). Striatal binding was displaced by 10 μM sulpiride. (B). Comparatively, ^{18}F -fallypride in vitro studies indicated significantly higher binding to the striata (ST) which was displaced by 10 μM sulpiride.

Fig. 6.

(A–C). In vivo PET image slices summed over the period of the 2 hr scan after iv administration of ^{18}F -norallypride (0.8 mCi) under isoflurane anesthesia. Coronal A, horizontal B and sagittal C sections of ^{18}F -norallypride in the rat brain showing uptake in the striatum (ST) and cerebellum (CB). (D). Time–activity curve of ^{18}F -norallypride in the striatum and cerebellum of the rat brain. (E–G). Ex vivo PET images of the excised brain of the same rat showing binding of ^{18}F -norallypride in various brain regions (dorsal striatum (DST), ventral striatum (VST), striatum (ST), hippocampus (HP), hypothalamus (HY), cerebellar nuclei (CN) and cerebellum (CB)).

Fig. 7.
 (A). ^{18}F -Norallypride MicroPET ex vivo rat brain slice, 0.796 mm thick (NA: nucleus accumbens; HP: hippocampus; CN: cerebellar nuclei). (B). Autoradiograph of horizontal brain slice, 0.04 mm thick obtained from ex vivo brain. (C). Brain slice of image B 0.04 mm showing the regions. (D,F,H). Midsagittal to lateral brain slices, 0.04 mm thick showing different brain regions, and (E,G,I). ^{18}F -Norallypride binding in hypothalamus (HY), striatum (ST), cerebellar nuclei (CN), DHP: dorsal hippocampus (DHP), ventral hippocampus (VHP) and cerebellum (CB).

Fig. 8. Ex vivo rat brain MicroPET images of (A). ^{18}F -fallypride and (B). ^{18}F -norallypride obtained 2 h after iv administration of ^{18}F -fallypride or ^{18}F -norallypride (0.7–0.8 mCi) under isoflurane anesthesia. Distribution of the two radiotracers in the entire brain, from dorsal to ventral regions shows distinct differences in the binding in the striatal and extrastriatal regions. ^{18}F -Norallypride exhibited a 10-fold lower uptake in the brain compared to ^{18}F -fallypride.

Fig. 9. Comparison of chemical structures of norfallypride (A) and zacopride (B). Energy minimized structure of norfallypride (C) and zacopride (D). Arrows show similarity of functional groups and overall similarity of their backbone structures.