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Radiosynthesis of [^{18}F]N-(4-Phenylbutyl)-4-(4-fluorobenzoyl)piperidine for Studying Serotonin 5-HT$_{2A}$ Receptors

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Summary

N-(4-Phenylbutyl)-4-(4-fluorobenzoyl)piperidine [4-PBFBP] shows highly selective binding to serotonin 5-HT$_{2A}$ receptors with high affinity. In this study, we prepared [^{18}F]4-PBFBP for *in vivo* study of 5-HT$_{2A}$ receptors in the brain using positron emission tomography (PET). Nucleophilic aromatic displacement of N-(4-phenylbutyl)-4-(4-nitrobenzoyl)piperidine by no carrier added [^{18}F]fluoride which was solubilized by Kryptofix 222 in DMSO produced [^{18}F]4-PBFBP with high specific radioactivity. The product was purified by reversed phase preparative HPLC and was extracted from the collected eluate by SEP-PAK® C18 cartridge prior to formulation. The radiochemical yield of the final product was 15 ± 1.8 % (mean ± S.D. of three experiments) with decay correction and the specific activity was 113 ± 27 GBq/μmol (mean ± S.D. of three experiments) at E.O.S. after a total preparation time of about 160 min. The radiochemical purity of [^{18}F]4-PBFBP was more than 99 %. The regional distribution of [^{18}F]4-PBFBP in mouse brain was also examined.

Key words: Serotonin 5-HT$_{2A}$ receptor, Fluorine-18, Radiolabeling, Positron emission tomography (PET)

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Introduction

The 5-hydroxytryptamine-$_{2A}$ (serotonin; 5-HT_{2A}) receptors plays a role in the regulation of several functions in humans as well as in different neurological and psychiatric diseases (1, 2, 3). Ketanserin, a prototypic 5-HT_{2A} receptor antagonist, has been the most widely used for preclinical pharmacological investigations. It is well known that ketanserin binds not only with 5-HT_{2A} receptors but also with 5-HT_{2C} receptors (previously the 5-HT_{1C} receptors). Owing to the lack of selective ligand for 5-HT_{2A} receptors little is known about its contribution to Alzheimer-type dementia, drug abuse disorders, and schizophrenia. Recently, polymorphism has been described for both 5-HT_{2A} and 5-HT_{2C} receptor genes (4). The availability of selective 5-HT_{2A} receptors radioligands should also make possible the integration of neuroreceptor imaging work with this new research area, pharmacogenetics (5).

Over the past years, several ligands labelled with positron emitters have been prepared for *in vivo* study of 5-HT_{2A} receptors in human brain using positron emission tomography (PET) (6). These include [^{11}C]ketanserin (7), [^{11}C]N-methylspiperone (8), [^{11}C]N-methyl-2-bromolysergic acid diethylamide (9), [^{18}F]spiperone (10), [^{18}F]setoperone (11), [^{18}F]altanserin (12, 13), [^{18}F]RP 62203 (14), [^{18}F]SR 46349B (15) and [^{11}C]MDL 100907 (16). It has been reported that N-(4-phenylbutyl)-4-(4-fluorobenzoyl)piperidine [4-PBFBP], a structurally related analog of ketanserin, binds with 5-HT_{2A} receptors nearly the same affinity (K_i = 5.3 nM) as ketanserin (K_i = 3.5 nM), and that 4-PBFBP binds at 5-HT_{2C} receptors with lower affinity than ketanserin (17). Therefore, it seems that 4-PBFBP is a more suitable ligand than ketanserin for *in vivo* study of 5-HT_{2A} receptors in the brain. In this paper, we report the radiosynthesis of [^{18}F]4-PBFBP and the biodistribution of [^{18}F]4-PBFBP in mouse brain.

Materials and Methods

Materials and equipments

The following drugs were obtained from the following sources: N-(4-phenylbutyl)-4-(4-fluorobenzoyl)piperidine (4-PBFBP) (Tocris Cookson Ltd., Langford, Bristol, U.K.), SR 42888B (Sanofi Recherche, France), ritanserin , SCH 23390, (-)-sulpiride, (+)-MK-801 (Research Biochemicals International Inc., Natick, MA, U.S.A.). Kryptofix 222 and ^{18}O-Enriched (98 %) water were purchased from Merck-Schuchardt Ltd. (Germany) and Enrichment Technologies Ltd. (Rehovot, Israel), respectively. N-(4-Phenylbutyl)-4-(4-nitrobenzoyl)piperidine was not commercially available but was synthesized by a literature procedure (11). Other reagents were purchased commercially.

IR spectrum was recorded as KBr disk on a Horiba FT-210 spectrophotometer. ^{1}H-(270 MHz) and ^{13}C-(67.5 MHz) NMR spectra were measured with a JEOL EX-270 instrument in $CDCl_3$ solution using TMS as internal standard. Melting point was measured on a Buchi melting point measuring apparatus and is uncorrected. High performance liquid chromatography (HPLC; JASCO, Japan) and column (Megapak SIL C18-10 (∅ 7.5 X 250 mm), Finepack SIL C18-10 (∅ 4.6 X 150 mm), JASCO, Japan) were used.

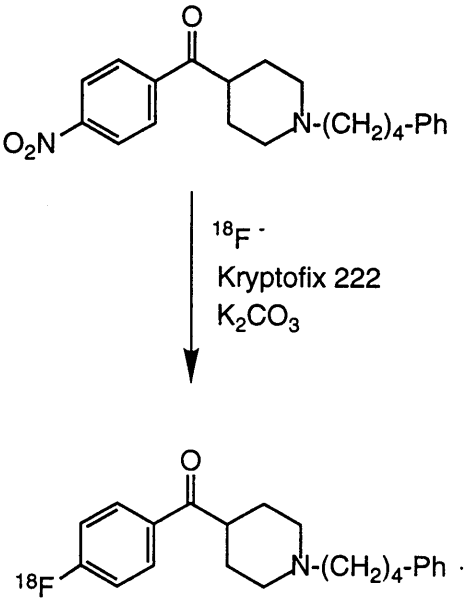

Figure 1. Preparation of [^{18}F]N-(4-phenylbutyl)-4-(4-fluorobenzoyl)piperidine (4-PBFBP)

Synthesis of N-(4-phenylbutyl)-4-(4-nitrobenzoyl)piperidine

A mixture of 4-(4-nitrophenylcarbonyl) piperidine hydrochloride (0.271 g, 1 mmol), 4-phenylbutyl tosylate (0.334 g, 1.1 mmol), and K_2CO_3 (0.304 g, 2.2 mmol) in DMF (5 ml) was stirred at 100-105°C for 14 hours. The reaction mixture was cooled to room temperature and saturated aqueous NaCl solution (50 ml) was added to the reaction mixture. The product was taken up in ether (30 ml x 4). The ether extract was washed with saturated aqueous NaCl solution (30 ml x 3) and dried over anhydrous K_2CO_3. Removal of the solvent gave a crystalline mass, which was extracted with hot hexane (50 ml x 4). The solvent was removed under reduced pressure to leave yellow crystals (0.26 g), which were recrystallized twice from a mixture of ether and hexane to afford pale

yellow crystals (0.108 g, 0.309 mmol, 29.5 %, mp 102-104°C). IR ν: 1680, 1524, 1375 cm^{-1}; ^{1}H-NMR δ: 1.50-1.70 (4H, m), 1.75-1.90 (4H, m), 2.03-2.13 (2H, m), 2.35-2.41 (2H, m), 2.38 (2H, t, *J* 7.5), 2.64 (2H, t, *J* 7.5), 2.99-3.02 (2H, m), 3.19-3.25 (1H, m), 7.16-7.19 (3H, m), 7.20-7.29 (2H, m), 8.06 (2H, d, *J* 8.9), 8.31 (2H, d, *J* 8.9); ^{13}C-NMR δ: 26.6, 28.5, 29.4, 35.8, 44.5, 53.1, 58.7, 124.0, 125.7, 128.3, 128.4, 129.2, 140.8, 142.4, 150.2, 201.1.

Radiosynthesis of [^{18}F]4-PBFBP

N-(4-Phenylbutyl)-4-(4-nitrobenzoyl)piperidine was prepared by the reaction of 4-nitrobenzoylpiperidine and 4-phenylbutyl tosylate. Aqueous [^{18}F]fluoride solution was produced by proton irradiation (18 MeV, 15 μA) of ^{18}O-enriched water (98 %) for 20 minutes using a small cyclotron (CYPRIS-HM18, Sumitomo Heavy Industries, Japan) and a static target chamber. [^{18}F]Fluoride was separated from $H_2{}^{18}O$ by the described method (18). A vial containing Kryptofix 222 (20 μmol, 6 mg), K_2CO_3 (10 μmol, 1.4 mg) and aqueous [^{18}F]fluoride ion was heated under a nitrogen flow at 90°C and any remaining water was removed by evaporation with dry acetonitrile. To the residue was added N-(4-phenylbutyl)-4-(4-nitrobenzoyl)piperidine (20 μmol, 7 mg) in dimethyl sulfoxide (DMSO) (0.5 ml) and the solution was heated for 30 minutes at 140°C. The vial was cooled and the DMSO mixture was diluted with water; the resulting solution was passed through a C18 SEP-PAK plus® cartridge (Millipore, U.S.A.) previously rinsed with ethanol (5 ml) and water (10 ml). After rinsing the cartridge with water (5 ml), [^{18}F]4-PBFBP and the nitro precursor were eluted with ethanol (1.5 ml). Following dilution of the eluate with 1.5 ml of water the whole solution was loaded on a high performance liquid chromatography (HPLC; CH_3OH : THF : 100 mM AcOH/100 mM $AcONH_4$ = 25 : 65 : 510, 5.0 ml/min, UV = 254 nm; retention time for 4-PBFBP = 102 min, retention time for NO_2 compound = 120 min) injector loop and injected on a HPLC column (Megapak SIL C18-10 (∅ 7.5 X 250 mm, JASCO, Japan)). The collected eluate was applied to a SEP-PAK plus® C18 cartridge prewashed as above and the final product that was eluted by ethanol was used in animal experiments. After removal of ethanol, the solution of [^{18}F]4-PBFBP for injection was prepared. The radiochemical purity, chemical purity and specific activity of [^{18}F]4-PBFBP was determined by analytical HPLC (Finepack SIL C18-10 (∅ 4.6 X 150 mm, JASCO, Japan); CH_3OH : 10 mM $(NH_4)_2HPO_4$ = 7 : 3, 1 ml/min, UV = 254 nm, retention time for 4-PBFBP = 19 min) with radioanalyzer (RLC-700, Aloka, Japan).

Animal experiments

Male ICR mice (25 ~ 30 g) were administered approximately 1 MBq of [^{18}F]4-PBFBP in 0.2 mL of saline intravenously *via* a tail vein. Mice were sacrificed by decapitation at seven time points after injection of radioligand, and the brains were quickly removed. To study the effects of several receptor ligands, vehicle (1 ml/kg), ritanserin (1 mg/kg), SR 42888B (5 mg/kg), SCH 23390 (1 mg/kg), (-)-sulpiride (50 mg/kg), and (+)-MK-801 (1 mg/kg) were administered intraperitoneally into mice 15 minutes before intravenous administration of [^{18}F]4-PBFBP. Thirty minutes after injection of [^{18}F]4-PBFBP, mice were sacrificed by decapitation. The frontal cortex, parietal cortex, occipital cortex, striatum, hippocampus, and cerebellum of the brain were grossly dissected. The tissue samples were weighed and radioactivity was measured by gamma counter (BSS-1, Shimadzu Corporation, Kyoto, Japan). The values of each sample were expressed as the percentage of the injected dose per gram tissue (% dose/g tissue).

Results and discussion

The radiolabeling of [^{18}F]4-PBFBP was carried out by the nucleophilic aromatic substitution of an activated nitro group by [^{18}F]fluoride, as shown in figure 1. [^{18}F]Fluoride was produced by the conventional (p, n) reaction on ^{18}O-enriched water. The precursor, N-(4-phenylbutyl)-4-(4-nitrophenyl)piperidine, allowed a one-pot fluorination step in the presence of K_2CO_3 Kryptofix 222 and DMSO using following azetropic drying. Rather high reaction temperature (140°C) and long reaction time (30 minutes) was required for the synthesis. Incorporation of radioactivity into 4-PBFBP ranged from 6 to 55% during preliminary investigations. Unexpected low yield was observed which could in part be due to remaining moisture in the reaction mixture. Lemaire reported that utilization of microwave heating led to higher, more reproducible yields (12), thus, the occasionally low yield with conventional heating might also be attributed to the other origins.

The HPLC purification was carried out as described in the experimental section. Rather long time was necessary for the complete separation of the [^{18}F]fluorinated product using a semi-preparative ODS column although it was eluted prior to the nitro precursor. Formulation of the final product was carried out with SEP-PAK plus® cartridge manifested as the alternative to rotary evaporator (19). Sterile solution of [^{18}F]4-PBFBP in ca. 10% ethanol in physiological saline was obtained with an overall radiochemical yield of 15 ± 1.8 % (mean ± S.D. of three experiments) with decay

correction and a specific activity of 113 ± 27 GBq/μmol (mean ± S.D. of three experiments) after a total preparation time of about 160 min. The radiochemical purity of [^{18}F]4-PBFBP used in the animal experiments was more than 99 %. We also utilized [^{18}F]fluoride from a circulating liquid targetry (20) (NKK, Japan) in the same reaction. We observed that the specific activity of the [^{18}F]fluoride from these two different sources was quite different. [^{18}F]Fluoride from circulating system contained much more cold fluoride, which is expected to be from the decomposition of fluorine containing material within the targetry.

The time course of radioactivity in the several regions of mouse brain after intravenous administration of [^{18}F]4-PBFBP was determined. High radioactivity in the mouse brain 1 minute after intravenous administration of [^{18}F]4-PBFBP was observed, as shown in table 1. Fifteen minutes after injection of radioligand, the maximum peak of radioactivity in the brain regions such as frontal cortex and cerebellum was observed. However, no significant regional differences in the frontal cortex, parietal cortex, occipital cortex, striatum, hippocampus and cerebellum could be determined. The effects of several receptor antagonists on the regional distribution of radioactivity 30 minutes after administration of [^{18}F]4-PBFBP was examined. The regional distribution of radioactivity was not altered by pretreatment with 5-HT$_{2A}$ receptor antagonists (ritanserin (1 mg/kg), SR 46349B (5 mg/kg)), dopamine D$_1$ receptor antagonist SCH 23390 (1 mg/kg), dopamine D$_2$ receptor antagonist (-)-sulpiride (50 mg/kg), or NMDA receptor antagonist (+)-MK-801 (1 mg/kg) (data not shown).

It has been shown that rank of order of density of 5-HT$_{2A}$ receptors in the brain is as follows (high to low): frontal cortex > parietal cortex > occipital cortex > striatum > hippocampus > cerebellum (21, 22). In this study, the radioactivity in the frontal cortex was slightly higher than that of cerebellum, although the difference was not statistically significant. Furthermore, *in vivo* specific binding of [^{18}F]4-PBFBP to 5-HT$_{2A}$ receptors in the mouse brain could not be detected from the pharmacological pretreatment study. As 4-PBFBP inhibits the *in vitro* binding of [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors preparations (17), some amount of cortical uptake of [^{18}F]4-PBFBP should be receptor mediated. The present result could be interpreted as low contrast between receptor specific uptake and nonspecific distribution. This is attributed to a low binding potential of [^{18}F]4-PBFBP in mouse cortex due to the rather low affinity of the compound and/or excess nonspecific distribution because of high lipophilicity. Further detailed studies will be necessary to evaluate this radioligand for *in vivo* labeling of 5-HT$_{2A}$ receptors in the brain.

Table 1. Time course of radioactivity in mouse brain after intravenous administration of [^{18}F]4-PBFPB.

Regions	1 min	5 min	15 min	30 min	60 min	120 min	180min
Frontal Cortex	7.93 ± 2.52	8.03 ± 1.02	9.31 ± 1.29	7.37 ± 1.18	4.82 ± 0.27	3.08 ± 0.90	2.38 ± 0.52
Parietal Cortex	7.71 ± 2.52	8.76 ± 1.86	9.16 ± 1.16	7.65 ± 0.84	4.74 ± 0.50	2.71 ± 0.23	2.41 ± 0.29
Occipital Cortex	8.67 ± 3.59	8.81 ± 1.40	8.54 ± 1.68	7.43 ± 0.88	4.54 ± 0.66	2.70 ± 0.25	2.13 ± 0.24
Striatum	6.97 ± 1.50	7.39 ± 1.75	7.84 ± 0.85	6.86 ± 1.34	4.47 ± 0.10	3.08 ± 1.37	2.31 ± 0.60
Hippocampus	5.97 ± 1.52	6.66 ± 1.52	7.09 ± 1.38	6.25 ± 0.74	4.61 ± 0.44	3.49 ± 1.49	2.77 ± 0.43
Cerebellum	6.78 ± 1.45	7.76 ± 1.28	7.84 ± 1.22	6.09 ± 0.95	4.07 ± 0.14	2.20 ± 0.22	1.97 ± 0.15

Values are the mean ± S.D. of three mice in each point.

In summary, [^{18}F]4-PBFBP with high specific activity could be prepared by nucleophilic substitution of [^{18}F]fluoride on the nitro precursor. In this animal experiments with [^{18}F]4-PBFBP, we could not determine *in vivo* specific binding of this radioligand in the mouse brain. Further detailed studies on the evaluation of this radioligand are necessary.

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